Australian Government
Commonwealth Environmental Water Office

FLOW Monitoring Evaluation Research

Commonwealth Environmental Water Office Monitoring, Evaluation and Research Project:
Lower Murray
2020-21 Technical Report

A draft report prepared for the Commonwealth Environmental Water Office by the South Australian Research and Development Institute, Aquatic Sciences

May 2022

Government of South Australia
Department of Primary Industries and Regions

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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This project was commissioned and funded by Commonwealth Environmental Water Office (CEWO) with additional in-kind support from the South Australian Research and Development Institute (SARDI) and South Australian Department for Environment and Water (DEW). The authors of this report as well as the CEWO respectfully acknowledge the traditional owners of Country in the Murray–Darling Basin, their Elders past and present, their Nations, and their cultural, social, environmental, spiritual and economic connection to their lands and waters. In particular, the Ngarrindjeri Nation and the First Peoples of the River Murray and Mallee as traditional owners of the land and water on which this publication is focused.

Cover photos: microinvertebrate (Cladocera) – the University of Adelaide; Coorong aerial shot – DEW; others – SARDI.

Disclaimer

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ACKNOWLEDGEMENTS

This project was funded by the Commonwealth Environmental Water Office (CEWO) from 2019–2022 with in-kind contributions from the South Australian Research and Development Institute (SARDI) and South Australian Department for Environment and Water (DEW). SARDI provided project leadership, undertook the fish and vegetation components of this study and sub-contracted other components to the University of Adelaide (UoA), Commonwealth Scientific and Industrial Research Organisation (CSIRO), University of Western Australia and Australian Limnological Services (ALS) (Wetland Research and Management, WRM). SA Water and DEW provided water quality data for the indicators of 'Matter Transport and Coorong Habitat' and 'Stream Metabolism and Water Quality'.

Thanks to numerous SARDI staff, including David Fleer, Arron Strawbridge, David Short and Kate Frahn for providing technical assistance in the field or laboratory. SA Water, Goulburn–Murray Water, Water NSW and NSW Department of Primary Industries staff assisted with collecting water samples for $^{87}Sr/^{86}Sr$ analysis. Dr Roland Maas from the University of Melbourne conducted water $^{87}Sr/^{86}Sr$ analysis, while Dr Aleksey Sadekov and Dr Peter Scott from the University of Western Australia (2018–2019) conducted otolith $^{87}Sr/^{86}Sr$ analysis. Dr Sarah Catalano from the SARDI Molecular Sciences Laboratory performed the DNA extraction, polymerase chain reaction (PCR) and gel electrophoresis steps. Positive amplicons were sequenced by the Australian Genome Research Facility (AGRF). Dr Daniel McCullough and Mahdi Montazeri (DEW) provided hydraulic model outputs that support the analysis undertaken for the Hydraulic Regime indicator.

Additional samples of Murray cod larvae and juveniles for analysis in the Murray cod recruitment indicator were accessed from sampling undertaken in the Katarapko, Pike and Chowilla Anabranches. This sampling was funded by the Murray–Darling Basin Authority (MDBA) and DEW.

Thanks to Anthony Moore, Lucy Carlile and Michelle Campbell (CEWO) for providing project management and support to the Lower Murray Selected Area. Jim Foreman and Aftab Ahmad (MDBA) provided detailed flow and environmental flow data. The Lower Murray Selected Area Working Group provided a forum for the exchange of information and intelligence that supported the CEWO MER Project, through effective coordination of environmental watering, and monitoring and evaluation. The authors also thank the Lower Murray Selected Area Working Group including staff from the CEWO, MDBA and DEW for providing most welcome and constructive feedback on the draft report.

EXECUTIVE SUMMARY

From 2019-20 to 2022-23, the Commonwealth Environmental Water Office (CEWO) Monitoring, Evaluation and Research (MER) Project will monitor and evaluate ecological outcomes of environmental water delivery in the Lower Murray, along with six other Selected Areas in the Murray–Darling Basin (MDB). This project, with an integrated research component, extends the monitoring activities of the Long-term Intervention Monitoring Project (2014-15 to 2018-19) and aims to demonstrate the ecological outcomes of Commonwealth environmental water delivery and support adaptive management.

During 2020-21, ~687 GL[a] of Commonwealth environmental water was delivered to the main channel of the Lower Murray River (LMR) in South Australia, in conjunction with ~247 GL of other environmental flows (e.g. the Murray–Darling Basin Authority's The Living Murray Initiative). Overall environmental water comprised 31% of the total annual flow volume to the LMR. Following winter unregulated flows, environmental water was delivered to the LMR from September to mid-December 2020, via coordinated watering events across the southern MDB, with return flows from the Murray, Goulburn, Murrumbidgee and Darling rivers. The water delivery promoted flow variability and contributed to a spring–early summer flow pulse, peaking at ~17,900 ML/d in late November at the South Australian border. Environmental water was delivered to the LMR during summer–late autumn via direct trades, mainly to support continuous flows to the Lakes and Coorong. Environmental water contributed to 71% of the total volume of barrage flows (including fishway releases), while Commonwealth environmental water contributed 65% to the total volume.

Nine indicators were used to evaluate the ecological response to Commonwealth environmental water in the Lower Murray. Three indicators (*Hydrology (channel)*, *Stream Metabolism and Water Quality*, and *Fish (channel)*) primarily aimed to evaluate Basin-scale objectives and outcomes, and in some instances, also local (Selected Area) objectives, following basin-wide standard protocols. Six indicators (*Hydraulic Regime*, *Matter Transport and Coorong Habitat*, *Littoral Vegetation Diversity and Productivity, Microinvertebrate Assemblage, Murray Cod Recruitment* and *Flow-cued Spawning Fish Reproduction*) aimed to address local evaluation questions, using area-specific methods. Additional assessment of the littoral zone soil seed bank was conducted in 2020-21 through contingency monitoring to establish a baseline for future evaluation of the contribution of Commonwealth environmental water to the resilience of littoral plant communities.

Key findings and ecological outcomes

Environmental water delivery contributed to some ecological improvements in the Lower Murray Selected Area in 2020-21:

- **Connectivity**: Environmental water improved (modelled) longitudinal hydrological connectivity via increasing annual flow by 31% in the LMR, meeting the Basin-wide environmental watering target of >30% increase in flow volume in the Murray River (calculated at the SA border). Commonwealth environmental water provided an additional 10,392 kilometre days (km d) of river distance and time period

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[a] Environmental water volumes and percentages provided here for the LMR are sourced from CEWO accounting data and exclude wetland pumping as this investigation focuses on the main channel of the LMR. Percentage contribution of environmental water to barrage flows are results from *Matter Transport and Coorong Habitat* modelling.

characterised by flow velocity >0.2 m/s (an increase of 58%), likely benefiting downstream transport of plankton and fish eggs/larvae, and contributed to lateral connectivity through increasing the maximum inundation area by 1,397 ha in late November 2020.

- **Hydraulic diversity**: Commonwealth environmental water increased the (modelled) duration and extent of 'flowing water' (lotic) habitat, with an extra 31 km (9%) of the LMR characterised by mean water velocities >0.3 m/s for at least 30 days. This hydraulic variability may have benefited native animals (e.g. Murray cod) adapted to lotic riverine environments.
- **Water level variability**: Commonwealth environmental water, in combination with weir pool manipulations, increased (modelled) water level variability (interquartile range[b]) by 0.12 m in the tailwaters (i.e. just downstream of each weir) of the LMR.
- **Littoral vegetation**: Native plant species diversity increased by 42–82% across all reaches following the inundation of littoral zones by spring–early summer flows. The above-ground biomass of understory vegetation also increased by 121–292% due to increased soil moisture, indicating increased productivity. River red gum survival was supported whereby the majority seedlings, geminated in 2019-20, developed into saplings in 2020-21. Variable water levels also produced conditions suitable for the recruitment of specialised riparian species, increasing plant functional diversity.
- **Water quality**: Commonwealth environmental water increased water mixing (velocities >0.2 m/s) and oxygen exchange at the water surface, reducing the potential low dissolved oxygen (DO) period by 52–79 days (varying among reaches) in the LMR. This mainly occurred within the spring–summer period, which corresponds with highest ecosystem respiration rates and the primary reproductive season of many species that generally favour DO >5 mg/L.
- **River productivity**: Primary production, which supports aquatic food webs (e.g. invertebrates and fish), slightly increased (0–3% across sites) in response to the physical changes in the LMR generated by Commonwealth environmental water. This was relatively low because the influence on channel volume is constrained by generally stable weir pool levels in the regulated LMR. However, decomposition rates increased by 5–13%, suggesting increased basal food resources to the river.
- **Microinvertebrates**: There was a 24% (modelled) increase in microinvertebrate density and 9% increase in taxa richness due to Commonwealth environmental water delivery. Further, the density of taxa transported downstream to the LMR increased by 40%, supported by improved longitudinal connectivity. However, the water delivery resulted in density decreases for floodplain/littoral habitat associated taxa (27%) and preferred prey species (40%) of large-bodied native fish larvae in this season.
- **Flow-cued spawning fish**: Substantial numbers of silver perch larvae were collected in the LMR during spring–summer. Silver perch and golden perch spawning, and downstream larval drift, were likely supported by environmental water delivery in the Murray River between Lock 1 and Lock 11 (mid-Murray to the LMR). However, no young-of-year (YOY, age 0+) of either species were detected in the LMR during autumn 2021, suggesting negligible or low-level recruitment, although future sampling will strengthen the assessment.

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[b] Interquartile range (IQR) is a measure of variability, as the difference between the 75th and 25th percentile values for water level over the year. If the IQR increases, the variability must have increased.

- **Murray cod recruitment**: There was poor recruitment of Murray cod in the LMR. During this year, enhanced lotic conditions occurred from late November to mid-December, after the key spawning period. While the increase of river experiencing lotic (velocities > 0.3 m/s) conditions were unlikely to benefit spawning as indicated by low numbers of larvae collected in November 2020, the increase in favourable (lotic) habitat during the larval/juvenile period may have helped support the survival and body condition of new recruits.
- **Fish assemblage**: With low in-channel flows (<18,000 ML/d) in the LMR since the 2016-17 flood, the current (2021) fish assemblage in the main channel represents one typical of low flows, with high abundances of small-bodied species, and a lack of recruitment of native, large-bodied flow-cued spawners. However, there was an increase in abundance of Murray cod, mainly driven by the survival of fish, now age 1+, from the 2019-20 cohort.
- **Barrage flows**: Commonwealth environmental water supported continuous barrage flows (including for fishway operations), which comprised 65% of the total volume in this year.
- **Salt export and reducing import**: Commonwealth environmental water substantially increased (modelled) salt export out of the Basin by ~1.1 million tonnes and reduced salt import into the Coorong estuary by ~4.2 million tonnes. Salt flux into the North and South lagoons was also reduced by ~3.6 million tonnes. These led to reduced salinity levels in the Coorong, which is crucial for maintaining ecosystem health and species diversity.
- ***Ruppia* and fish habitats**: Without Commonwealth environmental water delivery in 2020-21, the suitable habitat area for mulloway, congolli and smallmouth hardyhead would have decreased by 8%, 7% and 6% (modelled), respectively; whereas there was little effect on *Ruppia* habitat. However, the water delivery over four years (2017-18 to 2020-21) led to a 70% (modelled) increase in the suitable habitat area for *Ruppia* sexual reproduction and life-cycle completion in the Coorong.

Key learnings and management implications

- In the highly regulated LMR, environmental water can be used to help reinstate key features of the natural hydrograph to support hydrodynamic and ecosystem restoration; for example, in-channel spring–early summer flow pulses up to 18,000 ML/d have been reinstated during relatively dry years since 2014-15.
- It is increasingly evident that under regulated conditions, reaching and sustaining flows >20,000 ML/d in the LMR is challenging with existing volumes of environmental water and delivery constraints, and may only be possible via coordinating flow deliveries across the southern MDB, and through water delivery that is responsive and flexible to rain events. Under wetter scenarios, flows >20,000 ML/d may be achieved by delivering environmental water in conjunction with unregulated flows.
- In the LMR, increasing flows to >20,000 ML/d significantly improves hydraulic conditions (e.g. increased velocity and water level variability). Weir pool management, particularly lowering, could also complement flows to achieve hydraulic rehabilitation and promote lotic conditions. To inform flow management and maximise ecological outcomes, we need to better understand the effect of specific aspects of flow (e.g. timing, magnitude and duration) on ecological processes and the hydraulic requirements of flow-dependant species.

- Small to moderate increases in flowing water habitat within weir pools and increased riverine connectivity, associated with spring flows of 10,000–18,000 ML/d, supported a range of ecological outcomes in the LMR. This included reach-scale responses like mitigating the risk of low dissolved oxygen in water, benefiting littoral vegetation in tailwaters and Murray cod recruitment, and broader-scale responses like low-level spawning of flow-cued spawning fishes, and downstream transport of fish larvae and microinvertebrates. Notably, such ecological improvements associated with elevated flows may have been important, playing a 'maintenance' role for populations and the LMR ecosystem during periods between natural high flows.
- Larger flow pulses >20,000 ML/d, supported by environmental water, are required to substantially restore riverine characteristics to the LMR and achieve ecological outcomes of greater magnitude (e.g. significant spawning and recruitment of golden perch).
- The timing of flow delivery is important and should continue to align with ecological objectives and consider biological processes and species' life history requirements. To achieve multiple species outcomes, a holistic approach in flow regime design will be required.
- Environmental water delivery that promotes longitudinal and lateral connectivity will enhance productivity in the LMR. Lateral connectivity may increase carbon and nutrient inputs while longitudinal connectivity will help facilitate the transport and dispersal of matter and aquatic biota (e.g. aquatic plant propagules, microinvertebrates, larvae of flow cued spawning species).
- In the regulated LMR, the influence of environmental flows on riverine production is largely restricted by stable water levels regulated by weirs. To improve riverine productivity, water deliveries in conjunction with weir management to promote more natural water level variations are desirable.
- Flow management to increase connection and inundation of littoral habitats, wetlands and floodplain, and provide return flows to the main channel, may enhance food subsidy for riverine species via mobilising microinvertebrates, particularly microcrustaceans, important prey for large-bodied native fish larvae (e.g. Murray cod, golden perch, silver perch). A flow regime that promotes the delivery of abundant prey during and immediately following the spawning season (i.e. mid-October to January) of these fish species, along with favourable hydraulic conditions, are vital for their recruitment.
- Environmental flows are pivotal in maintaining barrage flows and end-of-system connectivity in the MDB, particularly during low flow periods, when there would otherwise be negligible water and matter exchange between the Lower Lakes and Coorong. This is critical for a functioning river system and supporting species' life history processes (e.g. migration of diadromous fish).
- Barrage flows play a key role in salt export out of the MDB and reducing salt import to the Coorong estuary. They also help reduce salt flux into the North and South lagoons. Reducing salt import and flux are essential for reducing salinity levels, maintaining estuarine habitat (e.g. for *Ruppia* and fish), ecosystem functions and biodiversity in the Coorong. Barrage flows also reduce the risk of Murray Mouth closure.

More specific management considerations are provided in Section 2, based on ecological outcomes and findings from indicators.

1 INTRODUCTION

1.1 Flow regimes and riverine ecology

River regulation and flow modification have severely impacted riverine ecosystems throughout the world, including the Murray–Darling Basin (MDB) (Maheshwari *et al.* 1995; Kingsford 2000; Grill *et al.* 2019). The southern MDB is highly regulated, where natural flow regimes have been substantially altered, leading to decreased hydrological (e.g. discharge) and hydraulic (e.g. water level and velocity) variability, and reduced floodplain inundation (Maheshwari *et al.* 1995; Bice *et al.* 2017). The Murray River downstream of the Darling River junction is modified by a series of low-level (<3 m) weirs (Figure 1), changing a connected flowing river to a series of weir pools (Walker 2006). The flow regime has been further exacerbated by upstream diversions and increased extraction. These have had profound impacts on riverine processes and ecosystems (Walker 1985; Walker and Thoms 1993; Wallace *et al.* 2014).

Flow regimes play a critical role in determining the distribution and abundance of native aquatic biota (Koehn *et al.* 2020a; 2020b), and the ecological integrity of floodplain rivers (Junk *et al.* 1989; Poff *et al.* 1997; Bunn and Arthington 2002). Lotic (flowing water) habitats, characteristic of the Murray River before weir construction, are integral to the ecological and life history processes of many native biota that are adapted to flowing riverine environments. For example, they provide stimuli for the spawning of flow-cued species (e.g. silver perch *Bidyanus bidyanus*) (Tonkin *et al.* 2019), facilitate downstream drift and transportation of plankton, microinvertebrates and fish larvae, and provide diverse hydraulic habitats that are suitable for a range of species (e.g. Murray cod, *Maccullochella peelii* and Murray crayfish, *Euastacus armatus*) (Mallen-Cooper and Zampatti 2018). Increased variability in water levels improve lateral connectivity and increase transport of material from off-channel habitats to enhance productivity and support food webs (Baldwin *et al.* 2016), benefit fringing and floodplain vegetation (e.g. Cooling *et al.* 2010), and assist in the regular "re-setting" of biofilms (Steinman and McIntire 1990), which are key components of riverine food webs.

In the MDB, environmental flows have been used to re-establish key features of the natural flow regime (MDBA 2012; Koehn *et al.* 2014; Webb *et al.* 2017). In South Australia, the main channel of the Lower Murray River (LMR) represents a significant ecological asset to be targeted for environmental watering (MDBC 2006; DEWNR 2015). To achieve the greatest ecological benefits from available environmental water, it is important to understand biological and ecological responses to flow regimes. This provides critical knowledge to underpin environmental flow management in the LMR.

1.2 CEWO Monitoring, Evaluation and Research Project

From 2019-20 to 2022-23, the Commonwealth Environmental Water Office (CEWO) Monitoring, Evaluation and Research (MER) Project monitors and evaluates ecological outcomes of Commonwealth environmental water delivery in the MDB. The project was implemented across seven Selected Areas throughout the MDB, including the Lower Murray, to assess and evaluate both Basin-scale and Selected Area (local) responses to environmental flows. The overall aim of this project is to demonstrate the ecological outcomes of Commonwealth environmental water delivery and support adaptive management. The current CEWO MER Project extends the monitoring activities commenced under the 2014–2019 Long Term Intervention Monitoring (LTIM) Project.

In the Lower Murray, the CEWO MER Project focuses on the main river channel between the South Australian border and Wellington (LMR), with one indicator (i.e. Matter Transport and Coorong Habitat) extending to the Lower Lakes and Coorong (Figure 1). The riverine monitoring sites (for indicators) cover three geomorphic zones (floodplain, gorge and swamplands) (Figure 1).

A total of nine indicators were established to assess ecological responses to environmental water delivery in the Lower Murray. Three indicators (***Hydrology (channel)***[c], ***Stream Metabolism and Water Quality*** and ***Fish (channel)***) followed standard protocols to support quantitative Basin-wide and Selected Area evaluation, where applicable (Hale *et al.* 2014). Six indicators (***Hydraulic Regime***, ***Matter Transport and Coorong Habitat***, ***Littoral Vegetation Diversity and Productivity, Microinvertebrate Assemblage, Murray Cod Recruitment*** and ***Flow-cued Spawning Fish Recruitment***) were developed to address Selected Area-specific objectives and test a series of hypotheses with respect to biological/ecological response to environmental flows. Under the MER Project, an integrated research project explores the links between key indicators to improve our understanding of how flow influences the ecological processes that drive recruitment of key fish species (Murray cod). Contingency monitoring activities (i.e. spawning and natal origin of flow-cued spawning fishes and soil seed bank composition of the littoral zone in 2020-21) are also being undertaken in response to opportunities as they arise to complement current monitoring and evaluation, and/or to inform environmental water use planning and management.

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[c] Hydrology (Channel) does not directly address any specific CEWO evaluation question, but provides fundamental information for analysis and evaluation of monitoring outcomes against hydrological conditions and environmental water delivery for all other indicators. Results for this indicator are presented in Section 1.4.

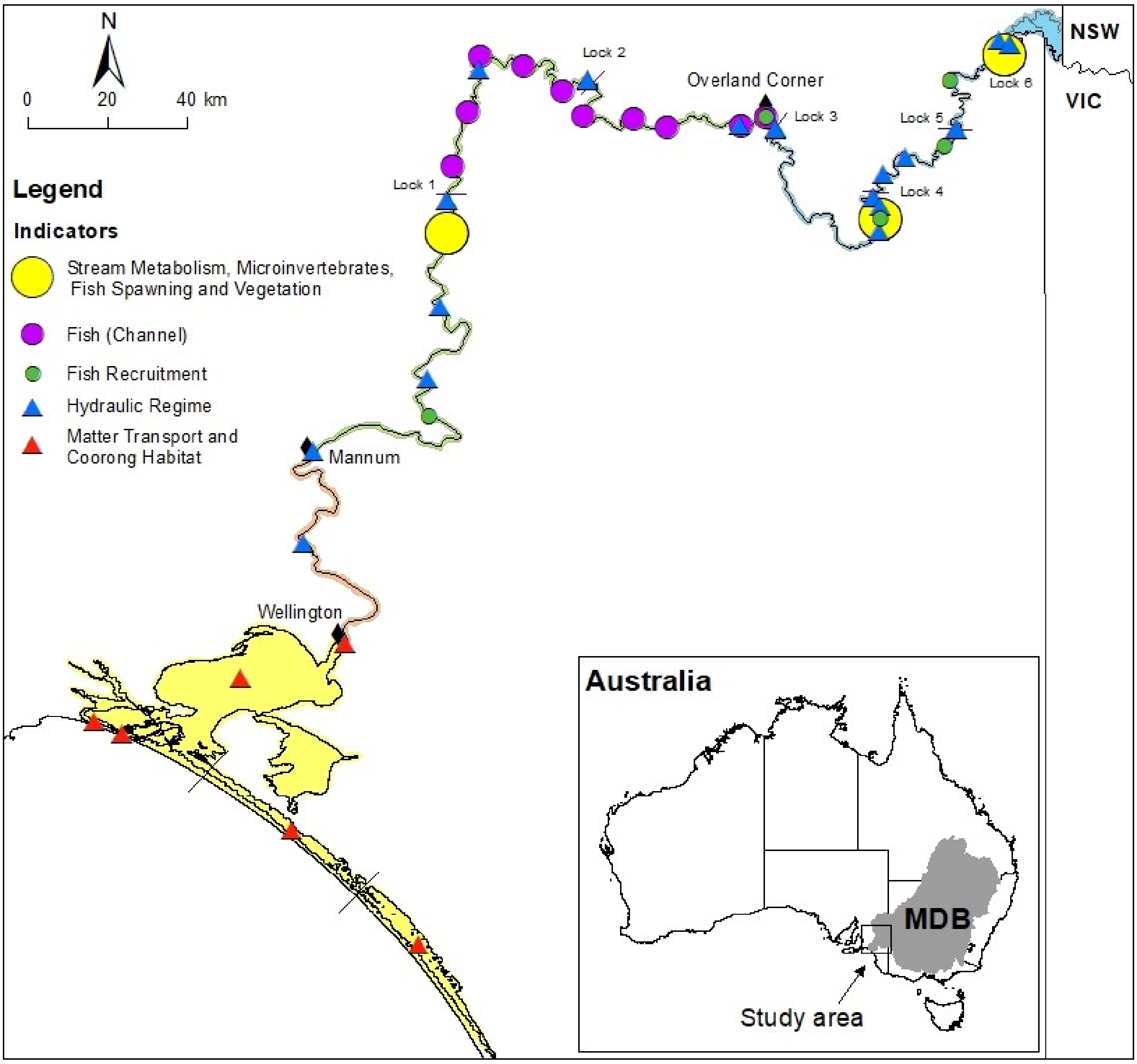

Figure 1. Map of the Lower Murray Selected Area showing the Lower Murray River floodplain (blue), gorge (green) and swamplands (orange) geomorphic zones, and the Lower Lakes and Coorong (yellow). Sampling sites are indicated by coloured circles (field monitoring) and triangles (modelling).

1.3 Expected outcomes in the Lower Murray

For the period of the MER Project (2019-20 to 2022-23), it is expected that most Commonwealth environmental water deliveries to the Lower Murray will contribute to base flows and freshes in the LMR channel (Figure 2), and maintain river flows to the Lower Lakes and Coorong. These particular flows aim to achieve a variety of environmental outcomes including those relating to fish, vegetation, birds, water quality and river function, Lower Lakes water levels, salt export and connectivity between freshwater, estuarine and marine environments (Appendix A), although only some of these are monitored through this project.

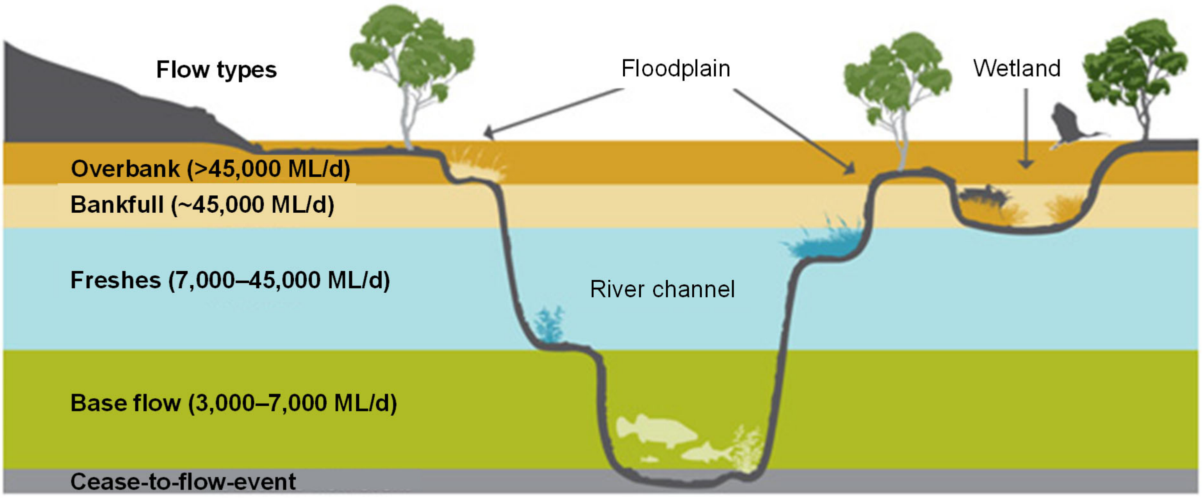

Figure 2. The various flow types of the Lower Murray River as described in the Murray–Darling Basin Plan (MDBA 2011). This diagram represents an idealised, unconstrained river reach.

In the Lower Murray, environmental water delivery that contributes to base flows and freshes increases stream velocity, mixing and dilution; increases variability in water levels; increases the inundated area of the littoral zone of channels, low-lying wetlands and floodplains; and improves connectivity between freshwater, estuarine and marine environments (Ye *et al.* 2020a). These changes to hydrological/hydraulic conditions in the LMR are expected to lead to:

- Maintained dissolved oxygen and water quality due to increased mixing and discharge;
- Increased productivity due to lateral transport of organic material;
- Increased transport of dissolved and particulate matter (salt and nutrients) downstream due to mobilisation and increased discharge;
- Increased littoral understorey vegetation diversity, productivity and community resilience due to increased water level variability;
- Increased microinvertebrates (and egg-bank) diversity and abundance due to increased inundated area in littoral and off-channel habitats from increased water levels and discharge;
- Increased abundance of microinvertebrate taxa common to upstream areas and those that have been found to proliferate during times of high velocity and longitudinal connectivity due to entrainment and downstream transportation;
- Increased larval abundance of flow-cued spawning fish species (golden perch *Macquaria ambigua* and silver perch) due to the provision of flow-cues for spawning and increased larval drift and dispersion;
- Increased recruitment of flow-cued spawning fish species due to increased spawning and larval drift, and enhanced survival rate due to increased productivity;
- Improved recruitment and population resilience of main channel specialist fish species (Murray cod) due to increase in lotic habitat and productivity;
- Increased salt export out of the MDB; reduced salt import into the Coorong, and reduced salinities; and

- Improved fish habitats in the Coorong, and also *Ruppia tuberosa* habitats at higher flow.

Over the long-term (decades), environmental water delivery is expected to make a significant contribution to achieving ecosystem outcomes in the Lower Murray, through restoring ecological processes and improving habitat for biota in the main channel and floodplain/wetlands. A consolidated view of the expected outcomes driven by flow for the Lower Murray is presented in Figure 3 below, which includes core monitoring indicators of the MER Project. The conceptual diagram demonstrates the inter-relationships between the changes of hydrological/hydraulic regime and riverine productivity (stream metabolism, vegetation, microinvertebrates) and matter transport, and how these may influence fish spawning and recruitment and the overall fish assemblage in the Lower Murray.

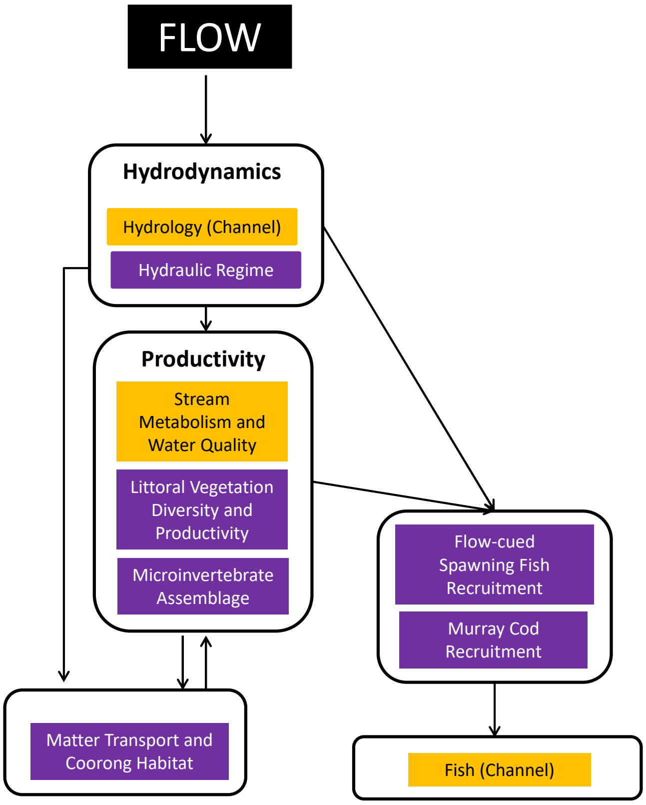

Figure 3. Cause and effect diagram of flow for the main channel of the Lower Murray with respect to the proposed indicators. Magnitude, timing and duration are factors of flow (in black). Yellow indicators followed standard protocols to support quantitative Basin-wide and Selected Area evaluation, where applicable. Purple indicators were developed to address objectives and test Selected Area-specific hypotheses with respect to biological/ecological response to environmental flows.

1.4 Environmental water delivery

Since 2011-12, environmental water has been delivered to the LMR to increase river flows (Figure 4) and improve ecological health (www.environment.gov.au/water/cewo). During the LTIM and MER project period (2014-15 to 2020-21), an average of ~697 GL/year of Commonwealth environmental water has been delivered to the LMR, in conjunction with other environmental flows (i.e. water from The Living Murray (TLM) Initiative, Victorian

Environmental Water Holder, River Murray Increased Flows, and New South Wales Department of Planning, Industry and Environment) (Table 1), with an average of ~631 GL/year of Commonwealth environmental water flowing through the barrages into the Coorong (Table 2). During this period, environmental water deliveries to the LMR largely occurred as return flows, during winter and spring–early summer, through coordinated watering events across the southern connected Basin to achieve multi-site environmental outcomes. Direct orders of environmental water to the South Australian border also occurred for specific purposes, often during summer–autumn, to provide flow for the Lakes and Coorong.

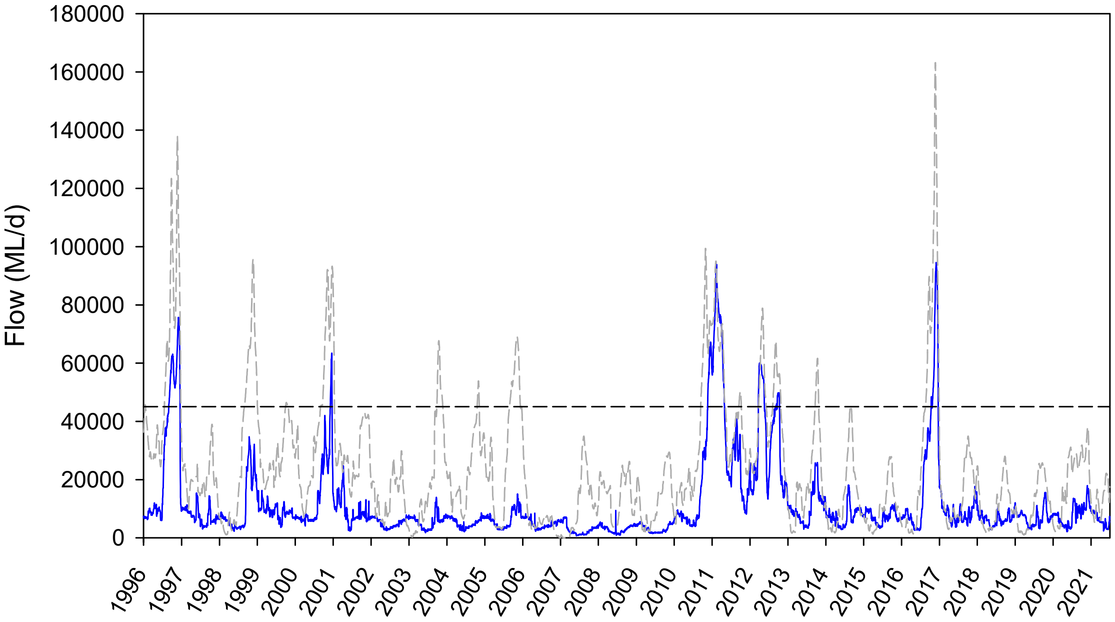

Figure 4. Daily flow (ML/d) in the Lower Murray River (LMR) at the South Australian border (blue solid line) from January 1996 to July 2021, compared to modelled flow under natural conditions (grey dashed line). Approximate bankfull flow in the main channel of the LMR is shown (black dashed line).

Table 1. Total annual volumes (gigalitres, GL) of environmental water (eWater), including Commonwealth environmental water (CEW), delivered to the Lower Murray River (LMR) channel (excludes wetland use*) and the proportion contribution towards total flow to the LMR (QSA). Accounted volumes are provided by the CEWO, include the environmental components of the South Australian entitlement (Ent.) and exclude environmental water delivered from the SA Minister for Environment and Water and Accolade Wines (predominantly for wetlands). TLM = The Living Murray, VEWH = Victorian Environmental Water Holder, RMIF = River Murray Increased Flows, NSW DPIE = New South Wales Department of Planning, Industry and Environment.

Water year	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21
CEW	581	798	618	898	549	750	687
TLM	107	101	234	176	96	68	175
VEWH	26	15	43	30	35	60	56
RMIF			100	53	111	52	5
NSW DPIE				9			
Total eWater	714 (25%)	914 (37%)	996 (11%)	1167 (43%)	791 (32%)	931 (39%)	922 (31%)

* A total of 12.5 GL of CEW was also delivered for wetland watering in 2020-21.

Table 2. Annual flow over the Murray barrages (total volume, GL) from 2014-15 to 2020-21, showing contribution by Commonwealth environmental water (CEW). CEW and total flow volumes are based on South Australian barrage dashboard accounting data. Matter transport results in Section 2.3.1 are based on barrage-specific modelled data, and may differ slightly compared to the accounted data presented here.

Water year	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21
Total	987	561	6484	854	377	685	1247*
CEW	454	561	802	757	377	685	808

*Other eWater during 2020-21 included 46.2 GL of TLM (incl. 13.1 GL of Ent.), 28.7 GL VEWH and 4.8 GL of RMIF (data source: CEWO). See Table 1 caption for abbreviations in full.

In 2020-21, flow remained in-channel and was similar to five of the previous six years, which were hydrologically dry (i.e. flow remained <18,000 ML/d at the South Australian border, Figure 4). During this year, ~922 GL of environmental water (excluding wetland use, 31% of the total flow), including ~687 GL of Commonwealth environmental water, was delivered to the LMR (Table 1).

Following unregulated flows in winter–early spring, environmental water delivered to South Australia between late September and mid-December 2020, supported by return flows from the Murray, Goulburn, Murrumbidgee and Darling rivers, increased flow variability and promoted multiple, in-channel spring flow pulses (Figure 5). Spring flow peaked at 17,900 ML/d in late November 2020, and flow was maintained >15,000 ML/d for a period of 18 days (Figure 5).

Environmental water delivery to the LMR from summer–late autumn comprised of direct trades[d] at the South Australian border (Figure 5a). During this period, Commonwealth environmental water played a critical role in maintaining barrage releases (Table 2). Approximately 65% of barrage flows in 2020-21 were Commonwealth environmental water.

The physical source of flows to the LMR during 2020-21are presented in Figure 5b. Flow to South Australia mainly comprised flow from the upper Murray and Goulburn rivers between July and November 2020, whereas flow from the Murray River and Lake Victoria was dominant after December 2020. Flow from Lake Victoria was delivered via direct trades. In 2020-21, the proportional flow from the Murrumbidgee (~10%) and Darling (~1%) rivers were low (Figure 5b). Most flow from the Darling River occurred during late June 2021.

Key watering events in the LMR during 2020-21 and the targeted expected outcomes of these deliveries are presented in Appendix A. Environmental water also supported other complementary management actions to achieve ecological outcomes in the Murray River; key activities from downstream of the Darling River junction to Wellington are summarised in Appendix B. These included manipulations of Weir Pools 1, 2, 4 and 5, operations of Katarapko and Pike floodplain regulators, and wetland watering by pumping.

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[d] "Direct trade" refers to an order for a specified volume of environmental water to be delivered at the South Australian border. Typically a timing and profile for the delivery is specified and river operators can meet the order by providing water from any available source.

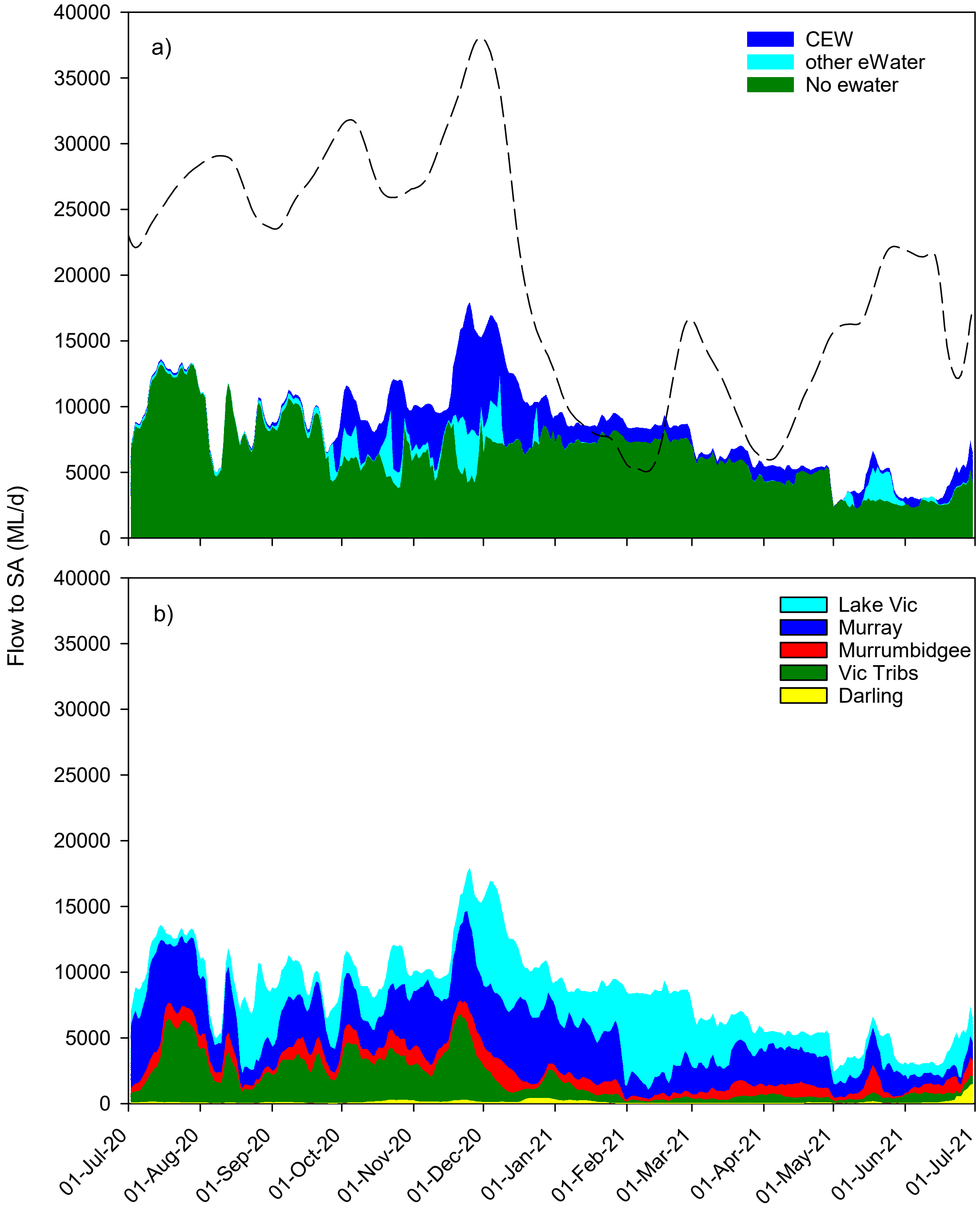

Figure 5. Flow to South Australia from July 2020 to June 2021 showing the (a) contribution of environmental water (eWater) and (b) source of all (environmental and consumptive) water (MDBA). CEW = Commonwealth environmental water. Modelled flow under natural conditions is shown by the dotted black line. 'Bigmod salinity routines' was used as a proxy for transport of biological matter, to estimate the proportion of the flow that originated at different upstream tributaries[e].

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[e] Molecules of water, nutrients, and the biological matter transported downstream often move slower than the wave front that is recorded as the change in flow discharge (Chow *et al.* 1988). To account for this, the MDBA has used Bigmod salinity routines as a proxy for transport of biological matter, to estimate the proportion of the flow at the South Australian border that originated at different upstream tributaries. While acknowledging potential difference in travel time between salt and other matter, this approach is preferred over estimating travel times based on observed changes in flow along the main channel.

1.5 Purpose of the CEWO MER report for 2020-21

This report presents the key findings from monitoring in the Lower Murray during 2020-21, and answers CEWO evaluation questions about ecological responses to Commonwealth environmental water deliveries (Sections 2 and 3). Refer to previous annual reports (Ye *et al.* 2016b; 2017; 2018; 2019; 2020; 2021) for Lower Murray monitoring findings from 2014-15 to 2019-20, and the *Monitoring, Evaluation and Research Plan for the Lower Murray* (SARDI *et al.* 2019) for a detailed description of methods for current activities. Specific management recommendations for environmental flows in the Lower Murray are provided in Section 2, with general management implications summarised in Section 4, based on monitoring and evaluation outcomes, and expert knowledge. Findings from the integrated research project will be presented in a separate report and be incorporated into the final MER technical report (2022-23). Monitoring and evaluation of Commonwealth environmental water delivery in the Lower Murray focusses on the main watering period of spring–summer; therefore, our findings and recommendations on environmental water management are most relevant to this period.

2 INDICATORS

2.1 Hydraulic Regime

The discharge, or *hydrology*, in the Lower Murray Selected Area was determined through routine monitoring. The hydrology expected to have occurred without environmental water components was determined by the Murray–Darling Basin Authority (MDBA) using operational records of environmental water delivered to the South Australian border, and modelled throughout South Australia accounting for travel time, losses and differences in diversions and environmental water use for the different scenarios with and without environmental water.

The *hydraulic* characteristics (e.g. depth/water level or flow velocity) of fluvial ecosystems result from the interaction of discharge and physical features (e.g. channel morphology, woody debris, man-made structures, etc.), and have a profound influence on river ecosystem structure and function (Statzner and Higler 1986; Biggs *et al.* 2005; Bice *et al.* 2017) (also see Section 1.1). It is these hydraulic characteristics that biota can sense and respond to, i.e. a change in velocity or water level, rather than a change in discharge.

The purpose of this indicator was to quantify the changes in hydraulics due to the delivery of environmental water using hydraulic models, to provide a basis to infer ecological changes caused by environmental water. This approach is particularly important in the LMR where a given discharge may not produce the same hydraulic response, as downstream structures (weirs) will also influence the hydraulics occurring.

<u>Hypothesis</u>

Commonwealth environmental water will promote greater extent of lotic habitat as evidenced by increased water velocities and variability in water levels.

Methods

A steady-state modelling approach was adopted, similar to that used in the Goulburn (Webb *et al.* 2015) and Edward-Wakool (Watts *et al.* 2015) Selected Areas. For each weir pool within the Lower Murray Selected Area, i.e. Weir Pools 1 to 5, as well as the river between Lock 1 and Wellington, a range of steady state flow scenarios were simulated in the hydraulic models (2,000–100,000 ML/d) and a range of weir pool levels required to cover the range of conditions experienced. Models used for this analysis are outlined in McCullough *et al.* (2017) and Montazeri and Gibbs (2019). For each steady state scenario, a range of hydraulic metrics were computed, including the 10th, 50th and 90th percentile velocities within the weir pool, the proportion of the weir pool exceeding 0.2 and 0.3 metres per second (m/s), and water levels at regular locations along the weir pool. The former water velocity (>0.2 m/s) represents favourable velocities that entrain and transport/disperse phytoplankton, zooplankton and fish larvae (Gibbs *et al.* 2020) and allow gas exchange at the water surface (Ye *et al.* 2021), and the latter (>0.3 m/s) represents flowing water (lotic) conditions for riverine biota (Bice *et al.* 2017).

To enable a consistent comparison of in-channel velocity changes due to environmental water, the same area was used for all velocity analysis. The area used for velocity analysis for each weir pool comprised the inundated area at flows of 5,000 ML/d and normal pool level conditions. As changes to in-channel velocities were the focus of this analysis, this approach of eliminating additional areas inundated was considered reasonable. Additionally, where the full inundated area was used, the disproportionate increase in area of slow flowing backwaters compared to in-channel area as discharge increased had a large impact on the overall proportion of the weir pool with low velocities.

Post-processing correction to the modelled water levels downstream of each lock was undertaken. A linear regression relationship between flow and the difference in modelled and recorded water level was used to correct for any systematic bias introduced by errors in the hydraulic model. Following this correction, the remaining residual error between the modelled and recorded data each day was applied to all scenarios, which represents random error introduced by other factors, such as wind setup. This results in modelled water levels that are the same as the observed water level downstream of each lock for the scenario representing observed conditions (*All Water*) with consistent corrections applied to the without environmental water scenarios. To calculate this correction, gaps in observed data downstream of Lock 4 and downstream of Lock 1 were interpolated based on the next water level station downstream and deriving a water level increase based on the flow occurring at the time and recent water level data. The water level and area results represent the main channel only and include the influence of increases in discharge as well as weir pool manipulation. However, the results do not include the influence of floodplain regulators at Pike, Katarapko and Chowilla or other watering activities such as pumping to wetlands.

Environmental water scenarios

With the lookup information derived from the hydraulic models, the time series of discharge for each of the environmental water scenarios presented in Section 1.4 and the downstream water level each day for each weir pool, time series of hydraulic parameters were interpolated using linear bivariate interpolation (R version 4.1.1 and akima package 0.6-2.2). Five scenarios were considered:

- ***All Water***, with all environmental water representing observed conditions,
- ***No CEW*** without Commonwealth environmental water,
- ***No eWater*** without any environmental water,
- ***No Locks*** the observed discharge as used for the *All Water* scenario, but the influence of the weir and locks across the Murray River removed, to provide an indication of the maximum hydraulic changes that could be expected for the delivered flow regime, and
- ***WoD***, or Without Development, a representation of natural conditions, which has the locks removed as used for the *No Locks* scenario, but also a modelled flow representing no storage or diversions across the Murray River.

The discharge time series for these scenarios were provided by the MDBA, and the data account for changes in diversions expected within South Australia by assuming full utilisation of the entitlements recovered for the environment in the without environmental water (*No CEW* and *No eWater*) scenarios. *WoD* and *No Locks* results were not simulated downstream of Lock 1, due to limited information on a suitable downstream water level for this scenario.

The observed water levels at each lock and at Wellington were used as inputs for the *All Water* scenario. For the without environmental water scenarios, the weir pool raisings at Locks 4 and 5 were removed, as these operations were supported by environmental water and associated with operation of the Katarapko and Pike floodplain infrastructure. Small weir pool lowering within operational ranges at Locks 1 and 2 were also removed, timed to coincide with the flow peak in early December 2020. The water level was assumed to be at normal pool level during these periods. For the Below Lock 1 reach, the influence of environmental water on the water level in the Lower Lakes was incorporated, based on MDBA water balance modelling and the recorded water level at Wellington. This modelling assumes that the observed lake water level is the highest priority for the no environmental water scenarios, and it is the barrage flow that is reduced when there is less water available. If there is not sufficient volume to maintain water level, the Lake Alexandrina water level reduces compared to the *AllWater* scenario and barrage flow is zero.

Results

A summary of the results at the Lower Murray Selected Area scale can be seen in Figure 6. For the velocity metrics, only results above Lock 1 were presented here to enable a comparison against the *WoD* and *No Locks* scenarios. Figure 6 includes the discharge at the South Australian border for the different scenarios, the resulting area inundated (from Lock 6 to Wellington), and length of the river between Lock 6 and Lock 1 experiencing lotic conditions, based on thresholds of velocity >0.2 m/s and >0.3 m/s.

Velocity

The modelling indicates that there were substantial short-term changes in the length of river with velocities exceeding 0.2 m/s in 2020-21 due to Commonwealth environmental water. An additional 166 km (49% of the reach) exceeded this threshold for 14 days, and 94 km of river (28% of the reach) for a duration of 30 days (Figure 6). Increases in the proportion of the river with a velocity exceeding the higher threshold of 0.3 m/s were also modelled, 84 km (25%) for 14 days and 31 km (10%) for 30 days due to Commonwealth environmental water. This can be compared to the without development hydraulic conditions, where the full reach was expected to experience cross section averaged velocities exceeding 0.3 m/s for the first 5.5 months of the year. The influence of the locks on the lotic habitat available can also be seen in Figure 6, where for the same flow to South Australia as the *All Water* scenario, without locks, half of the length of river considered had velocities exceeding 0.3 m/s over the period between August and the main flow pulse in November, whereas less than 15% of the river considered experienced velocities greater than 0.3 m/s with the locks in place.

The velocity magnitudes are presented at a weir pool scale in Figure 7, with the median velocity in the weir pool each day shown as a solid line, and the range in velocities (as the 10th and 90th percentiles) shown as the shaded band. The results suggest a relatively consistent response across the weir pools above Lock 1, and the *No Lock* median velocity (light grey line) is close to the upper end of the *All Water* velocity range (90th percentile) in most weir pools during the first six – eight months of the year (depending on the weir pool). Below Lock 1 the modelling indicated lower velocities due to the deeper river in this reach.

Water level and area

The inundation area expected for the different scenarios can be seen in the second panel of Figure 6, where flows were below bank full level (occurring at approximately 45,000 ML/d), and hence changes in inundation due to environmental water above Lock 1 were limited. The increase in area of approximately 1,396 Ha in late November is mainly attributable to the increase in environmental water raising water levels and hence riparian inundation along the LMR, whereas the increase in Lake Alexandrina water level at this time is small. From mid-December onwards, the increased inundation area is mainly due to the difference in Lake Alexandrina water level, raising water levels along the river below Lock 1 (as seen in Figure 8). For comparison, the total inundated area (including permanent water) from Lock 6 to Wellington for an 80,000 ML/d flow (representing the potentially managed floodplain) is 60,230 Ha, whereas the maximum inundated areas were 22,638 Ha and 21,242 Ha for the *All Water* and *No CEW* scenarios, respectively, in 2020-21.

The upstream end of the weir pool is the least influenced by the downstream weir and hence most responsive to changes in discharge when the weirs are controlling water levels (below 54,000–67,000 ML/d, depending on the weir). Environmental water created some variability in water levels at the upstream end of each weir pool that would not have occurred otherwise, particularly increasing water levels in late November and December, where without the environmental water the water levels would have receded (Figure 9). The weir pool raisings at Locks 4 and 5, supported by environmental water, also contributed to increased water levels when these events started in July.

The *No Locks* scenario removes the influence of the weirs on water levels at the upper end of each weir pool. For most locations the weirs can be seen to increase levels approximately 0.5 m over the first half of the year, and up to 1 m by the end of the water year during the lowest flows. The exception is in Weir Pool 3 (i.e. just downstream of Lock 4), where Lock 3 was modelled to have negligible influence on water levels (similar water levels from *All Water* and *No Locks* scenarios) (Figure 9). This indicates a free-flowing section of river and higher velocities are expected, which is supported by both the modelling results and monitoring undertaken at this location.

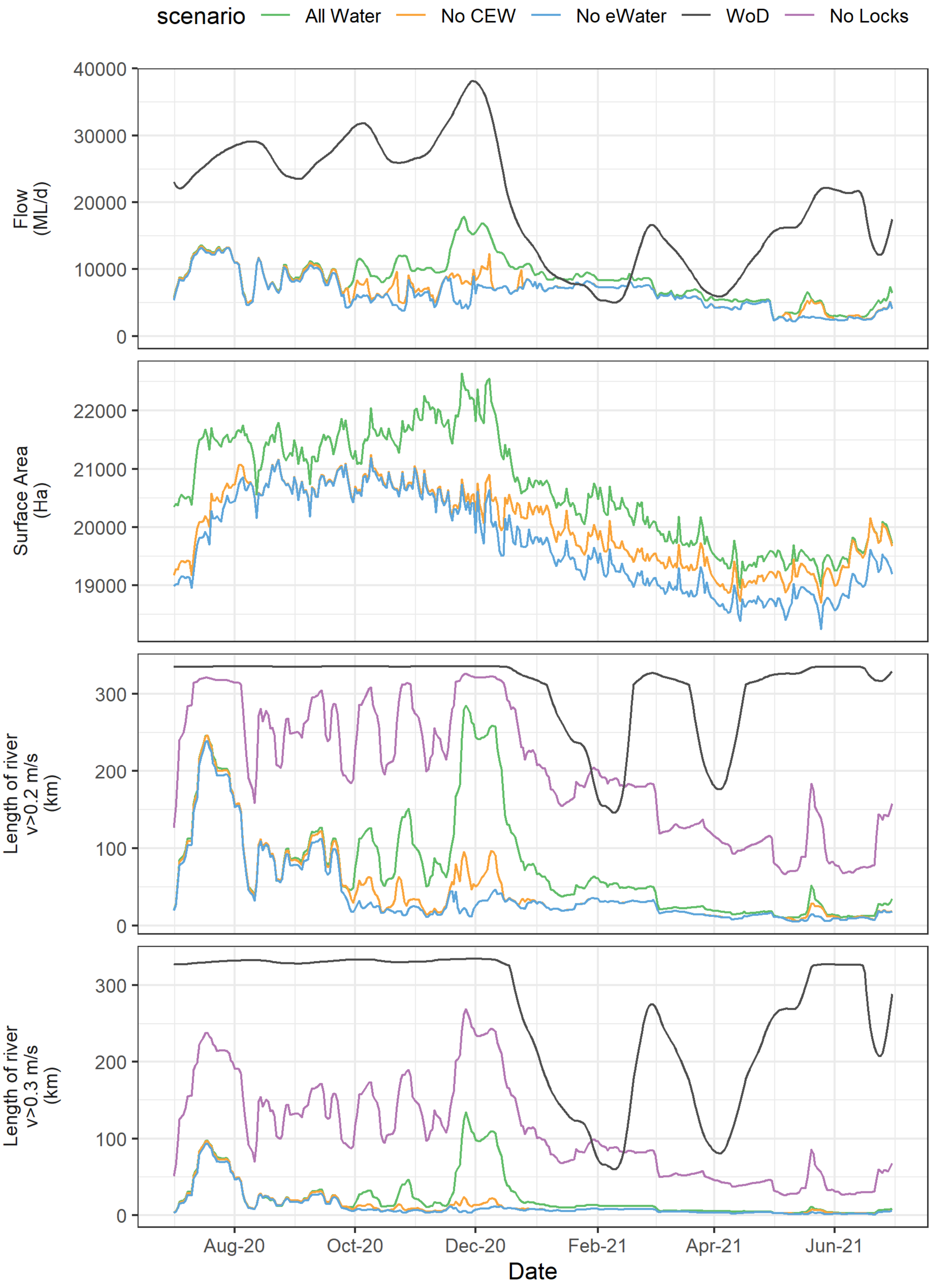


Figure 6. Discharge (flow to South Australia), surface area between Wellington and Lock 6, and length of river with faster flowing velocities (v>0.2 m/s and v>0.3 m/s) for the Lower Murray River (LMR) between Locks 1 & 6 (excluding anabranches). Total length of river assessed for the length of river metrics in the LMR = 345 km.

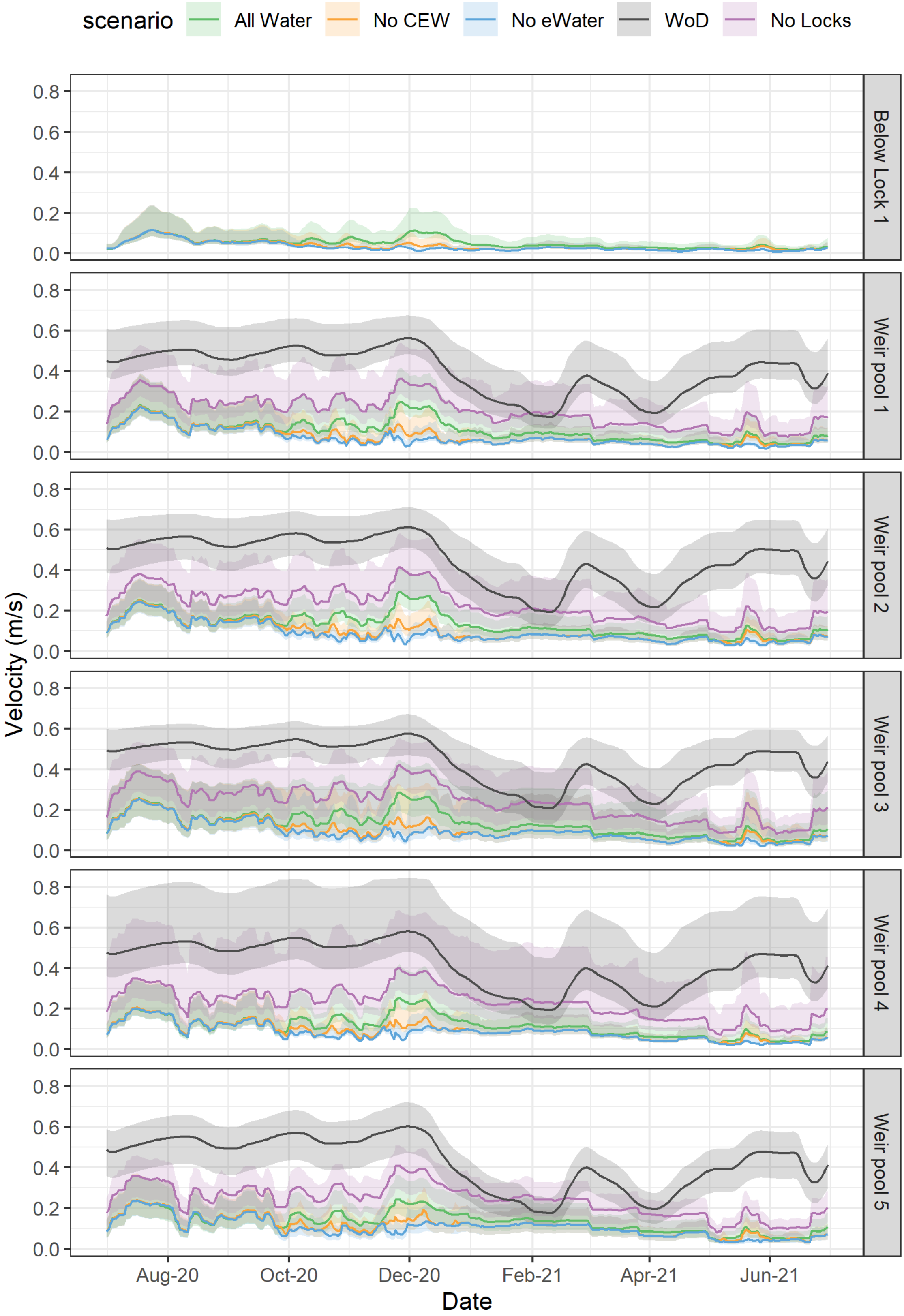


Figure 7. Median modelled velocity in each weir pool (line), with the range in velocities within the weir pool (the shaded area), defined by the 10th and 90th percentiles, in the Lower Murray River.

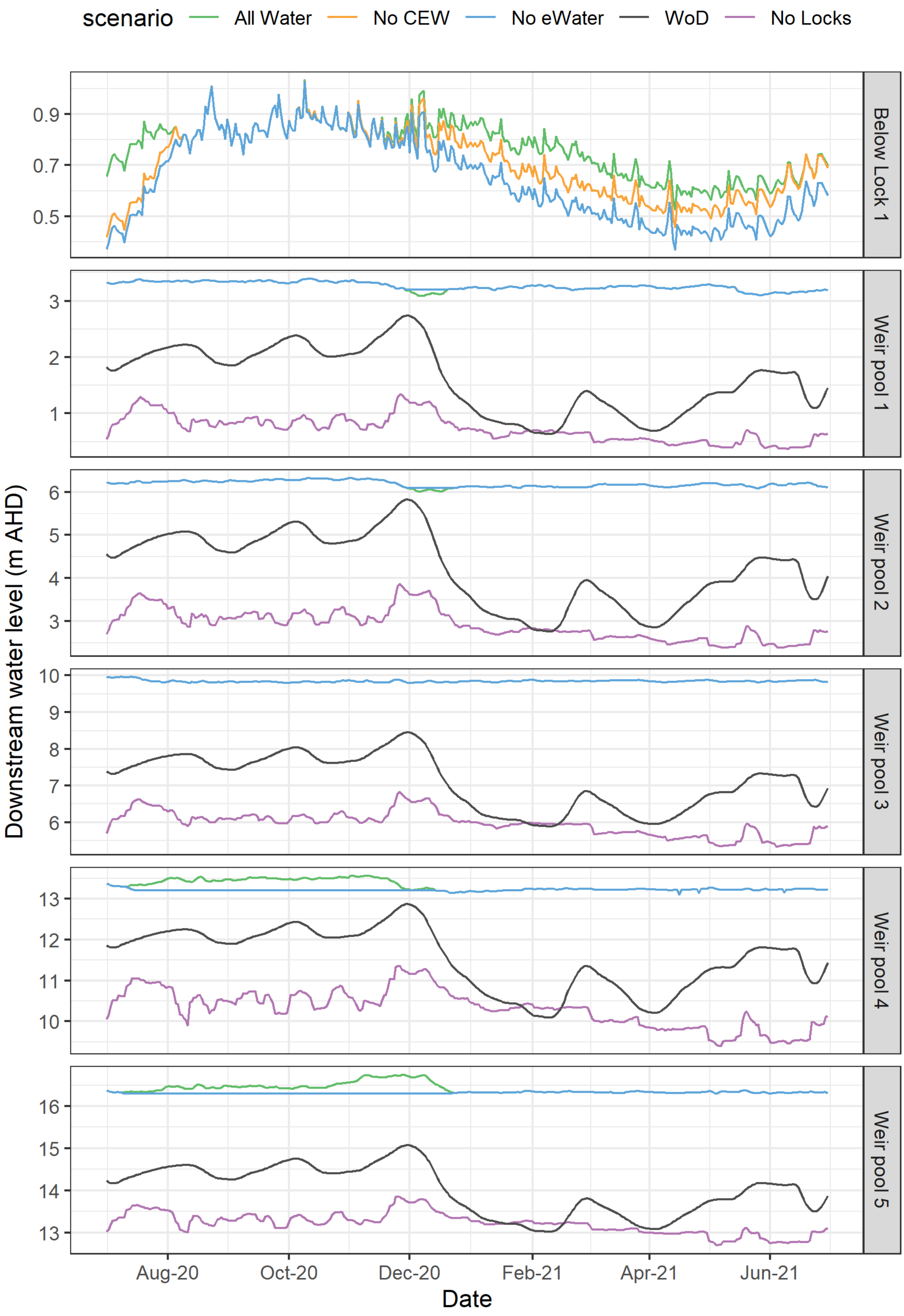


Figure 8. Modelled water level at the downstream end (i.e. above the Lock and Weir 1 for Weir Pool 1, and at Wellington for below Lock 1) of each weir pool in the Lower Murray River.

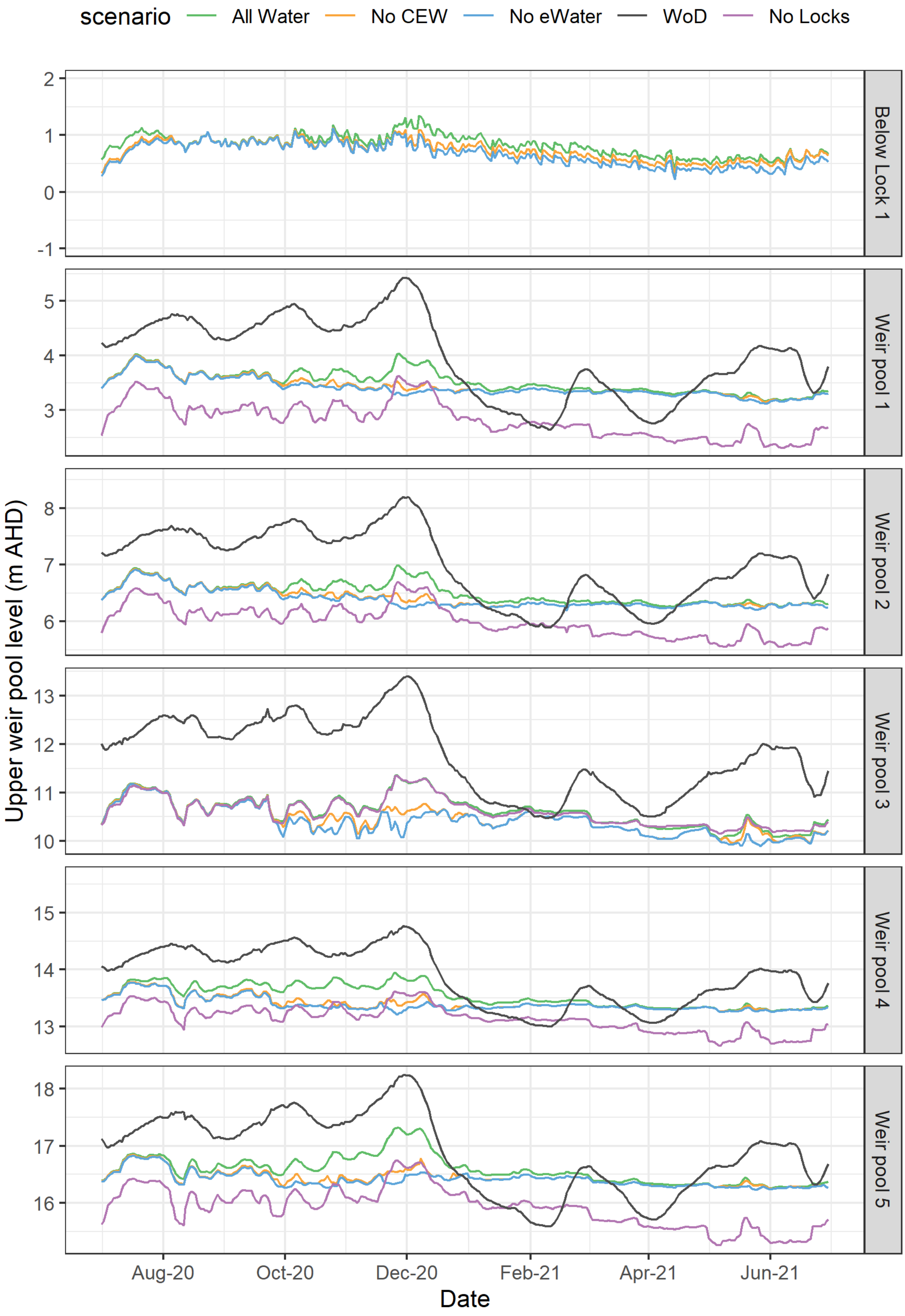


Figure 9. Modelled water level at the upstream end of each weir pool (i.e. downstream of Lock 2 for Weir Pool 1) in the Lower Murray River. The subplot for each Weir Pool has a y axis range of 3 m.

Evaluation

To evaluate the contribution of Commonwealth environmental water towards a short- (annual) or long-term (multi-year) outcome, a contribution significance level was assigned to each evaluation question. The level was viewed as 'to what extent Commonwealth environmental water contributed towards that observed outcome, with the ecological significance of the outcome considered where possible'. For example, the level assigned may be similar for an ecologically significant outcome of which Commonwealth environmental water had minor contribution towards versus an outcome that was considered minor of which Commonwealth environmental water had major contribution towards. The thresholds for assigning the significance vary among indicators and questions, ranging from using defined percentages or values of change, to qualitative assessment based on expert opinion. The thresholds for significance levels among the indicators were based on expert opinion and may be adjusted in response to developing understanding of these processes.

A new metric, total increase in longitudinal connectivity (in km days), has been added in this report. Previously, only the percentage increase in annual volume was used to represent longitudinal connectivity. However, this is a coarse measure of connectivity and does not represent the dynamics within the year. Similar to the increase in area, the increase in the length of river with velocity > 0.2 m/s each day has been summed over the year to produce a measure of longitudinal connectivity, measured in km days. The 0.2 m/s threshold was adopted as the indicator of connectivity given this velocity has been found to support entrainment and transport/dispersal of propagules such as phytoplankton, zooplankton and fish larvae (Gibbs *et al.* 2020).

With the addition of the new metric, and the addition of the reach below Lock 1 in the previous report (Ye *et al.* 2021), all evaluation metrics have been recomputed in this report to ensure consistency. This has introduced some differences to values presented in previous reports for a number of reasons, including: a change in methodology from complete hydrodynamic modeling to interpolation of steady state model outputs part way through the period and the reach below Lock 1 has been included for water level variability and lateral connectivity metrics for all years. The method to calculate water level variability has changed to be more representative of the increase in variability, where the difference in the interquartile range for the *All Water* and *No CEW* scenarios is calculated for each weir pool and then averaged across weir pools, where previously the interquartile range was calculated on the difference in water levels at each location. Given this different method, the values are different to previous reports. Water levels below Lock 1 are not included in these results, given the influence of the Lower Lakes on water levels. Previous reports were also found to not be consistent in the approach used to calculate longitudinal connectivity metric, which has been corrected in this report.

Table 3. Hydraulic Regime evaluation questions and answers. CEW = Commonwealth environmental water, eWater = environmental water.

CEWO evaluation questions	**Outcomes of CEW delivery**						
	2014-15	**2015-16**	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**
What did CEW contribute to hydraulic diversity within weir pools?	13 km, 4% (17 km, 5%)	18 km, 5% (22 km, 6%)	20 km, 6% (53 km, 15%)	36 km, 10% (49 km, 14%)	15 km, 4% (19 km, 6%)	33 km, 10% (73 km, 22%)	An additional 31 km or 9% of lotic conditions created by CEW for at least 30 days (An additional 84 km or 25% for at least 14 days)
	CEW provided a moderate contribute towards increasing lotic habitat in the LMR in 2020-21, similar to 2017-18 and 2019-20 for the period of one month, but the highest peak increase of 84 km over 14 days. Length of river with lotic conditions (velocity >0.3 m/s) has been used to represent hydraulic diversity. If there is some fast-flowing water, it is expected there will be greater hydraulic diversity due to changes in habitat complexity (bends, backwaters, benches, etc.). Length of river with lotic conditions exceeded for 30 days over the year is presented, and 14 days in brackets. The time periods represent a number of flow-related ecological or life-history processes that could occur over periods of ~2–4 weeks. Total length of river assessed in the LMR = 345 km.						
What did CEW contribute to variability in water levels within weir pools?	0.03 m	0.06 m	0.01 m	0.14 m	0.09 m	0.06 m	Interquartile range (IQR) in water level increased by 0.12 m due to CEW in the tailwaters (i.e. just downstream of each weir) across Weir Pools 1–5.
	CEW increased water level variability (IQR) in the tailwaters of weir pools during each year of the assessment. 2020-21 had the second greatest increase in variability, slightly below 2017-18. IQR is a measure of variability, as the difference between the 75th and 25th percentile values for water level over the year. If the IQR increases, the variability must have increased.						

CEWO evaluation questions	**Outcomes of CEW delivery**						
	2014-15	**2015-16**	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**
What did CEW contribute to hydrological connectivity?	28 Ha 20% (26%) 7,159 km d (41%)	1,287 Ha 37% (63%) 9,036 km d (123%)	122 Ha 5% (7%) 6,929 km d (10%)	904 Ha 39% (70%) 11,568 km d (118%)	1,398 Ha 20% (39%) 6,327 km d (77%)	1,621 Ha 33% (71%) 9569 km d (148%)	Maximum inundated area increased by 1,397 Ha due to CEW. 22% increase in volume at the SA border (34% increase at Lock 1). Total increase in connectivity (v>0.2 m/s) of 10,392 km d, an increase of 58%
	CEW contribution to connectivity has been assessed laterally, as the increase in maximum inundated area each year, and longitudinally, as the percentage increase in flow volume each year and as the total increase in river length over a number of days with velocity exceeding 0.2 m/s. The increase an annual flow volume at the SA border is reported (as used for Basin Scale assessment) as well as Lock 1, where the percentages increase further down the system due to the losses and consumption, as well as water recovery within SA. In 2020-21, CEW increased lateral connectivity throughout the water year. The total area increase can be driven by the difference in water level in the Lower Lakes, with an increase in level from 0.5 to 0.7 m AHD increasing the area inundated by 893 to 1197 Ha depending on the flow (based on 5,000 – 15,000 ML/d). The lower maximum inundated area in 2020-21 compared to 2019-20 is due to the Lower Lakes not expected to fall as low without CEW. The proportion increase in flow due to CEW was lower compared to some drier years (e.g. 2017-18 and 2019-20), due to the higher total flow volume to SA in 2020-21. The increase in connectivity, based on length-day of river with velocity > 0.2 m/s was the second highest over the evaluation period, resulting in a moderate contribution of CEW to hydrological connectivity in 2020-21.						

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):
⬜ Unknown 🟥 Negative ▫ None/negligible ▫ Minor 🟩 Moderate 🟦 Substantial

Discussion

2020-21 was the first year with some unregulated flow since the high flow year of 2016-17, and the second highest total volume entering the LMR since the start of LTIM in 2014-15. The volume of environmental water delivered was similar to the average, 922 GL of environmental water (average since 2014-15 of 921 GL, range 714 – 1167 GL), of which 687 GL was Commonwealth environmental water (average of 697 GL range 549 – 898

GL). With a similar total volume of environmental water delivered, the higher total volume to South Australia results in Commonwealth environmental water providing a slightly smaller proportion of the total flow, reducing from 33% of the total flow at the South Australian border in 2019-20 to 22% in 2020-21.

The evaluation of Commonwealth environmental water contribution to hydraulic diversity within weir pools is in line with previous years (Ye *et al.* 2021). Figure 10 presents the flow to South Australia over the past seven years to compare the events of flow delivery. It can be seen that 2020-21 was similar to 2017-18, with variable flow between 5,000 – 12,000 ML/d until November, before a flow pulse commencing in late November to approximately 18,000 ML/d. Peaks of similar magnitude and duration, albeit different timing, also occurred in 2019-20 and 2014-15.

A range of metrics has been considered to assess the evaluation questions in Table 3. Relevant velocity thresholds, proportions of the river, time of year and duration required for different ecological processes to be promoted are the focus of further research, as the empirical evidence relating the conditions occurring, and different ecological processes being promoted, continues to improve. It is expected that this hydraulic information, and the methodology developed to derive it for the future, will help to develop eco-hydraulic relationships.

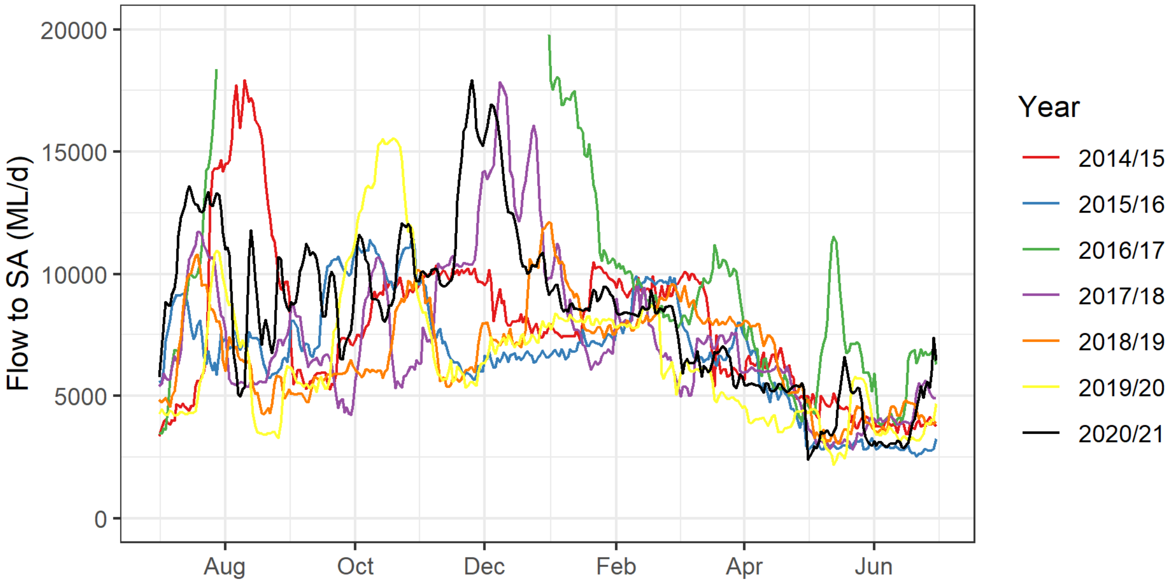


Figure 10. Flow to South Australia over the past seven years, where events of similar magnitude and duration have occurred in four of the seven years. The high flow year of 2016-17 peaked at 94,350 ML/d.

Management implications

Coordinated flow delivery

The flow event in late November 2020 was a highly coordinated event, synchronising delivery from multiple water holders and tributaries to create the flow peak and extend the duration. The source of the flow at the South Australian border modelled outputs provided by the MDBA are presented in Figure 11 to demonstrate this. The dashed lines indicate flow at the source location, with the solid lines the resulting component of the flow at the South Australian border from that location, after accounting for travel time, attenuation and losses. Both the Goulburn River (at McCoy's Bridge) and Murray River (at Yarrawonga) were at their current constraints in mid-October, coinciding to produce the flow peak at the South Australian border in late November. With a longer duration at the peak from the Goulburn River, earlier release from Lake Victoria, and higher base flows from the Murrumbidgee River, it may have been possible to create a slightly higher flow peak. But the 2020 coordinated flow pulse event is likely to represent close to the best possible case for the maximum peak flow that can be created under regulated river conditions based on: 1) current water holdings and availability; 2) Menindee Lakes being unavailable as an additional flow source; and 3) current maximum flow and duration constraints. Increasing any of these factors is likely to improve the ability to deliver larger coordinated flow pulses to the South Australian border.

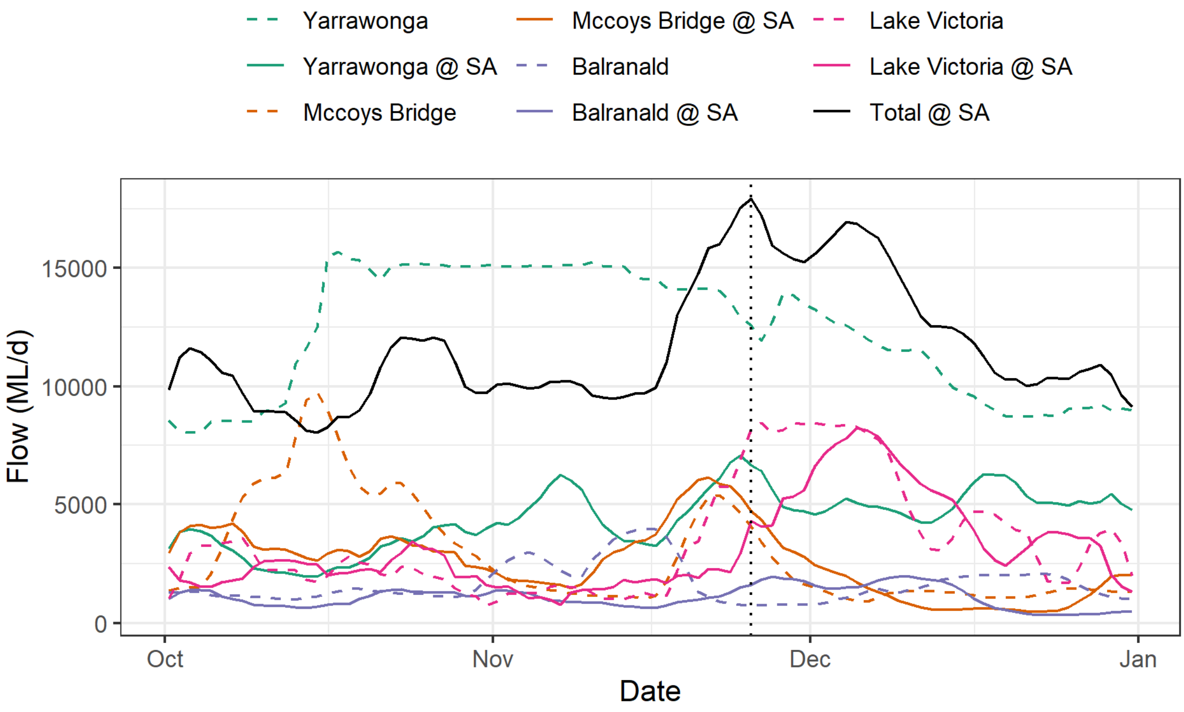


Figure 11. Source of flow at the South Australian border, coordinated water delivery with Murrumbidgee River (Balranald) providing base flow and peaks from upper Murray (Yarrawonga) and Goulburn Rivers (McCoy's Bridge) reaching the South Australian border to create the peak at 17,926 ML/d. Yarrawonga was at capacity from 16 Oct to 13 Nov (15,000 ML/d), and the Goulburn also reaching capacity at McCoy's bridge (9,000 ML/d) during a short peak around 15 Oct, arriving at the South Australian border over a month later. Lake Victoria commenced releases at outlet capacity (approximately 8500 ML/d) on Nov 26, the day of the peak flow.

Weir pool lowering

The *No Locks* scenario provides an indication of the influence of the weirs on the hydraulic conditions in the LMR, where it is not only the reduced flow (compared to *WoD*) that has resulted in a reduction in the degree of lotic habitat, but also the deeper water created by the weirs that also influences the hydraulic regime.

Based on this understanding of the influence of higher water levels reducing the velocities, the weir pool raising at Lock 4 that was undertaken to support the operation at Katarapko floodplain, was lowered earlier than originally planned to increase velocities in Weir Pool 4 during the 2020 flow pulse. Small weir pool lowerings (WPLs) within the operational range were undertaken to coincide with the flow pulse at Locks 1 and 2. Operations such as this are feasible under current river operations, and similar WPLs (0.08 m below NPL) were undertaken at Weir Pools 2, 5 and 6 in July 2017, and a WPL of up to 0.16 m lower at Lock 6 in June 2018.

To provide some context to the benefits to lotic habitat from WPLs smaller than full weir removal (i.e. the *No Locks* scenario), two additional scenarios have been considered:

- **2020 Planned** which continues the weir pool raising at Lock 4 undertaken in 2020 for an additional 3 weeks, returning to pool level on 25 December, the same as Lock 5. The WPLs at Locks 1 and 2 were removed, and the water level remains at pool level.
- **0.1 m below pool level** at all Locks 1–5 for the period of the flow pulse, from 15 November to 25 December 2020.

The results are presented in Figure 12, compared to the *No Locks* scenario. The discharge is the same in all scenarios and it is only the assumptions for the weirs that produce the differences. It can be seen that even a small WPL at all locks considered produced some benefits, with the maximum length of river with a velocity exceeding 0.3 m/s increasing by 24 km (7% of the length of river considered) from the *2020 Planned* scenario (131 km) to the *0.1 m WPL* scenario (155 km), but the *No Locks* scenario doubling the length of river with velocities greater than 0.3 m/s (269 km). Over the period of WPL (shown as the dotted vertical lines) the mean length of river with a velocity greater than 0.3 m/s (0.2 m/s in brackets) for each scenario was 68 (187), 81 (202) and 200 (299) km for the *2020 Planned*, *0.1 m WPL* and *No Locks* scenarios, respectively.

The results indicate that 2020 operations that were modified to be cognisant of the impact of weir pool raising on main channel river velocities slightly increased the amount of lotic habitat (reach with velocity greater than 0.3 m/s). Assuming all five weirs considered in this work were lowered by 0.1 m, the benefit was in the order of 20 km for the 2020 conditions considered, which included Locks 4 and 5 being raised. However, the degree of the weir pool operation has a direct relationship to the resulting benefits, with the *No Locks* scenario a substantial increase in length of lotic habitat compared to the *0.1 m WPL* scenario.

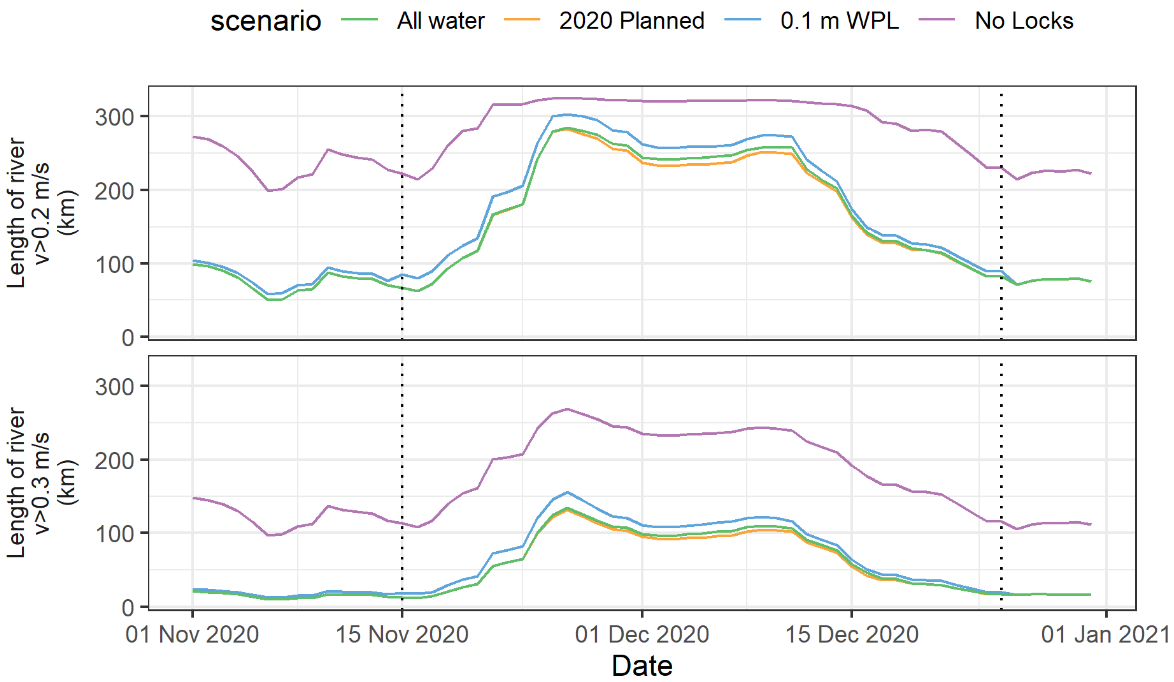


Figure 12. Length of river with velocities greater than 0.2 m/s (top) and 0.3 m/s (bottom) for scenarios that represent planned operations in 2020, a scenario that removed weir pool raising at Locks 4 and 5, and instead lowered Weir Pools 1–5 (0.1 m WPL), a scenario with full weir removal at Locks 1 – 5 (No Locks), and the actual conditions. The dashed vertical lines indicate the period with weir pools operations (raising or lowerings).

Conclusion

The current year (2020-21) was the first year with significant unregulated flow since the high flow year of 2016-17, and the second highest total flow volume to South Australia since the start of LTIM in 2014-15. Environmental water volumes delivered, both Commonwealth and total, were close to average over this period. However, the coordinated delivery of environmental water, both from different tributaries as well as different water holders, resulted in one of the largest increases in hydraulic diversity, water level variability and connectivity, over the evaluation period.

Due to Commonwealth environmental water, the flow pulse in late November 2020 resulted in the largest increase in lotic habitat with an additional 84 km of river (25% of the reach considered) for a period of 14 days or an additional 31 km of river (9% of the reach considered) for a period of 30 days. These durations relate to a number of flow-related ecological or life-history processes that could occur over periods of ~2–4 weeks (see Section 3). Without environmental water, flow to South Australia over November would have been at entitlement flow of 6,000 ML/d, compared to the peak of 17,926 ML/d created (November average of 12,477 ML/d). The operational changes to remove a weir pool raising at Lock 4, and small WPLs at Locks 1 and 2, made small, but discernible, contributions to the increases in lotic habitat.

2.2 Stream Metabolism and Water Quality

Background

River metabolism measurements estimate the in-stream rates of gross primary production (GPP) and ecosystem respiration (ER), providing information on the sources and utilisation of organic carbon in riverine food webs (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). Comparing rates of photosynthesis and respiration helps describe the fundamental trophic energy connections that characterise different food web types. It can indicate whether production or decomposition processes predominate, and whether the organic food materials have come from within the river (autochthonous sources) or from the surrounding landscape (allochthonous sources). The magnitude and characteristics of the metabolic processes indicate the size of the food web and its capacity to support higher trophic levels, including fish, which are key targets for ecosystem management (Odum 1956; Sellars and Bukaveckas 2003; Oliver and Merrick 2006; Oliver and Lorenz 2010). As concentrations of dissolved oxygen (DO) are monitored to estimate rates of stream metabolism, these measurements provide ancillary information on the suitability of oxygen levels to support aquatic biota.

Planktonic photosynthetic microbes, comprised of microalgae and cyanobacteria, fix dissolved carbon dioxide using the energy of sunlight to form organic materials for cell growth (Sellers and Bukaveckas 2003; Oliver and Merrick 2006; Várbíró *et al.* 2018). The availability of light for phytoplankton photosynthesis is influenced by the depth of light penetration, water depth, and the intensity of water mixing that circulates phytoplankton through the upper illuminated surface layers. The availability and concentrations of nutrients can also affect photosynthesis and the production of organic materials (Reynolds 1984). Flow rates, water depth and water quality, are influenced by the volumes of water delivered from the different catchment sources, including contributions of environmental water.

Net ecosystem production (NEP), the difference between GPP and ER, is considered a measure of the overall carbon balance and is frequently used as an estimate of the basal food resource supply (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). If GPP>ER, carbon is accumulating, while if GPP<ER, carbon is being lost from the system. However, this interpretation implicitly assumes that fixation of carbon through photosynthesis is the primary source of organic material for respiration. This is not usually the case, as ecosystem respiration also includes the decomposition of allochthonous organic carbon by heterotrophs, and this results in heterotrophic production, an additional food resource not accounted for in the NEP calculation. Terrestrial organic carbon enters rivers in particulate and dissolved forms, but dissolved organic carbon is most actively incorporated by heterotrophic microbes such as bacteria and is a major driver of heterotrophic respiration (Graeber *et al.* 2018). Flow patterns are important in determining the sources and supply of terrestrial organic carbon, and as environmental flows influence these patterns, they will impact the carbon supply to the river food web (Oliver and Merrick 2006; Baldwin *et al.* 2016).

Major hypotheses

To assess the influence of environmental flows on river metabolism the following major hypotheses were addressed by the monitoring.

In the spring/summer period, the increased flows and improved patterns of delivery into the LMR due to additions of environmental water will:

- Increase phytoplankton photosynthesis and the supply of autochthonous organic carbon to food webs if changes to flow and water quality improve growth conditions, particularly through enhanced light and nutrient availability.
- Enhance ER rates and heterotrophic production if flows improve primary production, or better connect the channel with riparian, wetland or floodplain areas, increasing the supply of allochthonous organic carbon.
- Reduce the likelihood of low DO concentrations by increasing water mixing and oxygen exchange at the water surface in the otherwise low flow zones.

Methods

Field sampling

Rates of stream metabolism were estimated from changes in the concentrations of dissolved oxygen (DO) over each day. These measurements also provided information on the suitability of DO concentrations to support the aquatic biota. Monitoring consisted of *in situ*, continuous logging at ten-minute intervals of DO, water temperature, and incident light at three river sites. The first and second sites were downstream of Lock 6 and downstream of Lock 4, respectively, in the floodplain geomorphic zone, and the third was downstream of Lock 1 in the gorge geomorphic zone (Figure 1). Monitoring occurred from 9 September 2020 to 18 February 2021, with occasional interruptions (ca. one day) due to probe maintenance on field trips.

Two metre, depth-integrated water samples were collected during the ten field trips (ca. fortnightly), and analysed for chlorophyll *a*, total nitrogen, combined nitrate and nitrite, ammonium, total phosphorus, dissolved forms of phosphorus, and dissolved organic carbon. The detailed monitoring and analytical protocols described in Hale *et al.* (2014) were followed, but with some minor adjustments as detailed in Ye *et al.* (2018). In addition, the vertical light attenuation for Photosynthetically Active Radiation (PAR) was measured on each occasion using LiCOR underwater sensors.

The sampling regime resulted in 10 sampling dates at each of the three sites when water quality measurements coincided with metabolism measurements. To increase the sample number, and to help account for daily variation in metabolism in some analyses, water quality measurements and vertical attenuation coefficients were considered relevant for three days before and three days after sampling, and this extended data set (174 points in 2020-21) was used to explore relationships with metabolic rates. Collected water quality data were supplemented with monitoring data from nearby sites provided by the Australian Water Quality Centre of South Australia Water.

Estimating metabolic rates

Daily volumetric rates for gross primary production (GPP) and ecosystem respiration (ER) were estimated over 24-hour periods from midnight to midnight with the *BASE* program

(Grace *et al.* 2015). This uses Bayesian regression routines to fit the measured changes in DO concentrations to a widely applied model describing the daily fluctuations in water column DO concentrations (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). Oxygen based metabolic rates were converted to carbon units by assuming photosynthetic and respiratory quotients were equal to one.

The volumetric rates of metabolism were integrated over river depth and width based on channel characteristics derived from a morphometric model linked to water level (Section 2.1). The morphometric data included the average cross-sectional areas, average depths, and average widths at different flow levels for the river reaches at each monitoring site. The influence of flow on average depth and cross-sectional area is a function of channel morphometry, and increased flows increase or decrease these depending on channel shape. Daily flows measured during the monitoring periods were adjusted using hydrological modelling to estimate the flows and water levels that would have occurred without Commonwealth environmental water, and without any environmental flows.

Cross-sectional metabolic rates were calculated as the product of the volumetric rate and the average cross-sectional area. Volumetric rates are reported as gm/m^3/day (numerically equal to mg/L/day), area as m^2 and so cross-sectional rates are for a 1 m length of river. Metabolic rates were integrated over time by summing daily rates.

The influence of light on photosynthesis was determined from the mean light intensity encountered by phytoplankton mixed through the water column. The mean intensity depends on the incident irradiance (*Io*), the vertical attenuation of light in the water column (*kd*), and the average depth (z_{ave}). If the average depth is greater than the depth of the illuminated surface layer, then the mean irradiance (*Im*) is given by (Oliver and Merrick 2006):

$$Im = \frac{Io}{kd * Zave} \qquad (1)$$

The amount of phytoplankton biomass present was estimated from the chlorophyll concentration. Rates of GPP were standardised to the chlorophyll concentration (GPP(b)) and compared with the corresponding mean irradiances *Im*. The derived relationship provided a means for estimating relative changes in GPP(b) due to additions of Commonwealth environmental water and total environmental water, based on the changes in *Im* resulting from these flows. Conversion of modelled GPP(b) to estimates of GPP required knowledge of the chlorophyll concentration and it was assumed that the concentration on each day remained the same with and without environmental flows, so that relative changes in GPP(b) and GPP were equivalent. This aligns with the general assumption that the supply of water to the Selected Area from upstream was of the same quality under all flow scenarios, as the likelihood of changes in the relative contributions of different water sources under scenarios without environmental flows could not be predicted.

The contributions to ER by photosynthetic and heterotrophic microbiota was investigated using multiple regression of potential respiratory sources. GPP provided a measure of the organic carbon produced by photosynthesis, which is a major source of phytoplankton respiration (Beardall and Raven 1990). Dissolved organic carbon (DOC) concentrations provided a measure of the resource supply for respiration of heterotrophic microbes, largely bacteria. These two sources were previously identified as major contributors to ER (Ye *et al.* 2020a).

The net ecosystem production (NEP) was calculated as the difference between volumetric GPP and ER, giving an integrated measure of the metabolic balance. The phytoplankton net production (PNP) was calculated as the difference between the measured GPP and the estimated phytoplankton community respiration (PCR). As metabolism measurements do not provide estimates of the gross production of bacteria, the bacterial net production (BNP) rates were determined from the relationship between BNP, bacteria community respiration (BCR) and bacterial growth efficiency (BGE):

$$BNP = \frac{BGE * BCR}{1 - BGE} \qquad (2)$$

The BGE is influenced by a range of environmental conditions including the chemical composition of DOC. An average value of 0.2 was considered reasonable for the Murray River with its typical temperature range and water quality attributes (Rivkin and Legendre 2001; Marra and Barber 2004; Berggren and del Giorgio 2015), and BNP was estimated as BCR/4.

Results

Dissolved oxygen

Time series of dissolved oxygen (DO) concentrations showed similar patterns across sites and were generally higher than saturation concentrations, indicating positive photosynthetic production (Figure 13). There was no consistent association with flow, even during the flow pulse from mid-November to late December 2020, and the seasonal decline in DO concentration was attributed to the increasing water temperature as it mirrored the calculated change in saturation concentration (Figure 13).

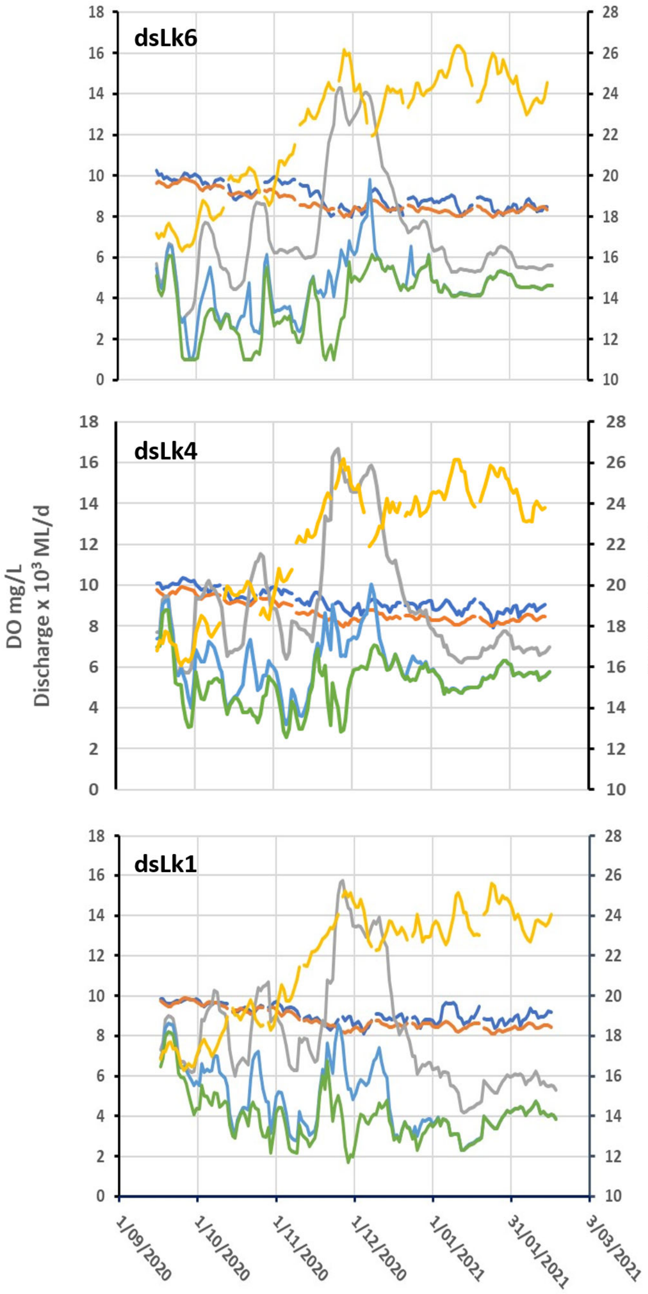


Figure 13. Dissolved (●) and saturated (●) oxygen concentrations, temperature (●), the daily discharge observed (●), and the daily discharge modelled without CEW (●) and modelled without environmental water (●) at monitoring sites downstream(ds) of Locks (Lk) 6, 4 and 1.

Water mixing, gas exchange and metabolism estimates

Low flows reduce water velocities and decrease the reliability of metabolism estimates because turbulent mixing declines and DO measurements no longer represent conditions throughout the water column. Previously it was demonstrated that the gas exchange coefficient (k) was correlated with water velocities above 0.18 m/s, increasing systematically at each site as velocity increased within channel (Ye *et al.* 2020a). The gas exchange velocity (K) is the exchange coefficient multiplied by the average depth, and accounts for differences in water depths, it also is correlated with water velocity (Figure 14). During the 2020-21 season estimates of K from downstream of Locks 1 and 4 showed similar relationships with water velocity as in previous years, but an unexpected result was observed downstream of Lock 6. Although many of the measurements downstream of Lock 6 fell within the bounds of previous data, the gas exchange velocity increased substantially above what was expected during the late spring/early summer flow pulse associated with Commonwealth environmental water delivery (Figure 14). The flow pulse was associated with a ca. 0.46 m water level raising in Weir Pool 5 (Appendix B). In contrast, during 2017-18 a similar flow peak, but of shorter duration, occurred during a period when the weir pool was at normal operating height and did not have enhanced gas exchange rates. A comparison of water levels between the two periods showed that the weir pool raising of 2020-21 had increased water level by ca. 0.2 m during the flow pulse and was associated with the enhanced gas exchange rates (Figure 15). The reasons for these differences in gas exchange are currently unknown but are presumably associated with increased turbulence during the 2020-21 flow, perhaps related to the increased water level variability.

The magnitude of the gas exchange velocity has an important influence on the likelihood of oxygen depletion. If the consumption of oxygen is greater than its resupply from the atmosphere, then the oxygen concentration falls until the consumption and resupply rates are in balance. Consequently, as flow and water velocity decline, the respiration rates that can be offset through gas exchange also decline and can lead to deleterious oxygen concentrations. Environmental water was considered to have contributed to the maintenance of observed DO concentrations during this period if it increased velocities to 0.18 m/s or greater. The periods, and scale of influence of these flows are depicted by the median average velocity timeline for each weir pool (Figure 7), and by the total river kilometres between Lock 1 and Lock 6 with velocities >0.2 m/s (Figure 6).

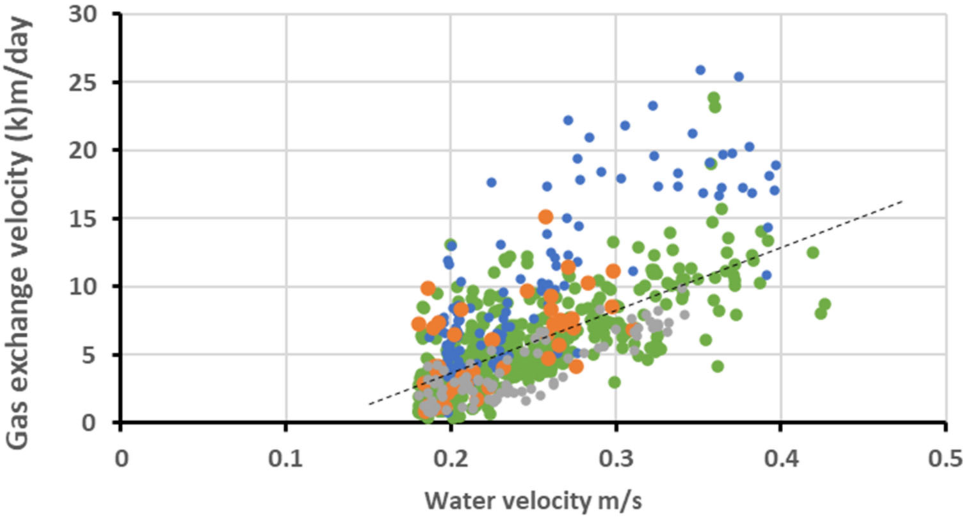


Figure 14. The relationship between the gas exchange velocity (K) and water velocity downstream of Lock 6 (●), Lock 4 (●) and Lock 1 (●) during 2020-21 and compared with previous years (●).

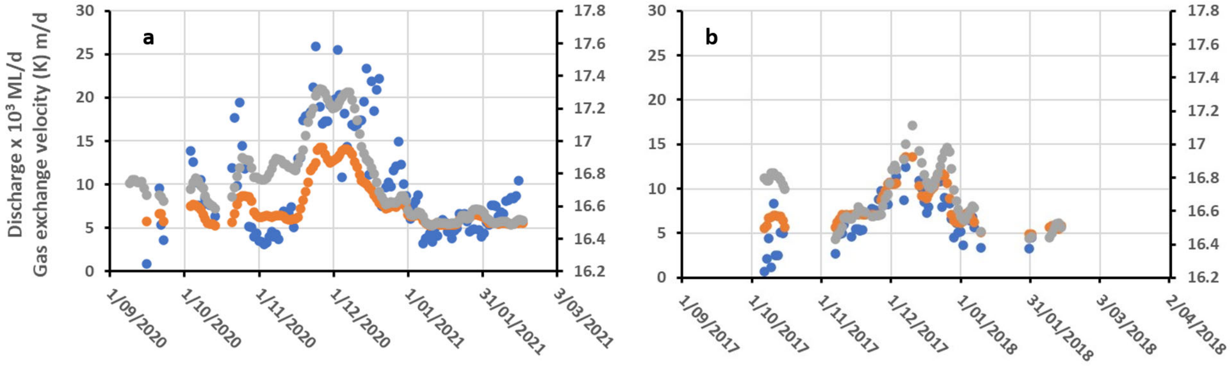


Figure 15. Time series of the gas exchange velocity (K) (●), water level (●) and discharge (●) during 2020-21 (a) and 2017-18 (b) at the downstream of Lock 6 site.

Daily metabolic rates

Patterns of daily GPP and ER were similar across sites over the monitoring period (Figure 16) and comparable with rates measured in previous years (Ye *et al.* 2020a). In response to the large increase in discharge in late spring/early summer 2020, volumetric rates of GPP reversed their seasonal increase and decreased until the end of the flow period when they again increased before levelling off (Figure 16). This pattern was mirrored by the changes in ER. If it is assumed that ER was largely associated with phytoplankton, then the average daily NEP (mgO_2/L/d) calculated as the difference in GPP and ER, was 0.78 downstream of Lock 6, 0.51 downstream of Lock 4, and 0.67 downstream of Lock 1. The cumulative NEP over the monitoring period was 115, 74 and 97 mgO_2/L respectively, or 43, 28 and 36 mgC/L.

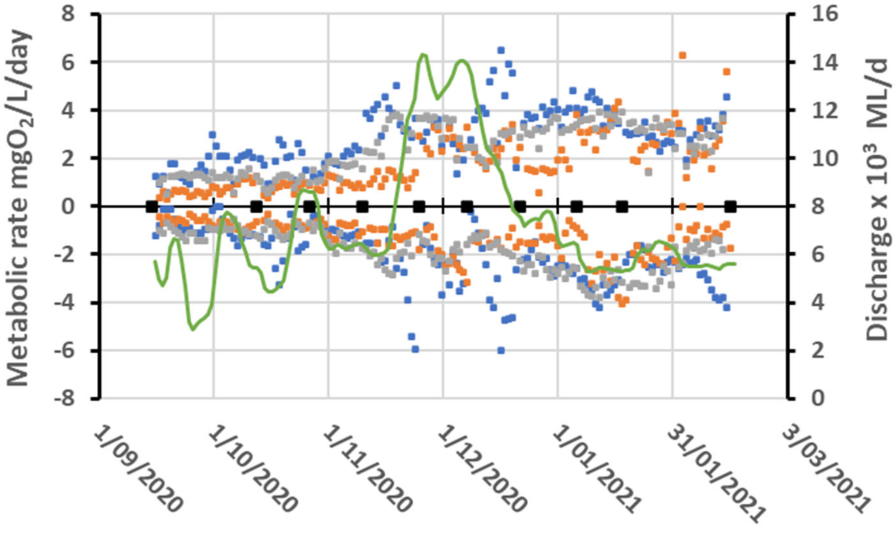


Figure 16. Daily gross photosynthesis (GPP, positive rates) and ecosystem respiration (ER, negative rates downstream of Lock 6 (●), Lock 4 (●) and Lock 1 (●) during 2020-21, with discharge at Lock 6 (●) and water quality sampling dates (●).

Determinants of gross primary production

Velocities of 0.18m/s or greater were used to identify periods when in-channel mixing was sufficient to ensure metabolism estimates were reliable (Oliver and Lorenz 2010; Ye *et al.*

2020a). Analysis of the 2020-21 GPP measurements meeting the reliability criteria supported previous findings of a significant linear correlation between the chlorophyll specific rate of gross photosynthesis (GPP(b)) and the mean irradiance in the mixed water column (*Im*) (Figure 17). The linear regression slope was equivalent to that reported for previous monitoring years (Ye *et al.* 2020a), and for channel reaches in up-stream sections of the river (Oliver and Merrick 2006).

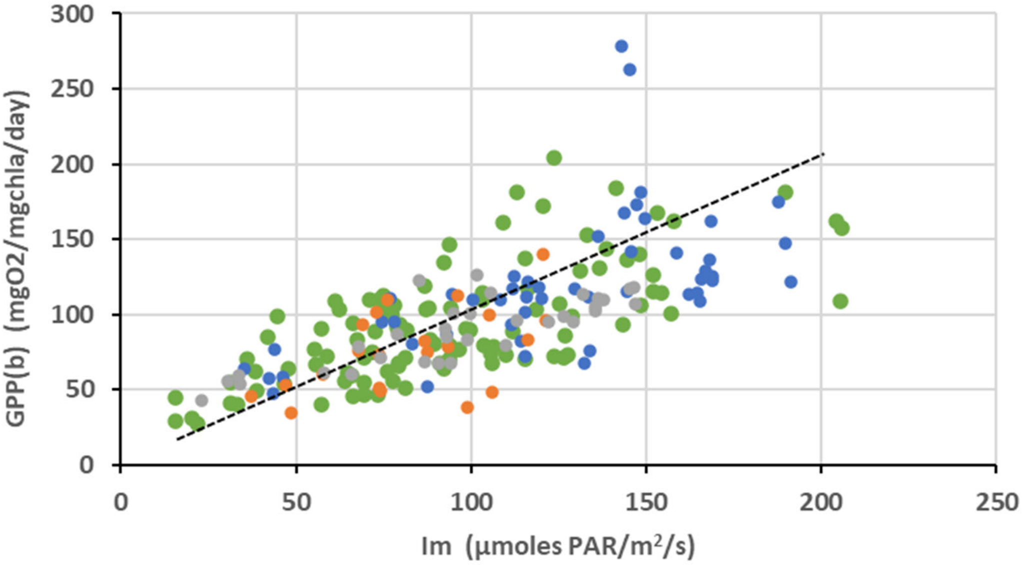


Figure 17. The relationship during 2020-21 of gross primary production per unit chlorophyll (GPP(b)) to the mean irradiance (Im) of the water column downstream of Locks 6 (●), 4 (●) and 1 (●), compared with all previous years and sites (●), for when water velocities were greater than 0.18 m/s, with an indicative line of the mean ratio of GPP(b)/Im (1.04 SE±0.03).

In naturally flowing rivers there is a relationship between flow and water depth, but within the weir pools of the LMR, this connection is decoupled. At these sites there is reduced change in water depth unless flows are sufficient to overwhelm the functioning of the weirs, or alternatively weir operations are managed to influence depth. The high flows associated with environmental water delivery between 16 November 2020 and early January 2021 resulted in water level and depth changes at each of the sites relative to modelled flows without environmental water (Figure 18). At the site downstream of Lock 6, the water depth change was enhanced by a 0.46 m raising of Weir Pool 5 in early November, approximately two weeks before the start of the flow pulse, resulting in a maximum depth change above the normal pool level of 0.6 m during the flow pulse (Figure 18). At the monitoring site downstream of Lock 4 the maximum depth change due to environmental water was 0.8 m above the normal pool level during the early peak of the flow pulse. The monitoring site downstream of Lock 1 is not in a weir pool but is connected by a long river reach to Lake Alexandrina and consequently water level is influenced not only by flow, but by levels in Lake Alexandrina that are determined by the barrages. Water level if also influenced by winds aligned with the long river reach pushing water upstream from the lake. At this site water depth slowly declined from 3.3 to 3.1 m over the monitoring period but increased during the high flow period by a maximum of 0.3 m (Figure 18).

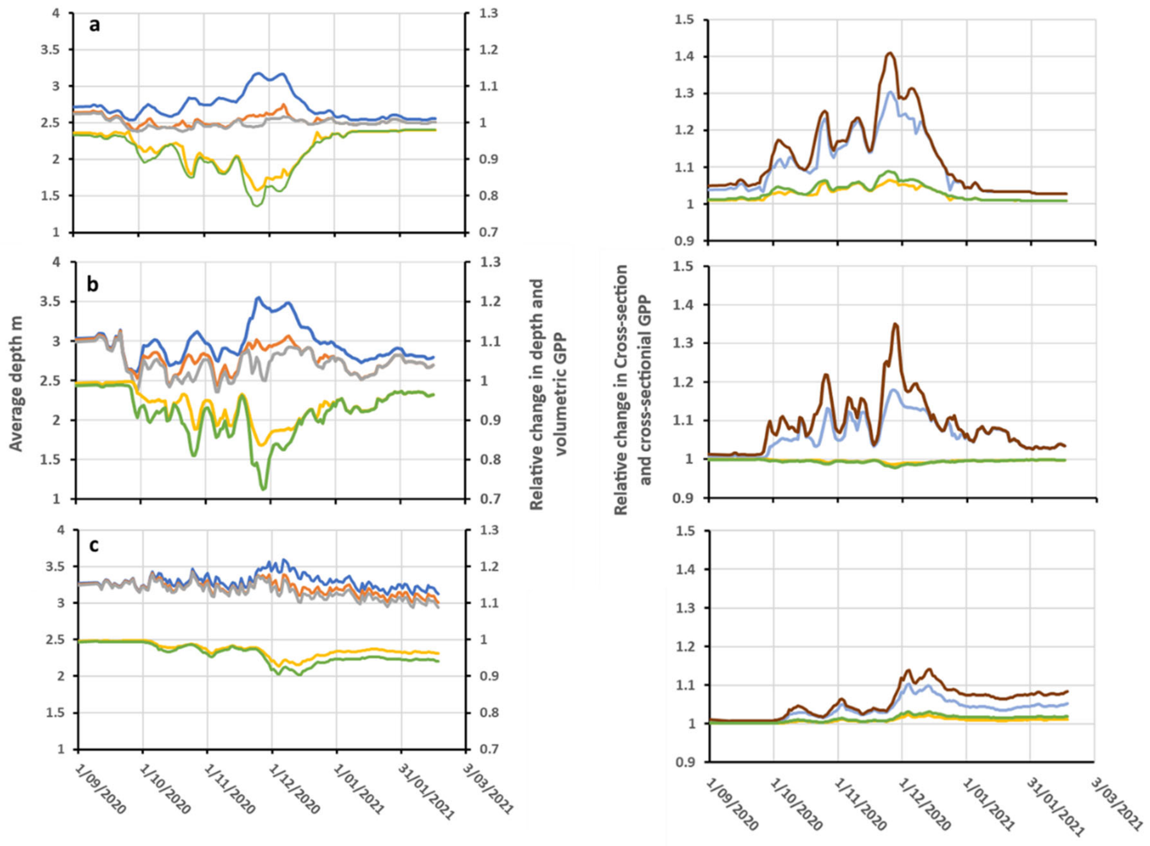


Figure 18. Changes downstream of (a) Lock 6, (b) Lock 4 and (c) Lock 1 in: (Left) the average depths observed (●), and modelled for flows without CEW (●), and without environmental water (EW) (●), and the fractional reduction in volumetric GPP predicted due to the increased depths associated with CEW (●) and EW (●). (Right) The fractional change in cross-sectional area due to additional flows modelled for CEW (●), and all environmental water (EW) (●), and the fractional change in cross-sectional GPP due to the combined change in depth and cross-sectional area for modelled flows of CEW (●) and all EW (●).

As *Im* is inversely related to the average depth of the water column (Equation 1), increases in flow that led to increases in depth reduced the mean light intensity, and reduced GPP(b) in inverse proportion to the relative change in depth (Figure 17). Assuming that the chlorophyll concentration remained unchanged between the actual and modelled flow conditions, then flow induced fractional changes in GPP(b) matched those of GPP. These relative changes were determined from the ratio of the modelled water depth without Commonwealth environmental water or without any environmental water to the observed depth with all flows (Figure 18). Downstream of Lock 6 in late November 2020, the increases in flow due to all environmental water were expected to reduce volumetric GPP by a maximum of 22%, while Commonwealth environmental water alone reduced GPP by a maximum of 20%. Downstream of Lock 4, environmental water had a larger effect, reducing GPP by a maximum of nearly 30% at the flow peak, while Commonwealth environmental water reduced GPP by 16%. Downstream of Lock 1 the flow influences were smaller and environmental water reduced GPP by a maximum of 10% in early December.

The cumulative effect of the depression in GPP due to additional flows was determined from the integrated production over the three-month peak production period from November to January. Due to the increased flows associated with Commonwealth environmental water, the cumulative production reduced at the monitoring sites, downstream of Lock 6 from 320 to 293 mgO_2/L (120 to 110 mgC/L), downstream of Lock 4 from to 274 to 251 mgO_2/L (103 to 94 mgC/L), and downstream of Lock 1 from 208 to 201 mgO_2/L (78 to 75 mgC/L). These relatively small differences reflect the short, one-month period of the flow pulse relative to the three-month integrating period. However, the impacts on river ecosystem functioning of 10% reductions in production rate are not well known, and the larger reductions during the actual period of the month-long flow peak might be expected to impact food resources at that time.

The volumetric rate of GPP measures the concentration supply of primary production but does not describe the total supply in the river, which will depend on the cross-sectional area of the flow (Ye *et al.* 2020a). Volumetric GPP rates were adjusted for the relative changes in average depth and multiplied by the corresponding cross-sectional areas. Despite reductions in the volumetric GPP at all sites, especially during the high flow period (Figure 18), the production per metre of river increased at sites downstream of Locks 6 and 1 due to larger relative increases in cross-sectional area (Figure 18). In contrast the cross-sectional production downstream of Lock 4 decreased slightly in response to the high flows (Figure 17) as the relative cross-sectional increase was of similar size to the relative volumetric decline in GPP. These opposite effects are a function of the relationship between depth and cross-sectional area at the different sites. The maximum changes in cross-sectional production due to all environmental water flows were increases of 9% below Lock 6 and 3% below Lock 1, and a decrease of 2% below Lock 4.

The effects of flow on the cumulative cross-section production were small within sites. Below Lock 6 Commonwealth environmental water increased production from 99 to 102 gO_2/m (37 to 38 gC/m) and below Lock 1 from 109 to 110 gO_2/m (40.9 to 41.3 gC/m). Below Lock 4 the change was negligible with the cumulative cross-section production being 123 gO_2/m (46 gC/m) over the monitoring period.

Although within site differences in the cumulative cross-sectional production due to environmental water were small, between site differences were larger, with below Lock 4 production 20% higher than below Lock 6. This reflected the differences in channel shape influencing average depths and cross-sections at the sites. In response to flow, larger changes in average depth and cross-sectional areas are expected in the absence of weirs as water level is not artificially maintained, implying that under unregulated conditions flow changes would have greater influences on the concentration and quantity of food resources.

Determinants of ecosystem respiration and net production

Rates of ER and GPP were converted to carbon units (mgC/L/day), and GPP and DOC were combined in a multiple linear regression to assess their contribution to ER. Analyses of data from all years, including from 2020-21, yielded a relationship with regression coefficients similar to previous estimates (Ye *et al.* 2020a):

$$ER = 0.688\ (\pm 0.05)*GPP + 0.058\ (\pm 0.011)*\ DOC - 0.202\ (\pm 0.065) \qquad r^2=0.51$$

The regression coefficients were used to estimate the separate contributions to respiration of phytoplankton (PCR) and bacteria (BCR) but are approximations due to the substantial unexplained variation (Figure 19a). Corresponding net production rates were estimated for phytoplankton (PNP) and bacteria (BNP) (Figure 19b). The overall respiration and net production rates attributable to bacteria were small relative to the phytoplankton, with BNP accounting for 20% of the combined net production (CNP), although contributions ranged from 8 to 47% over the season (Figure 19). The integrated CNP estimated over the three-month peak production period from November to January were 37, 29 and 23 mgC/L below Locks 6, 4 and 1 respectively. These are comparable with the estimates of NEP, 30, 21 and 22 mgC/L respectively, reflecting the low contribution from heterotrophic production, a result of the low DOC concentrations.

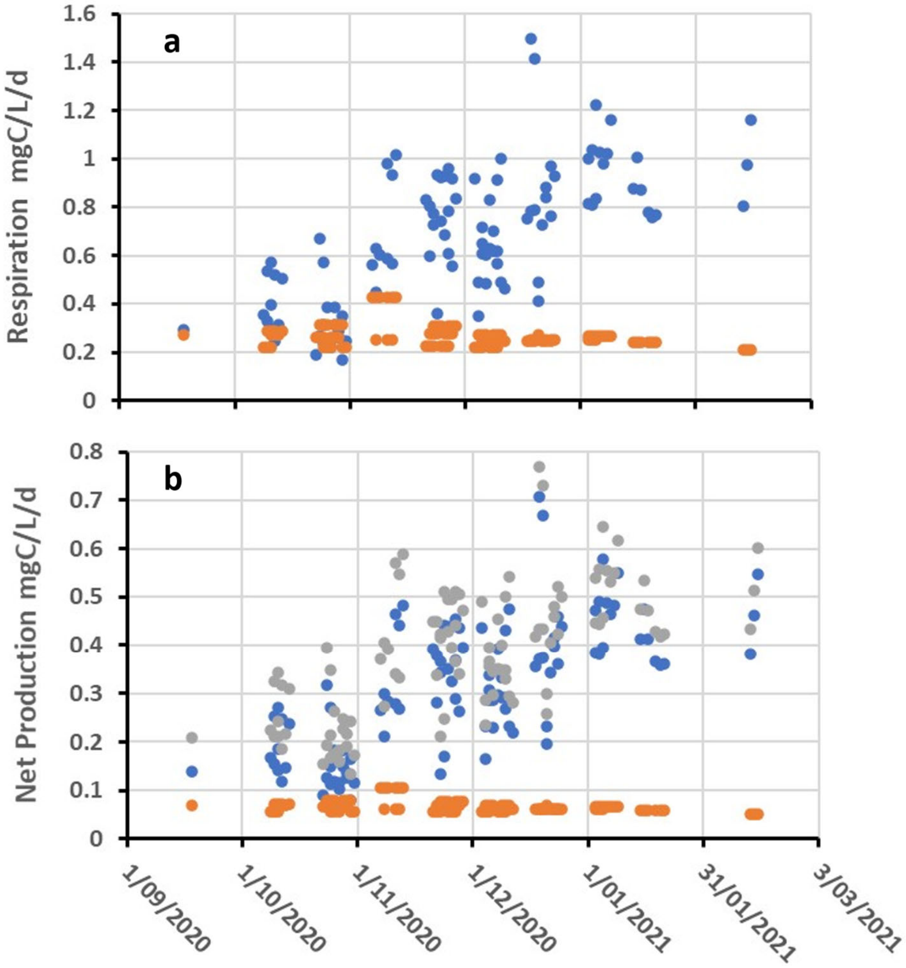


Figure 19. Respiration (a) and net production rates (b) of phytoplankton (●), bacteria (●) and net production of both combined (●) for all sites during 2020-21.

The effects of environmental flows on decomposition were estimated from BCR rates, but as daily DOC concentrations were considered equivalent with and without environmental water, daily volumetric rates of BCR were unchanged by environmental flows. Consequently, analyses were focused on cross-sectional rates which changed in proportion to the relative changes in cross-sectional area. The short-term changes were at times quite large, for example increasing BCR by up to 40% below Lock 6 due to environmental water during the flow pulse (Figure 18), suggesting a larger heterotrophic food resource per metre of river. However, the integrated effects were smaller as the period of the flow pulse was relatively short, as previously described in the GPP analyses.

Water quality

Chlorophyll concentrations varied between 15 and 44 mg/m^3 and generally increased until late November, then decreased across sites in response to the flow pulse, with a second peak below Locks 6 and 1 following the pulse (Figure 20). Turbidity decreased continuously following the arrival of the flow pulse and reflects the water quality of the upstream supply (Figure 20). The importance of turbidity to the vertical attenuation coefficient for PAR (*kd*) was previously reported (Ye *et al.* 2020a) and is evident from the matching responses (Figure 20). Concentrations of DOC averaged 4.5 mg/L across the three sites and showed little change except for sporadic increases at Lock 1, presumably related to local influences (Figure 20). Phosphorus and nitrogen can affect rates of metabolism and phytoplankton growth, but the data showed no clear evidence of this in 2020-21. Concentrations of total phosphorus (TP) declined over the monitoring period (Figure 20), matching the decline in turbidity and reflecting a close correlation between the two, indicating that much of the phosphorus was carried by suspended particles. In contrast, changes in total nitrogen (TN) concentrations mirrored those of chlorophyll, initially increasing, then declining during the flow pulse and with a peak following the pulse. This reflects a close association between TN and chlorophyll and may indicate a potential for nitrogen limitation of phytoplankton growth. However, this was not apparent in the relationship of GPP to light (Figure 17) which appeared constant under the changing TN conditions. The data suggested that phytoplankton metabolism was largely controlled by light, and heterotrophic metabolism by DOC concentrations. It is suspected that these resources constrained population sizes such that nutrients were not reduced to limiting concentrations.

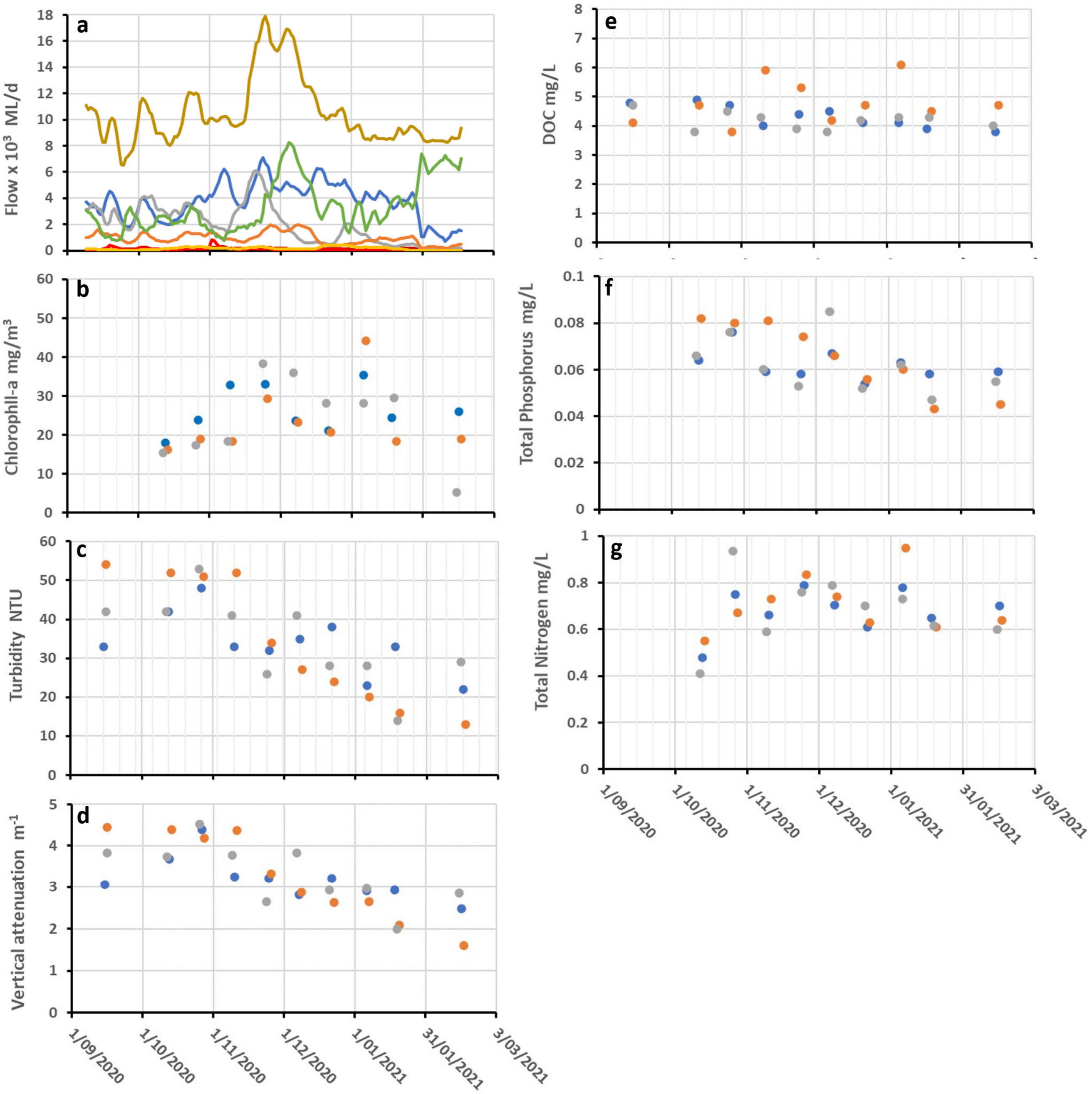


Figure 20. (a) Contributions of water sources to total flow (●) at the South Australian border due to: Darling River (●), Campaspe River (●) Broken Creek (●), Murrumbidgee River (●), Lake Victoria (●), Goulburn (●), and the Murray River (●). (b) Chlorophyll-a concentrations (c), Turbidity (d) vertical attenuation (k_d), (e) Dissolved organic carbon (f) Total phosphorus and (g) Total nitrogen below Locks 6 (●),4 (●) and 1 (●).

Evaluation

The contributions of Commonwealth environmental water to environmental outcomes associated with changes in dissolved oxygen, primary production and decomposition were assessed from a set of constructed metrics describing potential benefits. Significance levels were assigned to each evaluation question based on the extent that Commonwealth environmental water contributed towards the observed outcome, in one case the length of time of an enhanced effect, and in the other cases a percentage estimate of the extent of influence. The thresholds for significance levels among the indicators were based on expert opinion and may be adjusted in response to developing understanding of these processes.

Table 4. Stream Metabolism evaluation questions and answers relating to Commonwealth environmental water (CEW) and environmental water (eWater). DS = downstream.

CEWO evaluation questions	**Outcomes of CEW delivery**								
	Site	**eWater type**	**2014-15**	**2015-16**	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**
What did CEW contribute to dissolved oxygen levels?	**DS Lock 6**	All eWater	31	68	36	69	46	40	92
		CEW	0	52	22	51	26	30	79
		No eWater	0	0	9	12	1	0	53
	DS Lock 4	All eWater						57	100
		CEW						48	79
		No eWater						0	10
	DS Lock 1	All eWater	22	17	14	50	1	35	55
		CEW	22	17	9	35	1	35	52
		No eWater	0	0	10	0	0	0	7
	eWater/CEW decreased the likelihood of low DO by increasing water mixing and oxygen exchange at the surface. This was assessed as the extra days per year with water velocities >0.18 m/s due to eWater/CEW compared with No eWater. The contributions were significant, ranging from moderate to substantial across the sites in most years. A substantial contribution was considered greater than 30 days, moderate 15–30 days, minor 7–14 days and negligible < 7days. Note: Data for 2016-17 analyses commenced 6 January 2017 to avoid flood period.								

CEWO evaluation questions	Outcomes of CEW delivery								
	Site	eWater type	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21
What did CEW contribute to patterns and rates of primary productivity?	**DS Lock 6**	All eWater	1	2	1	2.5	1.5	1.5	3
		CEW	1	2	0	2	1.5	1	2.5
	DS Lock 4	All eWater						0	0
		CEW						0	0
	DS Lock 1	All eWater	0	0	1	0	0	1.5	1
		CEW	0	0	0	0	0	1	1
	Increased eWater delivery generally reduced the volumetric rate of primary production but increased the cross-sectional rate. This increased the overall “carrying capacity” of the river, although the implications of changes in the ratios of these two measures are unknown. The average percentage changes in cross-sectional GPP over the monitoring period are used to assess the influences of environmental flows on primary production. At the LMR sites, increases in cross-sectional GPP in response to eWater/CEW delivery were small because of the stable water levels induced by the weirs, but also because of the relatively short flow periods. However, changes during the flow pulses were larger than seasonal averages and likely to impact food web resources, but an evaluation of this is not yet developed. A substantial contribution was considered an increase in average cross-sectional GPP of 20% or greater, moderate 11–19%, minor 5–10%, negligible <5%.								
What did CEW contribute to patterns and rates of decomposition?	**DS Lock 6**	All eWater	5	4	8	10	3	1	15
		CEW	1	4	4	6	2	1	13
	DS Lock 4	All eWater						5	12
		CEW						5	9
	DS Lock 1	All eWater	2	0	10	4	2	7	8
		CEW	2	0	5	2	1	4	5

CEWO evaluation questions	Outcomes of CEW delivery								
	Site	eWater type	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21
	Bacterial respiration (BCR), a measure of decomposition, is directly related to DOC concentrations. Modelling of the influence of flows on BCR assumed that daily DOC concentrations were the same with and without eWater and so volumetric BCR was unaffected by flows. Evaluation was based on the percentage changes in river cross-sectional BCR due to the addition of CEW or eWater which were determined from the average change in cross-sectional area over the monitoring period. From 2014–2021, increases were generally small at all sites due to the constant water level maintained by the weirs and the short flow pulses except that, in 2020-21, there were moderate increases below Locks 6 and 4 due to eWater and a moderate increase below Lock 6 due to CEW, increasing basal food resources to the river. A substantial contribution was considered an increase in cross-sectional BCR of 20% or greater, moderate 11–19%, minor 5–10%, negligible <5%.								

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):

⬜ Unknown 🟥 Negative ⬜ None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

Dissolved oxygen concentrations remained near saturation levels throughout the 2020-21 monitoring period, reflecting the moderate flows (<18,000 ML/d) and low heterotrophic respiration rates. Environmental water delivery contributed to the maintenance of the DO concentrations by increasing water velocities above 0.18 m/s and sustaining critical surface gas exchange for an additional 55 to 100 days across the different monitoring sites (Table 4). These contributions are significant, helping avoid potential DO drawdown and the associated potential for fish kills.

The effects of environmental flows on volumetric GPP rates were substantial during the 2020-21 flow pulse with maximum reductions of 10–30% apparent in the time series (Figure 18). These reductions might be expected to impact planktivores dependent on the food concentration for their feeding efficiency (Rothhaupt 1990; Kiørboe *et al.* 2018), but the effect is difficult to assess. In part, this is because the flow pulse was relatively short, ca one month of the three-month monitored peak production period, such that the integrated impact was a 10% or less reduction in GPP. To assess the potential ecological impacts of volumetric reductions in GPP there is a need to develop evaluation criteria that consider the magnitude and patterns of responses to unregulated flows, where increases and decreases in production are normal occurrences. An estimate of integrated GPP for unregulated conditions (e.g. prior to river regulation or under conditions without weir impact), and the fluctuations in GPP in response to unregulated flows, would provide a context for assessment of the influence of the weirs, and of the regulated flow changes, on integrated GPP.

Increases in flow increased the cross-sectional areas at the monitoring sites by 10–40%, but because these increases were associated with similar, but opposite relative changes in water depth, increases in the overall cross-sectional rates of production were small, <10% below Locks 6 and 1 and slightly negative below Lock 4. The impacts of these changes on food resources are difficult to assess, and as discussed with respect to changing volumetric rates, evaluation criteria are required that set these findings in the context of responses to unregulated conditions.

Previously, the influences of flow on metabolism were modelled at an unregulated river site and demonstrated significant increases in the integrated cross-sectional production of up to 30% or more when flows were increased by environmental water (Ye *et al.* 2020a). Conversely, a reduction in average depth during low flows reduced the cross-sectional rate of GPP, but increased the volumetric rate by 20% in the modelled reach, a result that might not have occurred where water depth was controlled by a weir, such as in the LMR. This illustrates the major effect weirs have on metabolism through their disruption of the normal relationships between flow, water level and cross-sectional area. Understanding the relevance of these morphological disruptions to the functioning of food webs is important in order to manage flows for the benefit of the riverine ecosystem. Data to improve this understanding is being collected in the MER LMR Research Project "From Productivity to Murray Cod recruitment".

In addition to photosynthetic production, the food web was also supported by the supply of heterotrophic production through DOC utilisation. During most of the 2020-21 monitoring period, the bacterial contribution to ER was smaller than that of phytoplankton (Figure 19). The combined net production (CNP) of phytoplankton and bacteria was similar in magnitude to estimates of NEP reflecting the small bacterial contribution resulting from low and stable DOC concentrations. In previous seasons, when DOC concentrations were higher, the bacterial contribution to net production was larger and at times exceeded that of the phytoplankton (Ye *et al.* 2020a). Both heterotrophic and phytoplankton production are important sources of organic carbon to the river and supplies of DOC are critical to providing food webs with organic carbon through the heterotrophic pathway.

The effects of environmental flows on decomposition were estimated from BCR, but as daily DOC concentrations were considered equivalent between the flows with and without environmental water, daily volumetric rates of BCR were unchanged by environmental flows. Instead, analyses focused on responses of cross-sectional rates, and daily changes were substantial with increases of up to 40%, matching the changes in cross-sectional areas. As the period of the flow pulse was relatively short, integrated decomposition was less than the peak values, but rates were still 12 and 15% higher below Lock 4 and Lock 6 due to environmental flows. Increased BCR rates result in enhanced BNP, increasing basal food resources to the river.

Metabolism is influenced by water quality, especially turbidity and DOC as both affect the light available for photosynthesis, with DOC also critical to heterotrophic production. Data from the LMR have not demonstrated an influence of the major nutrients, nitrogen and phosphorus, as metabolism has appeared unresponsive to changing concentrations. This suggested that light and DOC were limiting the development of microbial populations and that nutrient limitation was not being induced.

Management implications

In general, Commonwealth environmental water deliveries increased the average water depth reducing volumetric GPP, but simultaneously increased cross-sectional areas which either increased or decreased the cross-sectional GPP depending on the channel shape. Shifts in the volumetric and cross-sectional rates of food production are likely to have fundamental effects on the composition and functioning of food webs. The empirical models demonstrated that changes in flow had a greater positive influence on these metabolic attributes in unregulated channels undergoing more ‘natural’ responses in water depth and cross-sectional area than in river reaches regulated by weirs (Ye *et al.* 2020a). What is not clear is how these different patterns influence overall food web productivity. Currently, there are no suitable ecological models to place the changes induced by river regulation into the context of unregulated river responses, or to assess the implications of these to food resources and food web size and structure. The empirical models derived from the metabolism monitoring data indicated that, compared to unregulated sites, weirs might be increasing total cross-sectional production by holding water levels above natural low flow levels, but reducing volumetric rates by sustaining greater depths. Data from unregulated sites would help to assess the impact of these production shifts on biotic community composition and help provide management targets that align more with natural responses. The need to identify suitable metabolic and food web targets is critical to the application of metabolic measurements to river management, and the most suitable template is likely to be the response to more natural, unregulated conditions.

Environmental flows which alter the attenuation of light through increased turbidity have a major influence on GPP. Turbidity in the LMR is substantially influenced by flows from the Darling River which naturally moved downstream into South Australia but are now often diverted into Lake Victoria for later supply. The impact of turbidity on river metabolism raises questions regarding the timing and volume of supply from these turbid sources and the benefits of mixing flows of different water quality to manage turbidity. The historical influence of turbidity on production in the LMR is unknown making target setting difficult. The empirical metabolism models provide a means for assessing the influence of turbidity on production, and for investigating historical influences, including their timing and duration, to help set suitable targets.

A major driver of metabolic activity is the external supply of organic carbon to the river. Estimates of the autotrophic, heterotrophic and combined net production (CNP) over all monitored years have demonstrated that both were important sources of organic carbon to the LMR, with autotrophs generally providing a larger contribution. However, the external DOC supply was critical in determining the response of the heterotrophic pathway and during floods when DOC increased, this became the major source of organic carbon (Ye *et al.* 2018). These changes influence the quantity, quality, and characteristics of the food supply to the food web, but the influences on riverine community structure, including higher trophic levels, are not well known and is a major impediment to linking basal food resources to desired ecological outcomes. Data to improve this understanding is being collected in the MER LMR Research Project “From Productivity to Murray Cod recruitment”.

Environmental flows can help reduce the likelihood of low DO concentrations in the LMR if they increase water velocities above a level of ~0.18m/s, below which surface oxygen exchange is poor. However, the level of flow required to generate adequate surface exchange to offset de-oxygenation is also influenced by the DOC concentration which affects heterotrophic respiratory oxygen depletion. If DOC is high, then surface exchange may not be able to re-supply oxygen at rates sufficient to sustain oxygen levels, potentially leading to “black water” events (Baldwin *et al.* 2016; Whitworth and Baldwin 2016). Currently there are no suitable models for critically assessing these interactions at the river scale.

The links between hydrology, water quality and metabolism are complex, and the developed empirical models that describe individual processes such as gas exchange, vertical attenuation, GPP, and ER, interact dynamically both in time and space. The individual models identify the magnitude of responses in metabolism that environmental flows may produce, but the application of these models would be greatly enhanced by incorporation into a dynamic, integrated, river scale framework. The Source model (Welsh *et al*. 2013) provides such a structure and would enable the models to be used in a more predictive way to assess the likely impacts of environmental water delivery. Such a model would also assist in the analyses of historical data to investigate and compare responses in regulated and unregulated river reaches, helping provide a framework for improved target setting.

Conclusion

The study has identified flow modified environmental conditions that influence GPP and ER including the:

- reliance of GPP on the mean light encountered by phytoplankton;
- effects on GPP of interactions between flow and channel morphometry;
- reliance of the mean light on turbidity and DOC;
- respiration rates of phytoplankton and bacteria;
- reliance of bacterial respiration on DOC concentration;
- contributions of phytoplankton and bacteria to net production;
- effect of water velocity on surface oxygen exchange; and
- contribution of environmental flow to improving velocities and reducing the likelihood of low DO.

Understanding these interactions is critical to predicting the likely changes in metabolism and net production within a particular river reach due to the delivery of environmental flows of given volume and water quality. The findings in relation to weir pools reflect their effect on river hydrology with a major impact on metabolism through disruption of the relationships between flow, water level and cross-sectional area. Knowledge of these links provides the means for assessing the effects of environmental flows on the basal food resources, an important target for river management. The dynamic nature of these interactions highlights the need for measurements at a range of different sites, and for the incorporation of the models into an integrating, river-scale framework to help provide a template for target setting.

2.3 Matter Transport and Coorong Habitat

2.3.1 Matter Transport

Background

Freshwater flows to estuaries shape habitat, transport nutrients to drive productivity, and generate a salinity gradient that impacts water quality and provides spawning cues for fish. Reduced flow may increase the intrusion of salt into the system from the ocean whilst simultaneously decreasing the export of salt from inland reaches. As there is continual deposition of salt onto the landscape (predominately from rainfall), it can accumulate if not transported by flow and exported from the system. Additionally, a change in the flow regime will alter the mobilisation of nutrients from the floodplain and change the subsequent primary productivity within the river. Environmental flows can be used to reinstate some of the natural processes or to increase the magnitude of the processes that control the availability and transport of dissolved and particulate matter. Salinity, dissolved and particulate organic nutrients, and chlorophyll *a* are often measured or modelled to understand the influence of flows on the concentrations and transport of matter.

In general, restoring river flow to an estuary is an important tool for salinity management by limiting seawater intrusion into the estuary. In the case of the Murray River, it is further complicated as seawater entering the Murray Mouth is highly dependent on how river water is released from the barrages, and these dynamics impact salinity in the connected Coorong. The Coorong is a 120 km long shallow reverse estuary that runs parallel to the coast and is separated from the Southern Ocean by Young Husband Peninsula. As water evaporates from the North and South lagoons, it is replaced with water coming from the north. When river water is exiting the barrages, it reduces sea-water intrusion and maintains fresher conditions around the Murray Mouth which enables fresher water to replenish water evaporated in the Coorong. When there is more seawater intrusion into the Murray Mouth, considerable amounts of salt are transported into the Coorong as this seawater flows south-east to replenish water evaporated from the lagoons. The salinity of the Coorong is a key determinant of habitat suitability for macrophytes, invertebrates and fish.

Nutrients drive system productivity and so understanding how they are transported between the various components of riverine ecosystems can offer insights into river and estuary productivity (assessed as Chlorophyll concentration). Dissolved inorganic nutrients are essential resources for the growth and survival of biota and are readily assimilated (Poff *et al.* 1997). In healthy ecosystems, mobilisation of nutrients can enhance productivity and support ecosystems, however, in poorly flushed or over-enriched systems, the addition of nutrients leads to eutrophication and numerous undesirable consequences.

To assess the contribution of environmental water delivery to matter transport in the LMR from 1 July 2020 to 30 June 2021, a hydrodynamic-biogeochemical model was applied for the region below Lock 1 to the Murray Mouth (Figure 1). The cumulative impacts environmental water delivery on salt and nutrient transport in the Coorong were assessed with the Coorong Dynamics Model (CDM) in the years 1 July 2017 – 30 June 2021. The model has been validated with water quality data, and is used to understand salt, nutrient and phytoplankton movement.

Major hypotheses

Commonwealth environmental water will:

- Increase the mobilisation of salt from the Basin and increase the transport of salt passing from Lock 1 through the Murray River Channel and through the Lower Lakes and Murray Mouth;
- Increase the mobilisation of nutrients from the Basin and increase nutrient loads passing from Lock 1 through the Murray River Channel and through the Lower Lakes and Murray Mouth;
- Increase suspended solid loads (including phytoplankton biomass) passing from Lock 1 through the Murray River Channel and through the Lower Lakes and Murray Mouth; and
- Decrease the rate of salt and nutrient accumulation in the North and South lagoons of the Coorong.

Through the modelling undertaken in the Long-Term Intervention Monitoring (LTIM) Project, it was well established that environmental flow diluted the concentration nutrients and salinity in the reach between Lock 1 and the barrages, including Lake Alexandrina and Lake Albert. Furthermore they increased the load (volume x concentration) of nutrients and salt that was exported from the reach. This report focuses on export from the basin which is the flow and matter that exit the barrages. This addresses the hypotheses above acknowledging that environmental flow increases the transport of salt and nutrients that pass from Lock 1 through the Murray Channel and through the Lower Lakes. The impact of water exiting the barrages on the salt flux and salinity in the Coorong was also assessed as salinity is a key determinant of suitable habitat for fish and *Ruppia*.

Methods

eWater modelling assessment scenarios

Five scenarios were considered in this modelling investigation:

- **All water (base-case scenario):** this scenario represents observed conditions and was run with the compiled barrage flows from 1 July 2017 to 30 June 2021;
- **No CEW 4 years:** this scenario was run with assumption that there was no Commonwealth environmental water (CEW) through the barrages from 1 July 2017 to 20 June 2021, aiming to investigate the continuous effect of CEW water on the Coorong in 4 years;
- **No eWater 4 years:** this scenario was run with assumption that there was not any environmental water (including no CEW) through the barrages from 1 July 2017 to 30 June 2021, aiming to investigate the continuous effect of all eWater on the Coorong in 4 years;
- **No CEW 1 year:** this scenario was run with assumption that there was no CEW water through the barrages in the last modelling year from 1 July 2020 to 30 June 2021, aiming to investigate the effect of CEW water on the Coorong in the last year only;
- **No eWater 1 year:** this scenario was run with assumption that there was not any environmental water (including no CEW) through the barrages from 1 July 2017 to

30 June 2021, aiming to investigate the effect of all eWater on the Coorong in the last year only

This report presents the outcomes from the modelling assessment, with deliveries of:

- Barrage flow analysis in the 'All Water', 'No CEW' and 'No eWater' scenarios;
- Time series and transect comparisons of salinity and nutrient concentrations between the five investigated scenarios;
- Detailed salt and nutrient fluxes analysis through the barrages, Murray Mouth, Long Point (North Lagoon) transect, and Parnka Point (South Lagoon) transect in all scenarios;
- Mean and median concentrations at the mouth, North Lagoon, and South Lagoon in all scenarios; and
- *Ruppia* and fish habitat analysis in all scenarios.

Hydrodynamic-biogeochemical models

The Coorong Dynamic Model (CDM), a high-resolution model of the Coorong that had been used in the 2019-20 environmental water assessment and undergone developments during the Healthy Coorong Healthy Basin (HCHB) project, was used to assess the effects of environmental water delivery on salt and nutrient transport. The modelling platform was consisted with a hydrodynamic model TUFLOW-FV, developed by BMT Global Pty Ltd., which is coupled with a biogeochemical model AED, developed by the University of Western Australia. The CDM had a high resolution mesh for better resolving the water quality conditions and habitat, and has been previously used to assess the contribution of environmental water to the transport of dissolved and particulate matter for water years 2017–2020 (Ye *et al*. 2021). The model has undergone development during the HCHB Trials & Investigation projects since the last environmental water assessment report (Ye *et al*. 2021), such as improvement in the sediment flux settings based on the newly available sediment survey data (Mosley et al. 2020), and updates in the barrage flows, tidal elevations and Salt Creek flow data (see Appendix D). Details of the CDM development, model sensitivity analysis and model performance have been summarised in the online CDM manual (http://aquaticecodynamics.github.io/cdm/index.html)

Barrage flow analysis

Analysis of the flow through the barrages is shown in detail in Figure 21–Figure 24. The contribution of environmental water to the total barrage flow is shown in Figure 21, which indicates that the environmental water dominated the barrage flows over the study period from 1 July 2020 to 30 June 2021, except a pulse of natural (unregulated flow) flow in July–October 2020. As the data provided for the environmental water accounting only contained the total flow for all the barrage combined, and do not resolve details of specific releases of each barrage (which were required for the model), we compared the environmental water data with the local estimates of barrage flow provided by the DEW barrage calculator (Figure 21–Figure 23), then used this to allocate the actual daily environmental flow to each barrage based on the overall proportion of barrage flow (Figure 24). The. The results indicate the dominance of flows through Goolwa and Tauwitchere over the period of interest, with some minor pulses through Mundoo (Figure 24).

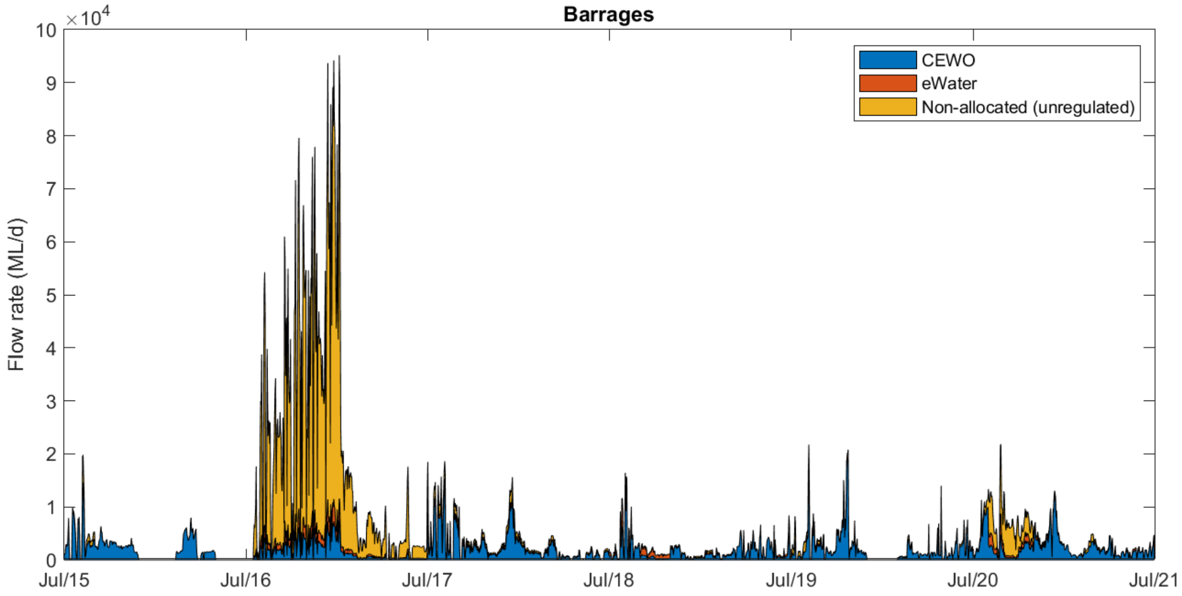


Figure 21. Summary of history of flow through the barrages, from July 2015 until June 2021, spanning the large 2016 flow event and a pulse of natural flow in July – October 2020, categorised based on water source.

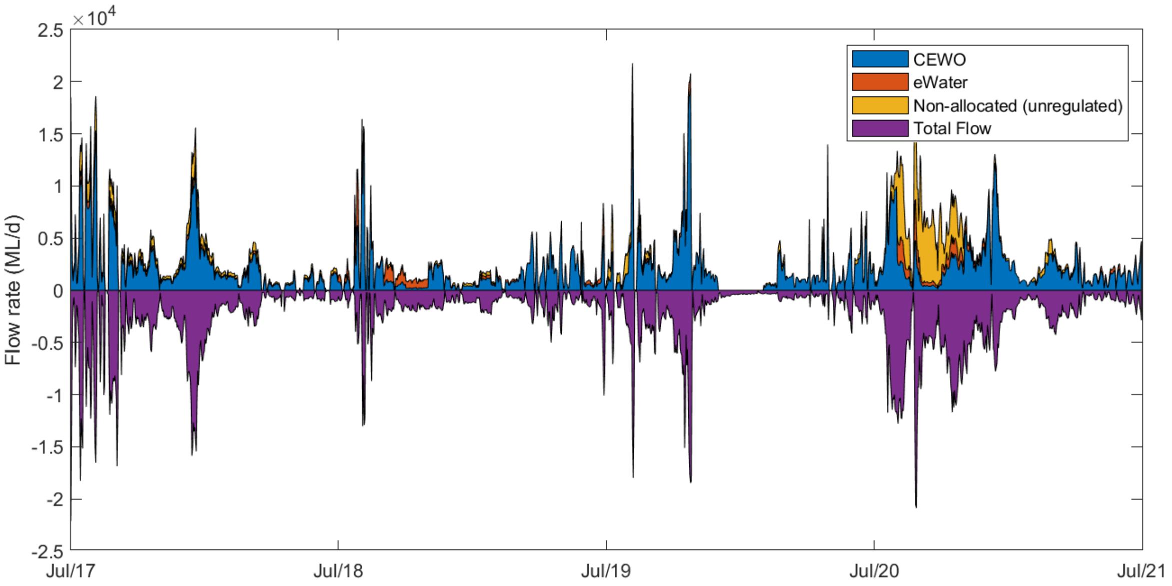


Figure 22. Comparison of *daily* barrage flow between the datasets from environmental water accounting (upper panel) and from local estimates of barrage flow (lower panel), spanning the modelling period from July 2017 to June 2021.

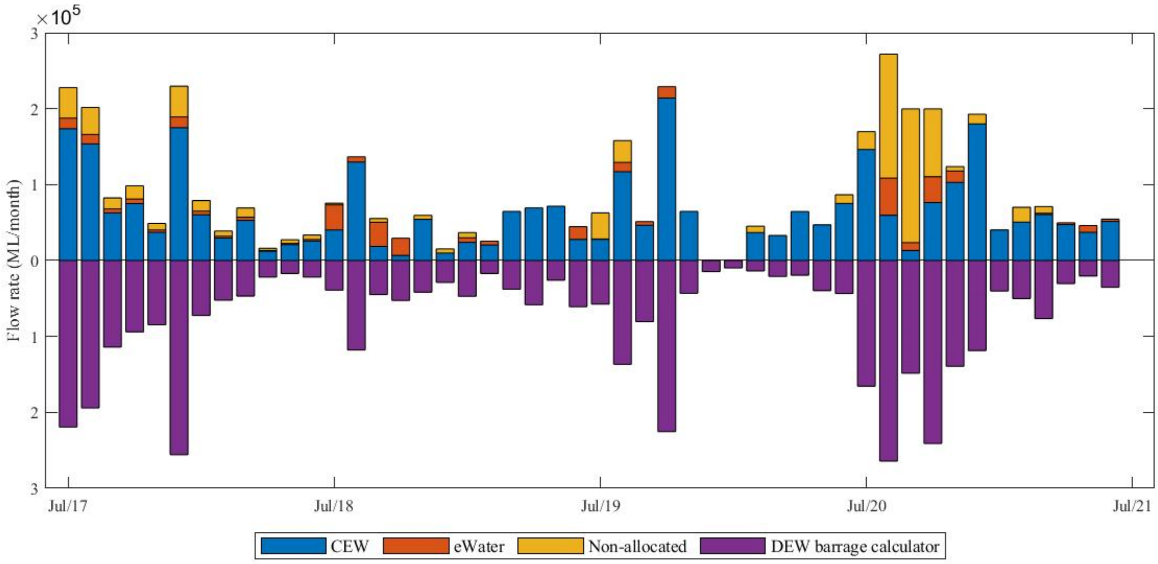


Figure 23. Comparison of *monthly* barrage flow between the datasets from environmental water accounting (upper panel) and from local estimates of barrage flow (lower panel), spanning the modelling period from July 2017 to June 2021.

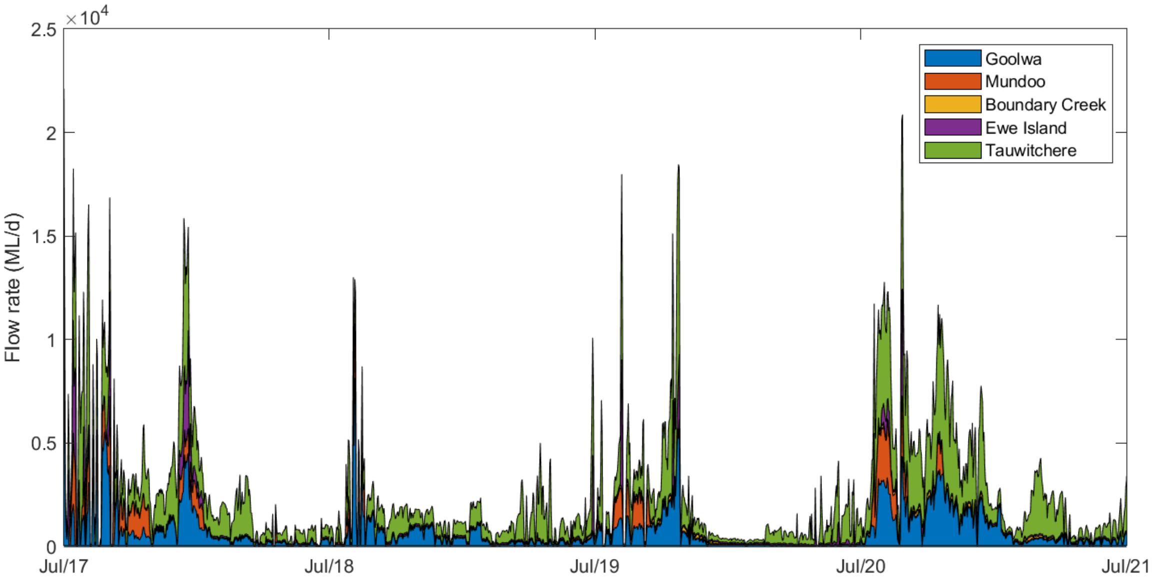


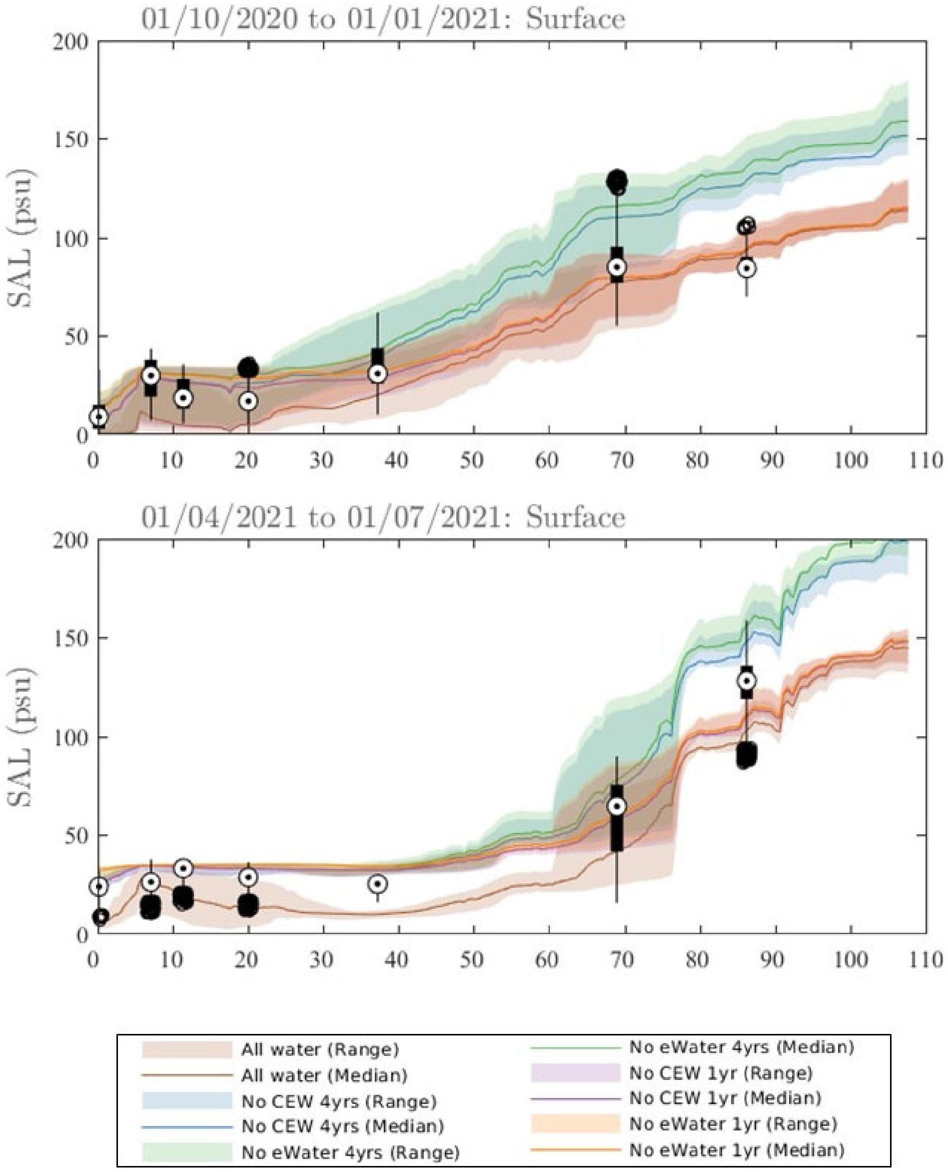
Figure 24. Summary of flow over each barrage, spanning the modelling period from July 2017 to June 2021.

Results

<u>Salinity dynamics in the Coorong</u>

Example transect plot of predicted salinity in all scenarios with comparison to the observations along the Coorong at the beginning of the season (1 July 2020 – 1 Oct 2020; Figure 25 upper panel) and at the end of the season (1 April 2021- 1 July 2021; (Figure 25 lower panel), which indicates higher salinity in the 'no CEW' and 'no eWater' scenarios. These time periods were chosen because they represented the cumulative effects of eWater on the salinity in the last year of modelling period; the upper panel showed the effect in the beginning season (1 July – 1 Oct 2020) and the lower panel showed the effect in the end season (1 April 2021 – 1 July 2021).

Predicted salinity changes in all scenarios at selected sites along the Coorong is presented below in Figure D5, Appendix D. The predicted salinity from the 'all water' scenario, which overlapped with the 'no CEW 1 year' and 'no eWater 1 year' scenarios in the first 3 years, showed good agreements with observations at most of the sites along the Coorong, except at Goolwa (A4261036) where the model generally underestimated the salinity. However, the salinity at the key areas of interest in the South Lagoon (sites A4260633, A4261209, and A4261165) have been well predicted. It is evident that the no CEW and no eWater conditions led to higher salinity at all sites.


01/10/2020 to 01/01/2021: Surface

01/04/2021 to 01/07/2021: Surface

Figure 25. Transect comparison of measured and simulated salinity along the Coorong lagoon in July-September 2020 (upper panel) and April-June 2021 (lower panel). The boxplots indicate the field observations; the solid lines indicate the median values and the shaded area indicate the 25th to 75th percentile from the modelling assessment.

Salt flux through the Murray barrages, the mouth and the Coorong

Salt loads for 2020-21 modelling year are summarised in Table 5. A total salt load of 1,846,289 tonnes was exported from the river basins into the Coorong in this year. If there was no environmental water in 2020-21 then a total salt load of 620,843 tonnes would have been exported from the basin. Commonwealth environmental water was responsible for the export of 1.07 million tonnes of salt, representing 87% of the total contribution by environmental water.

In 2020-21, there was a net export of 268,730 tonnes of salt from the Coorong into the Southern Ocean (through the Murray Mouth). Without environmental water, there would be a net import of 4,570,048 tonnes of salt from the Southern Ocean to the Coorong. Environmental water decreased salt import by approximately 4.8 million tonnes in 2020-21, most of which was attributable to Commonwealth environmental water (4.2 million tonnes). The environmental water also helped to reduce the salt flux to the North Lagoon and South Lagoon (Table 5; Figure 26). The southward salt fluxes to the north Coorong and south Coorong in the 'all water' scenario were 1,189,661 and 2,376,665 tonnes, respectively, which were much less than the 'no eWater 1 year' scenario of 4,416,836 and 3,331,846 tonnes.

Table 5. Summary of salt flux (tonnes) though barrages, Murray mouth, Long Point (North Lagoon) and Parnka Point (South Lagoon) in 2020-21.

Site	Scenario	Salt flux (tonnes)
Barrages (from lakes to Coorong)	With all water	1,846,289
	No CEW 4 years (07/2017 - 07/2021)	774,436
	No eWater 4 years (07/2017 - 07/2021)	620,843
	No CEW 1 year (07/2020 - 07/2021)	774,436
	No eWater 1 year (07/2020 - 07/2021)	620,843
Murray Mouth (from Southern Ocean to Coorong)	With all water	-268,730
	No CEW 4 years (07/2017 - 07/2021)	2,948,191
	No eWater 4 years (07/2017 - 07/2021)	3,512,169
	No CEW 1 year (07/2020 - 07/2021)	3,963,604
	No eWater 1 year (07/2020 - 07/2021)	4,570,048
North Lagoon (southward)	With all water	1,189,661
	No CEW 4 years (07/2017 - 07/2021)	3,540,085
	No eWater 4 years (07/2017 - 07/2021)	3,886,882
	No CEW 1 year (07/2020 - 07/2021)	4,030,524
	No eWater 1 year (07/2020 - 07/2021)	4,416,836
South Lagoon (southward)	With all water	2,376,665
	No CEW 4 years (07/2017 - 07/2021)	4,439,346
	No eWater 4 years (07/2017 - 07/2021)	4,672,910
	No CEW 1 year (07/2020 - 07/2021)	3,166,868
	No eWater 1 year (07/2020 - 07/2021)	3,331,846

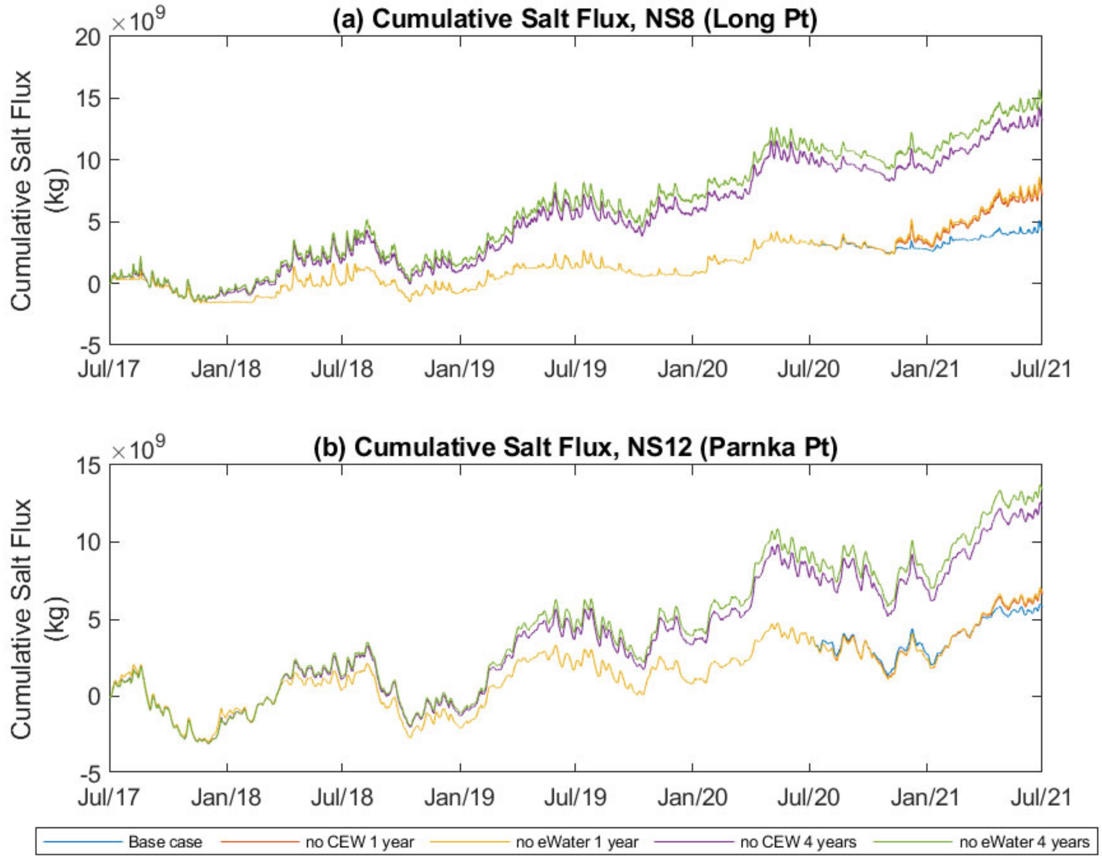


Figure 26. Cumulative salt flux with and without environmental water delivery through the Long Point and Parnka Point for July 2017–June 2021.

Environmental water reduced the salinity in the estuary especially in the South Lagoon (Table 6), where the median salinity in 2020-21 would have been 143.9 practical salinity units (psu) if there was no environmental water since 2017, compared to the median value of 95 psu in the ‘all water’ scenario. The mean nutrient concentrations and chlorophyll-a concentration in the south Coorong were also generally lower in the ‘all water’ scenario compared to the ‘no eWater 4 years’ scenario, indicating the cumulative effect of the environmental water helped to improve the water quality in this area.

Table 6. Summary of mean and median salinity, concentrations at the Murray Mouth, North Lagoon, and South Lagoon between July 2020 – June 2021.

Site	Scenario	Murray Mouth Salinity (PSU)	North lagoon Salinity (PSU)	South lagoon Salinity (PSU))
Mean Salinity	With all water	12.6	18.3	95.41
	No CEW 4 years (07/2017 - 06/2021)	24.3	38.5	135.2
	No eWater 4 years (07/2017 - 07/2021)	25.8	41.1	142.4
	No CEW 1 year (07/2020 - 07/2021)	23.1	30.31	98.7
	No eWater 1 year (07/2020 - 07/2021)	24.5	32.11	99.2
Median Salinity	With all water	12.7	18.1	95.0
	No CEW 4 years (07/2017 - 07/2021)	29.6	37.7	136.6
	No eWater 4 years (07/2017 - 07/2021)	30.5	39.4	143.9
	No CEW 1 year (07/2020 - 07/2021)	27.7	32.0	100.1
	No eWater 1 year (07/2020 - 07/2021)	29.5	33.7	101.4

Nutrients and chlorophyll a

The total nutrient loads in 07/2020 – 07/2021 are summarised in Table 7, which showed the barrage flow brought a large amount of nutrient from the river basin into the Coorong, most of which were then exported to the Southern Ocean through the Murray Mouth. The environmental water also helped to reduce the TN flux to the North Lagoon and South Lagoon (Figure 27). While environmental water has a very large and ecologically significant impact on nutrient export it does not impact the concentrations of nutrients very much (Table D1, Appendix D).

Table 7. Total loads of inorganic and organic matter (tonnes) in 2020-21. Chl-a= chlorophyll-a, ON = organic nitrogen, OP = organic phosphorus.

Site	Scenario	Ammonium	Nitrate	Phosphate	Silica	ON	OP	Chl-*a*
Barrages (from lakes to Coorong)	With all water	31.8	29.7	55.7	1256.0	2328.5	263.5	43.2
	No CEW 4 years (07/2017 - 07/2021)	15.6	13.8	17.6	530.0	926.1	66.1	17.8
	No eWater 4 years (07/2017 - 07/2021)	12.9	10.9	13.6	415.7	745.0	54.3	14.2
	No CEW 1 year (07/2020 - 07/2021)	15.6	13.8	17.6	530.0	926.1	66.1	17.8
	No eWater 1 year (07/2020 - 07/2021)	12.9	10.9	13.6	415.7	745.0	54.3	14.2
Murray Mouth (from Southern Ocean to Coorong)	With all water	-34.6	-15.5	-18.4	-1172.8	-1276.1	-110.6	-31.6
	No CEW 4 years (07/2017 - 07/2021)	-23.3	-9.6	-5.0	-373.3	-380.7	-18.9	-3.7
	No eWater 4 years (07/2017 - 07/2021)	-21.0	-7.7	-3.4	-250.2	-256.6	-12.7	0.4
	No CEW 1 year (07/2020 - 07/2021)	-24.8	-12.1	-5.0	-363.0	-383.2	-18.5	-4.0
	No eWater 1 year (07/2020 - 07/2021)	-22.6	-10.5	-3.4	-238.5	-258.9	-12.2	0.02
North Lagoon (southward)	With all water	-1.1	2.7	14.1	72.4	254.5	57.2	5.7
	No CEW 4 years (07/2017 - 07/2021)	2.1	2.6	4.0	149.4	181.0	19.8	6.0
	No eWater 4 years (07/2017 - 07/2021)	3.0	3.0	3.2	158.5	174.3	18.1	6.1
	No CEW 1 year (07/2020 - 07/2021)	1.0	1.2	4.0	155.7	177.3	20.0	5.9
	No eWater 1 year (07/2020 - 07/2021)	1.8	1.4	3.2	166.1	171.3	18.4	5.9
South Lagoon (southward)	With all water	9.7	2.1	-0.02	154.7	160.4	15.1	0.6
	No CEW 4 years (07/2017 - 07/2021)	15.0	24.4	0.2	207.1	169.0	13.8	1.2
	No eWater 4 years (07/2017 - 07/2021)	16.3	31.7	0.2	213.0	169.4	13.9	1.4
	No CEW 1 year (07/2020 - 07/2021)	11.2	3.5	-0.004	176.3	158.9	12.0	0.6
	No eWater 1 year (07/2020 - 07/2021)	11.7	3.9	-0.006	181.4	159.1	12.1	0.7

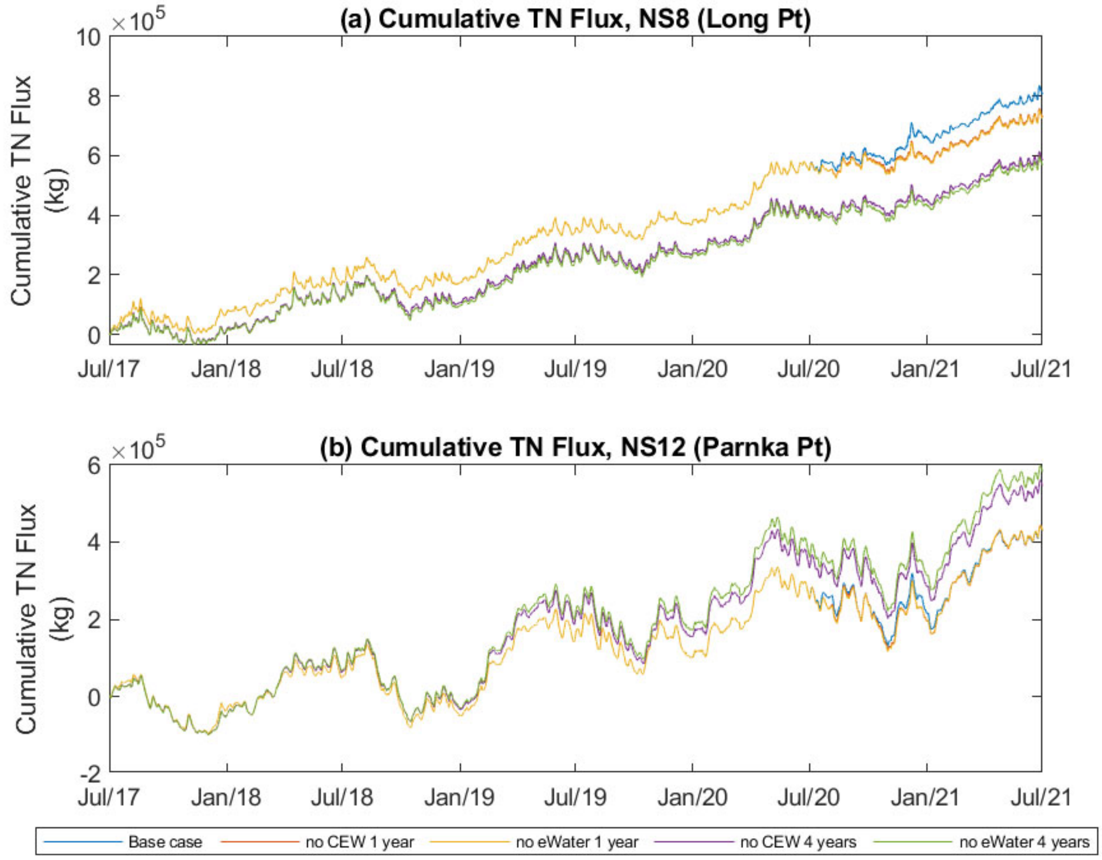


Figure 27. Cumulative TN flux with and without environmental water delivery through the Long Point and Parnka Point for July 2017–July 2021.

Evaluation

The evaluation approach, including assessment criteria, is described in the evaluation section for Hydraulic Regime (Section 2.1).

Table 8. Matter Transport evaluation questions and answers. CEW = Commonwealth environmental water.

CEWO evaluation questions		Outcomes of CEW delivery						
		14-15	**15-16**	**16-17**	**17-18**	**18-19**	**19-20**	**20-21**
What did CEW contribute to salinity:	Levels? Reduction due to CEW (Median salinity presented, PSU) At the Murray Mouth	From 34.6 to 24.0 *	From 35.1 to 29.7 *	From 21.6 to 11.3 *	From 28.9 to 15.9*	From 31.9 to 21.1*	From 31.9 to 21.3*	From 27.7 (without CEW) to 12.7 (with CEW)
	In the North Lagoon (Long Point)							From 32.0 (without CEW) to 18.1 (with CEW)
	In the South Lagoon (Parnka Point)							From 100.1 (without CEW) to 95.0 (with CEW)
	Transport? (tonnes salt per year) Additional export through the barrages due to CEW	285,064 *	251,632*	120,867 *	436,848*	532,222*	623,999*	1,071,853
	Reduction of salt import at the Murray Mouth	3,044,70 *	4,591,269 *	519,292 *	1,670,937*	1,847,273 *	1,997,037 *	4,232,334
	Reduction of southward salt flux at the North Lagoon (LP)						1,979,123 *	2,840,863

CEWO evaluation questions		Outcomes of CEW delivery						
		14-15	15-16	16-17	17-18	18-19	19-20	20-21
	Reduction of southward salt flux at South Lagoon (PP)	⬜	⬜	⬜	⬜	⬜	🟩 1.695,443 *	🟩 790,203
What did CEW contribute to the salinity regime?		🟩 CEW increased salt export through the barrages; and reduced salt intrusion into the Murray Mouth from the ocean, which reduced salinity in the Coorong. CEW played a key role in delivering flow to the Coorong, particularly during dry years with up to 100% of barrage releases being CEW. CEW has played a key role in salt export from the Basin, however, salt export remains below the Basin Plan target of 2 million tonnes of salt per year. In 2020-21 CEW contributed to over 1 million tonnes of salt export through the barrages which was 58% of all salt exported. CEW plays a significant role in reducing salt import through the Murray Mouth and reducing salt transport into the North and South lagoons of the Coorong. In the high flow year (2016-17), 1.5 million tonnes* was exported and CEW contributed 8% (120,867 tonnes) of salt export.						

* The salt export and salinity data reported for years 2014-15 to 2016-17 are from the large domain model previously used in the LTIM reporting. The numbers for 2017-18 to 2019-20 are from the new high-resolution Coorong only model, which uses a different method for barrage flow calculation and has a more accurate specification of salinity and salt flux. When we compared the two models, salt flux at the barrages was underestimated by the full domain model, and hence the 2014-15 to 2016-17 are systematically lower than the other years. The model has undergone further refinement as part of the Healthy Country Healthy Basin Project which predicts considerably more salt being exported through the barrages. The predictions of salt export reported for 2020-21 in this report (Table 5) are considered to be more accurate but they are larger than previously reported (Appendix D).

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):

⬜ Unknown 🟥 Negative ⬜ None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

Salinity

Environmental water continued to be a major contributor to salt export from the basin, fresher conditions at the Murray Mouth and a barrier to influx of salt from the Southern Ocean to the Coorong which keeps the Coorong fresher. Commonwealth environmental water accounted for 80–100% of annual environmental flow releases through the barrages during the dry years (between 2014-15 and 2020-21 except for 2016-17).

Salinity in the Coorong is primarily a function of riverine inflows and tidal movement. When barrage flows are low, seawater enters the Murray Mouth and more salt is then transported to the Coorong where it is subject to evapo-concentration. Commonwealth environmental water contributed 58% of the total salt flux from the Murray-Darling basin in 2020-21. Furthermore, environmental water through the barrages reduced the salt load to the South Lagoon measured as salt flux southward at Parnka point, by 4.67 million tonnes from July 2017 to June 2021. It is also evident that environmental water flowing through the barrages is required in every year to reduce excessive salt accumulation within the Coorong (Figure 29). If environmental water had not been delivered in 2020-21, an additional 3.1 million tonnes of salt would have accumulated in the South Lagoon.

During the Millennium Drought, and particularly from 2007-08 to 2009-10, flow through the barrages ceased and the import of salt into the Coorong resulted in salinity in the South Lagoon that was five times seawater, and demise of much of the aquatic life (Brookes *et al.* 2009). Environmental water provides freshening flows but also acts to inhibit seawater intrusions, thereby maintaining more appropriate salinity conditions for estuarine biota in the Coorong. Given that barrage releases almost entirely (up to 100%) depend on Commonwealth environmental water in dry years, environmental water is critical for limiting salt flux to the South Lagoon. Even one year without barrage flow can result in a large flux of salt southwards (Table 5; Figure 26). Salinity is a key determinant of *Ruppia* and fish habitat and is addressed in the following section.

Nutrients

During the LTIM monitoring period (2014–2019), the median concentrations of nitrogen and phosphorus did not vary considerably with or without environmental water. Environmental water, however, contributed considerably to the transport of nutrients, and this was primarily due to additional water volume not a change in the nutrient concentrations. It was evident that environmental flows contributed a considerable load of nutrients to the Murray Mouth between 2014-15 and 2020-21. From this evidence, it can be concluded that environmental flows are a key driver in promoting estuarine productivity. Environmental water does not have a significant effect on median nutrient concentrations in the North or South Lagoon. There is a general consensus that elevated nutrients in the South Lagoon of the Coorong are contributing to the formation and persistence of algal mats. The legacy nutrients in the sediments are contributing to this issue but it is apparent nutrients delivered by environmental flows do not alter nutrient concentrations significantly.

Management implications

There are approximately 10^{11} tonnes of salt in groundwater in the MDB and an additional 1.5 million tonnes of salt is deposited in the basin each year by rainfall (Herczeg *et al.* 2001). Unless salt is exported from the basin with flow, there will be a net accumulation of salt within the basin. The Basin Plan sets out a salt export objective (section 9.09) to ensure adequate flushing of salt from the Murray River system into the Southern Ocean. The Basin Plan's indicative target for salt export from the Basin is 2 million tonnes per year. The five years of salt export modelling enable the contribution of environmental flows to salt export to be scrutinised.

Commonwealth environmental water played a key role in salt export from the basin, accounting for 58–100% of total salt export, depending on the volume of unregulated flows and other environmental water. In the high flow year (2016-17), 1.5 million tonnes were exported and Commonwealth environmental water contributed 8%.

Maximum exports of matter from the Murray Mouth are likely to be achieved by delivering environmental water during periods of low oceanic water levels (e.g. summer). In contrast, environmental water delivery to the Murray River Channel at times of high oceanic water levels, which peak in the Austral winter, is likely to increase the exchange of water and associated nutrients and salt through the Coorong, rather than predominately through the Murray Mouth.

Conclusion

Though the numbers of salt and nutrient fluxes from this modelling assessment are a little different to those in the 2019-20 report (Ye *et al.* 2021) due to the changes in the scenario settings, barrage boundary conditions, and the biogeochemical model settings of the Coorong Dynamics Model, the conclusion from the current study remained similar to the ones from the 2019-20 report, that the contributions of environmental water appear to have significantly increased the export of salt from the Basin. Environmental water decreased salt import by approximately 4.8 million tonnes in 2020-21, most of which is attributable to Commonwealth environmental water (4.2 million tonnes). The environmental water also helped to reduce the salt flux southward to the north Coorong and south Coorong to 1,189,661 and 2,376,665 tonnes, respectively, which were much less than 4,416,836 and 3,331,846 tonnes if no environmental water was delivered in 2020-21.

2.3.2 Coorong Habitat

Background

The Coorong is an estuarine lagoonal system with a natural salinity gradient ranging from freshwater to hyper-saline at the extremity. Freshwater flows are important in maintaining estuarine habitat and ecosystem health and preventing extreme hyper-salinity (Brookes *et al*. 2009). *Ruppia tuberosa* is an important macrophyte in the Coorong that provides habitat for fish and food for herbivorous birds in the Coorong (Phillips and Muller 2006), and it can tolerate a salinity higher than natural seawater. The germination and growth of *R. tuberosa* is known to be governed in large part by changes in salinity and water level regimes, which are influenced by flows through the barrages (Kim *et al.* 2013). Other factors that influence *R. tuberosa* growth include nutrient availability, water temperature, sediment quality and interactions with algae, including shading of light and interference with flowers and fruits on the surface (Collier *et al*. 2017). Flows over spring and into early summer are likely to be particularly beneficial as they delay the drop in water level in the South Lagoon and can prevent extreme salinities emerging, thereby improving chances of completion of the reproductive cycle of *Ruppia*. In addition, salinity has also been identified as a key driver of fish distribution and assemblage structure by influencing the extent of estuarine fish habitat in the Coorong (Ye *et al.* 2011; 2016a; Bice *et al.* 2018). This sub-indicator aims to assess the benefits of environmental flows for the enhancement of *R. tuberosa* habitat, particularly those that are delivered in late spring and early summer, as the improvement of estuarine fish habitat throughout the year for several key species with different levels of salinity tolerance.

Major hypothesis

Increased freshwater flow through the barrages and into the Coorong due to environmental watering will prevent areas of extreme salinity and maintain water levels in the Coorong, thus expanding the extent of *R. tuberosa* and fish habitats.

Methods

The habitat extent of *R. tuberosa* ("*Ruppia*") and fish were estimated using previously reported environmental thresholds (Collier *et al*. 2017; Ye *et al*. 2016a), consistent with the 2019-20 MER report (Ye et al. 2021). The *Ruppia* ecological response model has capability to account for habitat suitability of critical life stages, and is designed to estimate the probability of replenishing the sediment seed-bank, turion sprouting, seedling development to juvenile plants, and adult plant flowering and seed setting (Collier *et al*. 2017). Each stage (adult, seed, flowering) is assigned a habitat suitability based on cell specific light, depth, salinity and temperature, which in the end results in a combined probability of sexual life-cycle completion. A suitability index for asexual reproduction was considered but it is not considered sufficiently robust to include. Where the index was above 0.4, we computed a suitable area of habitat, by multiplying the habitat score by the cell area, and summing over all cells within both North and South lagoons. The fish habitat model was implemented to calculate probabilities of habitat suitability for juveniles of key species based on known salinity thresholds, adopting a seasonal effect by account for temperature sensitivity to the salinity thresholds, according to the same functions and parameters described in the previous report (Ye et al. 2021).

Results

The *Ruppia* habitat areas in the Coorong in the modelling years are summarised in Figure 28, including the suitable habitat area for different stages. The comparison of the overall area of habitat, which is an index to complete a full sexual reproduction life-cycle, showed a general decrease of 44% the calendar year 2020 in the 'no CEW 4 years' and 'no eWater 4 years' scenarios when compared to the 'all water' scenario, and this appears to be mainly limited to the area suitable for successful flowering (Figure 28). The suitable habitat area for adults and seeds appears to be slightly higher in the scenario which has no environmental water delivery, but this was due to fresher salinity around the barrages and Murray Mouth (Figure 29), which is not the target area for *Ruppia* management or restoration.

The suitable habitat area for each fish species is summarised in Figure 30, which showed the environmental flows had led to fresher conditions in the Coorong and an expansion of suitable fish habitat area. Reduction of fish habitat areas of ~6–11% were predicted if there was no environmental water in 2020-21, and the reduced area focused on the middle and south Coorong (Figure 31). Reduction of 33–45% (species dependent) in the habitat areas for fish species were predicted in the Coorong if there is no environmental water for four years continuously (i.e. since July 2017) (Figure 30).

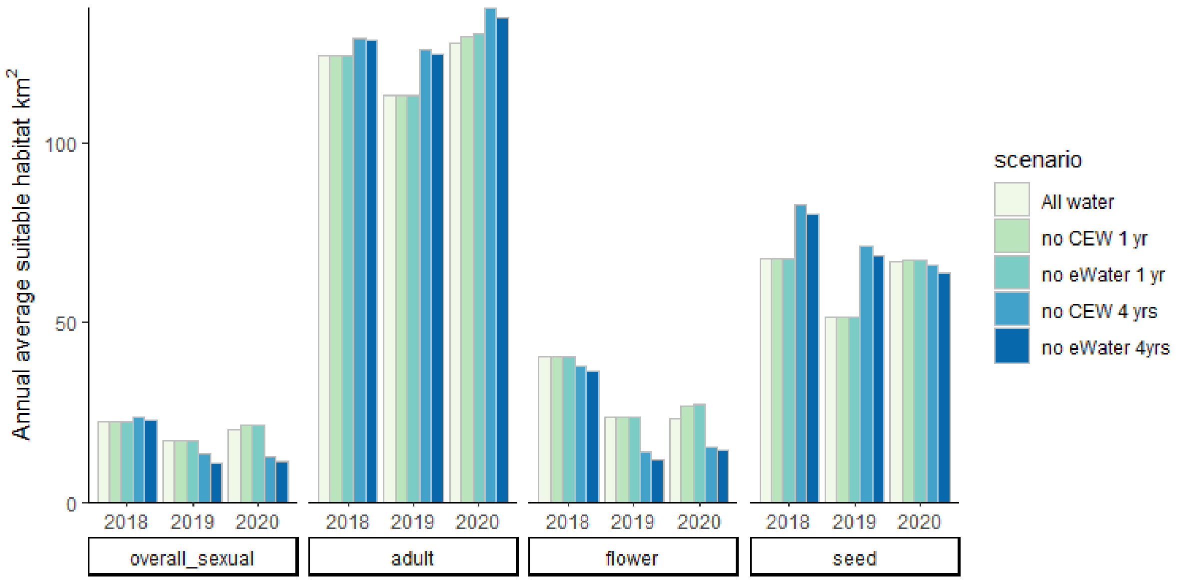


Figure 28. Habitat area suitable for *Ruppia tuberosa* (overall and individual life stages), comparing the "All water" (base-case) and "no CEW"/"no eWater" scenarios.

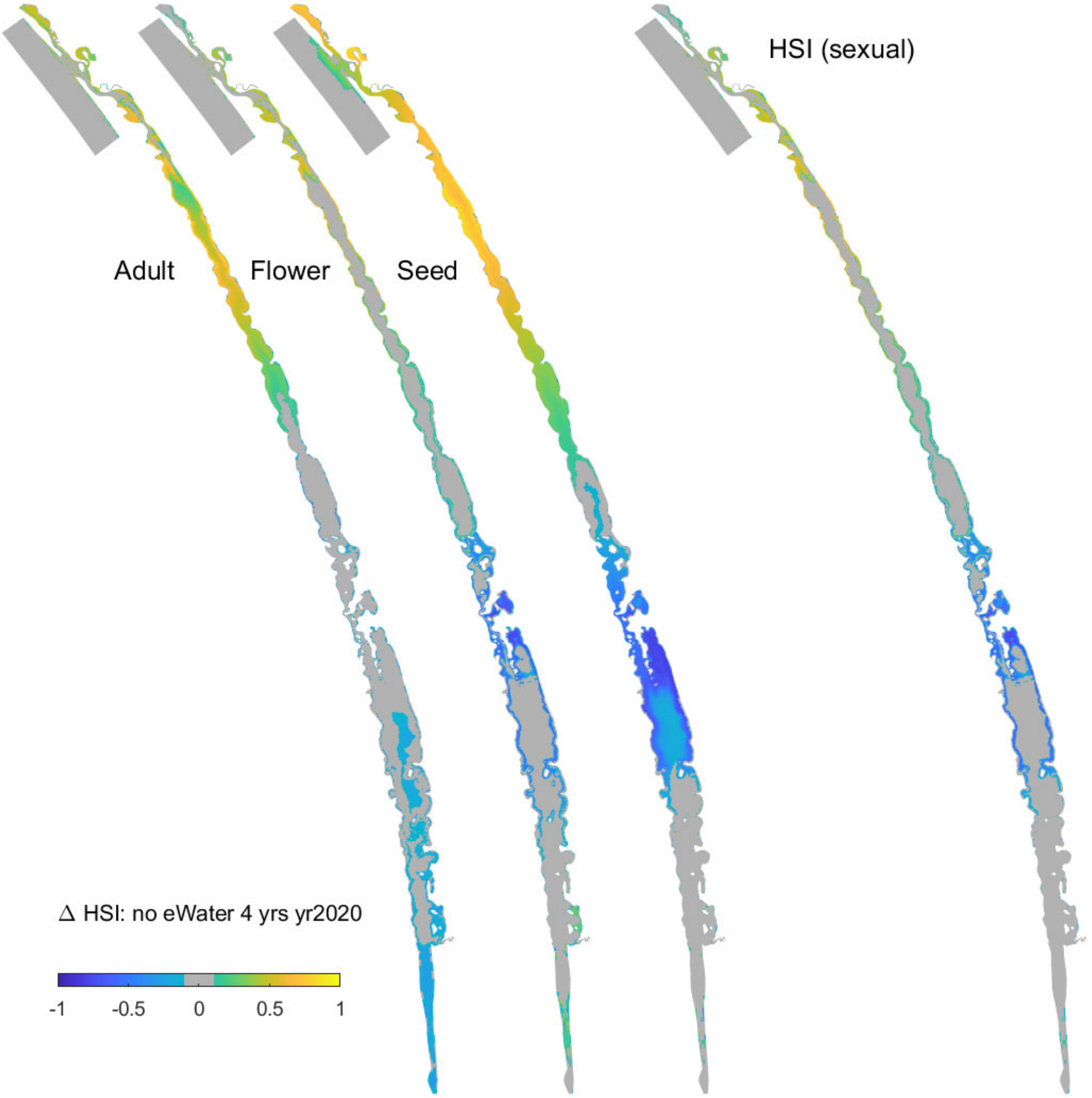


Figure 29. Differences of *Ruppia* HSI (habitat suitability index) values between the no environmental water since 2017 (No eWater 4 years) and the "All water" (base-case) conditions. The index is computed for each stage (adult, flower, seed) of the life-cycle, and the overall suitability (HSI sexual) is based on where all life-stage requirements are met. More saline conditions in the 'no eWater 4 years" scenario led to a loss of good habitat in the South Lagoon (blue indicates contraction, orange indicates expansion) that meets the requirements of all life-stages.

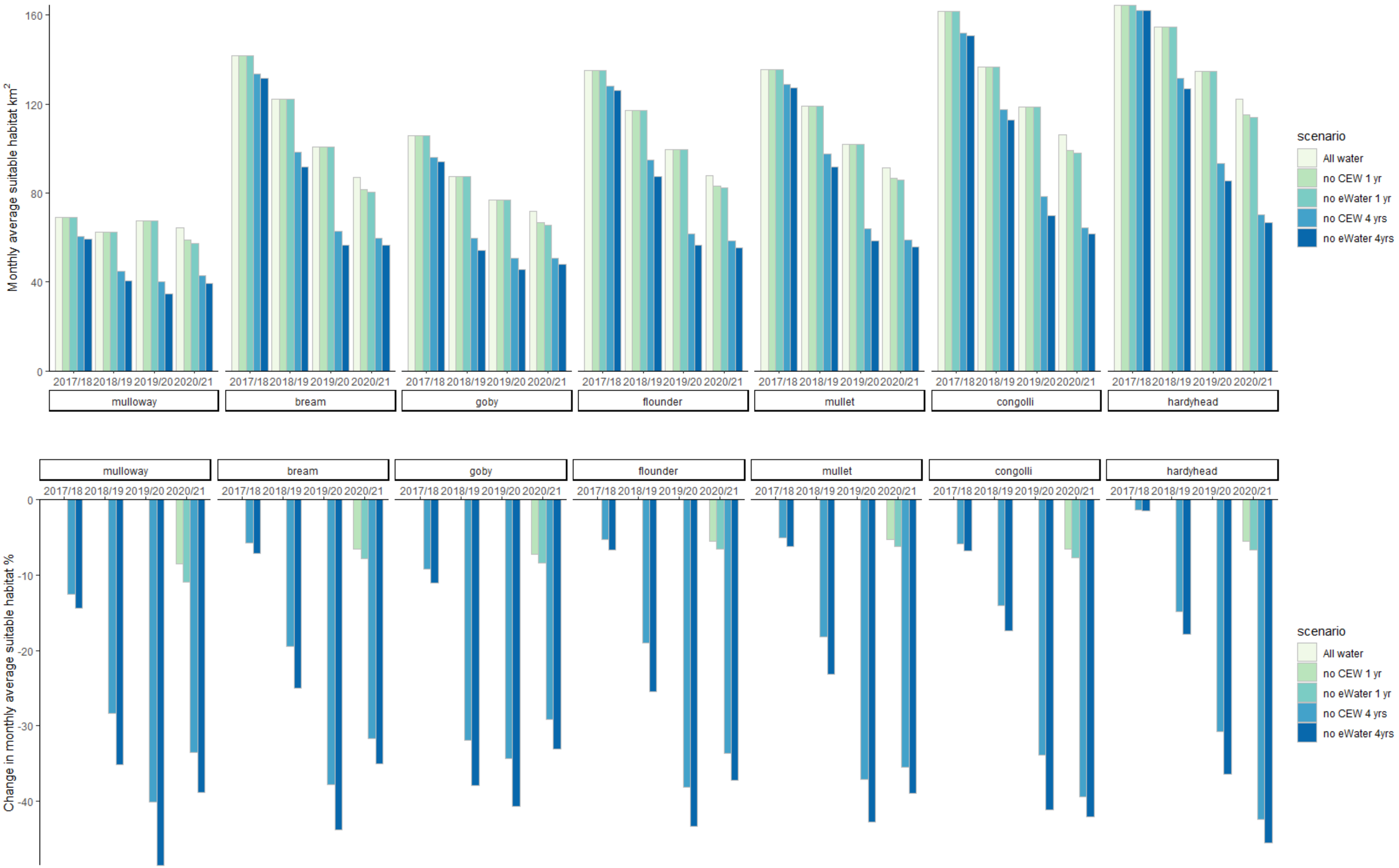


Figure 30. Habitat area of juvenile stages of key fish species for the scenarios (top). Change in area (%) that would have been in the case of no environmental water is shown in the bottom panel. Environmental water gives a large habitat expansion for all species and this increases year on year.

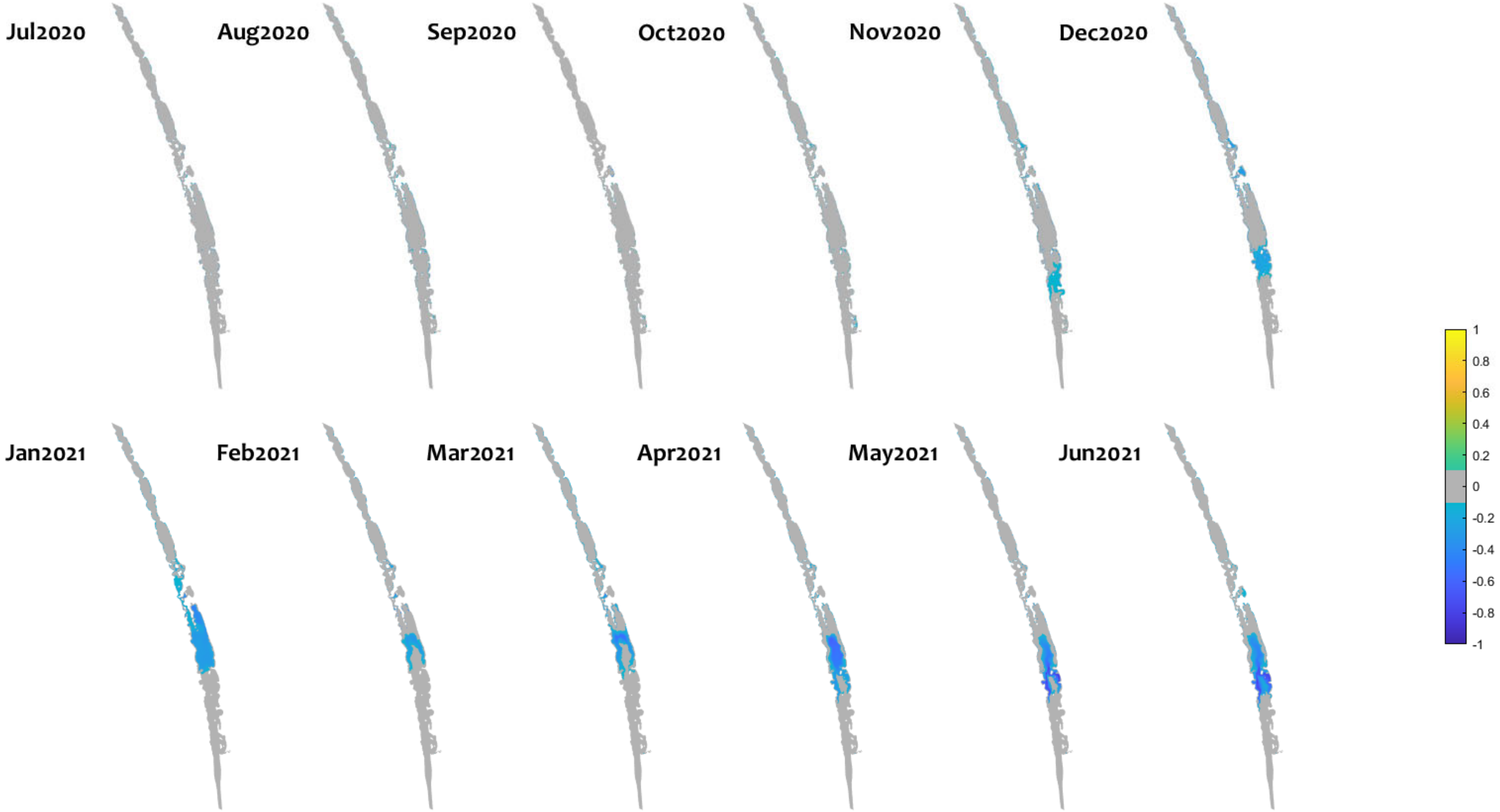


Figure 31. Monthly habitat area "lost" for smallmouth hardyhead during 07/2020 – 07/2021 if there was no environmental water delivery in 2020-21 (calculated as the difference between habitat in the "All water" (base-case) and "no eWater 1 year" scenario. Large areas of the South Lagoon have a decrease in habitat quality, highlighting areas that have benefited from environmental water since 07/2020.

Evaluation

The evaluation approach, including assessment criteria, is described in the evaluation section for Hydraulic Regime (Section 2.1).

Table 9. Coorong habitat evaluation questions and answers. CEW = Commonwealth environmental water, eWater = environmental water.

CEWO evaluation questions	Outcomes of CEW delivery			
	2017-18	**2018-19**	**2019-20**	**2020-21**
What did CEW contribute to improving *Ruppia tuberosa* habitat in the Coorong?	Habitat suitable for overall sexual life-cycle completion of *Ruppia* was significantly improved with eWater (including CEW). A single year with no environmental water did not have a significant impact on the area suitable for overall sexual productivity but the suitable area quickly contracted if CEW or other eWater was not delivered over consecutive years. Without CEW the area suitable for *Ruppia* would be 44% less after four years than the area of suitable habitat when CEW was delivered. This highlights the importance of eWater to the ecological restoration of the Coorong.			
What did CEW contribute to improving fish habitat in the Coorong?	eWater led to fresher conditions in the Coorong and an expansion of suitable fish habitat area throughout the year. Without eWater (or CEW) from 2017-18 to 2020-21, the area of suitable habitat for mulloway, congolli and smallmouth hardyhead would have declined by 39% (34%), 42% (39%) and 45% (42%) (modelled) respectively, in 2020-21. A similar trend is evident for black bream, Tamar goby, greenback flounder and yelloweye mullet. A major expansion of suitable habitat into the south Coorong is critically important to restore biodiversity in this recovering ecosystem.			

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):

⬜ Unknown 🟥 Negative ⬜ None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

Salinity is a key determinant of habitat suitability in the Coorong, and is primarily a function of riverine inflows, seawater ingress and tidal movement. When barrage flows are low, more seawater enters the Murray Mouth and more salt is then transported to the Coorong. It is evident that environmental water is critical for maintaining appropriate salinity in the Coorong and maintaining suitable habitat. If Commonwealth environmental water had not been delivered in 2020-21 (No CEW 1 year scenario), an additional 790,203 tonnes of salt would have accumulated in the South Lagoon (see Table 5).

The area of suitable habitat for *Ruppia* sexual reproduction increased through the three-year period (January 2018–December 2020) from approximately 11 to 20 km^2, largely attributable to an anticipated improvement in flowering success. The salinity at the commencement of the simulation is likely to have an impact on the habitat suitability area. The large flows in 2016-17 would have 'reset' salinity in the Coorong which progressively became more saline moving the salinity to above the minimum threshold ranges set for habitat suitability and creating suitable conditions. Without additional environmental water, this return above the minimum salinity would have in fact happened quicker, however, care needs to be taken when interpreting this result as prolonged conditions with no barrage flow are detrimental to the system in the longer term as conditions quickly go above the maximum tolerance. The key finding in this case is that the habitat suitability is highly dependent on the antecedent flow.

The comparison of the overall area of habitat for *Ruppia*, based on the completion of a full sexual reproduction life-cycle, indicated that without Commonwealth environmental water, there would have been a 44% reduction in habitat area in the calendar year 2020. The main reduction appears to be driven by the area suitable for successful flowering (Figure 29). It is evident from these modelling scenarios that environmental water is critical for *Ruppia* reproductive success in the South Lagoon of the Coorong.

The estuarine fish species that inhabit the Coorong vary slightly in their tolerance to salinity with yelloweye mullet, congolli and smallmouth hardyhead able to tolerate more saline conditions (McNeil *et al.* 2013). Fish differ from *Ruppia* in that they can move in response to changing salinity and habitat suitability. It is generally considered advantageous to have a greater area of habitat with suitable water quality (e.g. salinity) and abundant food resources to support the maintenance of fish populations. Estuaries are important feeding, spawning, nursery and refuge grounds for many estuarine-dependent fish species (Bice *et al.* 2018). Without environmental water, fish habitat contracts quickly and significantly. Even after the high flow year in 2016-17, if there was no environmental water in 2017-18, significant habitat contraction would have occurred. As mulloway and Tamar goby are less tolerant to high salinities and thus have the smallest area of suitable habitat, and thus their habitat contraction would have the most profound impact on these species, followed by greenback flounder, black bream and yelloweye mullet. Even for the more salt-tolerant congolli and smallmouth hardyhead, the reduction in their suitable habitat areas was substantial because without environmental water, a large area in the South Lagoon would have salinities beyond their tolerance thresholds. Consecutive years of no environmental water reduced suitable fish habitat by 33–45% within four years. In the scenario with no environmental water (Figure 30), it is evident that the greatest contraction in habitat occurs between 2018-19 and 2019-20, whereas the 2020-21 period shows a smaller contraction for all species except smallmouth hardyhead and congolli.

Management implications

Flow through the barrages is critical to maintain sexual reproduction of *Ruppia*. The starting salinity conditions are important and environmental flows could be tailored to improve *Ruppia* habitat suitability. Environmental flow management would need to consider the health of *Ruppia* in the system, the extent of *Ruppia* cover and the prevailing salinity. The model could potentially be used to optimise environmental water delivery through the barrages to maximise the area of suitable *Ruppia* habitat based on the volume of water available. Water level maintenance is also necessary for *Ruppia* to complete its lifecycle. If water level drops too quickly in early summer, then the plants can become stranded and desiccate. Environmental water delivered during spring and summer, if of sufficient volumes, can help maintain or reduce the rate of water level reduction in the South Lagoon and promote *Ruppia* reproduction.

The area of suitable estuarine fish habitat is very sensitive to river flow via the barrages. Environmental flows lead to fresher conditions in the Coorong and an expansion of suitable fish habitat area. Without environmental flows, the suitable habitat contracts for a range of fish species (e.g. mulloway, black bream, Tamar goby, greenback flounder, yelloweye mullet and congolli). Smallmouth hardyhead are more salt-tolerant and have been the most abundant prey fish species in the southern Coorong (Ye *et al.* 2020b). Their habitat also contracts with no environmental water but the major impact on habitat suitability took about one year longer to manifest than the other species. To maintain suitable habitat for the range of estuarine species, environmental flows should be maintained to support barrage releases every year. Without environmental flows in 2020-21, there would have been an 8% contraction in suitable habitat for mulloway in the Coorong, and four years (2017-18 to 2020-21) without environmental flows would have reduced mulloway habitat by 39%. This compares to an 18% contraction without environmental water for one year in 2019-20.

Conclusion

Environmental water is shown to be critical in reducing salt import into the Murray Mouth from the ocean, lowering salinity in the Coorong and maintaining estuarine habitat to support ecological functions and biodiversity. The overall area of *Ruppia* habitat showed a general decrease of up to 44% in the calendar year 2020 if there were no Commonwealth environmental water or other environmental water through the last four years (July 2017– June 2021). Reduction of fish habitat areas of ~6-11% were predicted if there was no environmental water in 2020-21, and the reduction area focused on the middle and south Coorong. Reduction of 33–44% in the habitat areas for key fish species were predicted in the Coorong if there was no environmental water for four years continuously since July 2017. Environmental flow delivery during periods when there would otherwise be negligible water exchange between the Lower Lakes and Coorong is critical for maintaining the connectivity between freshwater and marine environments and promoting fresher conditions in the Coorong with more favourable habitat for estuarine fish and plants.

2.4 Littoral Vegetation Diversity and Productivity

Background

Littoral (streambank) vegetation is an important component of the biota of riverine ecosystems. It is an important primary producer for both the riverine and terrestrial ecosystem (e.g. Roberts and Ganf 1986; Froend and McComb 1994), can improve water quality (e.g. Li *et al.* 2010; Borin and Salvato 2012), oxygenate the sediment and water column (e.g. Blom *et al.* 1990; Sorrell and Hawes 2010; Dickopp *et al.* 2011), provide habitat for water birds (e.g. Jansen and Robertson 2001; Kapa and Clarkson 2009) and invertebrates (e.g. Papas 2007; Walker *et al.* 2013) and stabilise banks (e.g. Abernethy and Rutherfurd 1998). Littoral zones are also hot spots for biodiversity because they contain a specialised group of species adapted to wetting and drying not found in aquatic or terrestrial systems and therefore, increase the species pool at the landscape scale (Sabo *et al.* 2005).

Littoral vegetation responds rapidly to changes in water level (e.g. Nicol *et al.* 2018a) as inundation changes the physicochemical environment and acts as an environmental sieve (*sensu* van der Valk 1981) producing conditions that are hostile to some species but favourable to others (e.g. Nicol *et al.* 2003). In riparian zones, inundation often acts as disturbance, removing the existing vegetation and providing an environment almost free of competition with high soil moisture (e.g. Pettit and Froend 2001; Bagstad *et al.* 2005; Beauchamp and Stromberg 2008). Due to the unpredictable nature of flooding regimes in arid Australian systems (Puckridge *et al.* 1998; Puckridge *et al.* 2000), many littoral species are short-lived annuals that are adapted to take advantage of these brief periods of favourable conditions germinating as water levels recede, completing their life cycle whist soil moisture is high and competition is low and replenishing the seed bank (e.g. Nicol 2004; Capon 2007). These species persist in the soil seed bank whilst conditions are unfavourable and are examples of Grime's (1979) r-selected species and have more in common with desert annuals than aquatic species (e.g. Nicol 2004; Capon 2007). Emergent and amphibious species (*sensu* Brock and Casanova 1997; Casanova 2011) are also present and are typically perennial taxa (Cunningham *et al.* 1992) adapted to persist or respond to inundation and exposure and often have a requirement of variable water levels to establish and/or reproduce (e.g. Brock and Casanova 1997; Casanova 2011; Nicol *et al.* 2018b).

Management of water levels in the LMR has typically focussed on maintaining stable water levels in lower weir pools, which can result in small increases in flow causing large water level rises in tailwaters immediately downstream of weirs (Maheshwari *et al.* 1995). Therefore, tailwaters represent areas where flood dependent and amphibious vegetation can persist in the absence of overbank flows over a wider range of the elevation gradient (Blanch *et al*. 1999; Blanch *et al.* 2000). This makes tailwaters an ideal location to assess the influence of Commonwealth environmental water as the volumes available for delivery will have a significant impact on water levels in these areas. The response of the vegetation along the elevation gradient in tailwaters can be exploited to evaluate the benefit of Commonwealth environmental water.

In 2020-21, Commonwealth environmental water was delivered to produce a spring pulse peaking at 17,917 ML/d (flow to South Australia) in the LMR arriving at the South

Australia/New South Wales border on 25 November. The aim of this study is to investigate the response of littoral vegetation to Commonwealth environmental water delivery by testing the hypotheses in the following section. This will be undertaken by comparing species composition and biomass between areas inundated and not inundated by the spring flow pulse.

Major hypotheses

- Increases in flow will result in increased water level variability in the littoral zone that will facilitate the recruitment of floodplain and amphibious understorey species and in turn increase biological and functional diversity of the littoral zone plant community.
- The increase in water level variability due to increases in flow will result in increased productivity of littoral vegetation compared to non-inundated areas.

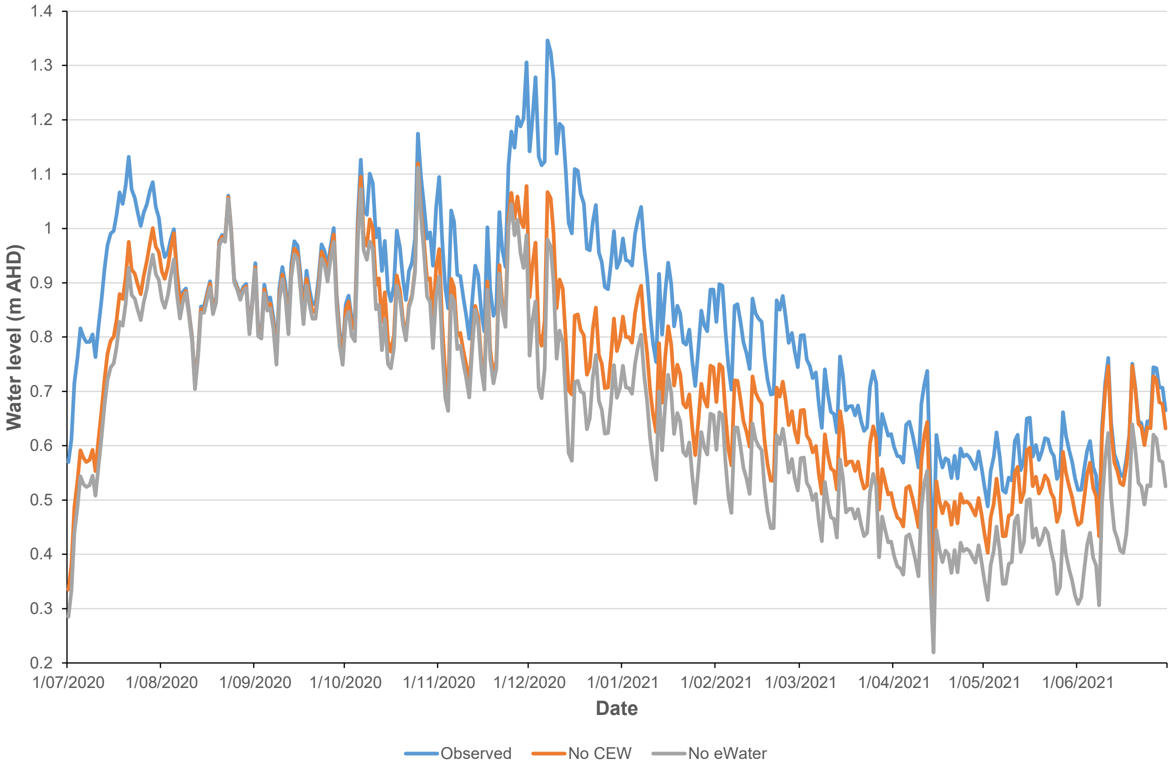
Methods

Study sites and hydrology

Vegetation surveys were undertaken on the banks of the LMR in weir pool tailwaters downstream of Lock 1, Lock 4 and Lock 6 (referred to herein as reaches) to correspond with the locations of other indicators (Figure 1).

Flow in 2020-21 was generally low with an in-channel flow pulse peaking in late-November 2020 that was comprised almost entirely of Commonwealth environmental water (Figure 32). The flow pulse resulted in an increase in water level of 76 cm downstream of Lock 6, 58 cm downstream of Lock 4 and 28 cm (downstream of Lock 1) compared to modelled levels without any environmental water (Figure 32). For a detailed description of the hydrology and environmental water delivery see Sections 1.4 and 2.1.

a.



1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
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0.5
0.4
0.3
0.2
Water level (m AHD)
1/07/2020
1/08/2020
1/09/2020
1/10/2020
1/11/2020
1/12/2020
1/01/2021
1/02/2021
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Observed
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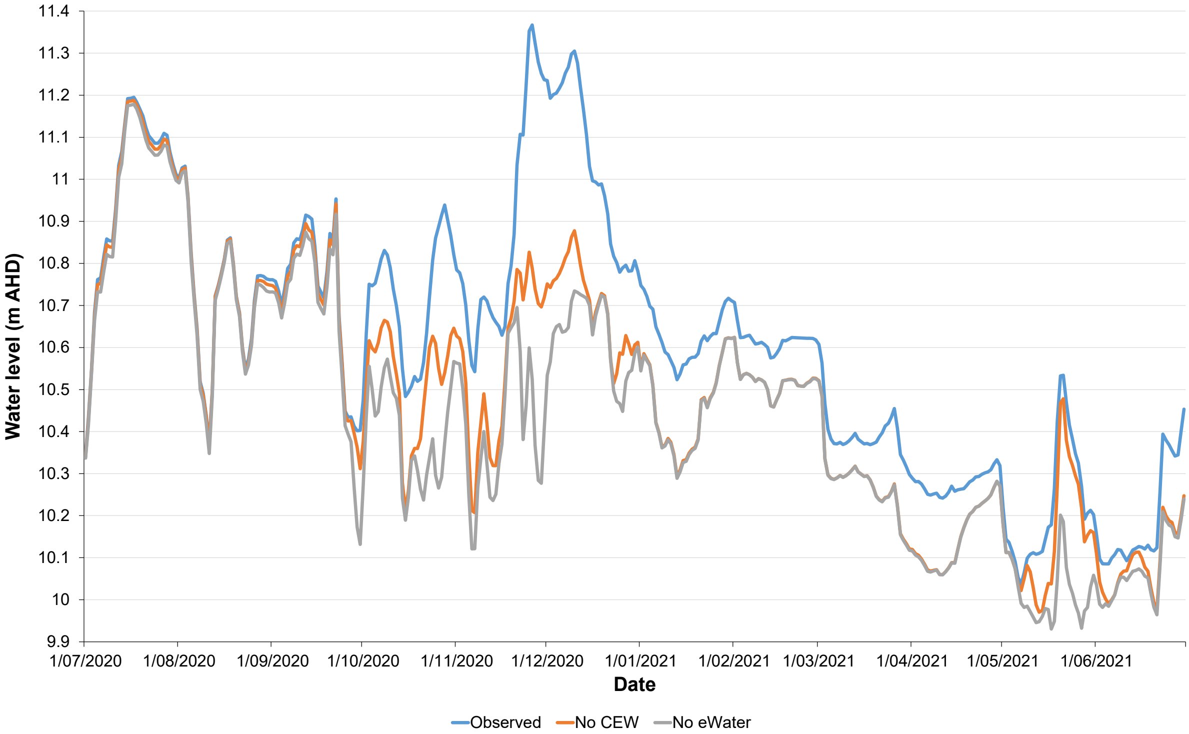
b.



11.4
11.3
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11
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Water level (m AHD)
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1/08/2020
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1/10/2020
1/11/2020
1/12/2020
1/01/2021
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Date
Observed
No CEW
No eWater

c.

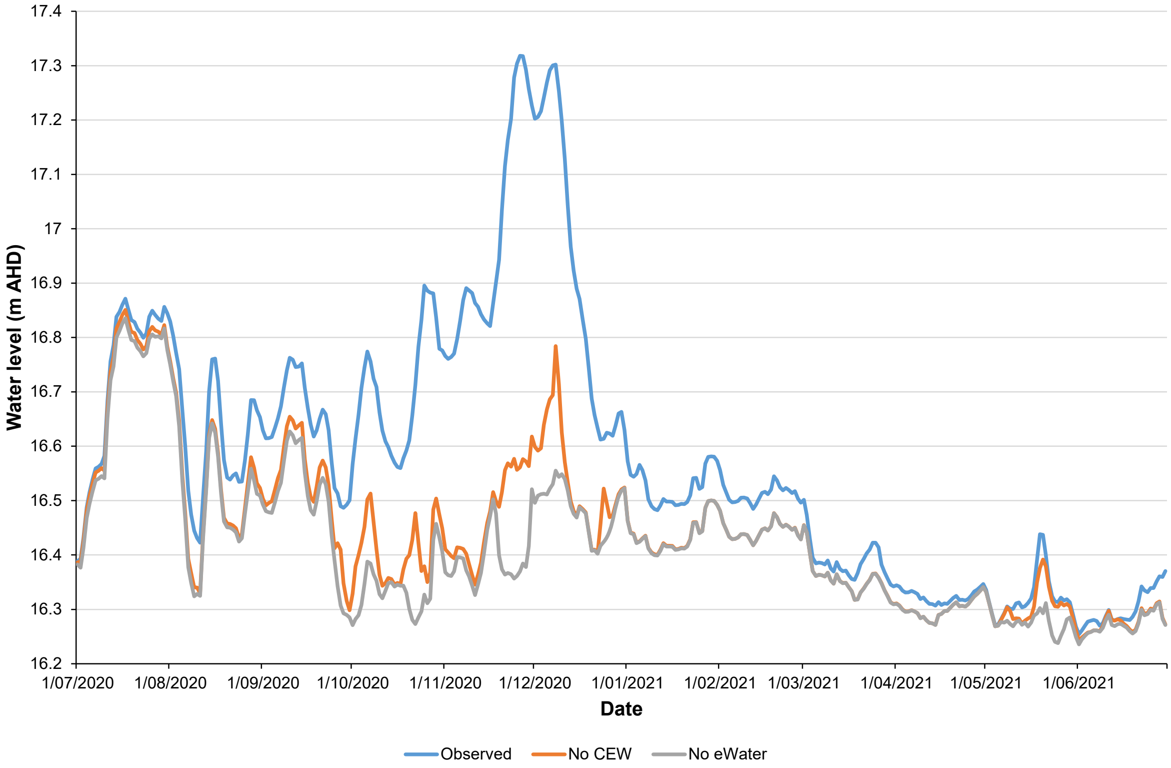

Figure 32. Observed and modelled (without Commonwealth environmental water and without any environmental water) water levels in upper weir pools (tailwaters) a. below Lock 1, b. Weir Pool 3 (below Lock 4) and c. Weir Pool 5 (below Lock 6).

Vegetation surveying protocol

Sampling of littoral vegetation occurred in February 2021. Transects established in December 2019 in each reach (Ye et al. 2021) were resurveyed (Table D3, Appendix D). Quadrats with dimensions of 15 x 1 m were positioned on each transect (the long side parallel to elevation contours) in relation to the maximum water level in spring 2020; one quadrat was located at normal pool level (herein referred to as *Pool level*), three in the area inundated by the spring flow pulse (*Inundated*), one at the spring 2020 high water mark (*Inundation extent*) and two above the high water mark (*Not inundated*) (Figure 33). Each quadrat was divided into 15, 1 x 1 m cells to determine species frequency in each quadrat (i.e. each species will have a score of zero (not present) and 15 (present in each cell). In addition, a 50 x 50 cm (0.25 m^2) section of each quadrat was harvested and dried at 40^o C to a constant weight to determine above ground biomass.

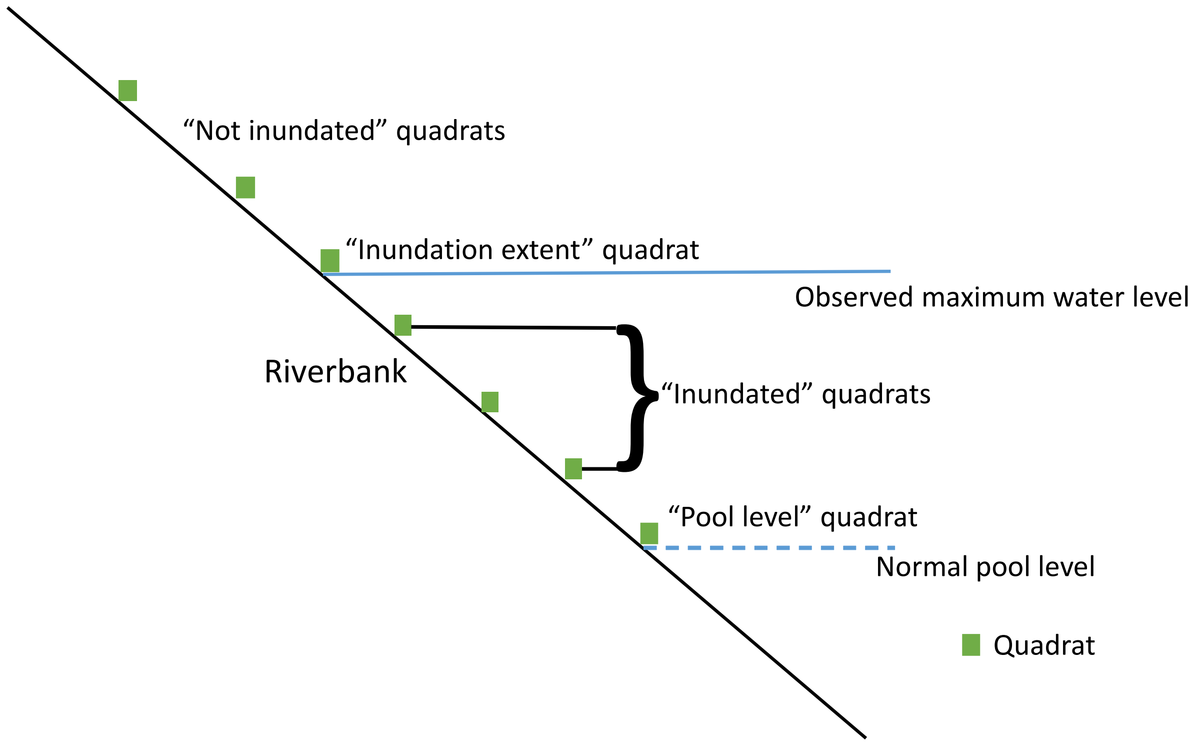

Figure 33. Position of quadrats in relation to maximum water level when sampled in February 2021.

The *Inundated* and *Inundation extent* zones were impacted by the delivery of Commonwealth environmental water. Modelling indicated that without Commonwealth environmental water, the *Inundated* zone would have remained dry and there would have been no increase in soil moisture in the root zone at the *Inundation extent* elevation (Figure 32). The *Pool level* quadrats would have been the only quadrats inundated with the delivery of other environmental water (Figure 32).

Plant identification and nomenclature

Plants were identified using keys in Jessop and Toelken (1986), Cunningham *et al.* (1992), Sainty and Jacobs (1981; 2003) and Jessop *et al.* (2006). In some cases, due to immature individuals or lack of floral structures, plants were identified to genus only. Nomenclature follows the Centre for Australian National Biodiversity Research and Council of Heads of Australasian Herbaria (2021). A comprehensive list of all species recorded, their functional classification, growth form, life history strategy and conservation status are presented in Table D4 in Appendix D.

Plant functional groups

The functional classification used to assess targets for the Chowilla condition and intervention monitoring programs was used in this study to assess the response of the littoral vegetation to environmental water. The functional classification was based on the classification framework devised by Brock and Casanova (1997), which was based on species present in wetlands in the New England Tablelands region of New South Wales and modified by Nicol *et al.* (2010) to reflect the vegetation of the Chowilla system.

The functional classification used for this study splits species into four groups (terrestrial, flood dependent, amphibious and emergent) based on their response to inundation. The description of each functional group is presented in Table D5, Appendix D.

Data analysis

Species composition between the different inundation zones (*Not inundated*, *Inundation extent*, *Inundated* and *Pool level*) and reaches (Lock 1, Lock 4 and Lock 6) were compared using nMDS Ordination, two-factor Multivariate PERMANOVA and Indicator Species Analysis. Bray-Curtis (1957) similarities were used to calculate the similarity matrix for nMDS Ordination and Multivariate PERMANOVA analyses, which were undertaken using the Package PRIMER version 7.0.12. (Clarke and Gorley 2015) and PERMANOVA+ for PRIMER (Anderson *et al.* 2008). Indicator Species Analysis (Dufrene and Legendre 1997) was undertaken using the package PC-Ord version 5.12 (McCune and Mefford 2006). Above ground biomass was compared between the different inundation zones and reaches using univariate PERMANOVA using the package PERMANOVA+ for PRIMER (Anderson *et al.* 2008). Euclidean distances were used to calculate the similarity matrix for the univariate PERMANOVA analysis.

Dufrene and Legendre's (1997) indicator species analysis combines information on the concentration of species abundance in a particular group (inundation zone) and the faithfulness of occurrence of a species in a particular group (McCune *et al.* 2002). A perfect indicator of a particular group should be faithful to that group (always present) and exclusive to that group (never occurring in other groups) (McCune *et al*. 2002). This test produces indicator values for each species in each group based on the standards of the prefect indicator. Statistical significance of each indicator value is tested by using a Monte Carlo (randomisation) technique, where the real data are compared against (in this case) 5,000 runs of randomised data (Dufrene and Legendre 1997). For this study, the groups were assigned according to inundation zone within a reach (planned comparison). A species that is deemed not to be a significant indicator of a particular group is either uncommon or widespread. An uncommon species is only found in one group but in low numbers, and a widespread species is found in more than one group in similar numbers (Dufrene and Legendre 1997). Whether a species was classed as a widespread or uncommon non-significant species was determined by examination of the raw data.

Results

Littoral plant communities

Native species richness was variable between reaches, with the reach downstream of Lock 1 having the highest native species richness (34) across all zones, followed by Lock 6 (28) and Lock 4 (24) (Table D6, Appendix D). Despite the variability among reaches, there were common patterns of native species richness within reaches. The inundated zone had the highest species richness in all reaches and the inundation extent the lowest downstream of Locks 1 and 6 (Figure 34).

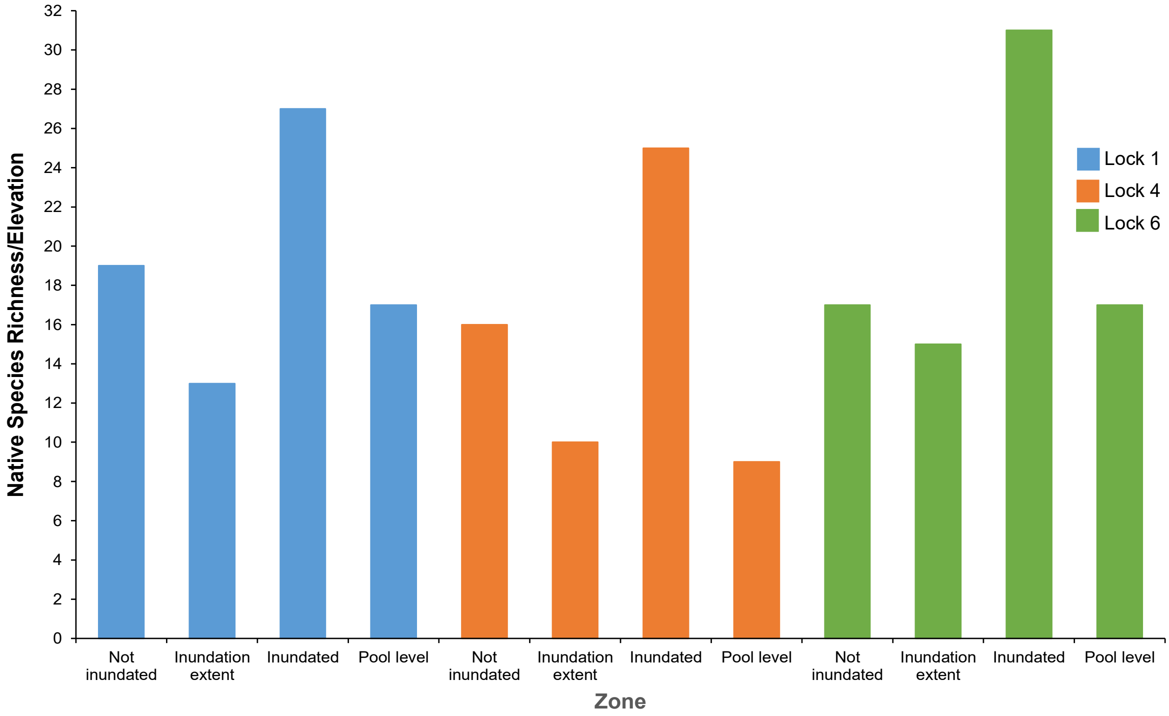

Figure 34. Native species richness in each inundation zone in each reach in February 2021.

The abundance of functional groups (percentage of cells occupied by species from each functional group) in each zone was variable between reaches with more of every functional group present downstream of Lock 1 (Figure 35). Nevertheless, there were similar patterns among reaches regarding the influence of inundation; terrestrial taxa were typically more abundant in non-inundated areas (the *Not inundated* and *Inundation extent* zones) and contrastingly, there was an increase in emergent taxa with decreasing elevation (Figure 35). Flood dependent and amphibious taxa were widespread and present in all zones in each reach (Figure 35). Amphibious species increased in abundance with decreasing elevation downstream of Lock 1 and Lock 6 but were most abundant in the *Inundated* zone downstream of Lock 4 (Figure 35). The abundance of flood dependent taxa varied between reaches with the highest abundance downstream of Lock 1 in the Inundation extent zone, at Pool level downstream of Lock 4 and in the Inundated zone downstream of Lock 6 (Figure 35).

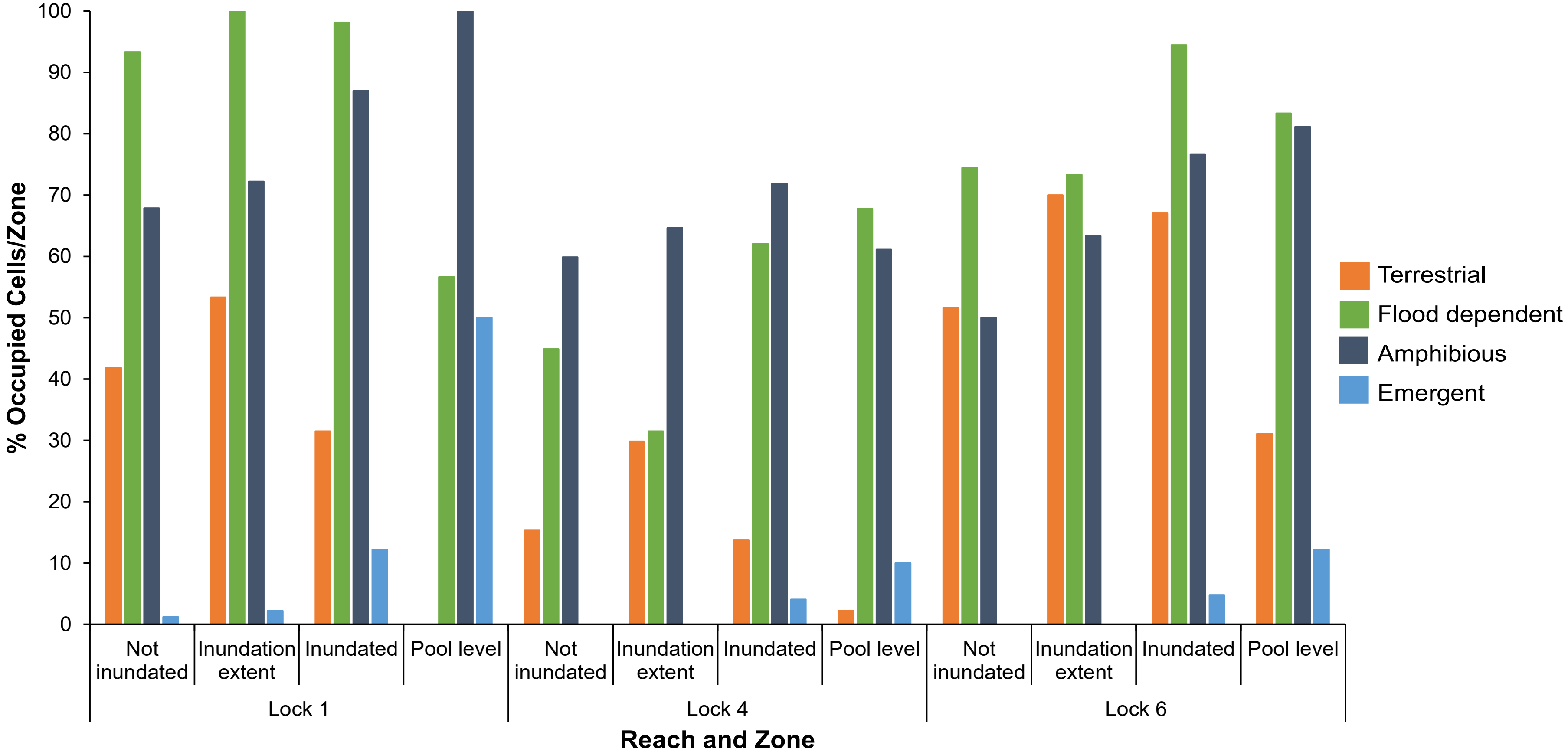

Figure 35. Percentage of cells occupied by each functional group in each inundation zone in in the Lower Murray River in February 2021

nMDS ordination showed that the vegetation was different between elevation zones and reaches (Figure 36). This was supported with PERMANOVA detecting significant differences in the plant community between reaches and inundation zones, with a significant interaction (Table 10). This provides evidence that the plant community responded differently to inundation between reaches. Multiple comparisons showed that there was no significant difference in the plant community between *Pool level* and the *Inundated* zone and the *Not inundated* and *Inundation extent* zones downstream of Locks 4 and 6 (Figure 36). In contrast, downstream of Lock 1 there was no significant difference in the vegetation in the *Not inundated, Inundation extent* and *Inundated zones*, whereas they were significantly different from the vegetation in the *Pool level* zone (Figure 36).

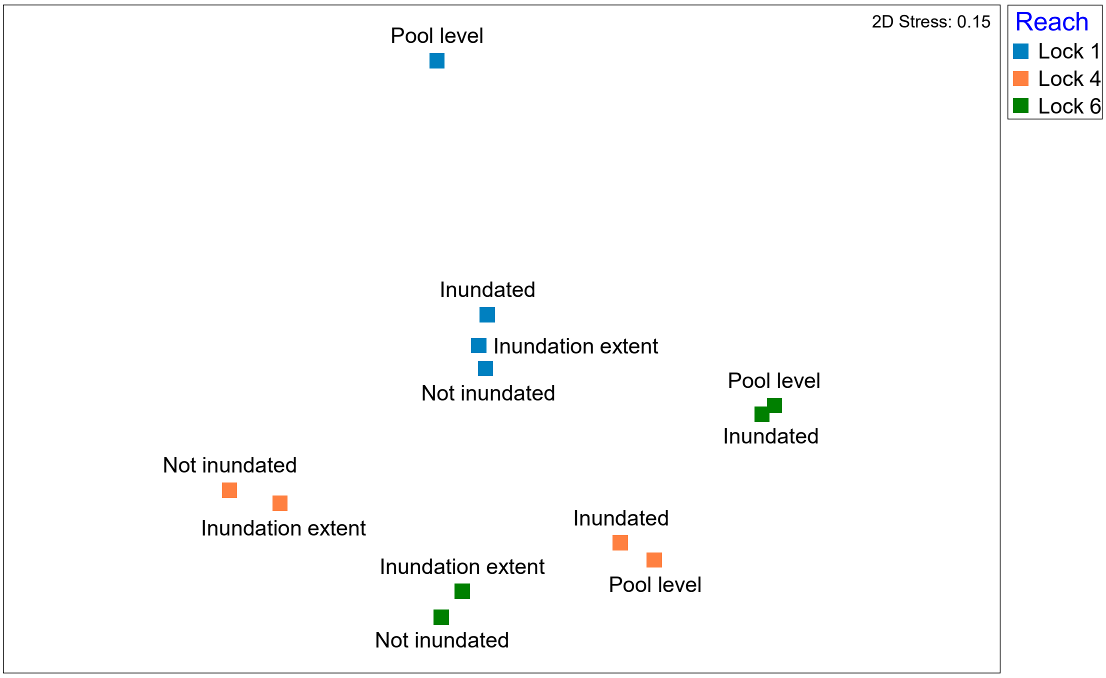

Figure 36. nMDS Ordination comparing the plant community in each inundation zone in each reach in February 2021.

Table 10. PERMANOVA results comparing plant community in each inundation zone in each reach in February 2021.

Factor	DF	*Pseudo F*	*P*
Site	2, 121	8.14	0.001
Inundation	3, 121	4.76	0.001
Site x Inundation	6, 121	2.08	0.001

Despite there being no significant difference between some of the zones, *Alternanthera denticulata* and *Sphaeromorphaea australis* were significant indicators of the *Inundated* zone and *Eleocharis acuta, Juncus usitatus, Limosella australis, Paspalum distichum* and *Schoenoplectus tabernaemontani* of *Pool level* downstream of Lock 1 (Table D6a, Appendix D). Downstream of Lock 4, *Brachyschome paludicola* was more abundant in the *Not inundated* zone, *Enchylaena tomentosa* in the *Inundation extent* zone and *Stemodia florulenta* at Pool level (Table D6b, Appendix D). Downstream of Lock 6 *Atriplex* was a significant indicator of the *Not inundated* zone, *Dittrichia graveolens* of the

Inundation extent zone, *Centipeda minima* and *Sphaeromorphaea australis* of the *Inundated* zone and *Ammania multiflora, Bolboschoenus caldwellii* and *Ludwigia peploides* of *Pool level* (Table D6c, Appendix D). Furthermore, many native species not deemed significant indicators of one inundation zone were present in two zones in similar abundances. In particular, several amphibious and flood dependent taxa were present in the *Pool level* and *Inundated* zones and absent at the *Inundation extent* and *Not inundated* zones (Table D6, Appendix D).

Several exotic species recruited in the *Inundated* and *Pool level* zones in response to environmental water delivery. *Xanthium occidentale* and *Symphyotrichum subulatum* were present in all reaches exclusively in the *Inundated* and *Pool level* zones (Table D6, Appendix D). *Cuscuta campestris* and *Hypochaeris glabra* was present downstream of Lock 1 and *Cuscuta campestris* and *Heliotropium europaeum* downstream of Lock 6 in the same zones (Table D6, Appendix D).

In contrast to the 2019 flow pulse, *Eucalyptus camaldulensis* seedlings were generally absent throughout the inundated zones of the Lock 4 and Lock 6 reaches. However, survivorship of the seedlings present in spring 2019 was high with large numbers of saplings now present in the areas inundated in the aforementioned reaches.

Above ground biomass

Above ground biomass was variable between reaches but there were similar patterns between zones with lower biomass downstream of Lock 4 compared to the other two reaches (Figure 37). Above ground biomass was highest in all reaches in the *Inundation extent* zone and decreased with decreasing elevation (Figure 37). Hence, there were significant differences between reach and inundation zone but no significant interaction detected by PERMANOVA (Table 11).

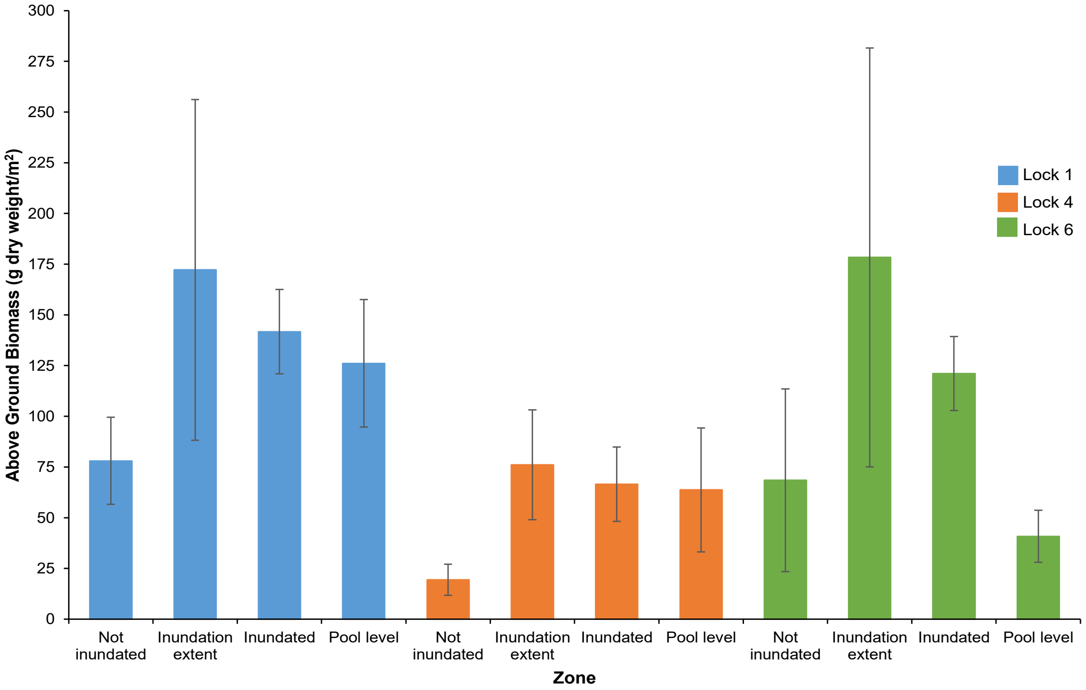

Figure 37. Above ground biomass in each inundation zone in each reach in February 2021 (error bars = ± 1 SE).

Table 11. PERMANOVA results comparing above ground biomass in each inundation zone in each reach in February 2021.

Factor	DF	*Pseudo F*	*P*
Site	2, 125	4.09	0.02
Inundation	3, 125	3.37	0.02
Site x Inundation	6, 125	0.42	0.866

Evaluation

The evaluation approach, including assessment criteria, is described in the evaluation section for Hydraulic Regime (Section 2.1).

Table 12. Littoral Vegetation Diversity and Productivity evaluation questions and answers. CEW = Commonwealth environmental water, eWater = environmental water.

CEWO evaluation questions	**Outcomes of CEW delivery**	
	2019-20	**2020-21**
What did CEW contribute to littoral understorey vegetation diversity?	CEW delivery increased native plant species diversity by 40–100% across all reaches in the LMR. This was evidenced by consistently higher native species richness in inundated zones in each reach compared to non-inundated areas, higher native species richness (albeit lower than inundated zones) on the edge of the inundation footprint, where there was increased soil moisture due to capillary action, and different plant communities in the inundated zones (despite there being few significant indicators) compared to non-inundated zones. CEW delivery in the form of a spring pulse produced conditions suitable for the recruitment of specialised riparian species that are adapted to fluctuating water levels and do not inhabit terrestrial or truly aquatic habitats, increasing plant functional diversity. Twenty-four native species responded positively to delivery of CEW either by recruitment or increased abundance (Ye *et al.* 2021).	CEW delivery increased native plant species diversity by 42–82% across all reaches in the LMR. This was evidenced by consistently higher native species richness in inundated zones in each reach compared to non-inundated areas and different plant communities in the inundated zones compared to non-inundated zones. CEW delivery in the form of a spring pulse produced conditions suitable for the recruitment of specialised riparian species that are adapted to fluctuating water levels and do not inhabit terrestrial or truly aquatic habitats, increasing plant functional diversity. Native species (*n* = 28) that responded positively to delivery of CEW either by recruitment or increased abundance: • *Alternanthera denticulata* • *Ammania multiflora* • *Bolboschoenus caldwellii* • *Calotis hispidula* • *Centipeda minima* • *Cyperus difformis* • *Cyperus gymnocaulos#* • *Dysphania pumilio* • *Eleocharis acuta* • *Eucalyptus camaldulensis##* • *Glinus lotoides* • *Goodenia heteromera* • *Isoetopsis graminifolia* • *Juncus usitatus* • *Lachnagrostis filiformis* • *Limosella australis* • *Ludwigia peploides* • *Lythrum hyssopifolia* • *Mollugo cerviana* • *Mukia maderaspatana* • *Myriophyllum verrucosum* • *Paspalum distichum* • *Persicaria lapathifolia* • *Pseudognaphalium luteoalbum* • *Polygonum plebeium* • *Schoenoplectus tabernaemontani* • *Senecio runcinifolius* • *Sphaeromorphaea australis* • *Stemodia florulenta* • *Tetragonia tetragonoides*

CEWO evaluation questions	Outcomes of CEW delivery	
	2019-20	2020-21
What did CEW contribute to above-ground biomass produced by understorey littoral vegetation?	Biomass production due to CEW was inconclusive and varied depending on reach with no clear pattern in relation to CEW delivery. The only reach that responded as hypothesised was Lock 6 with greater biomass in the *Inundated* and *Inundation extent* zones.	CEW delivery increased above-ground biomass by 121–292% via increasing soil moisture in the *Inundation extent* zone. Increased above-ground biomass was also observed in the *Inundated* zone across all reaches and at *Pool level* downstream of Locks 1 and 4, compared to the *Not inundated* zone.

#: *Cyperus gymnocaulos* was not exclusively present in the *Inundated zone* and/or *Pool level* in any reach; however, observations showed it benefited from increased soil moisture in the Inundation extent zone, where it was common in all reaches. ##: There was no large-scale germination of *Eucalyptus camaldulensis* in 2020-21 but the large number of saplings present showed that survivorship of seedlings from 2019 was high and probably due to the delivery of CEW.

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):
⬜ Unknown 🟥 Negative 🟩 None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

Results from monitoring in February 2021, after the spring 2020 flow pulse had transited the LMR, supported both hypotheses. As the spring pulse was almost entirely the result of the delivery of Commonwealth environmental water, this provided evidence that its delivery increased biological and functional diversity of the plant community and increased productivity.

The spring pulse (and in turn the delivery of environmental water) significantly changed the plant communities in areas that were inundated and where soil moisture in the plant root zone increased. This was not unexpected as inundation changes the physicochemical environment and acts as an environmental sieve (*sensu* van der Valk 1981) producing conditions that are hostile to some species but favourable to others (e.g. Nicol *et al.* 2003). There was evidence that many terrestrial taxa (e.g. *Atriplex* spp., *Sclerolaena* spp.) were extirpated as a result of inundation and, as water levels receded, there was recruitment of flood dependent and amphibious species. In the case of perennial amphibious species (e.g. *Cyperus gymnocaulos, Juncus usitatus, Ludwigia peploides*), there may not have been recruitment from seed but increases in cover and number due to growth or clonal expansion (rhizomes of *Cyperus gymnocaulos* and *Juncus usitatus* were widespread throughout sites).

The response was similar to 2019-20; however, more native amphibious and flood dependent species were present exclusively in the areas inundated by environmental water in 2020-21 (24 in 2019-20 compared to 28 in 2020-21). These results provided evidence of the benefits of follow up watering for vegetation and may improve resilience in the longer-term.

Whilst there were generally consistent patterns of floristic composition between zones in each reach there were differences between reaches, in particular between Lock 1 and the other two reaches (Figure 34; Figure 36). The reach below Lock 1 had higher species richness across all zones, which was probably due to the more variable water levels in comparison to the Lock 4 and Lock 6 reaches (Figure 32). The reach below Lock 1 is connected to the Lower Lakes and wind driven water level changes (seiches) driven by lakes Alexandrina and Albert impact this weir pool.

Whilst the highest species richness in all reaches occurred in areas that were inundated by the spring pulse, amphibious and flood dependent species were present across all zones in all reaches (Figure 34; Figure 35). Many amphibious species, such as *Eucalyptus camaldulensis*, *Duma florulenta* and *Cyperus gymnocaulos* are long-lived perennials that are tolerant to low soil moisture (Cunningham *et al.* 1992) and are widespread across the entire floodplain. In addition, there were many flood dependent and amphibious species present in the *Not inundated* zone that had recruited in response to the 2016 flood (J. Nicol *pers. obs.*). In future surveys the impact of the 2016 flood in the *Not inundated* zone will become less evident in the absence of large overbank floods.

The presence of *Eucalyptus camaldulensis* seedlings in 2019-20 was important because they are generally not recorded in the monitoring programs of other watering interventions such as pumping (Nicol *et al.* 2010, Nicol 2012), weir pool manipulation (Gehrig *et al.* 2015, Gehrig *et al.* 2016) and environmental regulator operation (Nicol *et al.* 2020). Whilst seedlings were generally absent in 2020-21, saplings were abundant and in good condition (J. Nicol pers. obs.) indicating high survivorship of seedlings from 2019. It is unclear why seedlings were not observed in this year; however, the flow pulse in 2020 was later than in 2019 and may not have coincided with *Eucalyptus camaldulensis* seed rain (sensu Pettit and Froend 2001a and Pettit et al. 2001). The locations of 100 *Eucalyptus camaldulensis* saplings were recorded by GPS downstream of Lock 4 and Lock 6 to provide future data on medium to long-term survivorship.

Patterns of above ground biomass at the different inundation zones were similar between reaches and could be explained due to environmental water delivery (Figure 37). Increased soil moisture and no extirpation of species intolerant of inundation in the *Inundation extent* zone resulted in increased above ground biomass in this zone in all reaches (Figure 37). Inundation also generally resulted in increased above ground biomass (compared to areas not inundated) (Figure 37), which may be due to increased abundance of perennial amphibious taxa from previous years that can tolerate or respond to inundation.

Management implications

Results from the 2020-21 water year showed that delivery of a spring pulse was beneficial for littoral vegetation diversity and productivity. Delivering environmental water to support a spring flow pulse in the river channel reinstates part of the natural hydrograph. The increase in water level is coupled with an increase in water velocity and in turn hydraulic complexity and lateral and longitudinal connectivity. This is in contrast to other watering interventions commonly undertaken in the LMR such as weir pool raising and environmental regulator operation, where the water level rise is often decoupled from the increase in flow velocity, or pumping where there is no connectivity with the river channel. Whilst these differences probably have less consequences for plants compared to other biotic groups, as water level is the key driver of plant communities and species typically have persistent (*sensu* Thompson 1992) or aerial seed banks (Nicol 2004), they may be significant. For example, a recent study indicated that water delivery as a flow pulse supported hydrochory (dispersal of propagules by water), which is a significant source of propagule input to inundated areas (Gibbs *et al.* 2020). These results provide evidence that water delivery as a pulse may be preferable compared to using environmental water to support weir pool raising, regulator operation or pumping. Whilst the aforementioned interventions have positive outcomes for vegetation (e.g. Nicol 2012), they should be seen as measures for maintaining perennial vegetation during extended periods of low flow.

One drawback of the flow pulse was the recruitment of several exotic species, in particular the proclaimed pest plants *Xanthium occidentale* and *Cuscuta campestris*. *Xanthium occidentale* is a common amphibious pest plant on stream banks, floodplains and in temporary wetlands (Hocking 1983; Nicol *et al.* 2018b) and is an unavoidable consequence of flooding or watering interventions in the LMR. The proximity to water prohibits the use of herbicides, which makes control of pest plants difficult in littoral zones, but the benefits of watering to native species as observed in this instance outweigh the negative impacts of pest plants. *Cuscuta campestris* is a parasitic plant that typically has agricultural crops as its host (Cunningham *et al.* 1992). However, *Xanthium occidentale* is also a common host (Cunningham *et al.* 1992) and *Cuscuta campestris* was only present when *Xanthium occidentale* was also present.

Conclusions

The 2020-21 flow pulse (and in turn environmental water delivery) resulted in changes to the littoral plant community. Areas that were inundated had increased native species richness and functional diversity. Water delivery also provided hydrological conditions to support *Eucalyptus camaldulensis* survival with development of many seedlings into saplings. Patterns of biomass could also be related to water delivery and showed increased soil moisture, resulting in increased productivity of understorey vegetation.

2.5 Microinvertebrate Assemblage

Background

To date, more than 400 species of planktonic and littoral microinvertebrates (protists, rotifers and microcrustaceans) have been identified in the LMR and Lower Lakes. This assemblage provides an important food resource for a range of higher order consumers such as macroinvertebrates, fish and birds (Shiel, Walker, and Williams 1982; Shiel and Aldridge 2011; Shiel and Tan 2013b, 2013a). Different habitat types support different assemblages of microinvertebrates within riverine ecosystems, and at the simplest level can be classified as either littoral, limnetic, lotic or benthic.

Microinvertebrates are rapid responders to flows. Within habitats that undergo wetting and drying cycles (e.g. the littoral zone and floodplains), these organisms start to emerge from an egg-bank and begin to reproduce within hours of inundation (Tan and Shiel 1993). Therefore, a healthy egg-bank, which is primarily a result of a long-term flooding regime, is an important driver of the magnitude of response to inundation (Boulton and Lloyd 1992). Once inundated, longer water residence times (WRT) will result in higher density and biomass of organisms and result in a shift from rotifer to crustacean dominated communities (e.g. Baranyi et al. 2002; Basu and Pick 1996; Obertegger et al. 2007). Therefore, habitats such as littoral zones and floodplains favour the development of abundant and diverse microinvertebrate communities which can then be transferred between habitats, through hydrological mixing and exchange. Once in the pelagic zone of the main river channel, only some organisms will survive, with a component of the persistent community, reproducing within areas of the main river channel. Which component persists will largely depend on factors such as season and hydraulics.

Therefore, Commonwealth environmental water can facilitate the maintenance and development of microinvertebrate assemblages within the LMR by:

- Inundating areas adjacent to the main river channel and therefore supporting populations that can act as a source to the main river channel community,
- Improving lateral and longitudinal hydrological connectivity which promotes the dispersal of organisms, and
- Improving the flow regime over the long-term to promote a more diverse and dense egg-bank and thus more diverse and dense community dispersed through lateral and longitudinal connectivity.

To determine the responses of the microinvertebrate community to Commonwealth environmental water deliveries in the LMR, their diversity and density is being assessed, with a focus on rotifers and microcrustaceans, every year over the spring–summer period. These data can be used to model the response of different microinvertebrate taxa to flow, floodplain inundation and water quality. The microinvertebrate response models can then be used with different modelled flow scenarios to evaluate the contribution of Commonwealth environmental water to microinvertebrate outcomes (for more details on the modelling approach see Appendix D in Ye et al. 2021).

Major hypotheses

- H1: Overall microinvertebrate density, and taxonomic richness, will increase with environmental water delivery due to the combined effects of longitudinal transport and improved lateral connectivity with off-channel habitats;
- H2: The density will increase for 'floodplain associated'[f] microinvertebrates during periods of greater floodplain inundation (H2a) and for those that are likely to be transported to the LMR from upstream during periods of high longitudinal connectivity (H2b); and
- H3: Environmental water delivery during spring and early summer will increase the density of preferred microinvertebrate prey species for large-bodied fish larvae.

Methods

Sampling sites and procedure

Microinvertebrate sampling was conducted four times during spring and three times during summer approximately two weeks apart between October and January (Table 13) at three core sites, concurrent with stream metabolism sampling (Table 14; Figure 1).

Table 13. Microinvertebrate sampling dates from 2020-21 in the Lower Murray River.

Trip	Sampling dates
1	11–13/10/2020
2	25–27/10/2020
3	9–11/11/2020
4	24–26/11/2020
5	7–9/12/2020
6	21–23/12/2020
7	6–7/01/2021

A Perspex Haney plankton trap (4.5 L capacity) was used mid-channel (by boat) to collect surface, middle and bottom volumes (9 L), which were filtered through a 37 µm-mesh plankton net suspended in a bucket and rinsed into a 200 ml PET bottle screwed to a purpose-built ferrule at the net end. The filtrate was then preserved in the field (100% ethanol) to a final concentration of ~75%, and a volume of <200 ml. In the laboratory, the sample was decanted into a measuring cylinder, the volume noted, the cylinder agitated, and a 1 ml aliquot withdrawn using a Gilson auto pipette. This aliquot was run into a Pyrex 1 ml Sedgewick-Rafter cell, and the microinvertebrates present were counted and identified. Counts for each sample were based on a single subsample.

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[f] 'Floodplain associated' microinvertebrates are those that are thought to benefit from floodplain environments.

Table 14. Details of microinvertebrate sampling sites in the Lower Murray River.

Zone	Site	Latitude	Longitude
Floodplain	5–15 km DS Lock 6	S-34.0190	E140.87572
Floodplain	15 km DS Lock 4	S-34.3892	E140.59477
Gorge	5–15 km DS Lock 1	S-34.4052	E139.61723

Modelling and statistical analyses

Both categorical and continuous predictors were used to build a model to predict the response of microinvertebrates to flow. Categorical predictors were month, water year, lock and site, while continuous predictors quantified: (1) flow, (2) flow variability, (3) water quality and (4) inundated floodplain area (Appendix D). For flow, we calculated both short-term (mean flow on the date of sampling) and long-term flow (mean flow over the preceding 12-week period) predictors. Flow variability was quantified as the change in flow over the preceding 10-days prior to sampling (denoted 'flow trend' or 'dQ10'). Water quality parameters used were daily temperature and electrical conductivity. Floodplain inundated area was quantified using the DEW MIKE model outputs (M. Gibbs, pers comm; see Appendix D for details). Values were summed over 28- and 60-day periods to estimate inundated floodplain area corresponding to an estimated minimum period required for the majority of taxa to emerge from the egg bank and complete one or two life cycles. A global model including all predictors was fit first, then optimised using an automated procedure. Model predictive performance was assessed prior to use in modelling scenarios (see Appendix D in Ye et al. 2021 for details).

To assess the influence of Commonwealth environmental water on microinvertebrate density, taxonomic richness and community assemblage structure (including rotifers, cladocerans and copepods) over the long-term (5 years), variation between sampling years (i.e. 2014-15, 2015-16, 2016-17, 2017-18 and 2019-20) and sites (2019-20, presented below) was investigated. First, models were fit to all existing data using observed flow conditions, and other predictors (see above) (for more details see Ye et al. 2021). Second, the predictors were re-calculated using the modelled flow data for scenarios of no Commonwealth environmental water contributions (hereafter **"*No CEW*"**) and no environmental water at all (**"*No eWater*"**). Finally, models were re-run using the predictors calculated on modelled scenarios and the predicted microinvertebrate parameters (see below) were compared between the three scenarios using paired t-tests to determine whether predicted changes in microinvertebrates differed from observed variability (i.e., whether such a result would be expected at random). The overall contribution of environmental water was calculated as the difference between the observed and modelled *No eWater* scenarios. The difference between the *No CEW* and observed scenarios was the contribution by Commonwealth environmental water. Models were built to test each hypothesis above using the following microinvertebrate parameters:

- H1: (a) density of microinvertebrates (estimated as the mean of three replicates) and (b) taxonomic richness (total taxa within the three replicates);
- H2: Density of microinvertebrate taxa with assumed greater dependence on: (i) lateral connectivity (due to the longer period of time to complete their life cycle),

and, (ii) longitudinal transport. Category (i) taxa were littoral and pelagic cladocerans, and calanoid and cyclopoid copepods, comprising 32 taxa (Table 15a). Category (ii) taxa (Table 15b) were primarily pelagic rotifers, with their dependence inferred from their known biogeographical range (R. Shiel, pers comm, 2020) along with prior reports indicating the importance of upstream taxa in determining the turnover of Lower Murray microinvertebrate assemblages (Ye *et al*. 2020a);

- H3: Density of microinvertebrate taxa identified as preferred fish prey species, based on findings from the LTIM (Ye *et al.* 2020a) and MER (SARDI unpublished data) (Table 16).

Table 15. Taxa used to quantify the influence of environmental water on.

Dependence group	Taxa
(i) Lateral connectivity	*Acanthocyclops* cf. *vernalis, Armatalona macrocopa, Australocyclops australis, Boeckella triarticulata, Bosmina meridionalis, Calamoecia ampulla, Calamoecia sp.,* calanoid and cyclopoid copepodites, *Ceriodaphnia cornuta, Ceriodaphnia* sp., *Chydorus* cf. *eurynotus, Daphnia carinata s.l., D. galeata, D. lumholtzi, Daphnia sp., Diaphanosoma excisum, Gladioferens* sp., *Ilyocryptus* sp., *Leberis diaphanus, Macrothrix* sp., *Mesocyclops notius, Microcyclops varicans, Moina* cf. *australiensis, Moina* cf. *tenuicornis, Moina micrura, Neothrix* sp.,*Pseudochydorus globosus, Pseudomonospilus diporus, Simocephalus* sp., *Thermocyclops* sp.
(ii) Longitudinal connectivity	*Anuraeopsis coelata, Anuraeopsis fissa, Brachionus bidens, B. calyciflorus amphiceros, B. diversicornis, B. falcatus, Cephalodella catellina, Collotheca* cf. *tenuilobata, Conochilus dossuarius, C. natans, C. unicornis, Filinia longiseta, F. pejleri, F. terminalis, Keratella australis, K. cochlearis, K. lenzi, K. procurva, K. slacki, Polyarthra remata, P. vulgaris, Synchaeta oblonga, S. pectinata, Trichocerca pusilla* complex*, T. similis, T. similis grandis*

Table 16. Microinvertebrate prey species for fish larvae of large-bodied native species (Murray cod, golden perch and silver perch) identified from gut content analysis used in modelling fish prey species density.

Taxon	Family	Phylum/Order
Bosmina meridionalis	Bosminidae	Cladocera
***Ceriodaphnia* species**	Daphnidae	Cladocera
***Daphnia* species**	Daphnidae	Cladocera
***Chydoridae* species**	Chydoridae	Cladocera
***Ilyocryptidae* species**	Ilyocryptidae	Cladocera
***Macrothricidae* species**	Macrothricidae	Cladocera
***Moinidae* species**	Moinidae	Cladocera

Taxon	Family	Phylum/Order
***Neotrichidae* species**	Neotrichidae	Cladocera
***Sididae* species**	Sididae	Cladocera
Boeckella triarticulata	Centropagidae	Calanoida
***Calamoecia* species**	Centropagidae	Calanoida
***Gladioferens* species**	Centropagidae	Calanoida
***Cyclopidae* species**	Cyclopidae	Cyclopoida

Results

Density patterns

Mean observed density ranged from 139 [67, 210] to 2,774 [2,542, 3,006] ind/L (mean [± 95% CI]). Generally, sites tended to have comparable densities prior to 22 December 2020 (e.g. near overlap in confidence intervals; Figure 38a). Including and after 22 December 2020, Lock 1 site samples tended to be much lower than upstream sites. The highest densities recorded for the study period occurred between 8 December and 22 December 2020 at all three sites where Lock 1 peaked at 2,045 [1,616, 2,474] ind/L on 8 December 2020 and Lock 4 and Lock 6 at 2,774 [2,542, 3,006] ind/L and 1,910 [1,618, 2,201] ind/L on 22 December 2020, respectively.

Diversity patterns (taxonomic richness)

Taxonomic richness ranged from 11 [10, 13] taxa to 30 [29, 30] and was similar at all locks early in the water year (Figure 38b). Concurrent with the >86% decline in density at Lock 1 from 8 to 22 December 2020, taxonomic richness declined considerably at this site, remaining lower than the two upstream locks.

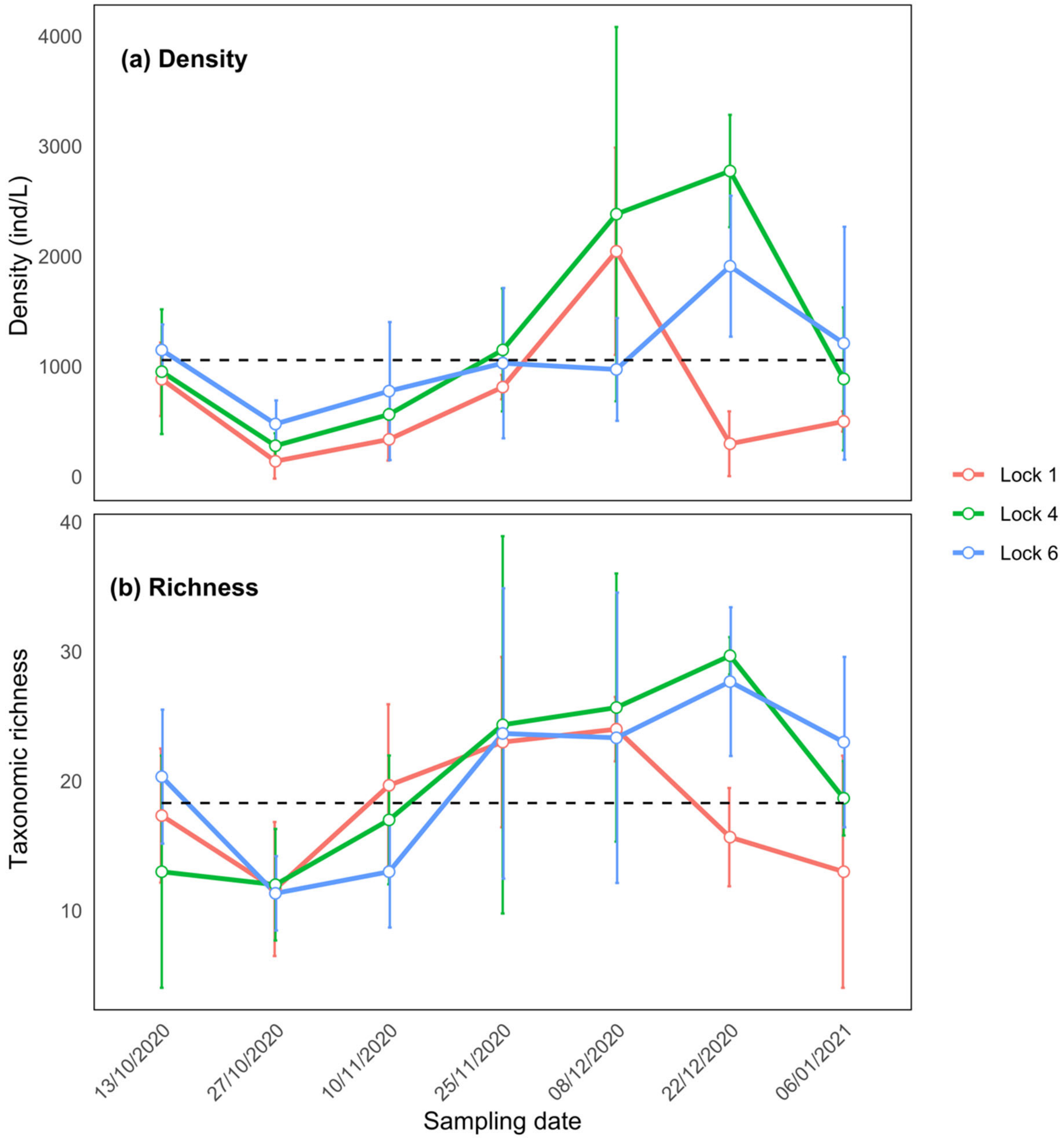

Figure 38. Observed density and taxonomic richness for 2020-21 sampling at each lock. (a) mean density of three sub-samples at each site (lock, indicated by colour) and date. Error bars show 95% confidence limits and horizontal black lines show mean for the water year.

Functional group density

The distribution of functional group density among sampling sites during 2020-21 suggests the dominance of primarily pelagic rotifers and secondly littoral (facultatively pelagic) rotifers throughout the year. The proportion of littoral (facultatively pelagic) rotifers increased at Lock 4 on 8 December 2020 and 22 December 2020 (Figure 39).

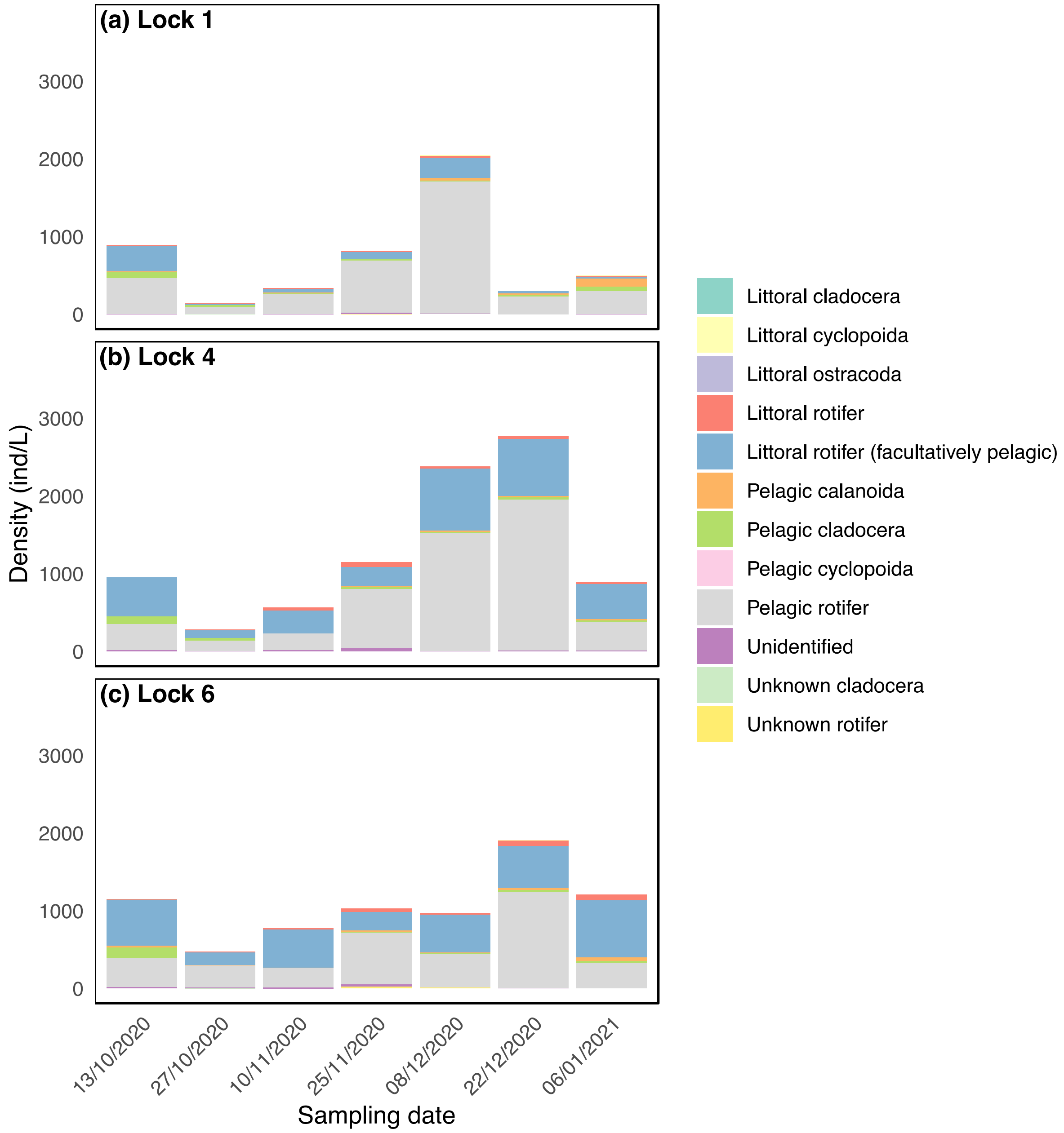

Figure 39. Functional group density by sampling date and lock for the 2020-21 season. Three sub-samples were collected at a single sample site located 5 km downstream of each lock shown in the panel caption.

Modelled responses to environmental flows

H1: Density and taxonomic richness

The modelling for 2020-21 estimated 33% of observed microinvertebrate density was due to environmental water delivery (mean difference between observed and no environmental water scenarios ±[95% CI] = 376 [258, 493] ind/L; *t* = 6.67, df = 20, *p*-value < 0.001) (Figure 40a). Commonwealth environmental water was estimated to have accounted for 73% of this (equates to 24%) (mean difference ± [95% CI] between observed and no Commonwealth environmental water scenarios = 273 [197, 350] ind/L; *t* = 7.48, df = 20, *p* < 0.001).

Around 16% of taxonomic richness for 2020-21 was predicted to have been due to environmental water (mean difference = 4 [2, 7]; *t* = 3.70, df = 20, *p* = 0.002), with Commonwealth environmental water estimated to have contributed around 57% of this (mean difference = 3 [1, 4]; *t* = 3.38, df = 20, *p* = 0.003), (Figure 40).

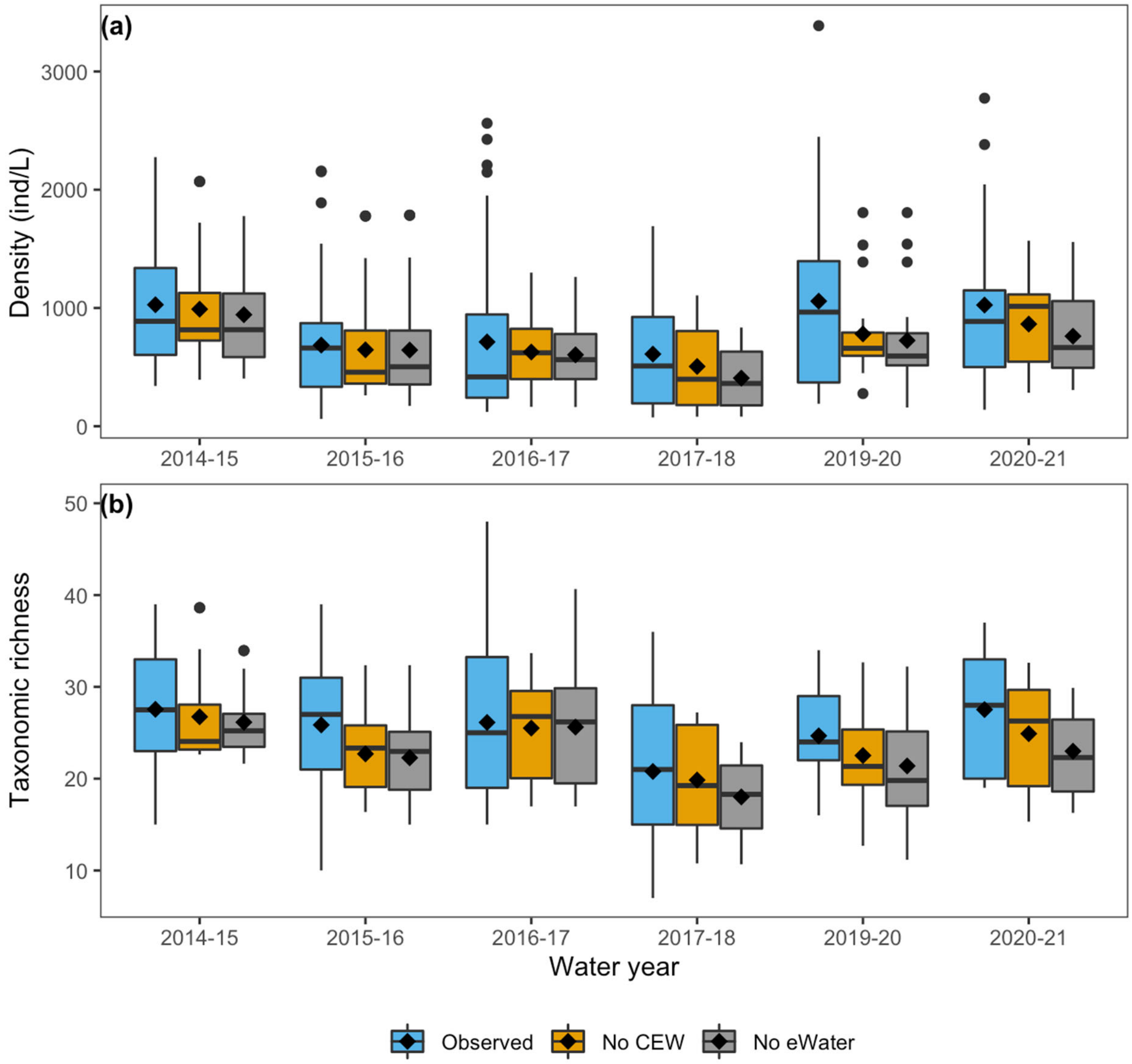

Figure 40. Observed and modelled (a) microinvertebrate density (individuals/litre, ind/L) and (b) taxonomic richness (number of taxa) by water year over the period 2014–2020 (2018-19 not sampled). In each panel, observed data ('*Observed*'; blue bars) for all samples in the water year shown on the x-axis are compared with modelled expectations under two environmental flow scenarios: Under no Commonwealth environmental water delivery ('*No CEW*'; orange bars) and if no environmental water were provided at all ('*No eWater*'; grey bars). Black diamonds represent mean values. Results of the generalised linear models used to run the scenarios are given in Tables D7 (density) and D8 (taxonomic richness) in Appendix D.

H2: Taxa associated with lateral and longitudinal connectivity

In 2020-21, modelling indicated that the density of floodplain dependent taxa was 20% lower than if no environmental water was delivered (mean difference = -7 [-17, 3] ind/L; *t* = -1.41, df = 20, *p* = 0.173) (Figure 41a). Furthermore, modelling indicated that the density of these taxa was 27% lower in density than if no Commonwealth environmental water was delivered (mean difference = -9 [-19, 1] ind/L; *t* = -1.93, df = 20, *p* = 0.068).

Conversely, in 2020-21, modelling indicated that the density of taxa common to upstream areas increased 54% due to environmental water delivery, improving longitudinal connectivity (mean difference = 330 [220, 441] ind/L; *t* = 6.23, df = 20, *p* < 0.001) (Figure 41b). Commonwealth environmental water was estimated to account for 74% of this (mean difference = 245 [157, 334] ind/L; *t* = 5.79, df = 20, *p* < 0.001) (Figure 41b).

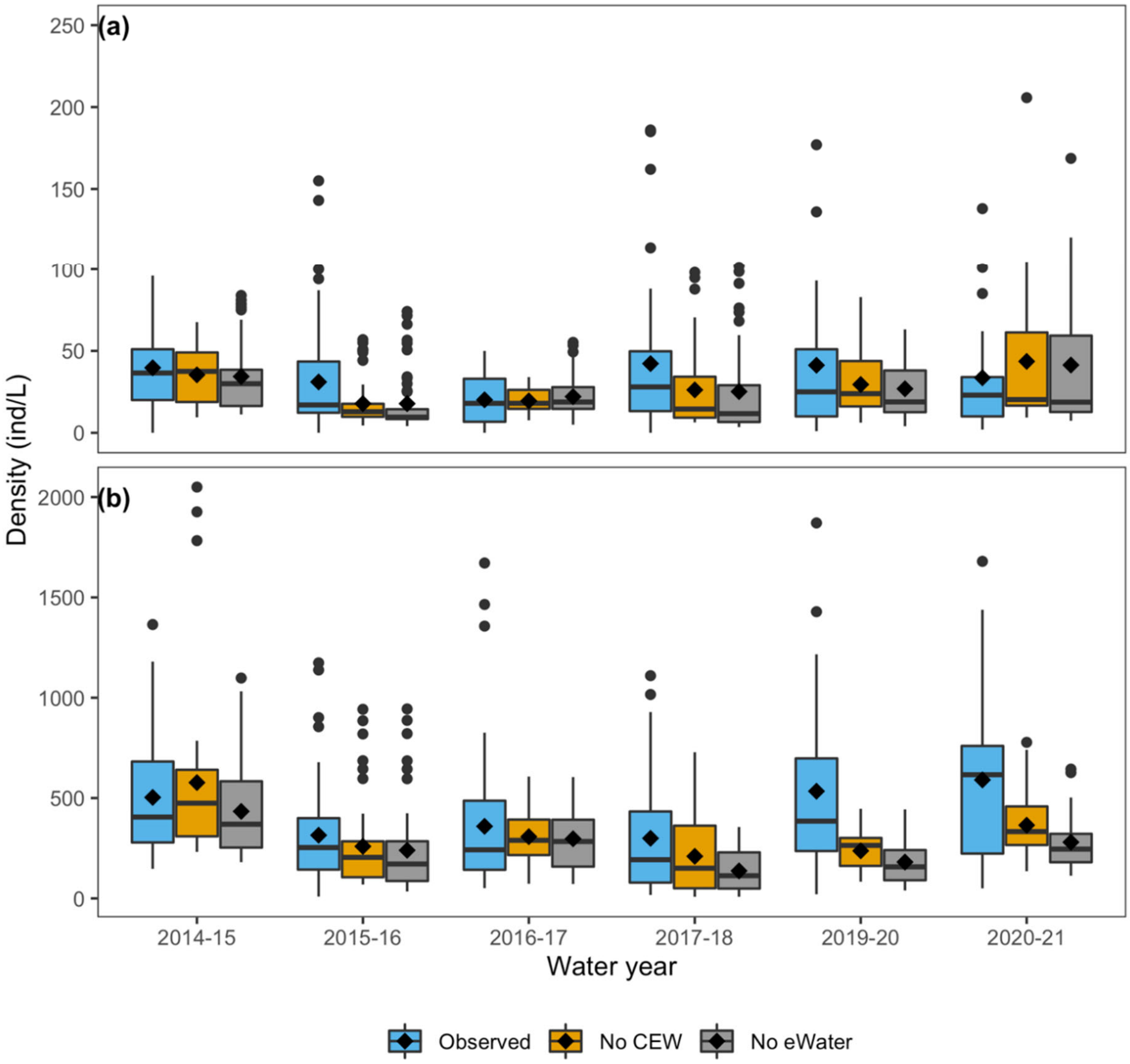

Figure 41. Observed and modelled density (individuals per litre, ind/L) with and without environmental water (a) microinvertebrate taxa associated with lateral connectivity to floodplains and backwaters and (b) microinvertebrate taxa associated with longitudinal transport and connectivity with upstream areas by water year over the period 2014–2021 (2018-19 not sampled). In each panel, observed data (‘*Observed*’; blue bars) for all samples in the water year shown on the x-axis are compared with modelled expectations under two environmental flow scenarios: Under no Commonwealth environmental water delivery (‘*No CEW*’; orange bars) and if no environmental water were provided at all (‘*No eWater*’; grey bars). Black diamonds represent mean values. Results of the generalised linear models used to run the scenarios are given in Tables D9 (taxa associated with lateral connectivity to floodplains and backwaters) and D10 (taxa associated with longitudinal transport and connectivity) in Appendix D.

H3: Spring-early summer microinvertebrate prey species density

For the 2020-21 sampling season, modelled spring prey species density was 41% lower (mean difference -13 ind/L ±[95% CI -23, -4] in observed than no environmental water scenarios (*t* = -2.95, df = 17, *p* = 0.009). Commonwealth environmental water was estimated to account for 99.5% of this difference (-13 ind/L [-22, -4]; *t* = -3.08, df = 17, *p* = 0.007) per sampling event.

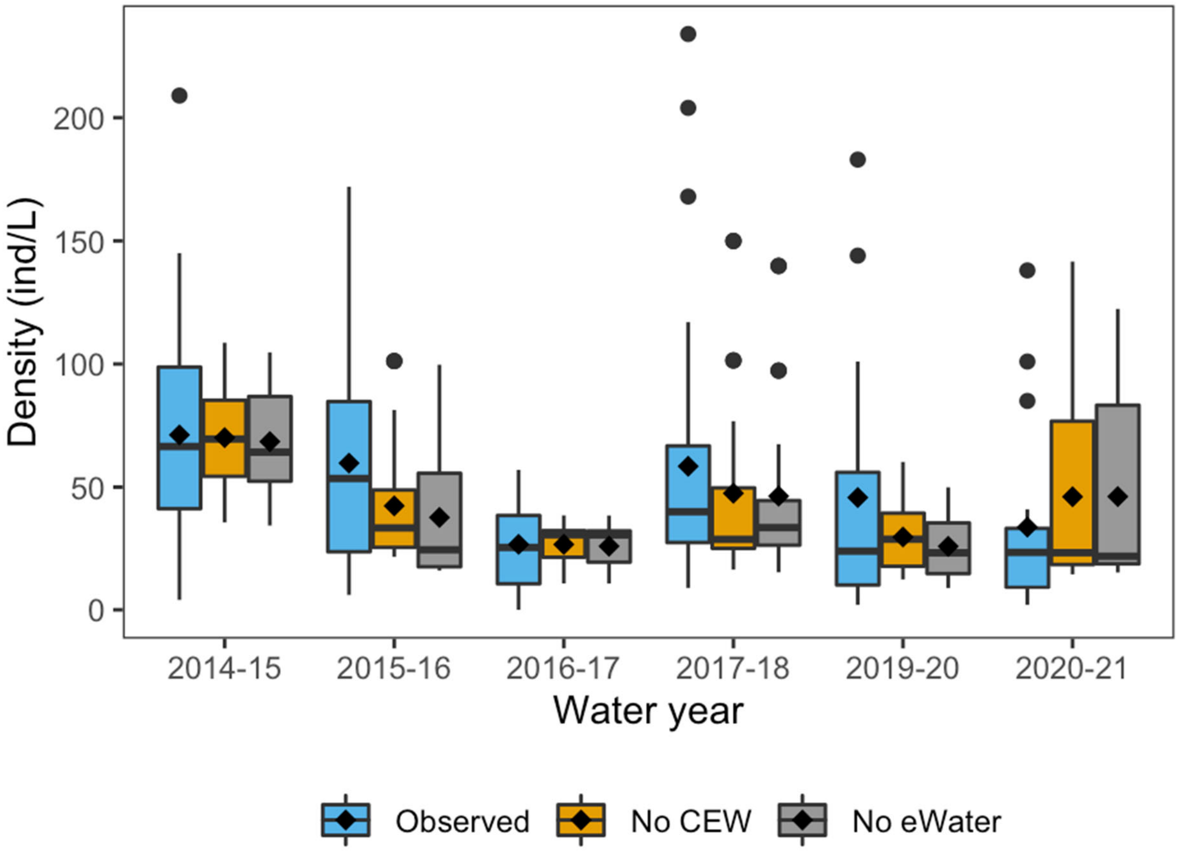

Figure 42. Observed and modelled density (individuals per litre, ind/L) of microinvertebrate prey taxa for large-bodied fish larvae in the Lower Murray during spring–early summer (mid-October to late-December). In each panel, observed data ('*Observed*'; blue bars) for all samples in the water year shown on the x-axis are compared with modelled expectations under two environmental flow scenarios: Under no Commonwealth environmental water delivery ('*No CEW*'; orange bars) and if no environmental water were provided at all ('*No eWater*'; grey bars). Black diamonds represent mean values. Results of the generalised linear models used to run the scenarios are given in Table D11 in Appendix D.

Evaluation

The evaluation approach, including assessment criteria, is described in the evaluation section for Hydraulic Regime (Section 2.1).

Table 17. Microinvertebrate Assemblage evaluation questions and answers. CEW = Commonwealth environmental water, eWater = environmental water. Hypotheses: H1a, H1b, H2a, H2b and H3. The mean [95% CI] and % attributable to CEW contributions for each water year are detailed for each evaluation question and each water year. The significance of the contribution was classified as none-negligible if the contribution was 0–9%, minor = 10–19%, moderate =20–29% and substantial = ≥30%.

CEWO evaluation questions	**Outcomes of CEW delivery**					
	2014-15*	**2015-16***	**2016-17**	**2017-18***	**2019-20***	**2020-21***
What did CEW contribute to microinvertebrate density (H1a)?	64 [42, 86] (+6%)	47 [23, 71] (+7%)	31 [12, 49] (+5%)	112 [80, 144] (+18%)	261 [170, 352] (+25%)	273 [197, 350] (+24%)
	eWater increased overall microinvertebrate density by an average of 19% across all years (2014-21), with CEW accounting for 68% of this.					
What did CEW contribute to microinvertebrate diversity (taxonomic richness) (H1b)?	1 [0, 2] (+2%)	1. [1, 2] (+4%)	1 [0, 2] (+3%)	2 [2, 3] (+11%)	4 [2, 6] (+14%)	3 [1, 4] (+9%)
	eWater increased overall microinvertebrate taxa richness by an average of 10% across all years (2014-21), with CEW accounting for 66% of this.					
What did CEW contribute to microinvertebrate communities of the LMR via lateral connectivity (H2a)?	5 [2, 7] (+12%)	13 [9, 17] (+42%)	0 [-1, 1] (-1%)	26 [15, 36] (+49%)	11 [5, 16] (+26%)	-9 [-19, 1] (-27%)
	eWater increased the density of taxa associated with lateral connectivity by an average of 26% across all years (2014-21), with CEW accounting for 97% of this.					
What did CEW contribute to microinvertebrate communities of the LMR via longitudinal connectivity (H2b)?	-43 [-102, 16] (-8%)	65 [48, 81] (+20%)	20 [8, 32] (+6%)	105 [72, 139] (+33%)	261 [176, 346] (+52%)	245 [157, 334] (+40%)
	eWater increased the density of taxa associated with longitudinal connectivity by an average of 34% across all years (2014-21), with CEW accounting for 58% of this.					
What did CEW contribute to spring-early summer microinvertebrate fish prey species density (H3)?	-5 [-10, 0] (-8%)	19 [15, 23] (+31%)	1 [0, 1] (+2%)	22 [13, 30] (+32%)	10 [4, 15] (+25%)	-13 [-22, -4] (-40%)
	eWater increased the density of microinvertebrate fish prey species during spring-early summer by an average of 19% across all years (2014-21), with CEW accounting for 79% of this.					

* = additional management levers were also used (Appendix B)

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):

⬜ Unknown 🟥 Negative 🟩 None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

A combination of raw data analysis and ecological modelling was used to assess the microinvertebrate community and its response to environmental water delivery in 2020-21. Data comparison showed that the microinvertebrate community in 2020-21 had high density and taxonomic richness during the spring-early summer flow pulse. This was supported by the modelling results which predicted a 33% increase in density and 16% increase in taxonomic richness due to environmental water, of which 73% and 57%, respectively, was due to Commonwealth environmental water across the study period. This response appeared greatest at Lock 4. However, it is important to note that multiple weir pools (including Weir Pool 1, 2, 4 and 5) were also manipulated, and managed inundation by operations of Katarapko and Pike floodplain regulators took place in the LMR during the study period and may have influenced density and taxonomic richness. Increases in taxonomic richness and density during environmental water delivery imply that organisms were being dispersed downstream, a critical process in the protection of genetic and species diversity (Aavik and Helm 2018). This was further supported by the modelling which predicted that environmental water resulted in a 54% increase in the density of taxa common to upstream areas and those that have been found to proliferate during times of high longitudinal connectivity, of which Commonwealth environmental water accounted for 74%. Microinvertebrates are also important prey items for a range of higher trophic organisms and therefore increases in density are also likely to have positive impacts on higher trophic organisms (Cooper and Goldman 1980; Vinyard 1980). Despite these multiple benefits detected in 2020-21, modelling predicted a 20% decrease in floodplain associated taxa and a 41% decrease in modelled prey species due to environmental water in 2020-21. These two findings however were mainly due to the decline in a single species (*Bosmina meridionalis*) that falls across both groupings.

Density and taxonomic richness and patterns

Observed patterns in density and taxonomic richness in 2020-21 highlighted similarities in general seasonal patterns with previous years and differences between sites in response to the delivery of environmental water. There was a consistent trend across years of a general increase in density and taxonomic richness over time as water temperature increased. This is not surprising as aquatic productivity often increases with temperature (Heinle 1969; White et al. 1991). In 2020-21, all three sites followed a similar trend in density and taxonomic richness until 8 December 2020. The delivery of environmental water (of which 76% was Commonwealth environmental water in 2020-21) occurred from late-November and throughout the month of December 2020, which coincided with large increases in microinvertebrate density at Lock 1 on 8 December 2020 and at Lock 4 on 8 and 22 December 2020. Numerous weir pools were being manipulated and managed inundation of two floodplains, the Pike and Katarapko floodplains, took place at the time, which could have also contributed to these results. These included the raising of Locks 1, 2, 4 and 5 of which Locks 1, 2 and 4 were being lowered and Lock 5 was at its peak through late November and December (Appendix B). It is during peak inundation and drawdown that weir pool raising is likely to influence in-channel communities (Furst 2019). However, another study investigating zooplankton productivity during this period did not detect a major influence of these weir pool manipulations and associated floodplain inundation on in-channel zooplankton communities, although the design of this study was only likely to pick up landscape scale changes rather than smaller scale influences (e.g. at the floodplain scale) (Dornan et al. 2021). Environmental water delivery also coincided with increases in taxonomic richness at all three sites on 25 November 2020 and 8 December 2020 and at Lock 6 and Lock 4 on 22 December 2020. These values (both density and taxonomic richness) were the highest recorded for the 2020-21 study period and were higher than those observed at the same sites in 2019-20 (a hydrologically similar year, although peak flow occurred earlier around mid-October 2019), suggesting that these increases were likely due to increases in flows rather than merely increases in water temperature. This was further supported by the modelling, which predicted a 33% increase in density, of which Commonwealth environmental water accounted for 73% and a 16% increase in taxonomic richness, of which Commonwealth environmental water accounted for 57%. Interestingly, Lock 1 became unique to the other two sites measuring generally lower densities and taxonomic richness from early December (this divergence also occurred in mid-November in 2019-20). The driver behind this divergence may be the emergence of different hydraulic conditions at different sites across geomorphic zones, e.g., variability in water velocity and water level, between weir pools as flows receded. Despite the differences in hydraulic conditions appearing relatively small (see Section 2.1 Hydraulic Regime), increasing water temperatures likely magnified the divergence of communities between Lock 6 and Lock 4, and Lock 1 later in the study.

Functional group density

Increases in density observed at the time of environmental water delivery were primarily due to increases in pelagic rotifers at Lock 1, and both pelagic rotifers and littoral (facultatively pelagic) rotifers at Lock 4. This may have implications for food-web function as these different functional groups are likely being sourced from a variety of habitats, which can result in different fatty acid compositions (e.g. Radnaeva et al. 2017). This is important as the fatty acid composition of rotifers is affected by both internally consistent features (e.g. cladocerans may accumulate EPA directly from their diet, or through transformation of dietary materials to facilitate rapid somatic growth and enhance reproduction due to their short generation time) and the fatty acid composition of their food resource (Brett et al. 2006; Smyntek et al. 2008). These different functional groups and/or taxa then provide more or less desirable fatty acid compositions for the predators feeding on them and can have implications for the predator's growth, health and reproduction (Adams 1999). How fatty acid composition differs between the different taxa and/or functional groups, is not well understood in the LMR. A better understanding of this will allow a more comprehensive comparison with responses detected during previous years and should be a focus of future research (for example see Perrsson and Vrede 2006).

Modelled effects of environmental flows for microinvertebrates

Overall, modelling demonstrated that environmental water delivery provided some benefits for the microinvertebrate community in the LMR. In 2020-21, one out of the three hypotheses under test were fully supported, one was partially supported, and one was not supported.

The first hypothesis was that *overall microinvertebrate density, and taxonomic richness, will increase with environmental water due to the combined effects of longitudinal transport and improved lateral connectivity with off-channel habitats*. Modelling indicated that environmental water represented a predicted 33% increase in density, of which Commonwealth environmental water accounted for 73%. Not unexpectedly, because of its correlation with density[g], taxonomic richness also increased due to environmental water with a predicted 16% increase in, of which Commonwealth environmental water accounted for 57%.

The second hypothesis was: *The density will increase for 'floodplain associated' microinvertebrates during periods of greater floodplain inundation (H2a) and for those that are likely to be transported to the LMR from upstream during periods of high longitudinal connectivity (H2b)*. H2a was not supported, with densities being 27% lower than if no Commonwealth environmental water was delivered. This decrease however was due almost solely to the decline in a single species of microcrustacean, *B. meridionalis*. *B. meridionalis* is a perennial species that often peaks in density in spring and early summer and as do all microcrustaceans, requires/prefers still or very slow flowing water to reproduce. However, unlike the majority of other microcrustaceans, it seems that *B. meridionalis* can exploit small areas of still and slow flowing habitats along the banks (littoral zone) of the main channel during low-medium in-channel flows. Thus, what possibly happened in this study is that prior to environmental water release, *B. meridionalis* was able to exploit these littoral habitats of the main channel. Lateral connectivity with these habitats meant that a proportion of these populations were being flushed into the flowing section of the channel. As the flow pulse arrived in late spring–early summer, these still and/or slow flowing littoral habitats of the main channel were flushed and eliminated.

H2b was supported, with a 54% increase in taxa common to upstream areas due to environmental water improving longitudinal connectivity, of which Commonwealth environmental water accounted for 74%. This longitudinal dispersal is an important process in the maintenance of species and genetic diversity, through the supply of new recruits to downstream habitats (spatial dispersal) and the egg bank (temporal dispersal).

The third hypothesis was that *environmental water delivery during spring and early summer will increase the density of preferred microinvertebrate prey species for large-bodied fish larvae*. This hypothesis was not supported, with a 41% decrease in modelled prey species due to environmental water, of which Commonwealth environmental water accounted for 99.5%. This was the first time since monitoring began in 2014-15 where a large decrease in these organisms was detected in response to the delivery of environmental water. However, as mentioned above, this was almost solely due to the decrease in a single species, *B. meridionalis*, whose numbers were possibly driven down by the in-channel flow pulse flushing and eliminating small areas of still or slow flowing water near the banks of the river channel. In situations where the flow pulse is of a greater magnitude and duration, at a point, large areas of ephemeral habitat would become inundated. Once this is achieved you may possibly see a return of this species, as well as a suite of additional microcrustaceans when they are flushed from these ephemeral habitats to the main channel. *B. meridionalis* was included in the list of prey species as it has been found in the guts of Murray cod and golden perch larvae (Gibbs et al., 2020; Ye et al. 2021; SARDI unpublished data). However, in the Chowilla anabranch there was also evidence that fish select against them in preference for other microcrustacean species when they are

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[g] Due to the high correlation between density and richness, the testing of other hypotheses focused only on estimating changes in density.

present (Gibbs *et al.* 2020). Therefore, it is important that flow or river management focuses on maintaining prey densities via the development of a diverse range of microcrustacean species.

Management implications

This project has highlighted key findings that may be used to inform future environmental water deliveries, aiming to promote a response in the microinvertebrate community in the LMR.

The year 2020-21 was hydrologically similar in magnitude, duration and timing to 2017-18. The measured densities during environmental water delivery in 2017-18 were generally lower than those detected during 2020-21. It is difficult to say why this was the case, however, antecedent conditions and/or weir pool manipulation and managed inundation of floodplains may have had some influence. The year prior to 2017-18 (2016-17) was a flood year that resulted in a large blackwater event. The highly deoxygenated water that flowed through the LMR throughout the months of November and December had significant negative impacts on the density and taxonomic richness of the microinvertebrate community (Ye *et al.* 2020a). Egg production in zooplankton has also been shown to be significantly impacted by low oxygen environments (Roman *et al.* 2019). Therefore, both the reduction in density and the impacts on egg production due to the blackwater event likely impacted the community's ability to replenish the egg bank and may explain the lower densities observed in 2017-18. During the year prior to 2020-21 (2019-20), a spring flow pulse was delivered that peaked at ~15,600 ML/day at the South Australian border. Therefore, the spring flow pulse in 2019-20 may have played some role in replenishing the microinvertebrate egg bank and driven the higher densities in 2020-21. Additionally, all weir pool manipulation in 2017-18 occurred early in the year, with weirs being returned to near pool level around the time that discharge began increasing in the main river channel. In comparison, numerous weir pools were being manipulated and the managed inundation of two floodplains was taking place at the time of environmental water delivery in 2020-21 including the raising of Locks 1, 2, 4 and 5 of which Locks 1, 2 and 4 were being lowered and Lock 5 was at its peak height. Therefore, it is possible that the concurrent operations of environmental water delivery and weir pool manipulation may considerably enhance the response observed in microinvertebrate density in the LMR. However, more long-term data collection and analysis is required to investigate this further.

The year 2020-21 was hydrologically similar in magnitude and duration to that seen in 2019-20. The community assemblage in 2019-20 was dominated primarily by littoral (facultatively pelagic) rotifers and secondly by pelagic rotifers, whereas the community assemblage in 2020-21 was dominated primarily by pelagic rotifers and secondly littoral (facultatively pelagic) rotifers. As mentioned above, this may have implications for food-web function as these different functional groups are likely being sourced from different habitats and can result in different fatty acid compositions (e.g. Radnaeva *et al.* 2017), affecting food quality for higher order consumers. In addition, the species that were responsible for the high numbers of organisms in the functional group 'littoral (facultatively pelagic) rotifer' were different between the two years with the species driving numbers in 2019-20 being very small, whereas those in 2020-21 were from the same genus however were a much larger species (estimated to be approximately three times the size). These larger organisms are more likely to equate to greater total biomass and therefore more food in general for higher trophic organisms. These contrasting results demonstrate how the timing of environmental water delivery may influence the magnitude and type of the response in the rotifer community generated by the delivery of environmental water.

The increase or decrease in microcrustaceans (comprise the majority of organisms that are categorised as 'floodplain associated' and 'fish prey species') in response to an in-channel flow pulse may depend on the antecedent conditions. An in-channel flow pulse of similar timing, magnitude and duration occurred in 2017-18 and 2020-21. In 2017-18, however, there was an increase in microcrustaceans in response to environmental water, yet a decrease in 2020-21. Additionally, an in-channel flow pulse of similar magnitude and duration to 2020-21 occurred in 2019-20, however again, there was an increase in microcrustaceans in 2019-20. The antecedent flow conditions may have influenced these differences between years. River flows were lower in 2017-18 and 2019-20 than those in 2020-21 (~5,000 ML/day in 2017-18 and 2019-20 and ~9,000 ML/day in 2020-21 at the South Australian border) prior to the in-channel flow pulse to the LMR. These lower flows likely created lake like conditions in the weir pools allowing open water lake microcrustacean communities (a community assemblage that overlaps with floodplain assemblages) to develop as they require slow/still water to reproduce. As flows increased, it is possible that these communities became mobilised along the channel, temporarily driving microcrustacean densities up in the more flowing sections of the river where we sampled. Furthermore, *B. meridionalis*, the species that drove the decreases in 2020-21, was in lower densities in 2017-18 than 2020-21 prior to the in-channel flow pulse reaching the LMR and may explain why a decrease in this species wasn't observed in 2017-18. Nonetheless, densities of *B. meridionalis* were high prior to the in-channel flow pulse in 2019-20 (as in 2020-21), yet initially increased in density as discharge increased (contrary to 2020-21), before decreasing at the peak of the flow pulse. However, the initial increases observed in 2019-20 may indeed have been due to dense populations from upstream of the LMR being washed downstream. Other factors like water quality and food resource availability may also have played a role. Further research to improve our understanding of what conditions promote or reduce certain taxa and whether certain taxa provide more important food resources than others will shed light on which flow regime is likely to produce a more desirable outcome for higher trophic organisms.

Responses to the delivery of environmental water in microinvertebrate density and taxonomic richness were consistently different between sites in both 2019-20 and 2020-21. Therefore, we need to reflect on what outcomes we want and where, when considering environmental water delivery.

Conclusion

The magnitude and duration of the resulting hydrograph in 2020-21 was similar to that seen in 2017-18 and 2019-20. The timing of the delivery between 2019-20 and 2020-21 was however quite different (see Figure 10). A comparison between these three years therefore provides insights into how the timing and antecedent conditions of environmental water delivery may produce different outcomes. Two key differences in microinvertebrate responses were detected between the three years that can be used to guide environmental water delivery in the future.

Firstly, increases measured in 2019-20 were of a similar density to 2020-21, however in 2020-21, these increases persisted for longer and consisted more so of larger bodied rotifers. Secondly, a density decrease in both ‘floodplain associated species’ and ‘prey species’ were detected in 2020-21, compared to modelled no CEW and no-eWater scenarios, whereas an increase in these two groups were detected in 2017-18 and 2019-20. These differences are likely to be due the antecedent conditions both within the LMR and upstream. Despite total microinvertebrate densities being one indicator of the magnitude of the response to environmental water, it is important that we also gain a much better understanding of the quality of these food resources for higher trophic organisms. This will allow a more comprehensive understanding of the ecological implication and significance of these different responses to inform environmental water management.

2.6 Flow-cued Spawning Fish Reproduction

Background

In the southern MDB, spawning and recruitment of golden perch corresponds with increases in water temperature and discharge, either in-channel or overbank (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b). Silver perch display similar life history characteristics and population dynamics, although in the lotic reaches of the Murray River, silver perch may spawn circa-annually (Tonkin *et al.* 2019). Annual increases in flow (spring flow pulses) were a distinct hydrological feature of the unregulated Murray River (Mallen-Cooper and Zampatti 2018). In regions where these features remain intact (e.g. the mid-Murray River), golden perch display more consistent recruitment (Zampatti *et al.* 2018). In the Murray River downstream of the Darling junction, however, spring flow pulses are compromised by river regulation. Commonwealth environmental water may be used, at least in- part, to restore these pulses.

Since 2012, >500 GL/year of Commonwealth environmental water has been delivered to the LMR to enhance the flow regime and rehabilitate the health of aquatic ecosystems. In the LMR, Commonwealth environmental water has been primarily used to contribute to increased base flows and freshes to 18,000 ML/d (Section 1.4). One of the ecological objectives of flow augmentation is to contribute to increased spawning and/or recruitment of flow-dependent fish species. Over the course of the CEWO's LTIM project (2014–2019) there was no substantial recruitment of golden perch in the LMR, despite spawning of golden perch coinciding with periods when environmental water was used to promote flow pulses in the LMR (Ye *et al.* 2020a). An absence of recruitment led to a population dominated by a few distinct and ageing cohorts. Evidence from these investigations suggest that greater flow rates (>20,000 ML/d) are likely required to significantly influence golden perch spawning and recruitment in the LMR. As part of the MER Project, the recruitment of golden perch and silver perch in the LMR is investigated through age structure analysis to assess the impact of the flow regime (including environmental water) on population dynamics. Contingency monitoring supported the evaluation of golden perch and silver perch reproduction (including spawning) in 2020-21 through larval sampling and otolith microstructure and chemistry. Data collected as part of the LTIM Project (Ye *et al.* 2020a) is integrated in the reporting and evaluation for this indicator.

Hypotheses

- Increased spring–summer flow (nominally >20,000 ML/d), either in-channel or overbank, will promote the spawning and recruitment (to YOY) of golden perch and silver perch; and
- Multiple years of enhanced spring–summer flow will increase the resilience of golden perch and silver perch populations in the LMR by promoting a more diverse age structure.

Methods

In 2020-21, to evaluate golden perch and silver perch reproduction (spawning and recruitment), and its association with flow, including environmental water, we: (1) sampled larval and young-of-year (YOY) fish in the LMR (Figure 1); (2) used otolith microstructure and chemistry, specifically strontium (Sr) isotope ratios (^{87}Sr/^{86}Sr), to retrospectively determine the time and place of spawning; and (3) used electrofishing to assess interannual variability in the relative abundance of golden perch, and collect representative sub-samples of golden perch and silver perch to determine population age structures in the LMR.

Analysis of water ^{87}Sr/^{86}Sr at sites across the southern MDB

To determine spatio-temporal variation in water ^{87}Sr/^{86}Sr over the spring–summer of 2020-21, water samples were collected weekly–monthly from nine sites across the southern MDB. At most sites, water samples were collected from early September 2020 to early February 2021. A subset of these samples (Figure 43; Table 18) were submitted for analysis.

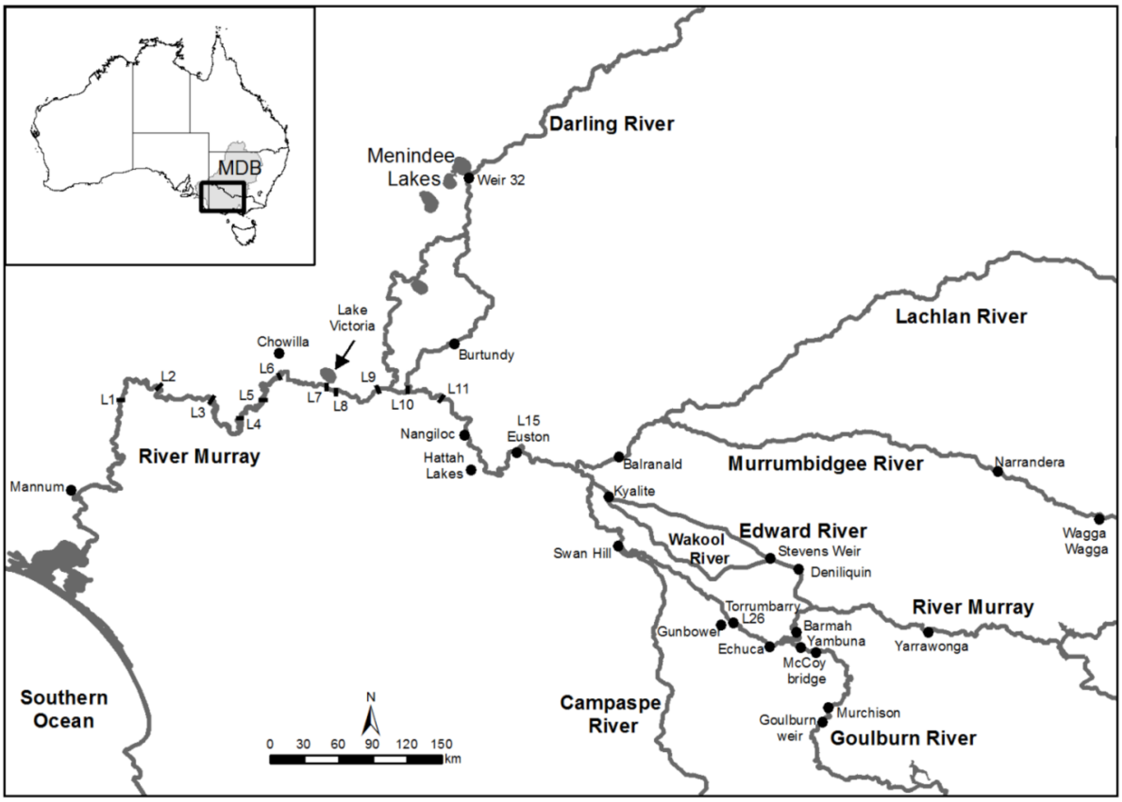

Figure 43. Map showing the location of the Murray–Darling Basin and the major rivers that comprise the southern Murray-Darling Basin, the numbered Locks (L) and Weirs (up to Lock 26, Torrumbarry), the Darling, Lachlan, Murrumbidgee, Edward–Wakool, Campaspe and Goulburn rivers and Lake Victoria, an off-stream storage used to regulate flows in the Murray River below Lock 9.

Table 18. Location of water sample collection for $^{87}Sr/^{86}Sr$ analysis in 2020-21.

River	Location	Sampling period	Total number of samples
Murray	Lock 1	2/11/20–11/1/21	6
Murray	Lock 6	3/11/20–12/1/21	6
Lake Victoria	Rufus River	22–24/12/20	3
Murray	Lock 9	3/11/20–12/121	6
Murray	Lock 11	6/11/20–12/1/21	6
Murray	Torrumbarry	2/11/20–11/1/21	6

Sampling eggs and larvae

In the LMR, larval fish sampling was conducted approximately fortnightly between 9 November 2020 and 20 January 2021. Weekly sampling, however, was undertaken between 24 November and 23 December 2020 to coincide with the ascending and descending limbs of the flow pulse (Figure 1). Sampling was undertaken at three sites, located 5–15 km downstream of Locks 1, 4 and 6, respectively (Figure 1). At each site, four day-time plankton tows were conducted using a pair of square-framed bongo nets with 500 µm mesh; each net was 0.5 x 0.5 m and 3 m long. The volume of water (m^3) filtered through each net was determined using a calibrated flow meter (General Oceanics™, model 2030R) placed in the centre of the mouth openings. Samples were preserved (70–95% ethanol) in the field and returned to the laboratory for processing. Samples were sorted using a dissecting microscope. Larvae and eggs were identified, and where possible, classified as pre-flexion (i.e. early stage larvae with notochord predominately straight) or post-flexion (i.e. the start of upward flexion of the notochord and appearance of fin rays and fin fold) following Serafini and Humphries (2004). We could not visually differentiate golden perch eggs from silver perch eggs. When eggs were present, golden perch and silver perch eggs were differentiated using DNA sequencing of a ~680 bp region of the cytochrome c oxidase subunit 1 mitochondrial gene (*COI*). DNA extraction, PCR and gel electrophoresis steps were performed by the SARDI Molecular Sciences Laboratory, with positive amplicons submitted to Australian Genome Research Facility (AGRF) for Sanger sequencing in the forward and reverse direction.

Sampling YOY and population age structure

Annual sampling was undertaken in May 2021 to complement Category 1 Fish Assemblage sampling and to maximise the likelihood of collecting YOY from the previous spring–summer spawning season. Adult and juvenile golden perch were sampled using a 7.5 kW Smith Root (Model GPP 7.5) boat electrofishing unit at a total of 14 sites in the LMR (Figure 1). Electrofishing was conducted during daylight hours and all available littoral habitats were surveyed. At each site, the total time during which electrical current was applied (on-time effort) ranged from approximately 573 to 2,880 seconds. All individuals were measured to the nearest mm (total length, TL) and a sub-sample of golden perch proportionally representing the length-frequency of golden perch collected was retained for ageing. In 2021, all silver perch captured (*n* = 4) were retained for ageing.

Abundance

Temporal variability in the relative abundance of golden perch was investigated by assessing changes in Category 1 electrofishing catch-per-unit-effort (CPUE) data (See Section 2.8, Fish Assemblage). Differences in the relative abundance (individuals per 90 seconds of electrofishing on-time effort) between years were analysed using univariate single-factor PERMANOVA (permutational ANOVA and MANOVA) in the software package PRIMER v. 6.1.12 and PERMANOVA+ (Anderson *et al.* 2008). These analyses were performed on untransformed, standardised relative abundance data.

Ageing

Golden perch and silver perch larvae were measured for TL to the nearest millimetre and sagittal otoliths were removed. To estimate the spawn date of larval and YOY fish, daily increments in otolith microstructure were examined. Otoliths were mounted individually in Crystalbond™, proximal surface downwards, and polished down to the primordium using a graded series of wetted lapping films (9, 5, and 3 µm). Sections were then polished using 0.3 µm alumina slurry to a thickness of 50–100 µm. Sections were examined using a compound microscope (x 200) fitted with a digital camera and Olympus Stream image analysis software (version 2.3). Estimates of age were determined by counting the number of increments from the primordium to the otolith edge (Zampatti and Leigh 2013b).

We used age-frequency distributions to assess the age structure and year-class strength of golden perch. Golden perch and silver perch retained for ageing were euthanised and sagittal otoliths were removed. Whole otoliths were embedded in clear casting resin and a single 400 to 600 µm transverse section was prepared. Sections were examined using a dissecting microscope (x25) under transmitted light. Estimates of age were determined independently by three readers by counting the number of discernible opaque zones (annuli) from the primordium to the otolith edge. YOY (<1 year old) fish were defined as individuals lacking clearly discernible annuli.

Otolith $^{87}Sr/^{86}Sr$ analysis

Sagittal otoliths were transferred to a master slide and fixed onto a thin smear of clear casting resin aligned in rows and allowed to set overnight before being placed in a drying oven at 500 °C for 4 hours and then sonicated in Milli-Q water. Individual locations of each otolith were recorded on a slide map along with preferred ablation path for each otolith. *In situ* microsampling analysis of $^{87}Sr/^{86}Sr$ in the otoliths of larval and juvenile golden perch and silver perch was achieved by laser ablation – inductively coupled plasma mass spectrometry (LA-MC-ICPMS). The laser ablation and mass spectrometry procedure is described in full in Zampatti *et al.* (2021). To investigate the natal origin and migration history of new recruits (larvae and YOY) and prominent cohorts of golden perch and silver perch in the LMR, whose origins had not previously been determined, we analysed $^{87}Sr/^{86}Sr$ from the otolith core to edge in a subsample of fish. We compared these transects to water $^{87}Sr/^{86}Sr$ measured at sites across the southern MDB during 2020-21 and from 2011–2019 (presented in Ye *et al.* 2020a). The origins of other prominent cohorts have been presented in Ye *et al.* (2020, 2021).

Results

Larval collection and spawn dates

From 26 November 2020 to 18 January 2021, a total of 20 golden perch and 142 silver perch larvae were collected at sites sampled downstream of Locks 1, 4 and 6 in the LMR (Table 19). The majority of silver perch (60%, *n* = 85) were sampled below Lock 6 in mid-December 2020, while the majority of golden perch (80%, *n* = 16) were sampled below Lock 1 from late November to early December 2020 (Table 19; Figure 44). Ages of golden perch larvae ranged 3–10 days, corresponding to spawn dates of 23 November–5 December 2020, while ages of silver perch larvae ranged 11–31 days, corresponding to spawn dates of 18 November 2020–2 January 2021 (Table 19; Figure 45). Golden perch eggs (later confirmed through DNA sequencing of the *COI* gene) were sampled below Lock 4 (*n* = 411) and Lock 6 (*n* = 5) on 24–25 November 2020 and below Lock 1 (*n* = 352) on 9 December 2020 (Figure 44; Figure 45).

Table 19. Capture details for larval golden perch and silver perch collected from the LMR by larval tows. * indicates that age was estimated based on historical ages of golden perch with similar total lengths. TL = total length. n = total number of larvae sampled.

Capture location	Capture date	Species	*n*	TL (mm)	Age (days)	Spawn date
Lock 1	26/11/20	Golden perch	5	4.2–4.6	*3	23/11/20
Lock 1	2/12/20	Golden perch	11	4.0–5.2	*3	29/11/20
Lock 4	2/12/20	Silver perch	3	9.5–10.5	11–14	18–21/11/20
Lock 6	3/12/20	Golden perch	1	8.2	10	23/11/20
Lock 4	7/12/20	Golden perch	2	5.5–6.0	5	2/12/20
Lock 6	8/12/20	Golden perch	1	4.5	*3	5/12/20
Lock 6	8/12/20	Silver perch	1	10.5	15	23/11/20
Lock 1	16/12/20	Silver perch	2	11.5–14.5	16–24	22–30/11/20
Lock 6	16/12/20	Golden perch	1	12.5	22	24/11/20
Lock 6	16/12/20	Silver perch	85	8.0–14.0	11–25	21/11–5/12/20
Lock 4	17/12/20	Silver perch	8	12.5–13.5	16–19	28/11–1/12/20
Lock 4	21/12/20	Silver perch	15	9.0–11.5	14–19	2–7/12/20
Lock 6	22/12/20	Silver perch	1	12.5	20	2/12/20
Lock 6	6/1/21	Silver perch	22	9.0–11.8	13–16	21–24/12/20
Lock 1	7/1/21	Silver perch	1	14.5	31	7/12/20
Lock 6	18/1/21	Silver perch	4	9.5–11.5	16–17	1–2/01/20

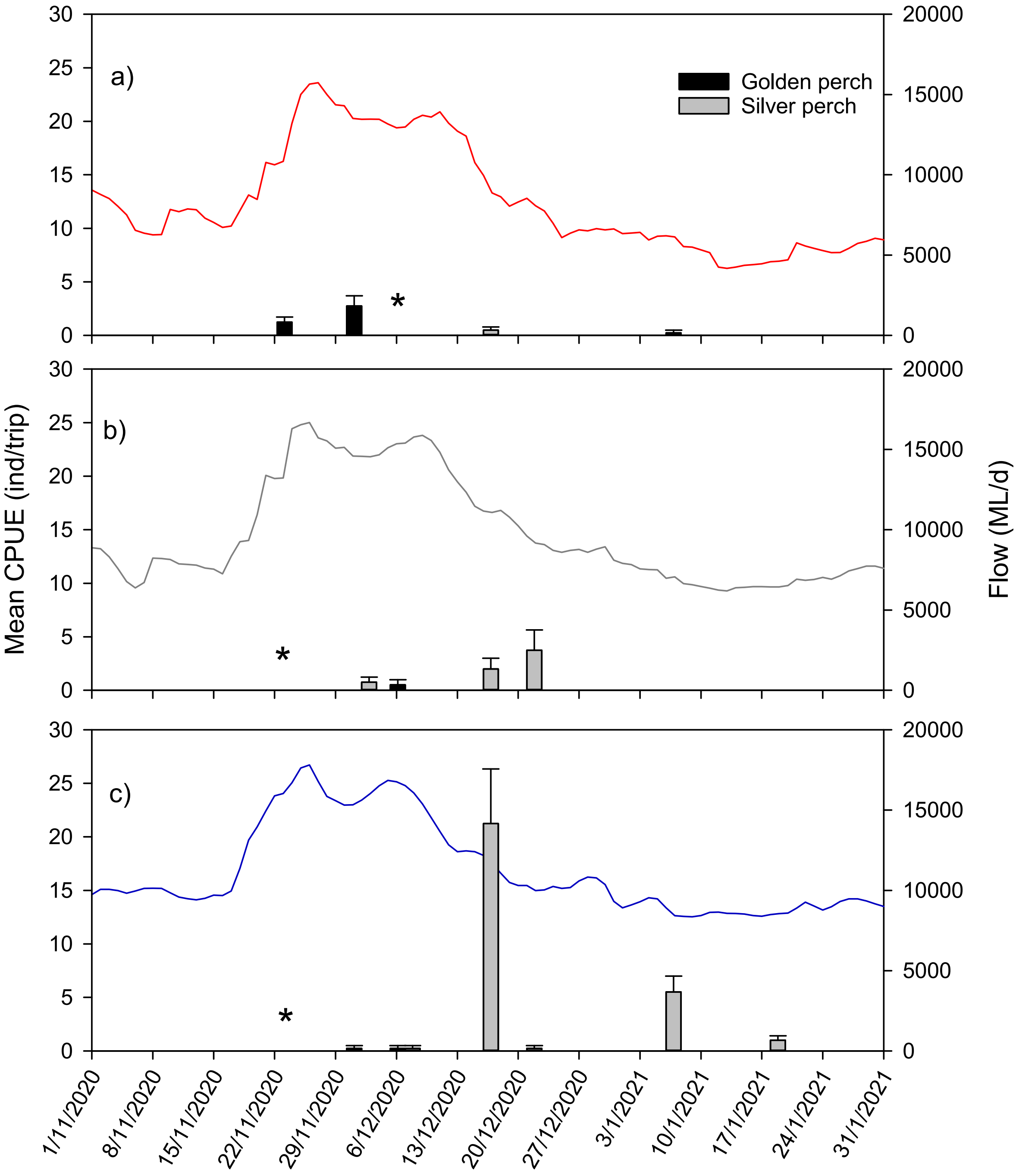

Figure 44. Mean catch-per-unit-effort (CPUE, individuals per trip) ± standard error of golden perch and silver perch larvae downstream of Locks 1 (a), 4 (b) and 6 (c) in the LMR from November 2020 to January 2021. Detections of golden perch eggs are indicated by asterisks. Daily flow at Lock 1 (red line), Lock 4 (grey line) and to South Australia (SA) (blue line) are shown.

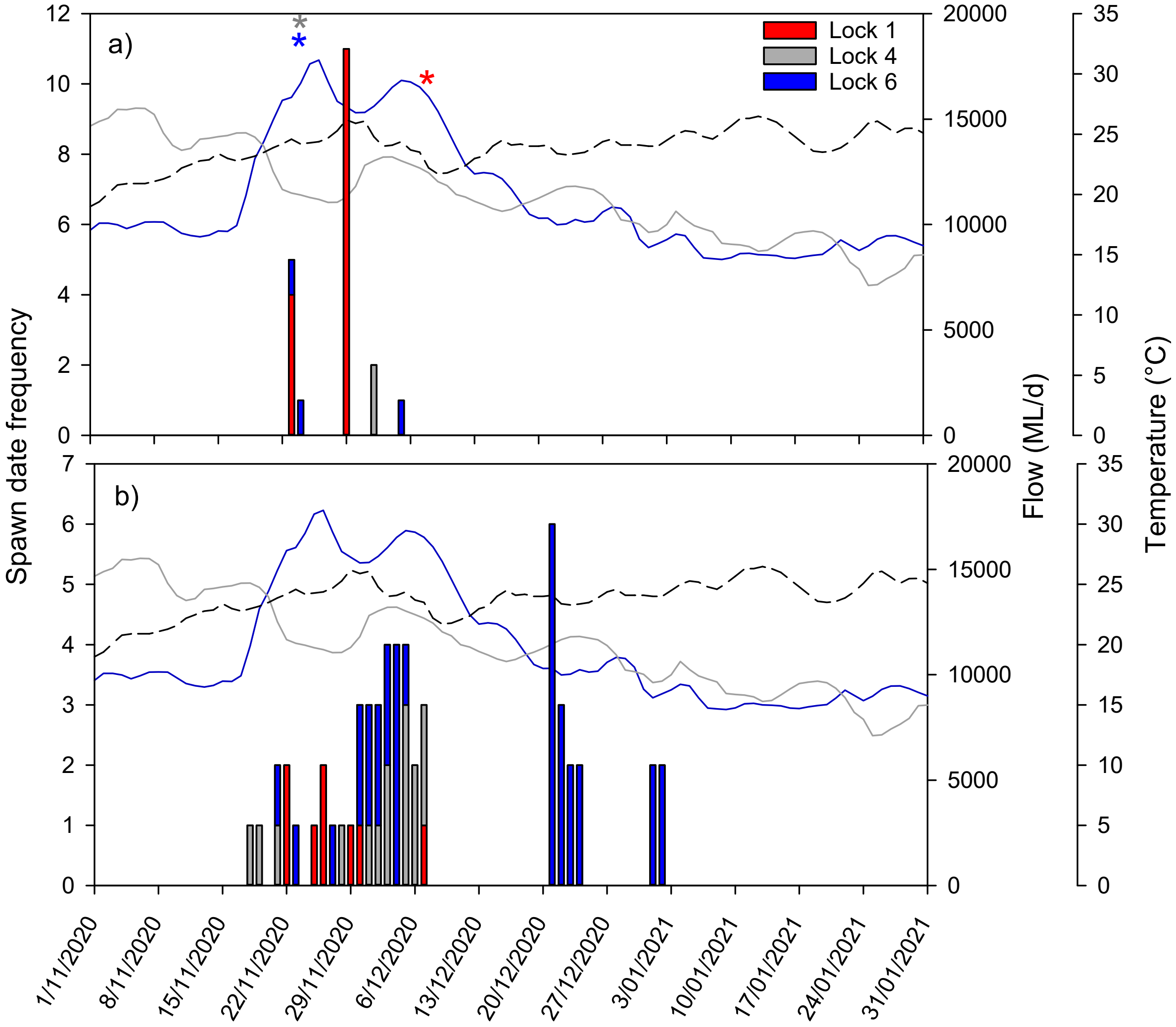

Figure 45. Back-calculated spawn dates for (a) golden perch larvae (*n* = 20) and (b) silver perch larvae (*n* = 56) captured below Lock 6 (blue bars), Lock 4 (grey bars) and Lock 1 (red bars) in the LMR during 2020-21, plotted against discharge (ML/d) at the South Australian border (solid blue line) and Euston (solid grey line), and water temperature (°C) (dotted black line). Golden perch egg collection dates are shown for Lock 6 (blue asterisk), Lock 4 (grey asterisk) and Lock 1 (red asterisk).

Water $^{87}Sr/^{86}Sr$, natal origin of larvae and YOY

From November 2020–January 2021, water $^{87}Sr/^{86}Sr$ was temporally stable at sites in the Murray River. Yet, along the Murray River, water $^{87}Sr/^{86}Sr$ gradually decreased in a downstream direction (Figure 46). Water $^{87}Sr/^{86}Sr$ was most variable at Lock 9 (0.7157–0.7164). Otoliths from a sample of 54 silver perch larvae and two golden perch larvae were analysed for $^{87}Sr/^{86}Sr$. The otoliths of the remaining larval golden perch were too small for analysis. The majority (85%) of silver perch and both golden perch larvae exhibited otolith core $^{87}Sr/^{86}Sr$ indicative of the mid-Murray River, above Lock 9 (i.e. 0.7158–0.7172) (Figure 46). The transects of $^{87}Sr/^{86}Sr$ from the otolith core to edges of these individuals indicated that these fish originated from the lower reaches of the mid-Murray River and subsequently moved (passively/actively) downstream to their capture location in the LMR (e.g. Figure 47a, b, d). The remaining silver perch larvae had otolith core $^{87}Sr/^{86}Sr$ (i.e. 0.7152–0.7160) indicative of the Murray River between Lock 6 and Lock 9. Transects of otolith $^{87}Sr/^{86}Sr$ indicated that these individuals were spawned in the Murray River, somewhere between Lock 6 and the Darling River junction, and remained in this region throughout their early life (e.g. Figure 47c).

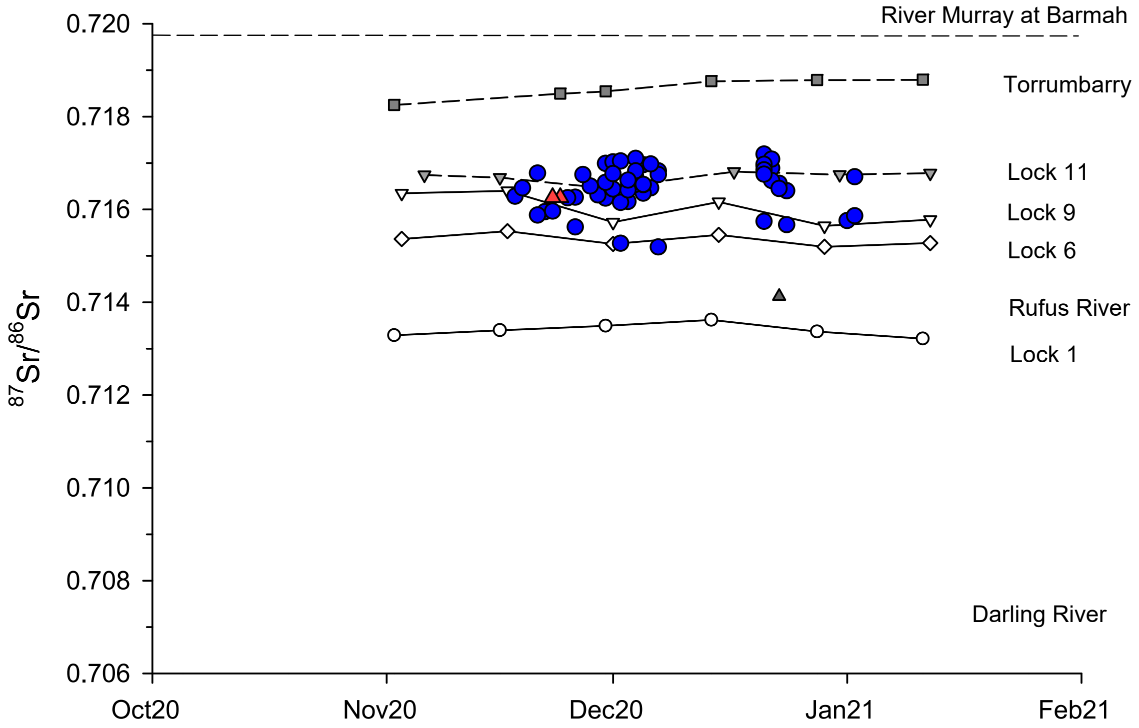

Figure 46. $^{87}Sr/^{86}Sr$ in water samples collected from early November 2020 to mid-January 2021 at sites in the southern MDB. $^{87}Sr/^{86}Sr$ in the Darling River (0.7076) and Murray River at Barmah (0.7192) are presented as dashed straight lines and based on long-term (2012–2019) averages (Ye *et al.* 2020a). Water from the Rufus River (upwards grey triangle) was only sampled on one occasion (3 replicates averaged) in late December 2020-21. Coloured symbols represent spawn date and otolith core $^{87}Sr/^{86}Sr$ of larval golden perch (red triangles) and silver perch (blue circles) collected in the LMR from November 2020 to January 2021.

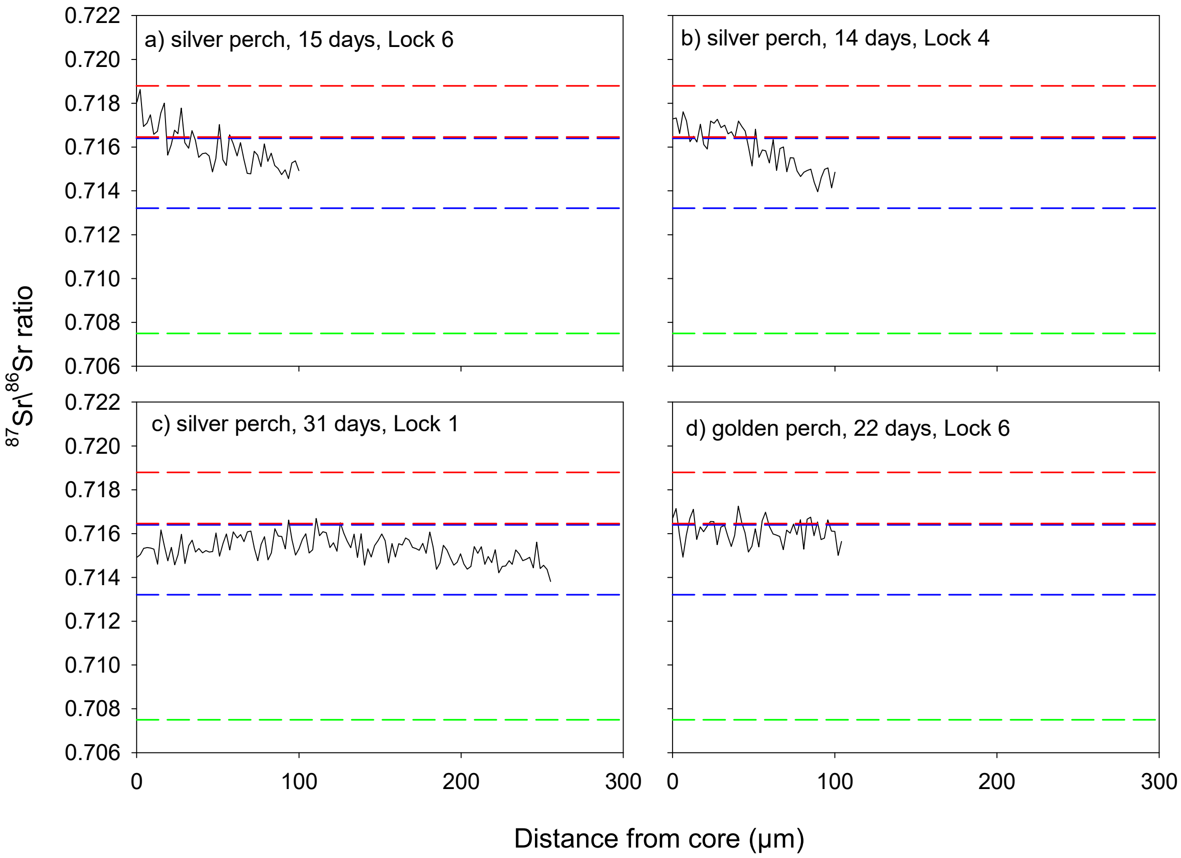

Figure 47. Individual life history profiles based on otolith Sr isotope transects (core to edge) for silver perch (a-c) and golden perch (d) larvae collected in the Lower Murray River. Age at capture and capture location are provide above each transect. Dashed lines denote minimum and maximum $^{87}Sr/^{86}Sr$ ratios in the Lower Murray River downstream of the Darling River junction (i.e. Lock 1 to Lock 9, blue) and mid-Murray River (i.e. Lock 11 to Torrumbarry, red) for the 2020-21 spring/summer period. Green dashed line indicates the temporally stable water $^{87}Sr/^{86}Sr$ of the lower Darling River (i.e. ~0.7075).

Juvenile/adult abundance

From 2015 to 2020, relative abundance of golden perch declined from 0.57 ± 0.08 ind./shot to 0.27 ± 0.03 ind./shot (Figure 48a). In 2021, abundance increased slightly to 0.38 ± 0.05 ind./shot. This was supported by PERMANOVA which demonstrated significant differences between years (*Pseudo-F*$_{6,64}$ =4.9726, *P*≤0.001). Pair-wise comparisons revealed significant differences between 2015 and 2019, 2015 and 2020, 2016 and 2019, and 2016 and 2020, but not between any other years (Table D12 in Appendix D).

Silver perch relative abundance was low (<0.02 ind./shot) and variable from 2015–2021 (Figure 48b). Low samples and variability in silver perch CPUE data meant no statistical comparisons were made.

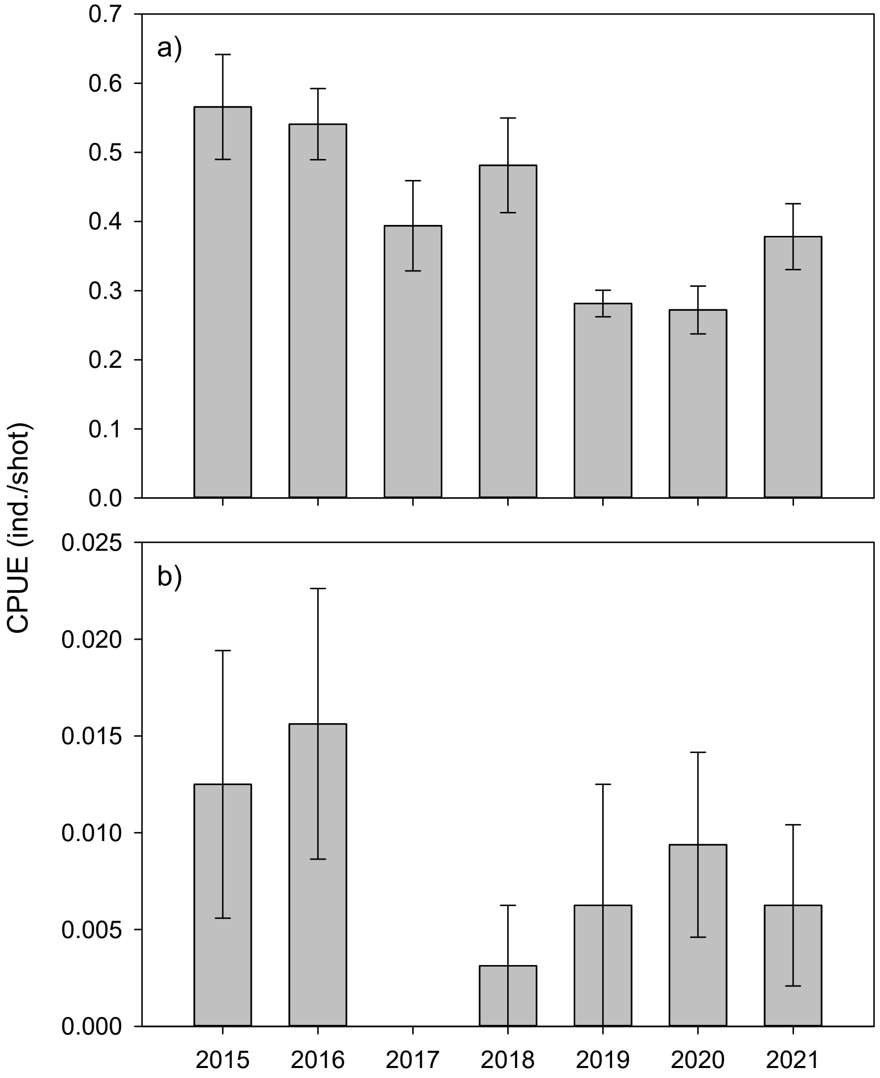

Figure 48. Mean catch-per-unit-effort (CPUE) ± standard error of (a) golden perch and (b) silver perch captured during Category 1 Fish Assemblage electrofishing (individuals per 90 second shot) in the gorge geomorphic zone (10 sites) of the LMR in autumn from 2015–2021. CPUE data from five sites are presented for 2017 as other sites were sampled during winter 2017.

Age structure

In 2021, YOY (age 0+) golden perch and silver perch were absent from electrofishing samples (Figure 49a), although two YOY golden perch were collected in the Pike and Katarapko anabranches through other projects. In 2021, golden perch collected in the LMR ranged in age from 2+ to 24+ years, with dominant cohorts of age 10+ and 9+ fish, born in 2010-11 and 2011-12, respectively, collectively comprising 49% of the sampled population (Figure 49a). In 2021, four silver perch were sampled in the LMR, ranging in age from 2+ to 4+ years (Figure 49b).

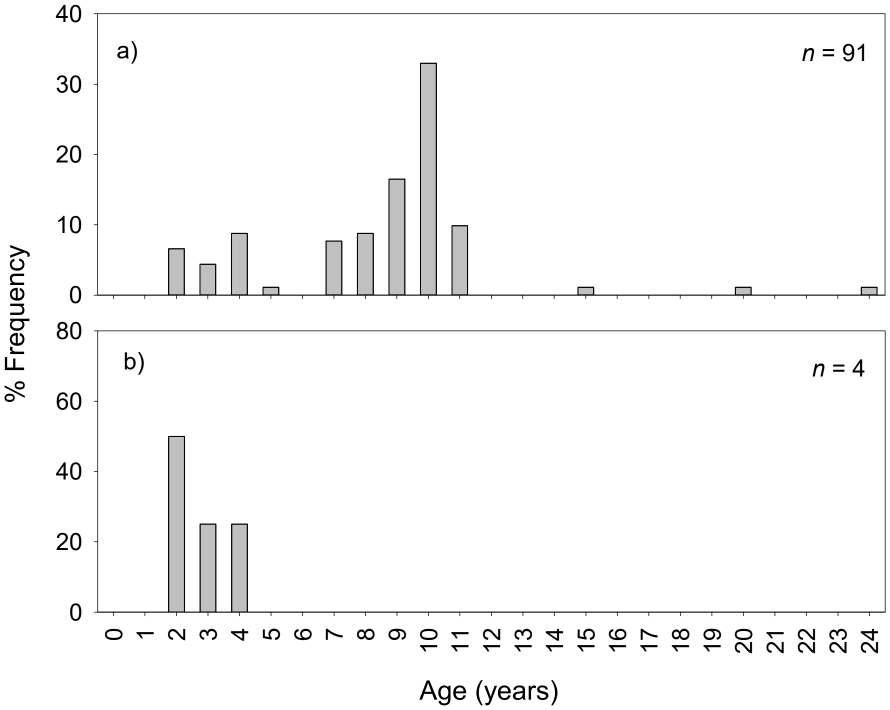

Figure 49. Age frequency distribution of (a) golden perch and (b) silver perch from the main channel of the LMR in 2021. Note the y-axis scales are different.

Natal origin and migration history of prominent cohorts

In 2021, natal origin and migration history were determined for 15 golden perch from age 0+ (*n* = 1), age 2+ (*n* = 5), 3+ (*n* = 4) and 4+ (*n* = 5) cohorts. In addition, natal origin and migration history were determined for seven silver perch from age 1+ (*n* = 1), 2+ (*n* = 5) and 3+ (*n* = 1) cohorts. Samples obtained through other Murray River projects (i.e. Pike and Katarapko Condition Monitoring and Fishway Assessments) were included in these analyses. All golden perch and silver perch from these cohorts exhibited otolith core ^{87}Sr/^{86}Sr comparable to water ^{87}Sr/^{86}Sr in the Murray River downstream of the Darling River junction (~0.7080–0.7140) and transects indicative of lifetime residence in this area (Figure 50).

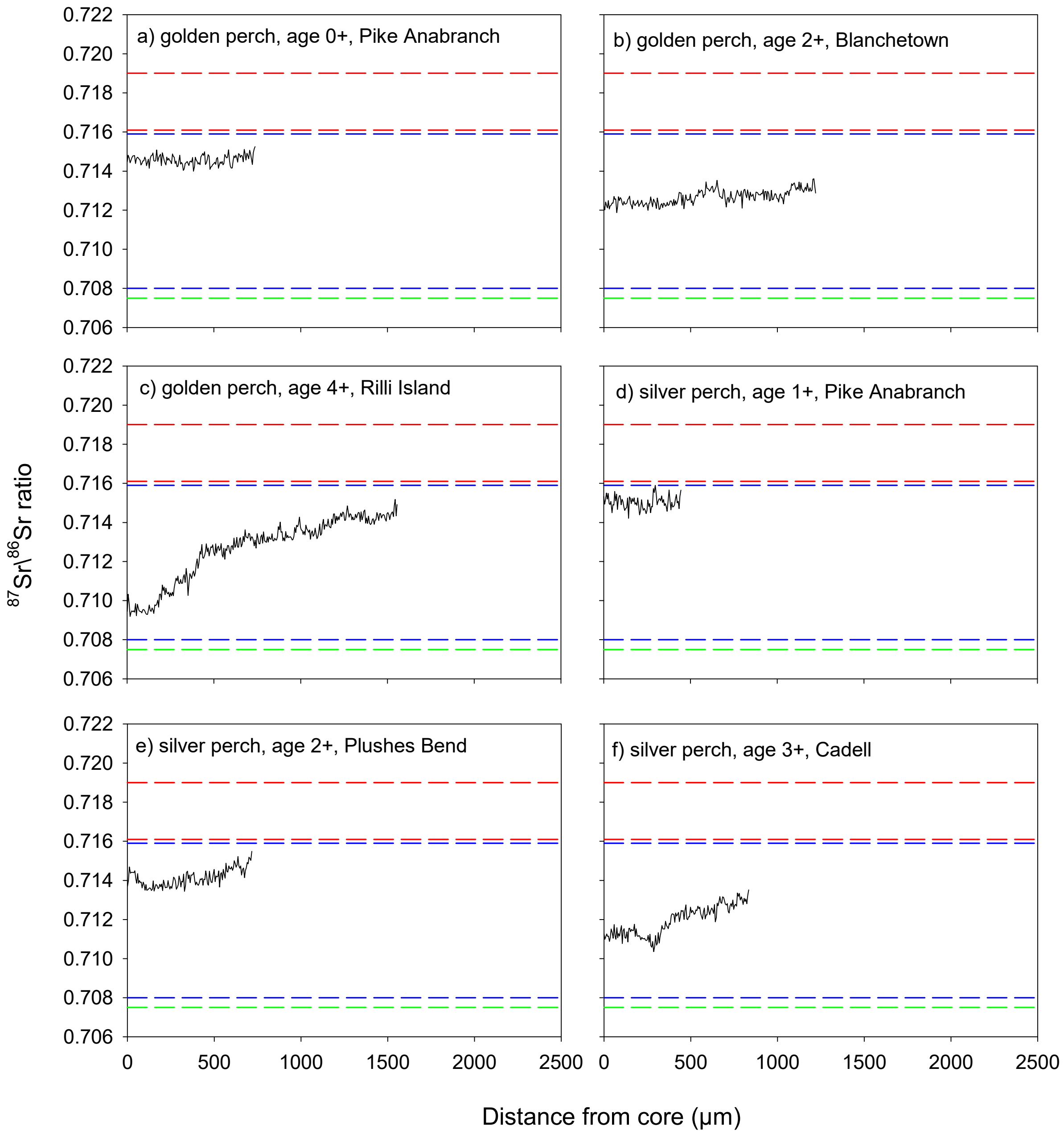

Figure 50. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from age 0+–4+ golden perch and silver perch captured in the Lower Murray River during 2021. Green dashed line indicates the temporally stable water $^{87}Sr/^{86}Sr$ of the lower Darling River (i.e. ~0.7075) and the blue dashed lines represent the range of water $^{87}Sr/^{86}Sr$ in the Lower Murray River downstream of the Darling River junction (i.e. ~0.7080–0.7160). Red dashed lines represent the range of water $^{87}Sr/^{86}Sr$ in the mid-Murray River (Lock 11–Torrumbarry, ~0.7160–0.7190).

Evaluation

The evaluation approach, including assessment criteria, is described in the evaluation section for Hydraulic Regime (Section 2.1).

Table 20. Flow-cued Spawning Fish Reproduction evaluation questions and answers. YOY = young-of-year.

CEWO evaluation questions	**Outcomes of flow delivery**		
	2014-15 – 2018-19	**2019-20***	**2020-21**
Did the flow regime (including environmental water) contribute to reproduction (spawning and recruitment) of golden perch and silver perch?	Coincident spawning, minor recruitment	Negligible recruitment	In 2020-21, spawning of silver perch and golden perch was coincident with environmental water delivery in the mid-Murray River and LMR, yet negligible recruitment (to YOY, age 0+) was evident in the LMR.
Did the flow regime (including environmental water) contribute to the resilience of golden perch and silver perch populations?	In the LMR in 2021, golden perch and silver perch ranging in age from 2+ to 5+ years can be associated with recruitment coincident with spring-summer flows that included environmental water. As such, the presence of these cohorts contributes to the resilience of populations of these species.		

*Contribution of the flow regime to spawning was not investigated in 2019-20.

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):
⬜ Unknown 🟥 Negative ⬜ None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

Over the CEWO LTIM and MER projects, we have aimed to identify potential associations between recruitment of golden perch and silver perch and flow, including environmental water delivery. We hypothesised that (1) increased flow (nominally >20,000 ML/d) in spring–summer would promote spawning and recruitment to YOY, and (2) multiple years of enhanced spring–summer flow would increase the resilience of golden perch and silver perch populations in the LMR by promoting a diverse age structure.

In 2020-21, golden perch and silver perch eggs and larvae were collected in the LMR. The presence of eggs, and estimated spawn dates of larvae, coincided with a flow pulse (peak flow at the South Australian border ~17,900 ML/d in late November 2020) in the Murray River but also extended through late December 2020 and early January 2021 in association with receding flow. Otolith chemistry analysis indicated that the majority of silver perch (85%) and the two golden perch larvae analysed exhibited otolith core ^{87}Sr/^{86}Sr indicative of an origin in the lower reaches of the mid- Murray River (i.e. upstream of the Darling River junction) and subsequently dispersal downstream to their capture location in the LMR. As such, it is appropriate to associate the spawning of golden perch and silver perch in the mid-Murray with flows in that region, rather than the further regulated and often distinct flows in the LMR, downstream of the South Australian border (Zampatti et al. 2018). The remaining silver perch larvae captured in the LMR, demonstrated otolith core $^{87}Sr/^{86}Sr$ indicative of a Murray River origin downstream of the Darling River junction, specifically in the reach between Lock 6 and Lock 10, thus spawning of silver perch also occurred in this region. The capture of golden perch eggs and young larvae (estimated ~3 days old) in the LMR also indicates spawning of this species in this area, but otoliths were too small for confirmation via otolith chemical analysis.

Our results again highlight that a variety of spatially distinct natal sources may contribute to golden perch and silver perch populations in the LMR (Zampatti et al. 2018; Zampatti et al. 2021). In 2021, however, early life stages (eggs and larvae) spawned in the Murray River between Lock 1 and Lock 11 did not contribute to measurable recruitment to YOY in the LMR. Indeed, from 2015–2021, golden perch recruitment in the LMR was generally poor, with no strong age classes added to the population. In 2015, the sampled population of golden perch ranged in age from 2+ to 18+ years, with dominant year classes from 2010-11, 2009-10, 2005–06, 2000-01 and 1996-97. These cohorts persisted in the population but by 2021 the contributions of older age cohorts (2005–06, 2000-01 and 1996-97) was low (~6% combined). In 2021, however, cohorts of 2+, 3+ and 4+ year old fish were apparent in the age structure, collectively comprising ~20% of the sample and indicating low-level recruitment in 2018–19, 2017–18 and 2016–17, respectively. Otolith chemistry analysis indicated these fish were born in the Murray River, downstream of the Darling River junction, and had spent their lives in this region. These age classes have become more prominent over the last few years, and it is possible that, when present in only low abundances, the detectability of these age classes may increase with age (Mahardja *et al.* 2017). Furthermore, decreasing strength of older cohorts, and lower general population abundance, may have contributed to the increasing prominence of these 2+ to 4+ cohorts. This reinforces the need for a long-term perspective (5–10 years) in detecting population responses of long-lived fishes to flow variability and management interventions.

In association with an ageing population, abundance of golden perch in the LMR steadily declined by almost half from 2015 to 2020. Over the same period, declines in golden perch abundance were also observed in other monitoring programs in the region, for example, in TLM C*ondition Monitoring* at the Chowilla icon site (Fredberg *et al*. 2019). Potential factors contributing to the decline are a lack of recruitment, mortality (fishing and natural) and upstream emigration of adults (Zampatti *et al*. 2018). Ultimately, a lack of younger cohorts, reduced abundance and diminished age structure diversity (e.g. population dominated by few cohorts) lead to a population that lacks resilience to environmental perturbations and other impacts (e.g. fishing).

Management implications

Over the past seven years, golden perch recruitment in the LMR was minimal. Evidence from the current investigation and allied studies suggest that greater flow (>20,000 ML/d) is likely required to significantly influence local golden perch spawning and recruitment in the LMR. Annual spring–summer in-channel flow pulses of this magnitude were a key feature of the hydrograph of the LMR prior to regulation (Mallen-Cooper and Zampatti 2018), and would have occurred in recent years under natural, unregulated conditions.

Nuanced understanding of the spawning, downstream dispersal and recruitment of silver perch in the LMR lags that for golden perch. Nevertheless, the collection of substantial numbers of silver perch larvae in 2020-21 and their natal origin suggests that the spring–summer hydrographs in the Murray River between Lock 1 and Lock 11 were suitable to promote spawning and downstream drift to the LMR. Furthermore, analysis of movement history of a small number of individuals 2+ to 4+ years of age suggested origin and residence in the LMR and the potential that some level of spawning and recruitment may occur in this region on a near annual basis.

Conclusion

Since 2013-14, there has been limited recruitment of golden perch in the LMR, leading to a population dominated by a few distinct and ageing cohorts. Yet, the appearance of small cohorts of age 2+ to 4+ fish appears to have mitigated decline in golden perch abundance. Silver perch abundance in the LMR remains low, although a low level of near-annual recruitment is evident. To improve the resilience of golden perch and silver perch populations in the LMR it is pertinent in the coming years to provide flows that may facilitate spawning and recruitment. Specifically, Commonwealth environmental water could contribute to spring/early summer in-channel flow >20,000 ML/d in the LMR to promote spawning, recruitment and subsequent downstream dispersal from upstream reaches (e.g. the lower Darling and mid-Murray rivers).

2.7 Murray Cod Recruitment

Background

Murray cod has great recreational and cultural significance in the MDB. Nonetheless, populations have declined as a result of human impacts including alteration of flow regimes, barriers to movement, overharvesting and habitat (hydraulic and physical) degradation (Lintermans and Phillips 2005), and the species is currently considered vulnerable under the Australian EPBC Act (1999). In the Murray River downstream of the Darling River junction, the fragmentation of the river by sequential weirs, alteration to hydraulics and loss of lotic habitats are considered primary threats to the persistence of Murray cod populations (Zampatti *et al.* 2014; Mallen-Cooper and Zampatti 2018). Indeed, under low flows, lotic environments that provide favourable juvenile and adult habitat, and support key life history processes (spawning and recruitment) are restricted to select anabranch systems (e.g. Chowilla) and lock and weir tailwaters. During elevated flows (e.g. >20,000 ML/d), however, lotic conditions are returned to considerable reaches of the LMR (Bice *et al.* 2017).

Murray cod spawn annually over a well-defined period from October–December, irrespective of flow (Davis 1977; Rowland 1998), but recruitment in the LMR main channel is positively associated with flow (Ye and Zampatti 2007; Zampatti *et al.* 2014). Indeed, from 2003–2010 during the Millennium Drought, discharge in the LMR was predominantly <10,000 ML/d, and Murray cod recruitment, measured as abundances of YOY in autumn, was limited in the predominantly lentic main channel. Subsequently, recruitment was observed in association with spawning that occurred in high flow years from 2010–2013 (Zampatti *et al.* 2014). In recent years (2015–2020), annual recruitment of Murray cod occurred in the LMR main channel, in concert with in-channel flow pulses (15,000–18,000 ML/d) and an overbank flow (>90,000 ML/d), but also years of low in-channel flows (10,000–12,000 ML/d) (Ye *et al.* 2020a; 2021). Furthermore, these recent cohorts have generally persisted in the population (Ye *et al.* 2020a; 2021).

The mechanisms that facilitate recruitment of Murray cod (to YOY) in the LMR likely relate to enhanced spawning habitat and survival of early life stages associated with improved riverine hydraulics and productivity. Survival is likely mediated by enhanced habitat availability, fish growth rates and condition, and ultimately determines recruitment and population abundance. This indicator will explore these mechanisms, together with a related research project (see SARDI *et al.* 2019), by assessing aspects of Murray cod recruitment (e.g. abundance, growth, condition) in association with flow. Understanding the magnitude of recruitment, and causal links between recruitment and flow, is critical for informing future environmental flow management and will help evaluate ecological outcomes of Commonwealth environmental water.

Hypotheses

- Elevated spring–summer flow, either in-channel or overbank, and associated increase in lotic habitat, will enhance recruitment (to YOY) of Murray cod;
- Elevated spring–summer flow, either in-channel or overbank, and associated increase in lotic habitat, will be associated with enhanced growth rates and morphometric condition of Murray cod; and

- Multiple years of enhanced spring–summer flow will result in broad size/age distributions of Murray cod and increased population resilience in the LMR.

Methods

To evaluate the contribution of flow, including Commonwealth environmental water, to the morphometric condition, growth and recruitment of Murray cod in the LMR during 2020-21, we used: (1) length–weight relationships and associated metrics to assess body condition; (2) otolith microstructure analysis (including daily otolith growth increments) to determine the time of spawning, along with seasonal and daily growth rates; and (3) electrofishing to quantify the abundance of YOY to assess recruitment, and to establish a length frequency distribution of the Murray cod population in the LMR to determine population structure. Reference data collected in numerous past monitoring projects and presented in Ye *et al.* (2021) were used and presented in this indicator section.

Sampling

Murray cod were collected from the main channel of the LMR at several stages during early ontogeny, from larvae through to YOY (>120 d of age) (Table 21). Sampling for larvae (<30 mm TL) in November 2021 comprised a combination of passive (light traps) and active (bongo net tows) methods. Sampling effort varied across sites, but included setting 20–30 quatrefoil light traps (225 x 225 x 255 mm, 5 mm mesh) and 2–4 drift nets (1.5 m long x 0.5 m diameter, 500 μm mesh) overnight for 2–3 nights (set time of 14–18 hours). Larval tows were performed opportunistically with a paired bongo net (0.5 x 0.5 m x 3 m long, 500 μm mesh) (Ye *et al.* 2018). Larvae were preserved (70–95% ethanol) in the field and returned to the laboratory for processing.

Electrofishing was used specifically to collect juveniles (~30–160 mm) and sample the broader population (Table 21). All sampling was performed using a 7.5 kW Smith Root (Model GPP 7.5) boat electrofishing unit during daylight hours. Sampling in January and February 2021 was targeted towards preferred habitat of early juveniles (e.g. root complexes of snags and rock crevices). Two complementary types of sampling were used to assess YOY abundance and population structure: 1) autumn (March/April) non-targeted Fish (Channel) electrofishing (see Section 2.8 Fish Assemblage) and 2) autumn (May) habitat-specific (targeted) electrofishing. Electrofishing during May targeted a wider range of Murray cod habitats, including snags in flowing habitat extending beyond the riverbank (preferred by sub-adults and adults). At each site, electrofishing 'on-time' effort was variable, and depended on the available habitat. All individuals were measured for TL (nearest mm) and weight (to 0.5 kg), and a sub-sample of juvenile (<160 mm) fish were retained for morphometric and otolith analyses.

Table 21. Sampling trips targeting Murray cod in the main channel of the LMR during 2020-21.

Date range	Methods	Indicator
8–11/11/2020	Light traps, larval tows	Growth, condition
5–8/1/2021	Light traps, electrofishing	Growth, condition
22–26/2/2021	Electrofishing	Growth, condition
29/3–8/4/2021*	Electrofishing	Condition, YOY abundance, population structure
3–6/5/2021	Electrofishing	Condition, YOY abundance, population structure

* Fish (Channel) sampling in the Gorge zone (see Section 2.8 Fish Assemblage for details)

Sampling for larvae (November) and early juveniles (January and February) was conducted in the tailwaters (<15 km downstream) of Lock 3 (Gorge zone) and Lock 4 (Floodplain zone) (Figure 1). Sampling to assess YOY abundance and population structure in May occurred at sites in the tailwaters of Locks 3 (Gorge zone), 4 and 5 (Floodplain zone), and was supplemented by non-targeted sampling in March/April at ten sites between Locks 1 and 3 in the Gorge zone (Fish (Channel) sampling). In addition to the sampling described above, Murray cod larvae and juveniles were opportunistically collected through other MER field sampling (e.g. Section 2.6 Flow-cued Spawning Fish Reproduction) and sampling from other projects (e.g. Chowilla, Katarapko and Pike/Mundic condition monitoring). Samples from the main river channel were included in all analyses, while samples from anabranches were used solely to supplement age analyses to determine the range of spawn dates for Murray cod in the LMR.

Otolith preparation and increment counts and measurements

Larval/juvenile Murray cod were measured for TL to the nearest millimetre, weighed to 0.001 of a gram, and sagittal otoliths were removed. Sagittae were embedded in Crystalbond™, then ground and polished from the anterior margin towards the core. For larvae and early juveniles (from January), we used 9 and 3 µm lapping film, while for otoliths of larger juveniles from February, used 240-grit sandpaper and 15, 9 and 3 µm lapping film sequentially. The ground surface was adhered to the centre of a microscope slide with crystal bond™ and then further ground and polished from the posterior side, to produce sections of approximately 50 µm thickness.

Prominent hatch marks were evident on most sectioned otoliths, providing a reliable reference point to begin increment counts. As such, daily increment counts were made from the hatch mark along the maximum growth axis towards the ventral apex. Two readers examined each otolith on separate occasions and each reader performed two counts of the increments. Counts from each reader were compared and if they differed by more than 10%, the otolith was rejected; but if count variation was within 10%, the mean of all counts was accepted as the daily increment number and estimate of age. Hatch dates were determined by subtracting estimated age from capture dates. Prior to increment counts, otolith readers were calibrated by reading a reference collection of otoliths from known-age Murray cod larvae and achieving 90% agreement. Otolith sections were examined using a compound microscope (x 200) fitted with a digital camera and Olympus Stream image analysis software (version 2.3). YOY Murray cod collected in late April and May were omitted from age analyses due to unreliability in daily increment estimates.

<u>Data analyses</u>

Morphometric condition

The length–weight relationships for juvenile (0+ year old, <160 mm) Murray cod were described using linear regression, following the methods outlined in Tonkin *et al.* (2008). Linear and non-linear relationships were examined with non-linearity modelled by fitting polynomial terms of increasing order (up to 4) to length. The relative support for each of the models was assessed using Akaike's Information Criterion, corrected for small sample size (AICc) (Burnham and Anderson 2002). AICc values were rescaled as differences between the model and the model with the lowest AICc value, with the likelihood of the model calculated (see Tonkin *et al.* 2008 for equation). Morphometric condition was estimated by calculating the relative condition factor (K*rel*) of an individual fish, using the estimated weight calculated from the selected model equation: $Krel = Wt_{actual}/Wt_{estimated}$. To determine if the condition of Murray cod differed among years (i.e. 2015–2021) of different flow regimes, differences in K*rel* of individuals sampled between March and August were assessed using a single-factor univariate PERMANOVA (permutational ANOVA and MANOVA) in the software package PRIMER v. 6.1.12 and PERMANOVA+ (Anderson *et al.* 2008). A significance value of $\alpha = 0.05$ was adopted for all statistical comparisons, acknowledging an increased likelihood of type 1 errors for multiple comparisons. Comparisons of morphometric condition, seasonal growth rate and CPUE between weir pools were not explored as they were not of interest in this study.

Growth rate

Mean seasonal growth was described by fitting linear and nonlinear models to length-at-age data. Three models were considered for fish growth rate: linear, Gompertz and Von Bertalanffy. Model selection procedures using AICc, as described above, were used to select the model with most support. Mean seasonal growth rate was calculated as the function of TL and age (days) at capture. To determine if seasonal growth rates of Murray cod differed between years (2019-20 and 2020-21), mean seasonal growth rates were compared using a single-factor univariate PERMANOVA.

Recruitment

To compare recruitment and abundance of Murray cod across years (2013–2021), temporal variability in the relative abundance of YOY (i.e. <160 mm TL) and all Murray cod was assessed using two datasets: 1) March/April non-targeted Category 1 electrofishing CPUE data (see Section 2.8 Fish Assemblage); and 2) May targeted electrofishing CPUE data. For the first dataset, sites (*n* = 10) were grouped and represented as two reaches, namely downstream of Lock 3 and downstream of Lock 2. For the second data set, numerous 'shots' (*n* = 1–5) were undertaken across broad sites downstream of Locks 3, 4 and 5, and CPUE data presented as per the first dataset. Differences in relative abundance of YOY (individuals per minute of electrofishing on-time effort) among years at each reach were analysed using univariate single-factor PERMANOVA. These analyses were performed on untransformed, standardised relative abundance data. For statistical analyses, in the first dataset, YOY CPUE data from downstream of Lock 2 and Lock 3 were amalgamated to represent the gorge geomorphic zone because there were no significant effects of reach or the interaction of reach and year. For the second dataset, data from downstream of Lock 4 were used to represent the floodplain geomorphic zone because sampling downstream of Locks 5 and 3 had low replication in multiple years ($n < 3$) and/or were not sampled across all years.

Results

Catch summary

During sampling in the main channel of the LMR and associated anabranches (e.g. Chowilla) from November 2020 to May 2021, a total of 37 age 0+ Murray cod were collected (Table 22). The majority of Murray cod (n = 35) suspected to be age 0+ were retained for morphometric, age and growth assessments.

Table 22. Catch summary of age 0+ Murray cod in the main channel of the Lower Murray River and Chowilla anabranch from sampling of the MER project (Table 21) and other projects (e.g. Chowilla and Katarapko monitoring) in 2020-21. Data are not comparable between sites due to varying sampling effort. NS = not sampled.

Date range	**Main channel**			**Chowilla anabranch**		
	n	**TL (mm)**	**Age (days)**	***n***	**TL**	**Age (days)**
3–25/11/2020	4	11–13	12–13	14	10–13	11–17
5–8/1/2021	0			NS		
22/2–17/3/2021	2	67–68	96–100	3	62–87	96–112
29/3–21/4/2021	7	83–109	125–129	NS		
3–20/5/2021	2	95–121		4	103–135	
Total	**15**			**21**		

Length and hatch dates

Murray cod larvae and age 0+ juveniles sampled from the LMR main channel and anabranches ranged in TL from 10–135 mm. From November 2020 to May 2021, length frequency distributions indicated progression in length between sampling events (Figure 51). Estimated ages during November, February/early March and late March/April ranged from 10–17, 96–112 and 125–129 days, respectively (Table 22), corresponding to hatch dates from 18 October to 1 December 2020. Assuming an incubation period of 7 days (Ingram *et al.* 2012), spawning is estimated to have occurred from 11 October to 24 November 2020, with spawn date frequency distributions influenced by the timing of larval sampling (Figure 52a).

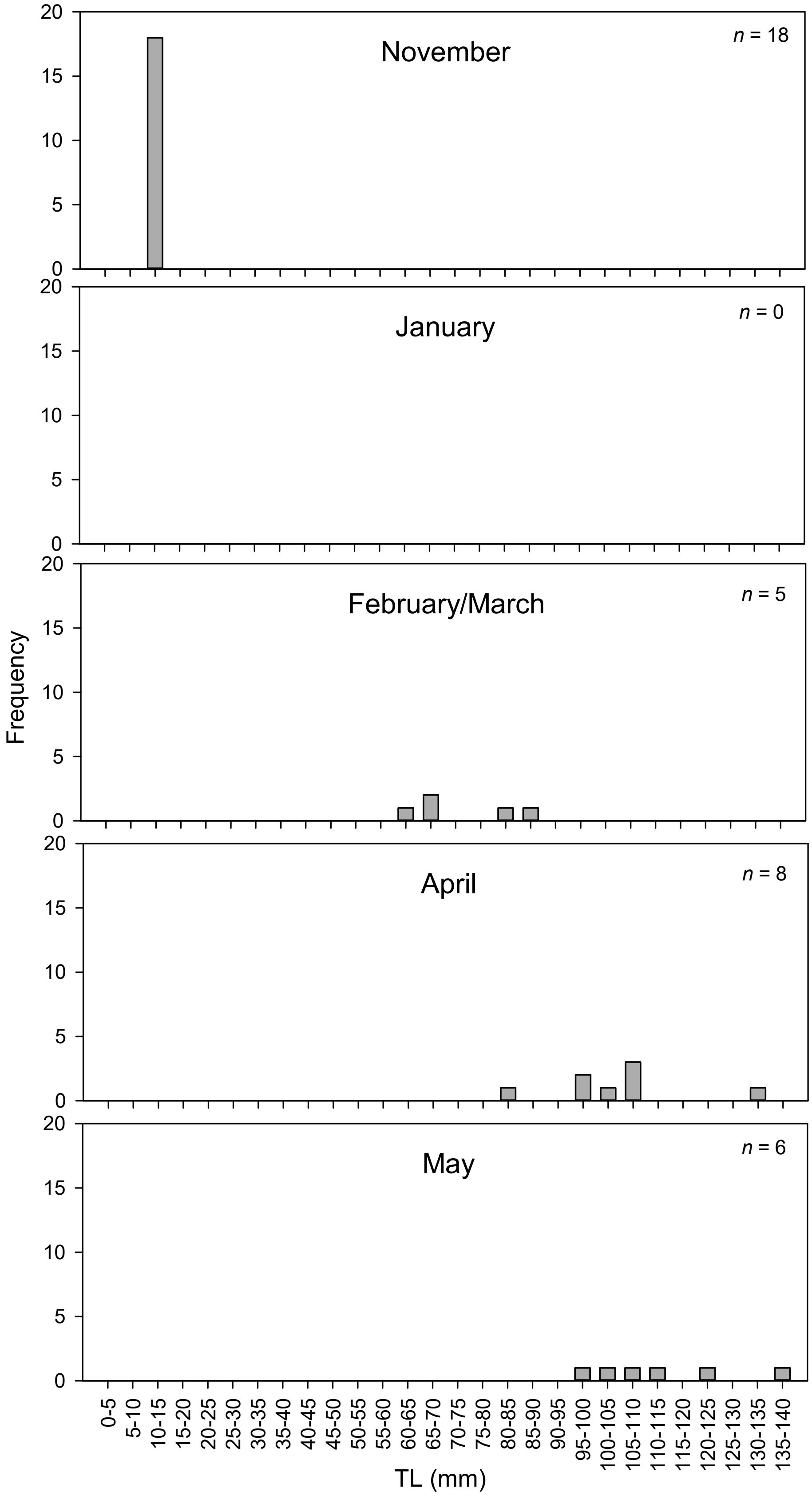

Figure 51. Length frequency distributions of larval/juvenile Murray cod collected in the Lower Murray River main channel and anabranches (sites pooled) from November 2020 to May 2021.

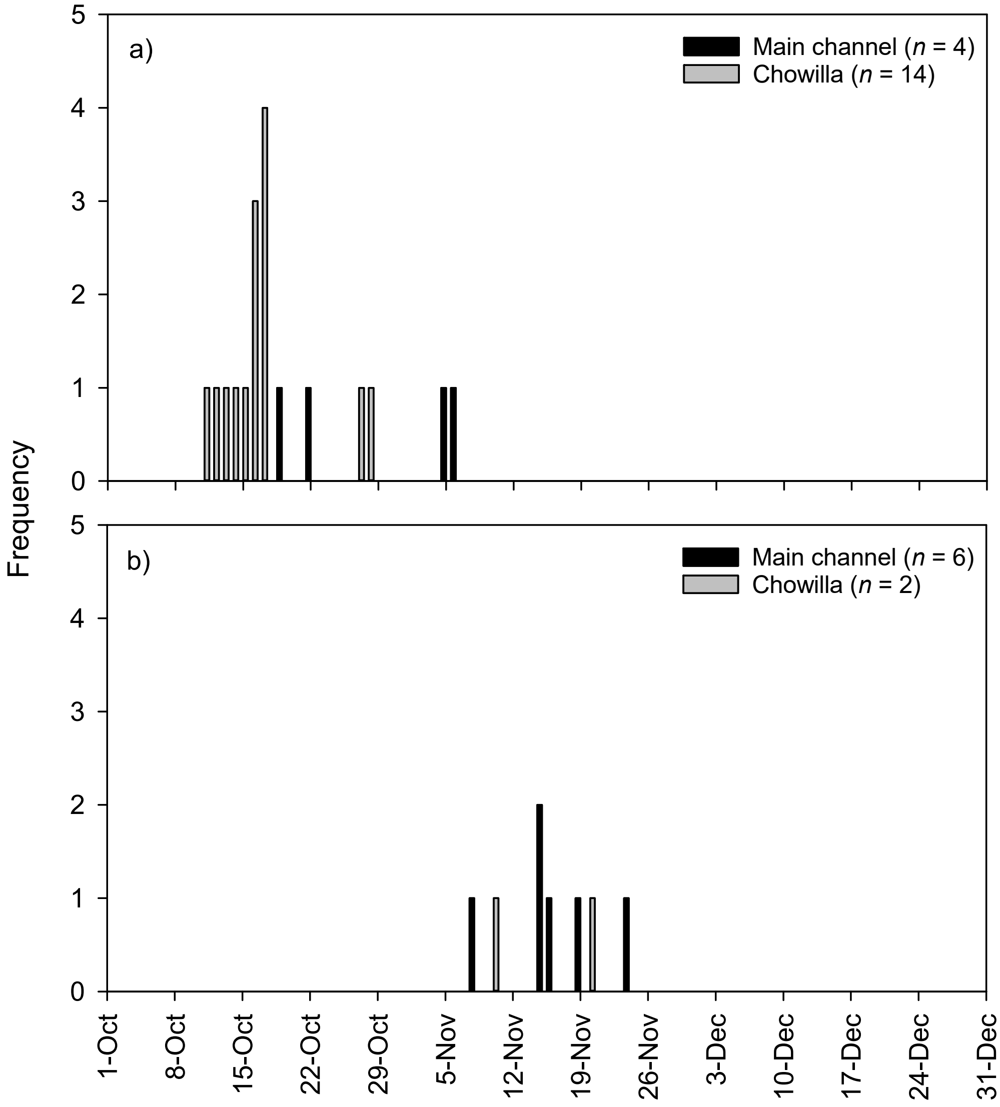

Figure 52. Estimated spawn date frequency distributions of (a) larval Murray cod captured in November 2020 and (b) juvenile Murray cod captured from February to April 2021, in Lower Murray River main channel and anabranches.

Morphometrics and seasonal growth

Using the model selection procedure, a cubic polynomial of TL was applied to model length–weight relationships for larval/juvenile (<160 mm) Murray cod for all years (reference data, 2004–2021), and for 2019-20 and 2020-21 (Figure 53). Comparison of the 2020-21 length–weight relationship to the reference curve indicates that the condition of Murray cod during 2020-21 was similar to the 'average' condition. To further investigate this, differences in the relative condition index (K*rel*) of YOY (sampled autumn/winter) Murray cod among sampling years (2015 to 2021) (Figure 54) were assessed using PERMANOVA. There was a significant effect of sampling year on K*rel* (PERMANOVA, $Pseudo\text{-}F_{6,120}$ = 3.5548, p = 0.0033), with pairwise comparisons suggesting the K*rel* of YOY was significantly greater in 2017 than in 2015, 2016, 2018 and 2020, and significantly greater in 2019 compared to 2018 and 2020 (Figure 54). The relative condition of YOY Murray cod in 2021 (mean ± S.E. K*rel* = 1.07 ± 0.02) was similar to 2019, and significantly greater than 2016 and 2018.

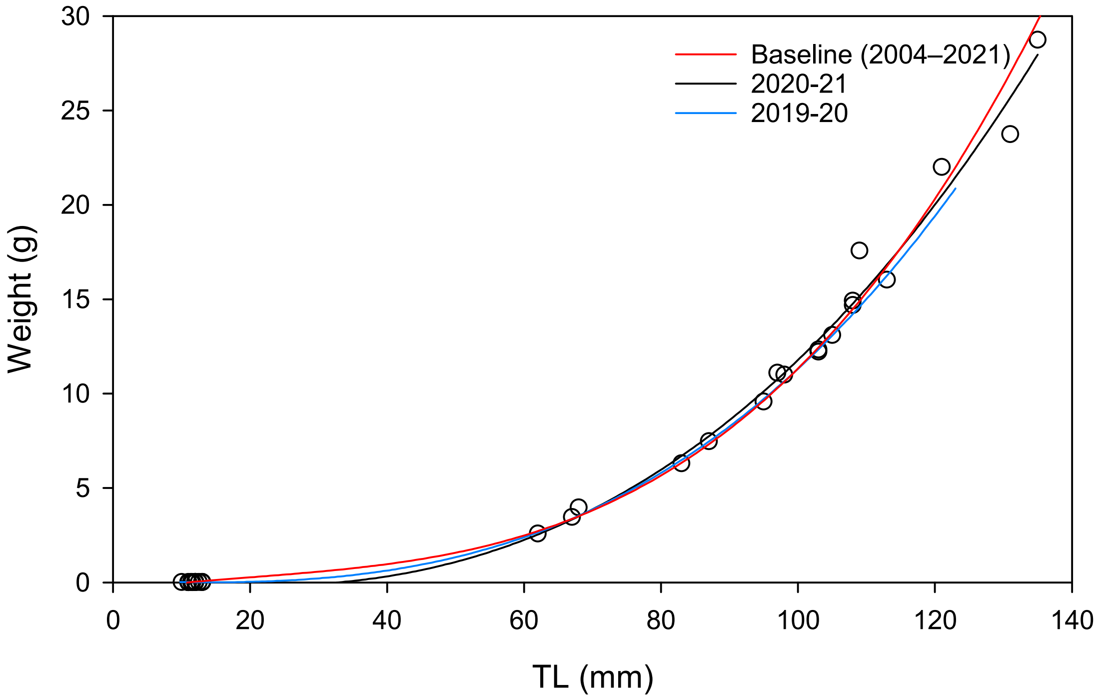

Figure 53. The relationship between weight and total length (TL) of YOY (<160 mm) Murray cod in the Lower Murray River predicted by a cubic linear regression model ($y=-0.5326-0.1716x-0.0010x^2-0.0000066155x^3$) for 2020-21 (black line and circles, *n* = 154), 2019-20 (blue line, Ye *et al.* 2021) and all years (2004–2021, 'stocked' fish removed, red line). See Table D13 in Appendix D for statistics.

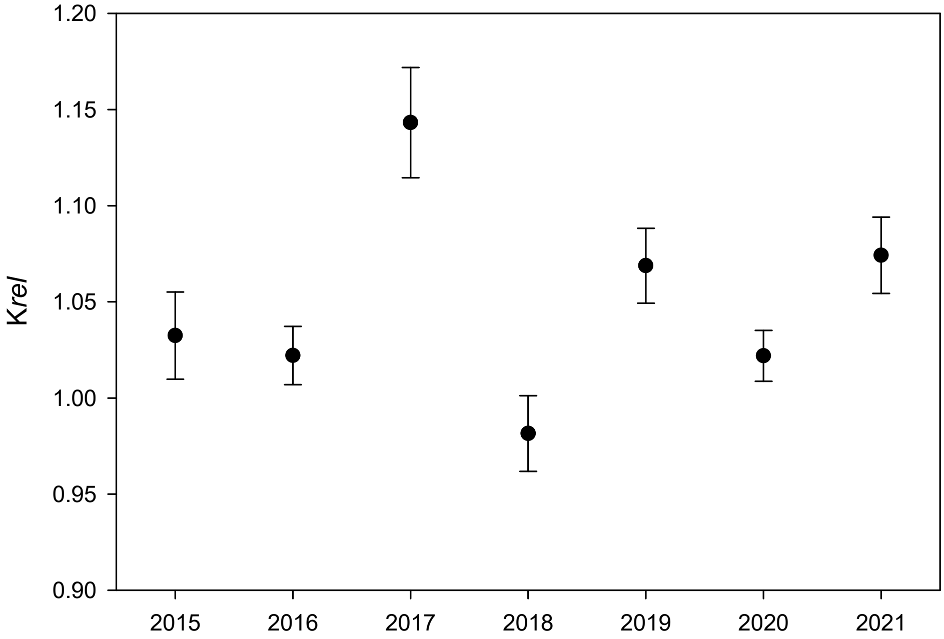

Figure 54. Mean relative condition index (K*rel*) ± S.E. of YOY (<160 mm) Murray cod in the Lower Murray River (excludes Chowilla anabranch) during autumn/winter 2015 (*n* = 11), 2016 (*n* = 14), 2017 (*n* = 6), 2018 (*n* = 11), 2019 (*n* = 25), 2020 (*n* = 51) and 2021 (*n* = 9). See Table D14 in Appendix D for statistics.

Model selection indicated that the Gompertz model was the best fit for the 2017–2021 Murray cod age–length data (Figure 55). Mean seasonal growth rate in 2020-21 (0.846 mm/d ± 0.038 S.E.) was slightly higher than in 2019-20 (0.781 ± 0.018), but differences were not significant (PERMANOVA, $Pseudo\text{-}F_{1,\,80} = 1.7251$, $p = 0.1886$). The lack of fish 20–90 mm TL in 2020-21 may have influenced this result.

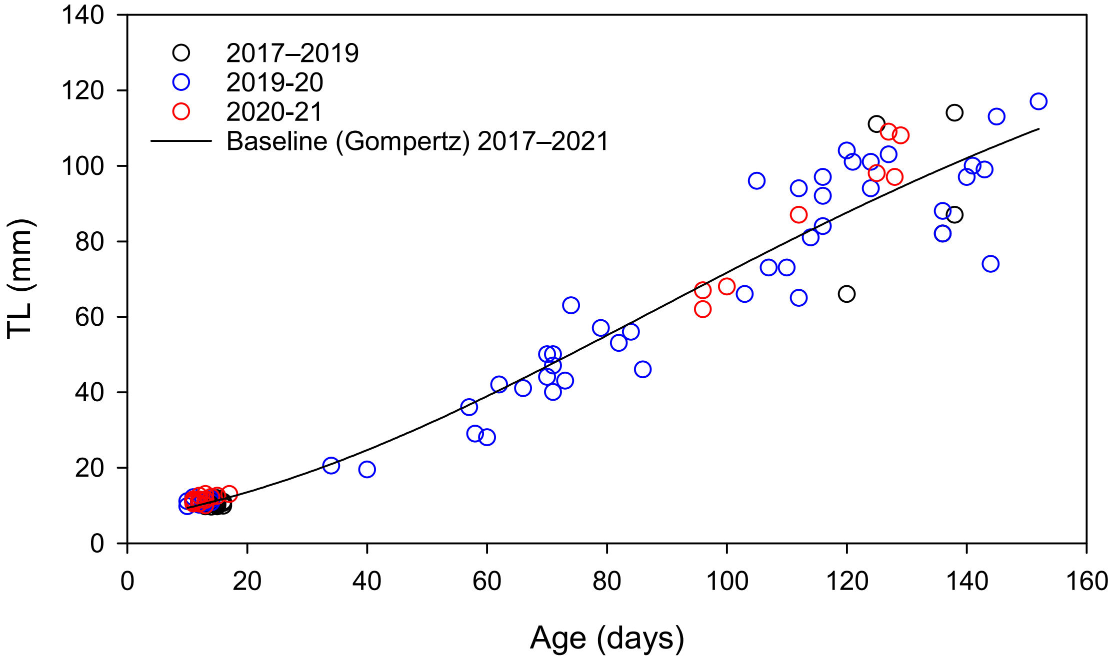

Figure 55. The relationship between total length (TL) and estimated age of YOY (<160 mm) Murray cod in the Lower Murray River from 2017–2021, predicted by the Gompertz model. See Table D15 in Appendix D for statistics.

Recruitment and population structure

Mean relative abundances (non-targeted CPUE) of Murray cod in the weir pools below Locks 2 and 3 varied among years. Abundance remained relatively consistent from 2015 to 2019 (<0.05 fish/min). In 2020, there was a four-fold increase in mean abundance below Lock 3, driven by an increase in the abundance of YOY fish (Figure 56 and Figure 57). From 2020 to 2021, mean abundance below Locks 2 and 3 declined. Nonetheless, abundance in both reaches in 2021 remained slightly higher than the pre-2020 period. The proportional abundance of YOY from these sites throughout the seven-year period was relatively high (>50%), with the exception of 2021 (20%) (Figure 56 and Figure 57). Relative abundances of YOY between Locks 1 and 3 varied among years (PERMANOVA, $Pseudo\text{-}F_{6,\,69} = 2.359$, $p = 0.041$). PERMANOVA pair-wise comparisons revealed significantly higher recruitment (YOY abundance) in 2015, 2019 and 2020 compared to 2017, but not for any other comparisons between years (Figure 56; Table D16, Appendix D).

Relative abundances (targeted CPUE) of Murray cod in the tailwaters of Locks 4 and 5 varied among years and showed a general increasing trend in abundance from 2013 to 2016, before a decline in abundance in 2017. This was followed by an increase in relative abundances in 2020. Consistent with the non-targeted CPUE data from below Lock 3, the 2020 increase in relative abundance below Lock 4 was driven by an increase in abundance of YOY (Figure 56 and Figure 57). In 2021, high relative abundances below Locks 4 and 5 were maintained and not driven by an increase in the abundance of YOY.

Relative abundances of YOY downstream of Lock 4 varied among years (PERMANOVA, *Pseudo-F*$_{7, 29}$ = 7.318, $p < 0.001$). PERMANOVA pair-wise comparisons revealed significantly higher recruitment (YOY abundance) in 2020 compared to years 2013–2015, 2017 and 2021, but not for any other comparisons between years (Figure 56; Table D17, Appendix D).

Excluding years with low sample sizes ($n < 10$; 2013 and 2014), Murray cod generally exhibited broad length frequency distributions, comprised of juveniles (including YOY), sub-adults (300–600 mm) and adults (>600 mm) (Figure 57). YOY fish (i.e. <160 mm) dominated the sampled population in 2019 (69 %) and 2020 (67 %). In 2021, YOY fish contributed towards the lowest proportion (7 %), across all years, of the sampled population, and age 1+ individuals (~150–300 mm) dominated the population (49%). From 2015, new (YOY) cohorts can be seen to persist and progress through the population each year to adult size (>600 mm).

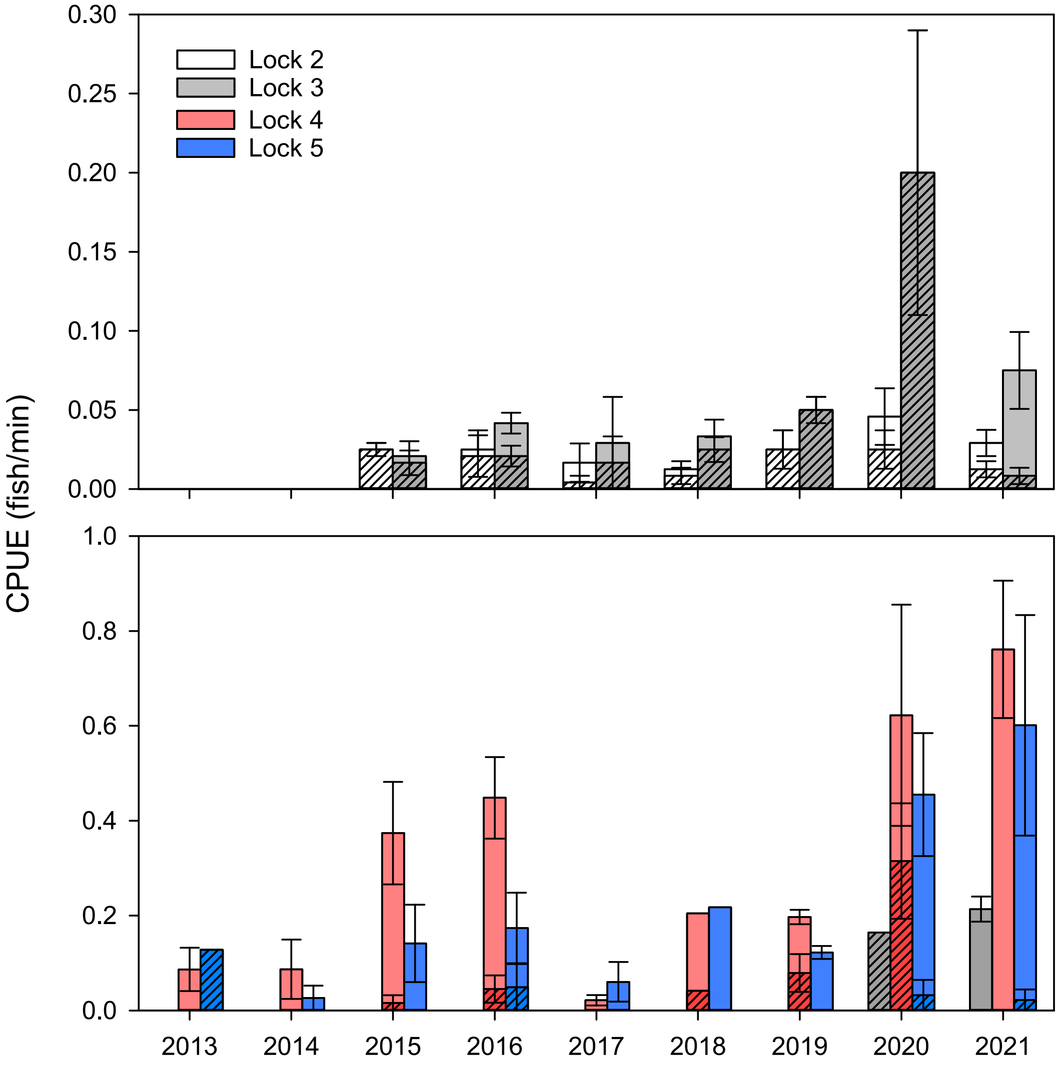

Figure 56. Mean non-targeted (top) and targeted (bottom) electrofishing catch-per-unit-effort (CPUE) ± S.E. of Murray cod in the weir pools downstream of Locks 2, 3, 4 and 5 of the Lower Murray River. Dashed bars represent the proportion of YOY to the total CPUE. Note the different scales on the CPUE-axis for the different methods of electrofishing. See Tables D16 and D17 in Appendix D for statistics.

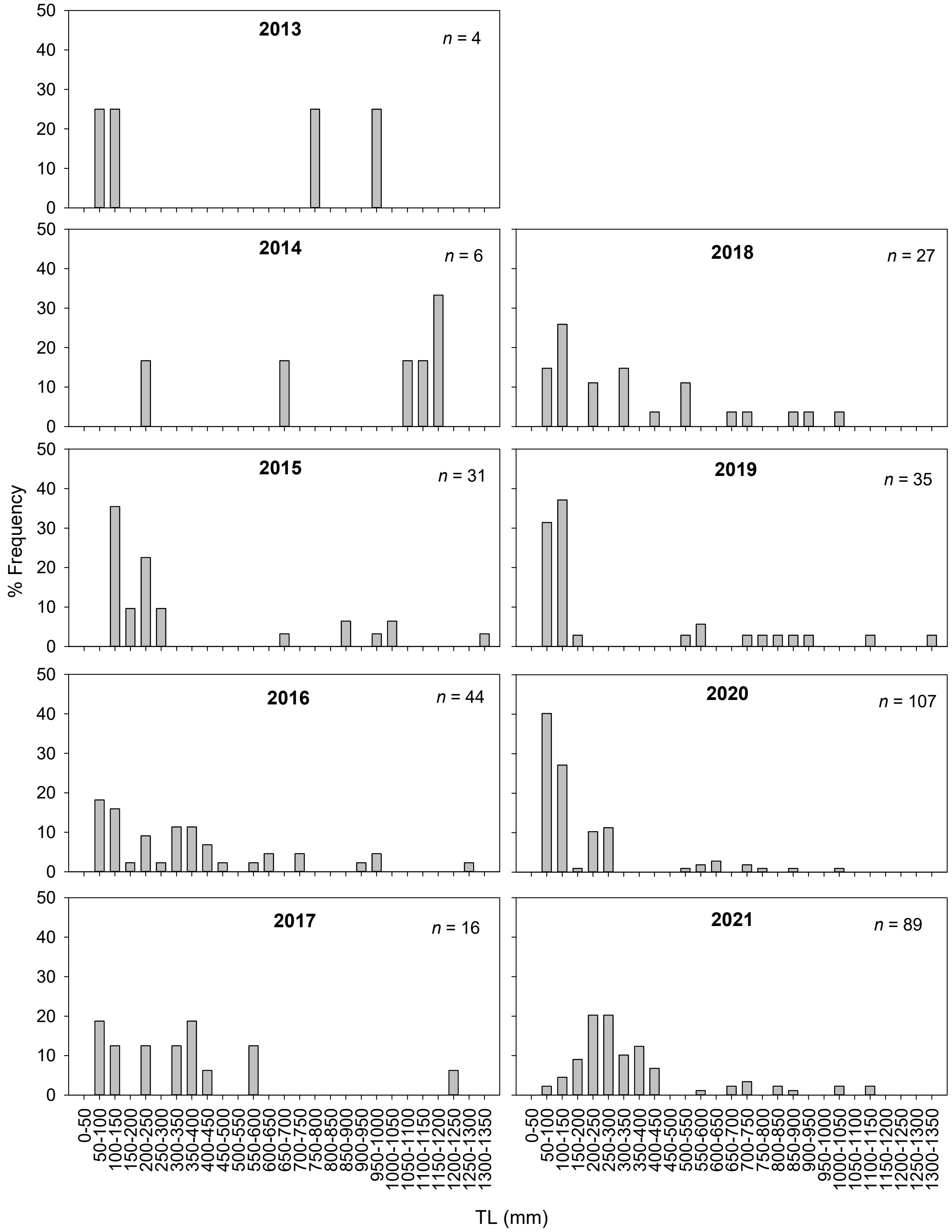

Figure 57. Total length (TL) frequencies of Murray cod in the main channel of the Lower Murray River during autumn/winter from 2013–2021.

Evaluation

The evaluation approach, including assessment criteria, is described in the evaluation section for Hydraulic Regime (Section 2.1). The assessment for Murray cod recruitment in 2019-20 and 2020-21 took a conservative approach and is likely to vary in future based on further analysis as additional annual data are added and findings from the allied research project are integrated.

Table 23. Murray Cod Recruitment evaluation questions and answers. CEW = Commonwealth environmental water, eWater = environmental water.

CEWO evaluation questions	**Outcomes of CEW delivery**	
	2019-20	**2020-21**
What did CEW contribute to the growth and morphometric condition of Murray cod?	The influence of CEW on the growth and condition of Murray cod in 2019-20 is unknown. Increased flows (including CEW) during the spawning/early larval period, however, may have improved food resources (e.g. microinvertebrates) that in part supported survival of larvae. Nonetheless, CEW likely had negligible or minor influence on later growth as there was minimal CEW delivery post-November 2020.	The influence of CEW on the growth and condition of Murray cod in 2020-21 is unknown. However, morphometric condition was greater than many previous years, and the seasonal growth rate was greater than that in 2019-20. Increased flows (including CEW) from late November to mid-December may have improved food resources (e.g. decapods) for early juveniles.
What did CEW contribute to recruitment of Murray cod?	The influence of CEW on the recruitment of Murray cod in 2019-20 remains unquantified. Nevertheless, increased flows (including CEW) during the spawning/early larval period (October–November) likely benefited Murray cod by increasing the extent and duration of lotic habitat, potentially enhancing spawning habitat area and survival of early life stages.	Recruitment of Murray cod in 2020-21, as indicated by YOY abundance, was poor relative to previous years. The influence of CEW on the recruitment of Murray cod in 2020-21 remains unquantified.
What did CEW contribute to the resilience of Murray cod populations?	In 2020-21, the 2019-20 cohort persisted and represented 49% of the sampled population as age 1+ individuals. As the influence of CEW on the recruitment of Murray cod in 2019-20 remains unknown, the contribution to resilience also remains unknown.*	

*The influence of CEW on the resilience of Murray cod populations will be assessed based on its contribution to length/age structure in future years.

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):

⬜ Unknown 🟥 Negative ⬜ None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Discussion

Over the course of the MER Project (2019-20 to 2022-23), we aim to identify associations between hydrology and hydraulics, and the recruitment of Murray cod in the LMR, and ultimately evaluate the benefit of environmental water delivery. Flow may influence the spawning and early life history of Murray cod in the MDB via several mechanisms, and responses to flow likely differ among river systems (Tonkin *et al.* 2018). For the LMR, we hypothesise that elevated spring–summer flow, either in-channel or overbank, and an accompanying increase in lotic habitat, may: (1) enhance the area of suitable spawning habitat; (2) be associated with enhanced growth rates and morphometric condition of Murray cod; and (3) enhance recruitment (to YOY). Further, we hypothesise that multiple years of enhanced spring–summer flow will contribute to broad size/age distributions of Murray cod and increased population resilience in the LMR.

Across the Basin, Murray cod spawn annually over a well-defined period from October–December, irrespective of flow (Davis 1977; Rowland 1998) and in the LMR the key period appears to be from mid-October to mid-November (Ye *et al.* 2021). Broad-scale recruitment in the LMR, however, is positively associated with spring–summer flow and lotic habitats (Zampatti *et al.* 2014). In recent years (2015–2020), in association with predominately in-channel flows, peaking at 10,000–18,000 ML/d during spring–summer, regular recruitment of Murray cod was evident in the LMR. In the Upper Murray River, spring flows and antecedent maximum (e.g. bankfull) flow conditions (i.e. flows preceding and during the spawning period) increase recruitment strength (Tonkin *et al.* 2018). Potential mechanisms driving this could be increased extent and quality of spawning and nursery habitat (e.g. Koehn 2009; Baumgartner *et al.* 2014; Stuart *et al.* 2019) or provision of additional food resources for larvae. In 2019-20, strong recruitment, indicated by an increase in the relative abundance of YOY, was evident in the LMR and throughout the MDB (e.g. Goulburn, Lachlan and Edward-Wakool rivers, Hladyz *et al.* 2021). During this year, a spring flow pulse, supported by Commonwealth environmental water, occurred from late-September to early November, flow (QSA) peaking at 15,600 ML/d around mid-October. This coincided with the period immediately preceding and during the key spawning period from mid-October to mid-November (Figure 58). The spring flow pulse in 2019-20 increased the extent of lotic habitat (i.e. water velocities >0.3 m/s) favoured by Murray cod for spawning and early life history processes (e.g. drift and residence) (Gibbs *et al.* 2020).

Alternatively, during 2020-21, there was poor recruitment of Murray cod in the LMR. The period from late-September to early November 2020 was characterised by flow typically <10,000 ML/d, with the majority of environmental water delivered and promotion of lotic conditions (Figure 58) occurring after the key spawning period, from late November to mid-December 2020 (peak at = 17,900 ML/d in late November) (Figure 5). Our data suggest elevated flow in early spring, immediately prior to and during spawning, may be positively associated with strong recruitment in Murray cod. Flows delivered mostly after the spawning season may not elicit the same response. Nonetheless, multiple years of data and further modelling is required to identify the drivers of recruitment, and to better evaluate the influence of environmental water on Murray cod recruitment.

To assess morphometric condition and growth during 2020-21, and the influence of flow (including Commonwealth environmental water) on these parameters, data from other projects including LTIM were used to develop ‘reference’ values/relationships for comparison. During 2020-21, low numbers of Murray cod were sampled throughout their early life history from larvae (November) through to YOY (April/May). This made it difficult to compare with 2019-20, which was the first year early juvenile Murray cod were successfully sampled in summer (January and February). Additional years of data are required to provide robust comparison and evaluation. The addition of YOY body condition data from 2020-21 further supports the theory that condition may be influenced by large-scale (i.e. overbank) flow events, while small-scale seasonal flow deliveries (e.g. winter or spring–summer freshes 10,000–18,000 ML/d) may have a lesser influence. For instance, condition of YOY in 2021 was marginally but significantly greater than observed in 2016 and 2018, years characterised by lower total flow volumes in spring–summer. Potential mechanisms driving this may be related to increases in productivity/food resources (allochthonous sources) and/or hydraulic changes that improve habitat quality and increased feeding efficiency.

Through 2013–2021, abundance (CPUE) of Murray cod in the LMR was variable. During 2017, a decline in abundance, notably adults (>600 mm TL), was associated with a hypoxic blackwater event in the LMR (Ye *et al.* 2018). Following this widespread blackwater event, declines in the abundance of adult Murray cod were also observed throughout the Murray River and some of its tributaries (e.g. Lachlan River, Murrumbidgee River, Edward–Wakool River system) (Dyer *et al.* 2017; Wassens *et al.* 2017; Watts *et al.* 2017; Stoffels *et al.* 2018). After 2017, abundance of Murray cod in the LMR increased to a peak in 2021, driven predominantly by strong recruitment in 2019-20 and the persistence of this cohort as age 1+ in 2021. Length frequency distributions showed a broad size distribution from 2015–2020, comprised of juveniles (including YOY), sub-adults and adults. In 2019 and 2020, YOY dominated the sampled population (≥67%) and cohorts from recent recruitment events (e.g. age 1+ and ~3+) were also apparent in 2020. As a result of poor recruitment in 2020-21, age 1+ fish spawned in spring 2019 continued to dominate (49%) the sampled population. These findings contrast with the Millennium Drought when length frequency distributions in the main channel of the LMR were dominated by adults >800 mm TL, indicating a lack of recruitment from 2003–2010 (Zampatti *et al.* 2014). Contemporary length frequency distributions in the LMR main channel will likely infer population resilience to future environmental perturbations such as hypoxic blackwater events.

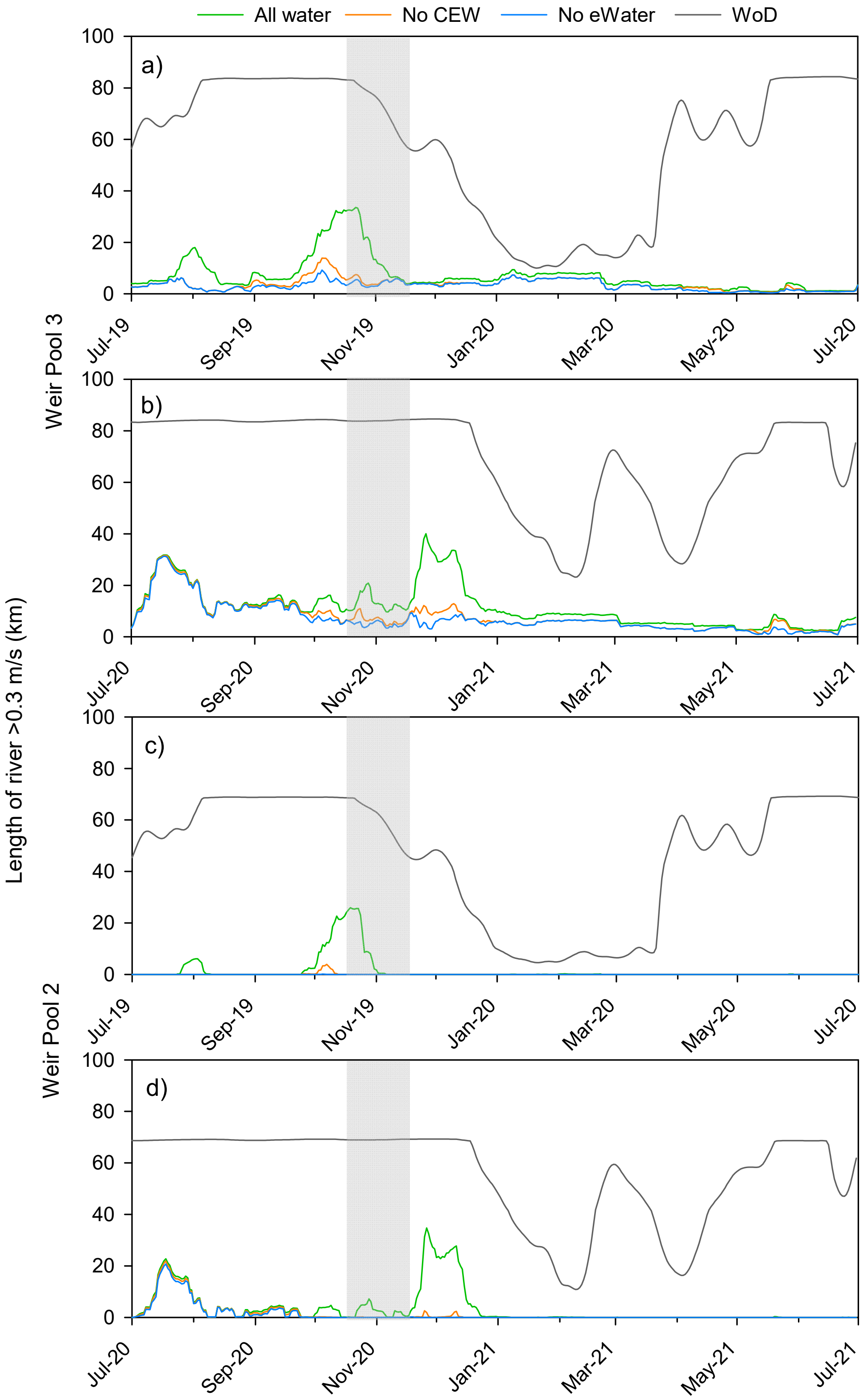

Figure 58. Increases in lotic habitat (length of river with velocities >0.3 m/s) in the weir pools below Lock 3 (a,b) and Lock 4 (c,d) from July 2019 to June 2021. The Murray cod peak spawning period for South Australia (i.e. mid-October to mid-November) is indicated by a grey shaded bar. Simultaneous improvements of lotic conditions also occurred in the other weir pools (Section 2.1). WoD = Without Development, a representation of natural conditions, which has the locks removed and a modelled flow representing no storage or diversions across the Murray River.

Management implications

Murray cod show a preference for lotic habitats and in the LMR, the extent of lotic habitat available during spawning and early life stages may be important for the magnitude of spawning and subsequent recruitment. In 2019-20, environmental water delivered as freshes during October–early November increased the longitudinal extent of lotic habitats - defined as mean cross-sectional velocity >0.3 m/s - in the LMR by 10% for at least 30 days. Specifically, downstream of Locks 4 (Weir Pool 3) and 3 (Weir Pool 2), the peak of the spring flow pulse was associated with an increase of lotic habitat area, relative to without Commonwealth environmental water, two-fold and five-fold, respectively (Figure 58). The hydrograph and timing of the flow pulse during 2019-20 was considerably different to any other years during the CEWO LTIM/MER period (Figure 10). In contrast, in 2020-21, environmental water contributed to a fresh later in the season (late November to mid-December), following the spawning period of Murray cod, and was associated with poor recruitment.

Murray cod morphometric condition appeared to be most influenced by large-scale flow events (e.g. floods), while smaller scale events (e.g. small freshes) appearing to elicit commensurate minor improvements in condition. As such, flow pulses may support enhanced recruitment of Murray cod more so through expansion of lotic habitat favoured for spawning and by early life stages rather than substantial productivity benefits. Nonetheless, further analysis and comparison of otolith daily growth increments will inform on finer temporal-scale influence of flow on growth.

In addition to the delivery of environmental water, other actions that support the maintenance/enhancement of lotic habitats in the LMR (e.g. weir pool lowering) may further benefit Murray cod recruitment and population resilience.

Conclusion

Poor recruitment of Murray cod to YOY was observed in the LMR during 2020-21. However, an increase in the extent of favourable (lotic) habitat by the spring–summer flows during the larval/juvenile period may have played a role in supporting the survival of new recruits in 2020-21. Evaluation of the contribution of Commonwealth environmental water towards growth, condition and recruitment in 2019-20 and 2020-21, was conservatively designated as unknown. In future years, a more robust evaluation will be informed by multiple years of data (including otolith increment analysis), and results of the Selected Area research project and Basin-scale research and evaluation.

2.8 Fish Assemblage

Background

In 2021, we collected fish assemblage data in the main channel of the LMR to inform Basin-scale evaluation of fish community responses to Commonwealth environmental water. The evaluation under the MER Project (2020–2022) is being undertaken by a team of fish ecologists and biometricians, led by the Arthur Rylah Institute for Environmental Research (ARI) (Hladyz *et al.* 2021).

Objectives

In this section, our objectives are to: (1) provide summary statistics of the catch rates and population demographics for nominated species; (2) describe temporal variation in fish assemblage and population structure from 2015–2021; and (3) discuss key findings based on published research and a contemporary understanding of fish life histories and population dynamics in the LMR. Our interpretations of the data for this indicator do not infer association with environmental water delivery.

Methods

During March–April 2021, small- and large-bodied fish assemblages were sampled from the gorge geomorphic zone of the LMR (Figure 1) using fyke nets and electrofishing, respectively. Prescribed methods (Hale *et al.* 2014) were used to obtain population structure data for seven target species (Figure 59). Refer to SARDI *et al.* (2019) for detailed sampling design and methodology.

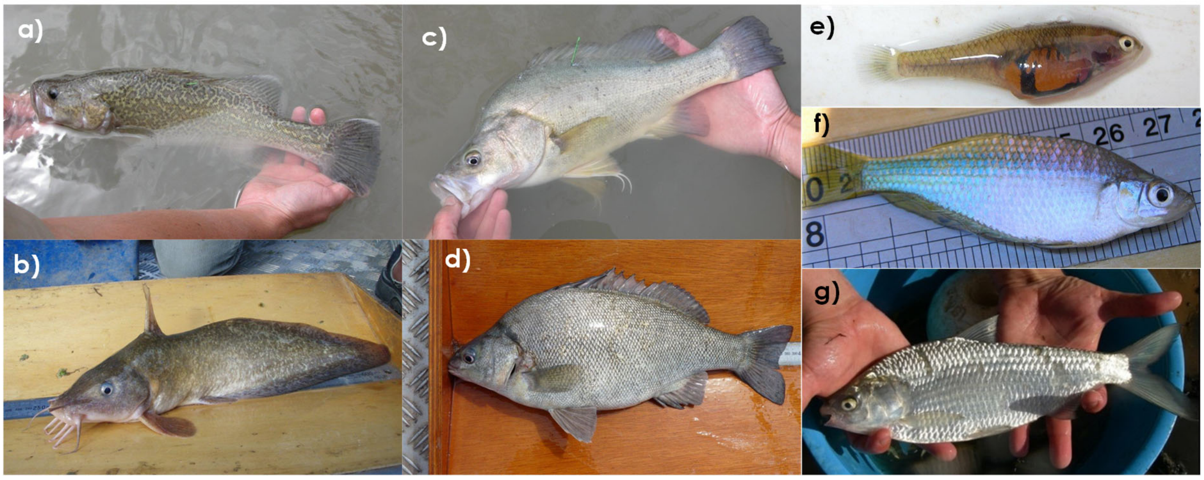

Figure 59. Target species for the Lower Murray River: (a) Murray cod and (b) freshwater catfish (equilibrium life history); (c) golden perch and (d) silver perch (periodic life history); and (e) carp gudgeon, (f) Murray rainbowfish and (g) bony herring (opportunistic life history).

Temporal variation in fish assemblage structure (species composition and abundance), between sampling years (i.e. 2015–2021), was investigated using Non-metric Multi-Dimensional Scaling (MDS), permutational multivariate analysis of variance (PERMANOVA) and Similarity Percentages (SIMPER) analysis in the software package PRIMER v. 6.1.12 (Clarke and Gorley 2015) and PERMANOVA + v.1.02 (Anderson *et al.* 2008). To determine temporal variation in population structure, length frequency histograms were qualitatively compared among sampling years.

Results

Catch summary for 2021

A total of 6,424 individuals (ind.) from eight large-bodied fish species were collected by electrofishing. Bony herring (*Nematalosa erebi*) was the most abundant species (90% of the catch by number), followed by common carp (*Cyprinus carpio*) (6%) (Figure 60a).

A total of 20,915 individuals from seven small-bodied species were collected using fyke nets. Carp gudgeon (*Hypseleotris* spp.) was the most abundant species (89% of catch by number), followed by Murray rainbowfish (*Melanotaenia fluviatilis*) (5%) and unspecked hardyhead (*Craterocephalus stercusmuscarum fulvus*) (4%) (Figure 60b).

Temporal variability in fish assemblage structure

MDS ordination of electrofishing data demonstrated separation of 2017 from all other years (Figure 61a). PERMANOVA indicated that large-bodied fish assemblages were significantly different among years ($Pseudo\text{-}F_{6,65} = 5.7663$, $P \leq 0.001$). Pairwise comparisons revealed significant differences between 2017 and most other years (i.e. 2015, 2018, 2019 and 2020), and between 2015 and 2021, 2016 and 2020, and 2020 and 2021, but not for any other pairs of years (Figure 61a).

For small-bodied fish assemblages, there were significant differences among years ($Pseudo\text{-}F_{6,69} = 4.0111$, $P \leq 0.001$). PERMANOVA pair-wise comparisons revealed significant differences in small-bodied fish assemblages between 2017 and most other years (i.e. 2016, 2018, 2020 and 2021), and between 2020 and 2021, but not for any other pairs of years (Figure 61b).

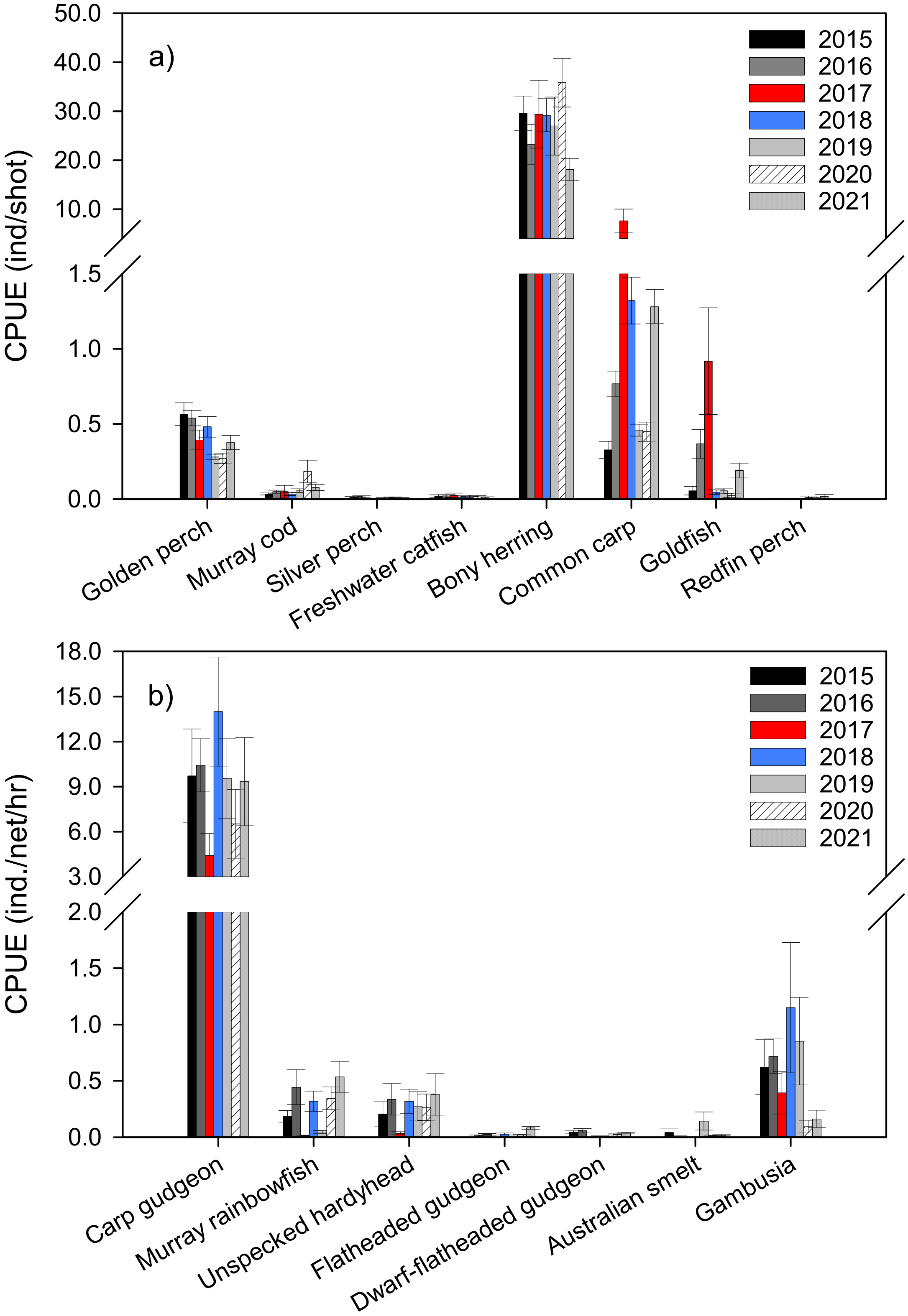

Figure 60. Mean catch-per-unit-effort (CPUE) ± standard error of (a) large-bodied fish species captured using electrofishing (individuals per 90 second shot) and (b) small-bodied fish species captured using fine-mesh fyke nets (individuals per net per hour) in the gorge geomorphic zone (10 sites) of the Lower Murray River in autumn from 2015–2021. Electrofishing CPUE data from five sites are presented for 2017 as other sites were sampled during winter 2017. See Table D18 in Appendix for statistics.

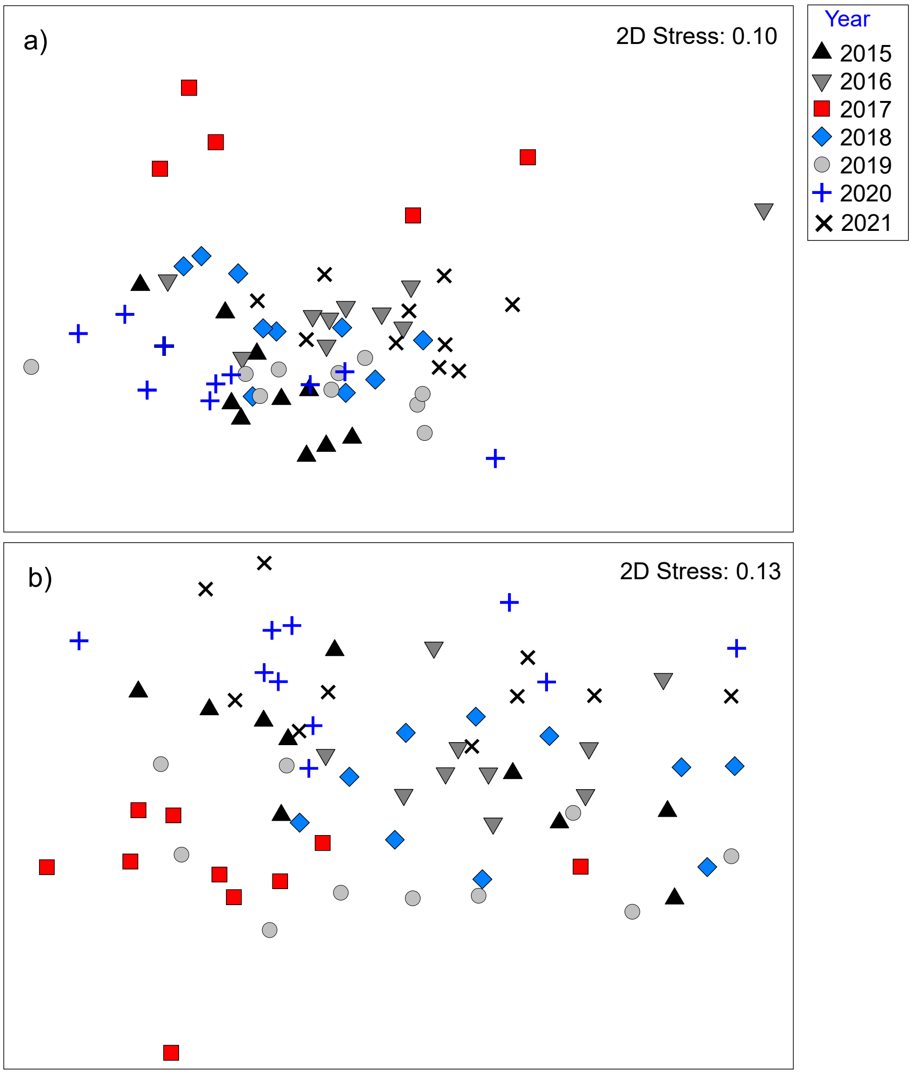

Figure 61. Non-metric multi-dimensional scaling (MDS) plot of (a) large-bodied fish assemblages sampled by electrofishing and (b) small-bodied fish assemblages sampled by fyke netting in the gorge geomorphic zone of the Lower Murray River from 2015–2021. Sites (*n* = 5) sampled in winter 2017 were removed from the ordination.

SIMPER indicated that differences between years for large-bodied fish assemblages were primarily driven by higher abundances of common carp and goldfish in 2017 and 2021, and lower abundance of bony herring in 2021 (Figure 60). SIMPER indicated that differences between years for small-bodied fish assemblages were driven by lower relative abundances of carp gudgeon in 2017 and 2020 (Figure 60).

Temporal variation in length/age structure of large-bodied species

In 2021, the sampled golden perch population in the LMR ranged in age from 2+ to 24+ years and was mostly comprised of age 9+ (16%) and 10+ (33%) fish (Figure 49a). In 2020, silver perch (*Bidyanus bidyanus*) in the LMR ranged in age from 2+ to 4+ years (Figure 49b). In 2021, the length distribution of golden perch (217–500 mm, Figure 62), silver perch (236–347 mm, Figure 63) and freshwater catfish (425–465 mm, Figure 64) in the gorge geomorphic zone of the LMR indicated an absence of new recruits and an ageing population.

In 2021, the sampled Murray cod population consisted of individuals 97–1140 mm (Figure 57). Unlike other years, individuals 150–300 mm (suspected age 1+) dominated (49%) the length distribution of Murray cod. In 2021, the sampled bony herring population ranged in age from 0+ to 7+ years, but was dominated by age 0+ fish (83%)(Figure 65).

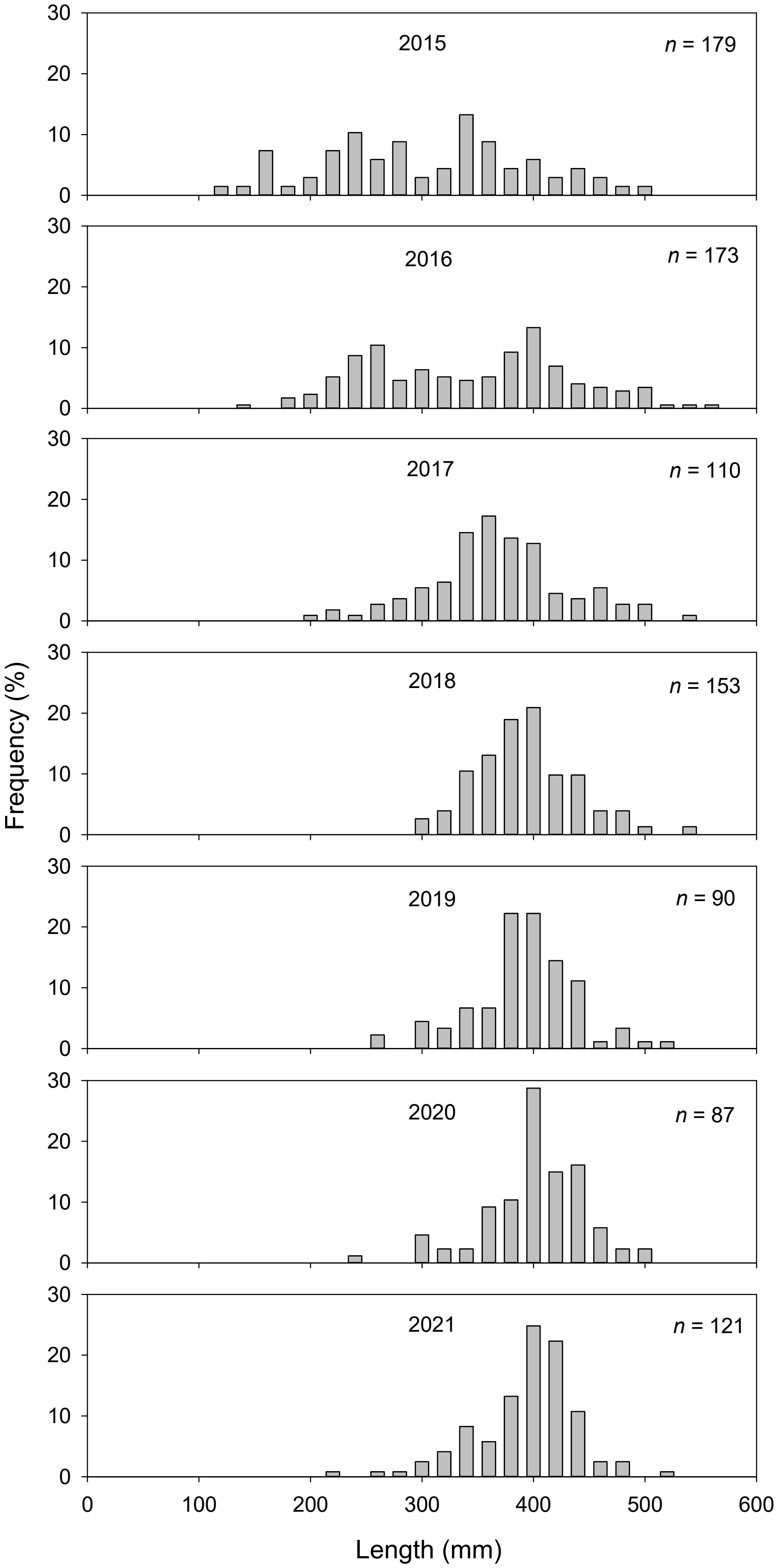

Figure 62. Length frequency distributions of golden perch collected from the gorge geomorphic zone of the Lower Murray River from 2015–2021.

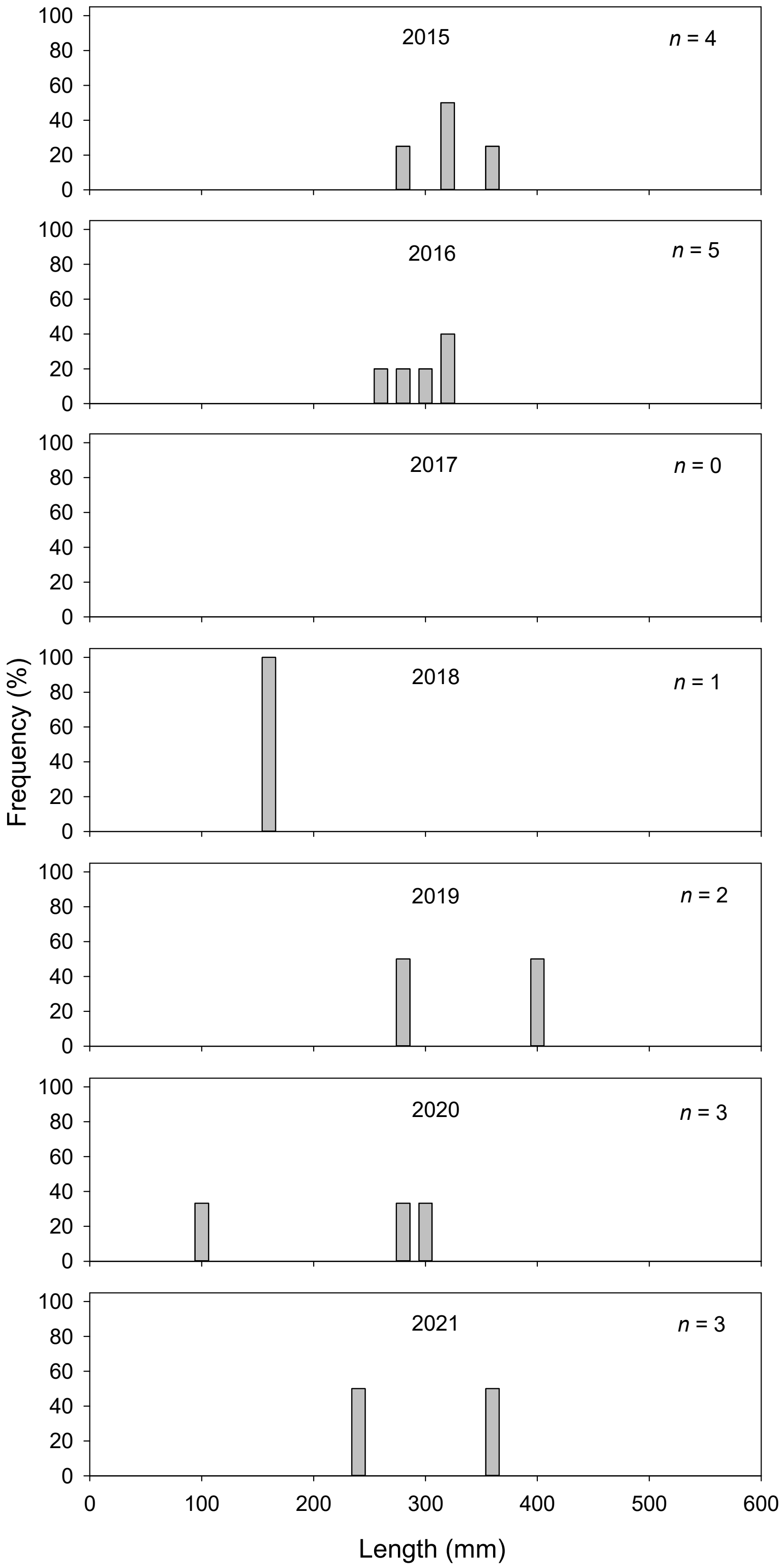

Figure 63. Length frequency distributions of silver perch collected from the gorge geomorphic zone of the Lower Murray River from 2015–2021.

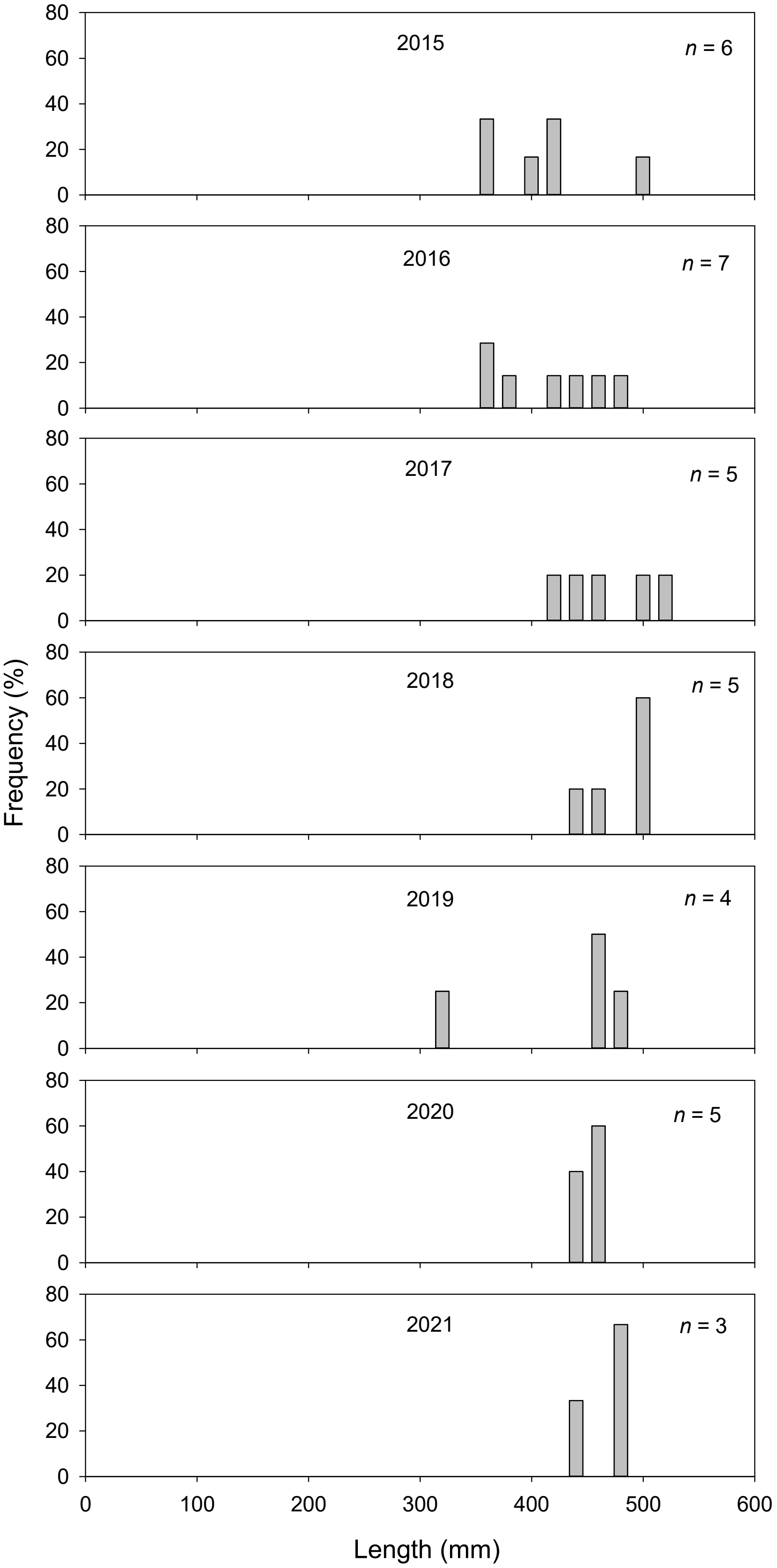

Figure 64. Length frequency distributions of freshwater catfish collected from the gorge geomorphic zone of the Lower Murray River from 2015–2021.

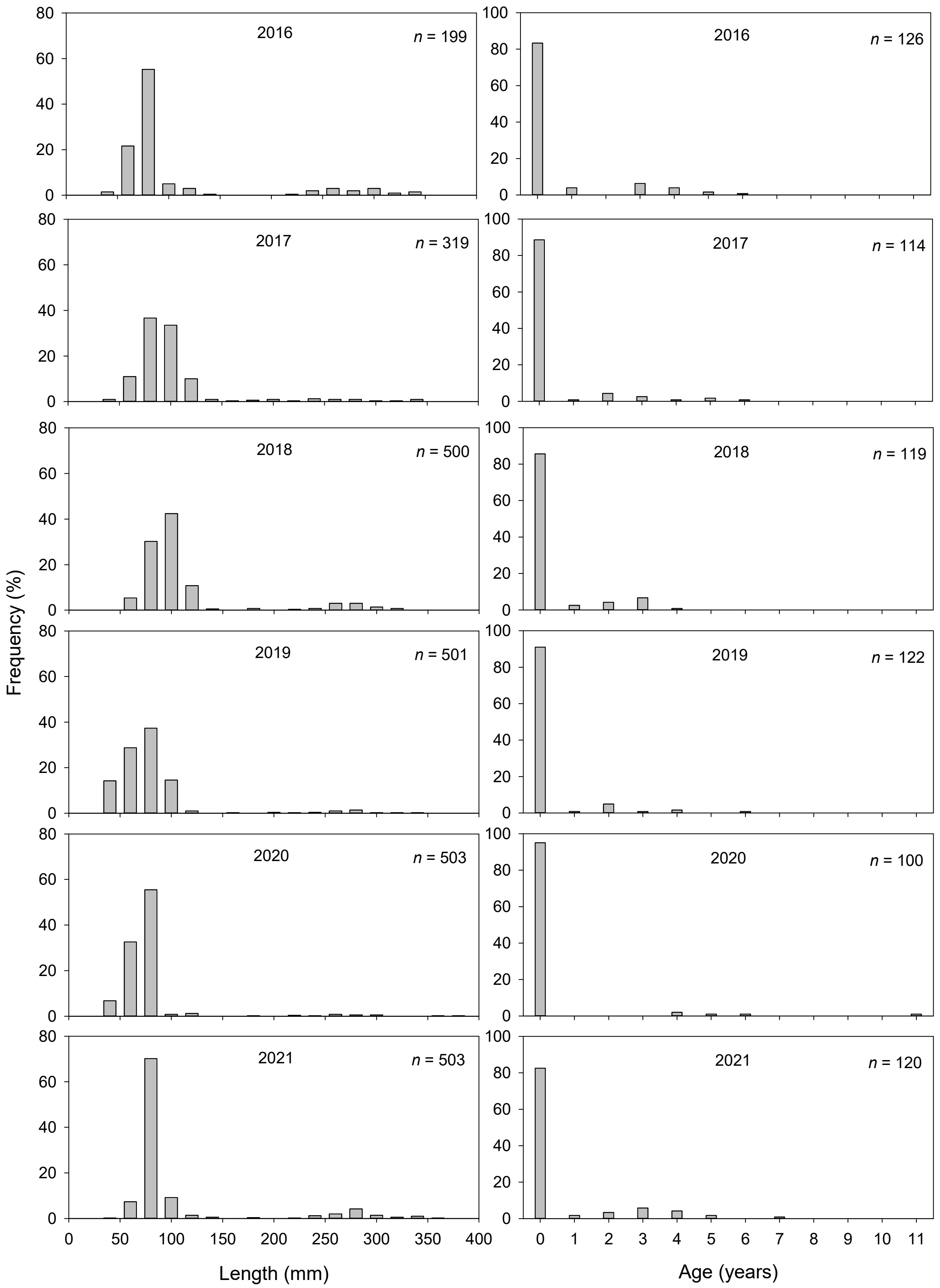

Figure 65. Length frequency distributions and age structures of bony herring collected from the gorge geomorphic zone of the Lower Murray River from 2016–2021.

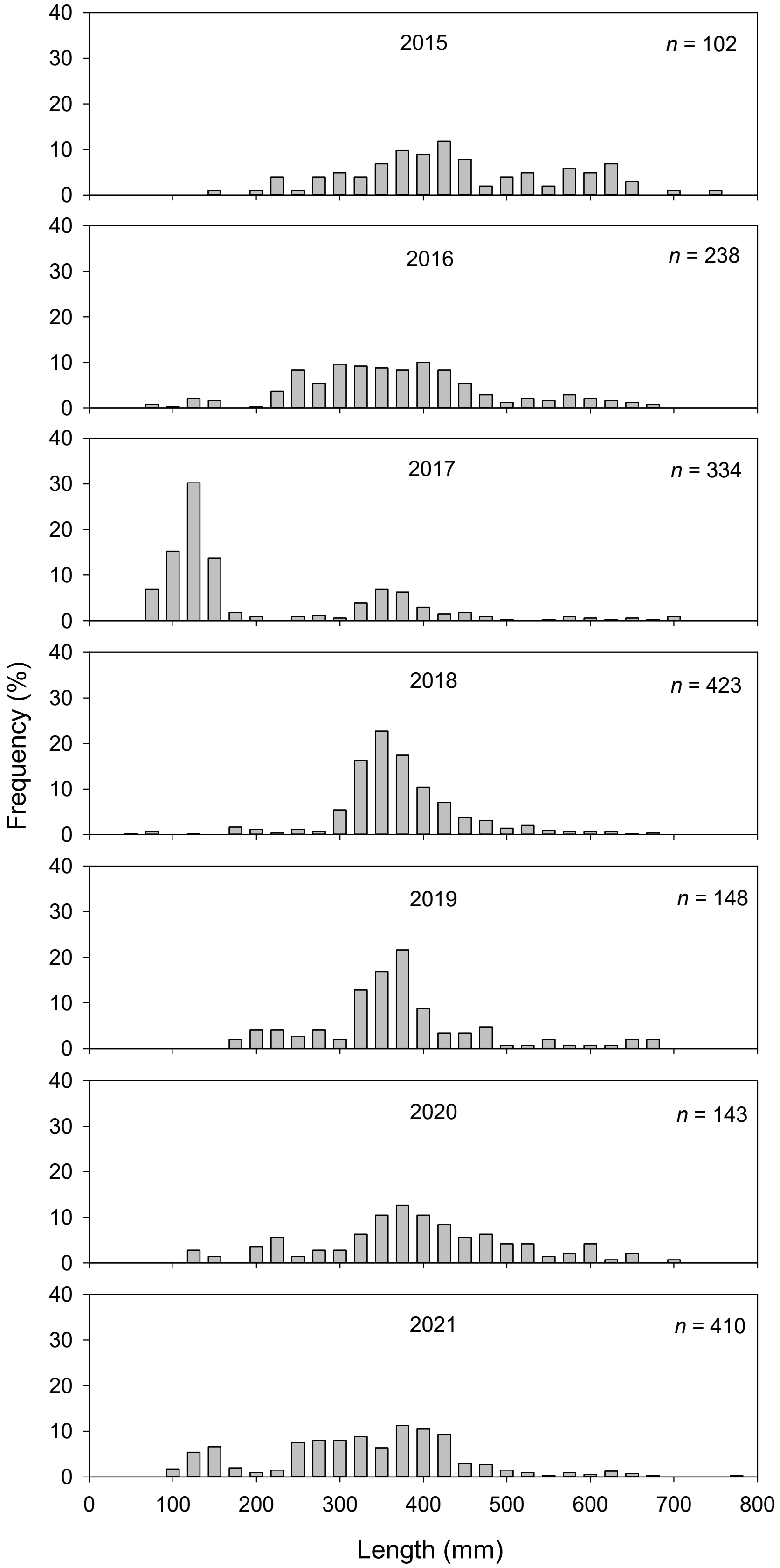

Figure 66. Length frequency distributions of common carp collected from the gorge geomorphic zone of the Lower Murray River from 2015–2021.

Evaluation

There are no CEWO evaluation questions for this indicator for the Lower Murray Selected Area. For this report, fish monitoring data from this standardised sampling, and additional targeted sampling for 'Flow-cued Spawning Fishes Reproduction' (Section 2.6) and Murray Cod Recruitment (Section 2.7), were consolidated to evaluate a number of fish targets of the Long-Term Environmental Watering Plan (LTWP) for the South Australian Murray River (DEWNR 2015) and the Basin Plan Matter 8 Report (DEW 2020) in Appendix C.

Discussion

During 2014-15 and 2015-16, relatively low (<15,000 ML/d), stable flows predominated in the LMR. In these years, small-bodied fish abundance and diversity were high. Abundances of flow-cued spawning species (i.e. golden perch and silver perch) remained similar in both years and overall, fish assemblage structure was characteristic of low flows in the LMR and similar to that during drought in 2007–2010 (Bice *et al.* 2014).

In 2017, following flooding in spring–summer 2016 (peak flow ~94,600 ML/d), there was a significant change to the small- and large-bodied fish assemblages, with an overall decrease in the abundances of small-bodied species and an increase in the abundance of common carp. A reduction in submerged vegetation in the main channel of the LMR during 2016-17, due to a combination of increased water depth/decreased light penetration and physical scour, likely resulted in a decrease in habitat availability and decreased abundance of small-bodied fishes (Bice *et al.* 2014). In 2017, increased abundance of common carp was driven by a large recruitment event associated with flooding in 2016-17. Following a recession in water levels in summer 2017, large numbers of YOY (age 0+) common carp likely entered the main channel from off-channel floodplain and wetland habitats (their typical spawning and nursery habitat) and were captured during sampling in autumn and winter 2017.

In general, the fish assemblage in 2017 was typical of high flow conditions and similar to assemblages in high flow years from 2010–2012, with the exception of low abundances of golden perch (Bice *et al.* 2014). In 2016-17, recruitment of native, large-bodied flow-cued spawners (e.g. golden perch) was negligible, despite a flow regime that was potentially conducive to spawning of these species (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b) (also see Section 2.6). It is possible that recruitment in 2016-17 was compromised by hypoxia associated with blackwater during the spring–early summer spawning season (Ye *et al.* 2018), impacting the survival of eggs and larvae.

Following in-channel flows (up to 17,800 ML/d) in spring–early summer 2017-18, small-bodied fish species composition and abundance in 2018 reverted back to that of pre-flood conditions (i.e. 2016 and 2015), likely due to structural and hydraulic habitats (i.e. submerged vegetation and stable water levels) conducive to small-bodied fish recruitment in the main river channel. Abundances of small-bodied fish have been variable from 2018–2021, following comparable hydrological conditions (i.e. low, in-channel flows <18,000 ML/d) in 2018-19 and 2019-20. From 2017 to 2020, the large-bodied fish assemblage trended back towards one typical of 'low flows' (e.g. 2016, 2015 and 2008, Bice *et al.* 2014) due to a reduction of common carp and goldfish abundance in 2019 and 2020 to levels similar to 2015 and 2016. Abundance of common carp and goldfish, however, increased in 2021. This was in part driven by recruitment in 2020-21

(Figure 66) which coincided with the operation of Pike and Katarapko regulators and associated floodplain inundation (Appendix B).

Based on electrofishing length frequency data, no recruitment (to YOY) was observed for freshwater catfish in the LMR from 2014–2021. In the LMR, the spawning biomass of freshwater catfish is low (Ye *et al.* 2015) and their recruitment dynamics are poorly understood.

From 2015–2019, regular recruitment and similar inter-annual abundances of Murray cod (i.e. fish <160 mm TL) were observed in the LMR, during years characterised by an in-channel flow pulse (15,000–18,000 ML/d) and a high, overbank flow (>90,000 ML/d), but also during three years of low, stable, in-channel flows (<12,000 ML/d). Furthermore, based on length frequencies, there was evidence of some cohorts from 2015–2019 persisting in the population. In 2020, in association with an in-channel flow pulse (<18,000 ML/d), Murray cod abundance increased significantly, driven by the increased abundance of new recruits. In 2021, however, poor recruitment was observed following an in-channel spring pulse of similar magnitude to 2020, but later timing (late November/early December). The mechanisms that facilitate recruitment of Murray cod (to YOY) in the LMR are being explored as part of the Murray Cod Recruitment indicator (Section 2.7) and associated research project (not presented in this report).

Management implications

Prolonged low, in-channel flows (<20,000 ML/d) promote hydraulic (e.g. lentic) and structural (submerged plant) habitat conditions suitable for low flow generalist fishes such as small-bodied carp gudgeon and unspecked hardyhead. Conversely, in-channel flows >20,000 ML/d facilitate a shift towards more natural riverine hydraulic characteristics that benefit flow-dependant species such as golden perch and Murray cod. Increased variability in the annual flow regime that includes large, overbank (>45,000 ML/d) flows will lead to increased recruitment of a range of species with different life histories (e.g. flow-dependant), promoting diverse fish assemblages and resilience in populations.

Conclusion

Since high flows in 2016-17, low, in-channel flows (<18,000 ML/d) have predominated in the LMR. Small-bodied fish abundances were variable from 2018–2021 but have returned to that of 2015 and 2016. While there has been a lack of recruitment from native, flow-cued spawners, strong recruitment of Murray cod in 2020 and the survival of these fish as age 1+ in 2021 has led to an increase in abundance of this species, relative to 2015 and 2016.

3 SYNTHESIS AND EVALUATION

To assess ecological responses to Commonwealth environmental water in the Lower Murray, a series of evaluation questions were investigated (SARDI *et al.* 2019). The contribution of environmental water to Hydraulic Regime, Matter Transport and Coorong Habitat was assessed throughout the year using a modelling approach, whereas indicators of specific ecological responses (Stream Metabolism and Water Quality, Littoral Vegetation, Microinvertebrates, Flow-cued Spawning Fish Reproduction, and Murray Cod Recruitment) were assessed through empirical monitoring during targeted seasons. This annual report for the MER Project focuses on the evaluation of Commonwealth environmental water delivery during 2020-21, with ecological outcomes for all years (2014-15 to 2020-21) presented, building on the findings from the LTIM Project (Table 24).

In 2020-21, a total of 687 GL of Commonwealth environmental water was delivered to the LMR in conjunction with other sources of environmental water (~247 GL; i.e. TLM, VEWH and RMIF). Environmental flow delivery during spring–early summer was supported via multi-site watering events, with return flows from the Murray, Goulburn and Murrumbidgee rivers, whereas flows during summer–late autumn were largely via direct trades. Commonwealth environmental water contributed to a substantial increase in the total flow volume in the LMR (22% at South Australia border and 34% at Lock 1), and improved longitudinal hydrological connectivity, providing additional 10,392 km d (modelled length x duration) of flowing river (velocity >0.2 m/s). Such flow conditions are critical for key ecological processes including supporting entrainment and downstream drift/transportation of plankton, invertebrates and fish larvae.

Following winter unregulated flows, environmental water delivered to the LMR from late September–mid December 2020 increased flow variability and supported a distinct spring–early summer flow pulse, peaking at 17,900 ML/d in late November 2020 and maintaining flow at >15,000 ML/d for a period of 18 days. The increased flow improved (modelled) hydraulic diversity in the LMR, with an extra 34 km (10%) of river characterised as lotic (mean water velocity >0.3 m/s) for at least 30 days due to environmental water delivery. Greater extent and duration of lotic habitat may benefit Murray cod during their reproductive season (spring–early summer) by increasing spawning habitat area and survival of early life stages. This was evidenced by strong recruitment of Murray cod in the LMR in 2019-20, when a spring flow pulse (peaking at 15,600 ML/d in mid-October) was delivered immediately prior to and during spawning. In contrast, poor recruitment of Murray cod (to YOY, age 0+) was observed in 2020-21, associated with variable spring flows (generally <10,000 ML/d). During this year, enhanced lotic conditions occurred from late November to mid-December, after the key spawning period. While these lotic conditions were unlikely to benefit spawning as indicated by low numbers of larvae collected in November 2020, the increase in favourable (lotic) habitat during the larval/juvenile period may have helped support the survival and body condition of new recruits, although in low numbers. The mechanisms that influence Murray cod recruitment in the LMR need further investigation and are currently being explored via the Selected Area research.

Environmental flows, in combination with weir pool manipulations, also increased (modelled) water level variability (IQR) by 0.12 m in the tailwaters across Weir Pools 1–5.

Periodic increases in water levels could increase biofilm diversity (Steinman and McIntire 1990), which is a key component of riverine food webs, and improve the condition of littoral vegetation (Gehrig *et al.* 2016). Vegetation monitoring in February 2021 demonstrated increased native plant species diversity at multiple spatial scales following the inundation of littoral zones by spring–early summer flows in the LMR, supported by environmental water. The above-ground biomass of understory vegetation also increased due to increased soil moisture, indicating increased littoral vegetation productivity. Furthermore, environmental water delivery also provided hydrological conditions to support river red gum (*Eucalyptus camaldulensis*) survival, with many seedlings geminated in 2019-20, developing into saplings in 2020-21.

Increased flows supported by environmental water promoted water mixing (velocities >0.2 m/s) and oxygen exchange at the surface, which was considered to have made a significant contribution to reducing the risk of low dissolved oxygen (DO) in the LMR. During 2020-21, the potential low DO period in the main channel was reduced by 55–100 days (varied between reaches) due to environmental water, primarily associated with the spring–early summer flow pulse. Without environmental water, the period of low risk was short, for example, only 7 and 10 days at downstream of Locks 1 and 4, respectively. Maintaining DO is particularly important during spring–summer as this period corresponds with high ecosystem respiration rates and is the primary reproductive season of many species that generally favour DO >5 mg/L, noting warmer water also has a lower saturation concentration of DO. The consequences of low DO on survival of aquatic biota are evident by extensive fish kills during the 2016-17 hypoxic black water event in the Murray River, and more recent fish kills in the Darling River during 2018–2020.

As in the previous six years, the estimated increase in primary production (average over the monitoring period) by environmental water remained low (e.g. 1% and 3% at downstream of Locks 1 and 6, respectively) in 2020-21, thus indicating negligible effect on the carrying capacity of organisms in the LMR. The influence of environmental water delivery on riverine production in the heavily regulated LMR is likely restricted by the stable water levels that characterise weir pool environments during in-channel flows. In contrast, in 'less regulated' river reaches of the Murray River (e.g. adjacent to the Hattah Lakes), environmental water could lead to substantial increases in riverine production (e.g. 31% in cross-sectional GPP, during 2017-18) (Ye *et al.* 2020a). Furthermore, the relatively short duration of the flow pulse (i.e. 18 days >15,000 ML/d) would have mitigated seasonal (six months) average of the production response. In fact, larger productivity increases occurred during the flow pulses than the seasonal average, and their implication on riverine food web warrants further investigation. Similarly, the effect of environmental flows on seasonal decomposition rates, estimated as bacterial respiration (BCR), has generally been small in the LMR. However, in 2020-21, there were moderate increases in BCR by 12% and 15 % downstream of Locks 4 and 6, respectively, due to environmental water inundating the littoral zone of the LMR, suggesting increased basal food resources to the river.

During spring–summer 2020-21, environmental flows were estimated (through modelling) to contribute to a 33% increase in microinvertebrate density, dominated by larger-bodied rotifers, and 16% increase in taxa richness. This included a substantial increase (54%) in the density of taxa transported downstream to the LMR through improved longitudinal connectivity (i.e. increased river length with flow velocity >0.2 m/s x days) of 4,019 km d. The longitudinal dispersal is a vital process in maintaining microinvertebrate genetic and species diversity in downstream communities. Further, as microinvertebrates are important prey items for a range of higher trophic organisms, increases in their density and diversity are likely to have positive impacts on food webs (Cooper and Goldman 1980; Vinyard 1980). In 2020-21, however, modelling estimated density decreases of 20% for floodplain/littoral habitat associated taxa and 41% for preferred prey species of large-bodied native fish larvae (including Murray cod) due to environmental water, although the decreases were mainly driven by a single microcrustacean species (*Bosmina meridionalis*). Such impact by environmental water on the microinvertebrate prey was the first recorded since 2014-15. Microcrustaceans, as the preferred prey for large-bodied fish larvae, generally require slackwater to reproduce, with many species preferring spring–early summer conditions (Shiel *et al.* 1982). Large areas of slackwater are present during low flow periods due to lack of flow in the main channel, or during higher flow periods when large areas of ephemeral habitat become inundated. However, during the flow pulse (moderate flow) in late spring–early summer 2020, much of the slackwater habitat that was likely present prior may have been flushed/eliminated, while little additional ephemeral habitat was inundated, leading to a reduction in microcrustacean prey species. Notably, the mean larval prey density during spring–early summer 2020 has been among the lowest since 2014-15 (except 2016-17), which suggests that reduced food resources during early life stages could be a contributing factor to poor recruitment of Murray cod in 2020-21, relative to other years.

Spawning of silver perch and golden perch coincided with environmental water delivery in the mid-Murray River to LMR during late spring–early summer in 2020-21. Substantial numbers of silver perch larvae were collected in the LMR, most of which had a natal origin from the lower reaches of the mid-Murray (i.e. upstream of the Darling River junction), while a small proportion were from the Murray River between Lock 6 and Lock 10. This suggested that the flow regimes during spring–summer in the mid-Murray River to LMR were conducive to promote silver perch spawning and downstream larval drift. Golden perch eggs and a limited number of larvae were also collected in the LMR during this year, with spawning occurring from the mid-Murray River to the LMR. Nevertheless, in autumn 2020-21, no YOY silver perch or golden perch were detected in the LMR, suggesting negligible localised recruitment. Future sampling will help detect the presence and assess relative strength of recruitment from this year. With low in-channel flows (<18,000 ML/d) prevailing since the 2016-17 flood, the current (2021) fish assemblage in the main channel of the LMR continued to represent one typical of low flows, with high abundances of small-bodied species, and a lack of recruitment of native, large-bodied flow-cued spawners. However, there was an increase in abundance of Murray cod in 2020-21 mainly driven by the survival of fish, now age 1+, from the 2019-20 cohort.

Similar to other dry years, in 2020-21, Commonwealth environmental water continued to play an important role in supporting barrage flow and maintaining connectivity between the river and estuary. The increased end-of-system flow by environmental water (91% Commonwealth) increased (modelled) salt export by ~1.2 million tonnes out of the Basin and reduced salt import by ~4.8 million tonnes into the Coorong estuary. Environmental water also reduced salt flux into the North and South lagoons by ~4.2 million tonnes (modelled). This resulted in substantially reduced salinity levels and helped maintain fish habitat for estuarine-dependent species in the Coorong. Without environmental water delivered to this region from 2017-18 to 2020-21, the area of suitable habitat for mulloway, congolli and smallmouth hardyhead would have declined by 39%, 42% and 45% (modelled) respectively, in 2020-21. Over the four years, environmental flows also led to a substantial increase (modelled 78%) in the area of suitable habitat for *Ruppia* sexual reproduction and life-cycle completion in the Coorong. These outcomes highlight the importance of environmental water to the ecological restoration of the Coorong.

Table 24. CEWO evaluation questions by indicators for the Lower Murray, which includes the Lower Murray River (LMR) and the Coorong, Lower Lakes and Murray Mouth (CLLMM). Evaluation questions are sourced or adapted from Gawne *et al.* (2014). Evaluation of CEW for hydraulic and matter transport questions is based on modelled data. CEW = Commonwealth environmental water. Refer to the evaluation in respective indicator sections (Section 2) for more detail.

CEWO evaluation questions	Outcomes of CEW delivery						
	14-15	**15-16**	**16-17**	**17-18**	**18-19**	**19-20**	**20-21**
What did CEW contribute to hydraulic diversity within weir pools? (LMR)	▒	▒	▒	🟩	▒	🟩	🟩
What did CEW contribute to variability in water levels within weir pools? (LMR)	▒	▒	▒	🟩	▒	▒	🟩
What did CEW contribute to hydrological connectivity (lateral and longitudinal)? (LMR)	▒	🟩	░	🟩	▒	🟩	🟩
What did CEW contribute to dissolved oxygen levels? (LMR#)	░	🟦	🟩	🟦	🟩	🟩	🟦
What did CEW contribute to patterns and rates of primary productivity? (LMR#)	░	░	░	░	░	░	░
What did CEW contribute to patterns and rates of decomposition? (LMR#)	░	░	░	▒	░	░	🟩
What did CEW contribute to salinity levels at the Murray Mouth? (CLLMM)	🟩	▒	🟩	🟩	🟩	🟩	🟦
What did CEW contribute to the salt transport? (CLLMM)	🟩	🟩	▒	🟩	🟩	🟩	🟦
What did CEW contribute to improving *Ruppia tuberosa* habitat? (CLLMM)	■	■	■	🟩			
What did CEW contribute to improving fish habitat? (CLLMM)	■	■	■	🟦			
What did CEW contribute to littoral understorey vegetation diversity and productivity? (LMR)	■	■	■	■	■	🟩	🟩
What did CEW contribute to above-ground biomass produced by understorey littoral vegetation? (LMR)	■	■	■	■	■	□	🟩
What did CEW contribute to microinvertebrate density? (LMR)*	░	░	░	▒	■	🟩	🟩
What did CEW contribute to microinvertebrate diversity? (LMR)*	░	░	░	▒	■	▒	░
What did CEW contribute to spring microinvertebrate fish prey species density? (LMR)*	🟥	🟦	░	🟦	■	🟩	🟥
What did the flow regime (including CEW) contribute to the spawning and recruitment of golden perch and silver perch? (LMR)	▒	▒	▒	▒	▒	░	░
Did the flow regime (including CEW) contribute to the resilience of golden perch and silver perch populations? (LMR)	▒						
What did CEW contribute to growth, morphometric condition and recruitment of Murray cod? (LMR)*	■	■	■	■	■	□	□

* = not all evaluation questions are presented here for this indicator. # = evaluation results presented for downstream of Lock 6 in the LMR.

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered). See respective indicator evaluation sections (Section 2) for selection criteria.

■ Not assessed □ Unknown 🟥 Negative ░ None/negligible ▒ Minor 🟩 Moderate 🟦 Substantial

4 GENERAL MANAGEMENT RECOMMENDATIONS

During dry years, environmental water delivery to the Lower Murray has typically involved contributing to base flows (~South Australian entitlement flows), promoting in-channel flow pulses (i.e. increasing magnitude, duration and/or frequency of freshes), and supporting barrage flows. The overall aims of the water delivery are maintaining/improving habitat and populations and restoring ecosystem health in the LMR and CLLMM region. General recommendations for flow management in the LMR are provided below, based on monitoring outcomes from the LTIM and MER projects, in conjunction with our contemporary understanding of flow-related ecology in the LMR. More specific management considerations are provided in Section 2, based on ecological outcomes and findings from indicators. Our findings and recommendations on flow management are most relevant to the spring–summer period as this is the primary period driving biological response of selected indicators in the LMR.

Spring–early summer in-channel flow pulses

Spring–early summer in-channel flow pulses were key features of the natural hydrograph in the LMR but are conspicuously absent or reduced from the contemporary flow regime. These flow pulses improve hydrological connectivity, increase hydraulic diversity, and support a broad range of ecological processes and outcomes in riverine and estuarine ecosystems (e.g. lotic habitat, matter transport, food webs, spawning cues for riverine fishes, zooplankton/fish larvae drift, littoral vegetation diversity and productivity, fish recruitment). Such outcomes have been demonstrated to different levels via the ecological indicators in the Lower Murray over the last seven monitoring years.

Under the predominantly dry conditions from 2014-15 to 2020-21 (except 2016-17), environmental water represented 25–43% (31% in 2020-21) of the annual total volumes (mean ~2,700 GL/y) in the LMR, noting the total volume would have been 89% (modelled) greater prior to river regulation. In these years, environmental flows, typically delivered via return flows from upstream watering events, have supported multiple in-channel flow pulses (10,000–18,000 ML/d) in the LMR during spring–early summer (Figure 10). These small freshes elicited minor to moderate improvement in hydraulic conditions in the LMR. For example, increasing lotic habitat (velocity >0.3 m/s) by 35 km (11%) and 34 km (10%) for at least 30 days in 2019-20 and 2020-21, respectively. Due to the limited magnitude and/or duration of the flow pulses, even under the best case in 2020-21 (see Table 25), compounded by the impact of weirs, the spatiotemporal scale of hydraulic improvements and overall riverine ecosystem responses were restricted in the LMR. This has been exemplified by poor recruitment of golden perch from local spawning and small increases in riverine primary productivity in the LMR since 2014-15. Higher flow rates (>20,000 ML/d) and/or greater durations are likely required to substantially reinstate flowing river characteristics to achieve greater ecological outcomes (e.g. riverine production and recruitment of flow-cued spawning fishes) in the LMR. With existing volumes of environmental water and delivery constraints, reaching and sustaining flows >20,000 ML/d in the LMR during dry years is largely reliant on coordinating flow deliveries across much of the southern MDB, including flows from the Murray, Goulburn, Murrumbidgee and Darling rivers. Such concerted effort has been increasing in recent years. Under wetter scenarios, flows >20,000 ML/d may be achieved by delivering environmental water in conjunction with unregulated flows.

Nevertheless, in recent years, spring–early summer flow pulses up to 18,000 ML/d in the LMR have led to positive ecological responses at 'reach scale'. For example, increased water level variability by environmental flow supported the recruitment of native littoral understorey vegetation and the germination (2019-20) and survival (2020-21) of river red gums in river reaches downstream of weirs; improved hydrological connectivity, indicated by increased river length and duration with flowing velocity >0.2 m/s, promoted microinvertebrate downstream transport and facilitated larval drift of flow-cued spawners (e.g. silver perch larvae from the lower mid-Murray to the LMR in 2020-21). Furthermore, spring flows of 10,000–18,000 ML/d have been associated with recruitment of Murray cod in most years since 2014-15. Flows of this volume result in an increased extent of lotic habitat and may promote prey abundance in individual weir pools. The life history of Murray cod operates over these smaller spatial scales (10s of km), and as such, their recruitment may have been benefited by improved lotic habitat at 'reach scale', along with appropriate food supplies, resulting from environmental water delivery.

Timing of flow delivery

The timing of flow delivery is important and should continue to be considered with regard to its effect on achieving ecological objectives and meeting biological processes and species' life history requirements. For example, in recent years (2017-18, 2019-20 and 2020-21), the spring–early summer flow pulses were of a similar magnitude in the LMR, but the flow pulse in 2019-20 occurred earlier in the season (peak in mid-October) (Table 25), coinciding with the spawning period of Murray cod (i.e. October to November). Stronger recruitment of Murray cod was evident in 2019-20, compared to 2017-18 and 2020-21, suggesting that elevated flow in early spring, immediately prior to and during spawning, may be beneficial to Murray cod reproduction, potentially by improving the extent/quality of spawning habitat and providing favoured food resources for early life stages. Alternatively, flow pulses delivered mostly after the spawning season may not elicit the same response by Murray cod. Nonetheless, further research and modelling using multiple years of data is required to identify key drivers of Murray cod recruitment in the LMR. Furthermore, later flow pulses generally coincide with higher water temperatures (>20°C) and, if of sufficient magnitude, may promote spawning of golden perch. While the spawning and recruitment of this species has been poor in the LMR since 2014-15, peak spawning of golden perch typically occurs between November and January (based on data from 2010–2013 wet years). To achieve multiple species outcomes, a holistic approach in flow regime design will be required. This can be informed by better understanding of the effect of specific aspects of flow (e.g. timing, magnitude and duration) on life-history processes, the hydraulic requirements of flow-dependant species, and the availability of food resources. Whilst the timing of flow is important, a large proportion of environmental water is delivered to the LMR as return flows (e.g. in winter/spring/early summer). This reinforces that a collaborative and coordinated approach to environmental water planning and delivery across the southern Basin is essential. This includes aligning the timing and delivery of water, that is responsive and flexible to rain events, to achieve multi-site ecological outcomes.

Table 25. The magnitude, timing and duration of the main spring–summer flow pulses from 2014-15 to 2020-21.

Water year	Peak (ML/d)	Peak date	Duration		Season
2014-15	10,200	23/11/2014	>10,000 ML/d: 16 days	05/11–26/11/2014 (except 6 days)	Mid-–late spring
2015-16	11,600	28/10/2015	>10,000 ML/d: 36 days	18/09–29/10/2015 (except 6 days)	Early–mid-Spring
2016-17	94,600	29/11/2016	>45,000 ML/d: 66 days	12/10–19/12/2016 (except 3 days)	Mid-spring–early summer
2017-18	17,800	08/12/2017	>15,000 ML/d: 11 days	06/12–25/12/2017 (except 9 days)	Early summer
2018-19	12,101	1/1/2019	>10,000 ML/d: 17 days	22/12/2018–07/01/2019 (continuous)	Mid-summer
2019-20	15,600	19/10/2019	>15,000 ML/d: 11 days	12/10–22/10/2019 (continuous)	Mid-spring
2020-21	17,900	25/11/2020	>15,000 ML/d: 18 days	21/11–08/12/2020 (continuous)	Late spring–early summer

Restoring riverine hydraulics

Improving riverine hydraulics (e.g. water velocity and turbulence) is fundamental for ecological restoration in the LMR. Flows of >20,000 ML/d can significantly improve hydraulic conditions, by transforming >50% of a weir pool from lentic (slower flowing water, median velocities ≤0.3 m/s) to lotic habitat (faster flowing water, >0.3 m/s) (Ye *et al.* 2018). Restoring such hydrodynamic conditions will promote riverine ecological processes and support the rehabilitation of many declining biota that are adapted to a flowing environment in the LMR (Mallen-Cooper and Zampatti 2017). Pre-regulation, the LMR was characterised by lotic, riverine habitats, with water velocities ranging ~0.2–0.5 m/s, even at flows <10,000 ML/d (Bice *et al.* 2017). With limited volumes of environmental water, infrastructure management such as weir pool lowering could be considered to complement flows to achieve hydraulic restoration in this region. Modelling indicates a substantial hydrodynamic impact by weirs in the LMR (Figures 7 and 12), and even a small lowering (~0.1 m) at multiple weir pools can elicit commensurate benefit in lotic habitat improvements (Figure 12). A Weir Pool Operations Plan has been developed for Lock 1 to 6 (Muller and Creeper 2021) and small weir pool lowering within the operational range have been undertaken in recent years including 2020-21.

Longitudinal and lateral connectivity

Overall, environmental water delivery that promotes longitudinal and lateral connectivity will enhance the productivity in the LMR through increased carbon and nutrient inputs and matter transport. Water delivery, in conjunction with weir pool manipulation, to promote more natural water level variability are desirable to promote in-channel productivity, although the capacity of productivity influence has been limited with current infrastructure and operational arrangements in the LMR. Increasing water level variability may also improve seed banks and thus the resilience of littoral vegetation, noting the seed bank is currently depauperate in the LMR (Appendix E). Furthermore, flow management to increase connection and inundation of littoral habitats, wetlands and floodplain, and provide return flows to the main channel, may enhance food subsidy for riverine species via mobilising microinvertebrates. In particular, slow flowing slackwaters in littoral and off-channel habitats provide conducive environment for the reproduction of microcrustaceans, which have been reported as the main prey for large-bodied native fish larvae (e.g. Murray cod, golden perch). A flow regime that promotes the delivery of abundant prey during and following the reproductive season of these fish species, along with favourable hydraulic conditions, are important for their recruitment. Furthermore, longitudinal connectivity of river flow is important for the transport and dispersal of aquatic biota (e.g. microinvertebrates, larvae of flow-cued spawning fish) to and throughout the LMR, which can be supported by environmental water delivery. As demonstrated by LTIM and MER, downstream transport of microinvertebrates increased species diversity and/or abundance in the LMR. These increases likely contributed to improved productivity and community resilience in the LMR, which is important for aquatic food webs. However, further research is required to: (1) confirm specific taxa group(s) that provide important food resources for higher trophic organisms; and (2) determine how flow regime, including antecedent conditions, may influence food abundance and quality in the LMR. Some of these questions are currently being explored via Lower Murray MER research.

Flow integrity

Managing environmental water releases across broad 'riverscape' scales is critical not only to achieving desired flow volumes in the LMR, but also considering the effects on water quality and the transport of biota. Indeed, water quality (e.g. turbidity, DOC, the amount and form of nutrients) and entrained biota (e.g. plankton, fish larvae/juveniles) may vary from different sources of water (dependent on ecological processes occurring at those sources), therefore, influencing ecological outcomes (e.g. microinvertebrate assemblage composition, recruitment of golden perch or silver perch) in downstream ecosystems in the LMR. Consequently, maintaining flow integrity from upstream (e.g. Darling River or mid-Murray) to the LMR is important to support broad-scale ecological processes and promote positive outcomes (e.g. improving productivity, supporting spawning and recruitment of flow-dependent fishes). In this regard, consideration for environmental flow management needs to include: (1) maintaining hydrological integrity (i.e. magnitude, variability and source) of flow from upstream; and (2) the potential effects on water quality and biological attributes by river operations that re-route (e.g. through floodplains or wetlands) or fragment the flow (e.g. by diversions or water storages), which could lead to changes in ecological response and the structure and function of aquatic food webs.

Barrage flows

During dry years, Commonwealth environmental water is pivotal in maintaining barrage flows and end-of-system connectivity to support a functioning river system and species' life history processes (e.g. migration/recruitment of diadromous and estuarine-dependent fishes). Barrage flows increase salt export out of the Basin, contributing to the Basin Plan salt export objective/target. They also play an important role in reducing salt import from the ocean into the Coorong and reducing salt flux into the North and South lagoons. Environmental flow through the barrages is required every year for salt reduction in the Coorong and for maintenance of fish habitat. Even one year without flow through the barrages results in millions of tonnes of salt entering the Coorong and a contraction of fish habitat (predicted to be 11% reduction of mulloway habitat in 2020-21). Consecutive years of environmental water delivery to this region is essential to support *Ruppia* recruitment and maintain ecosystem health. Without environmental water, extensive reductions of estuarine habitat (44% for *Ruppia* and up to 45% for fish) would have occurred within four years, leading to a detrimental impact on the ecological functions and biodiversity in the Coorong. Barrage flows also reduce the risk of Murray Mouth closure. Our work highlights the critical role of water for the environment to the restoration of the Coorong ecosystem.

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6 APPENDICES

APPENDIX A: EXPECTED OUTCOMES OF COMMONWEALTH ENVIRONMENTAL WATER IN THE LOWER MURRAY RIVER, LOWER LAKES AND COORONG

Table A1. Summary of specific watering actions and expected outcomes for the Lower Murray River, Lower Lakes and Coorong in 2020-21 (Source: CEWO). Volumes of Commonwealth environmental water (CEW) are given at the South Australian (SA) border.

Watering action and target	Expected outcomes	Delivery details
Winter pulse (July to September 2020) CEW volume: LMR 49,467 ML, barrages 241,494 ML.	• Coorong water quality/habitat suitability • Salt export, • Ecosystem function, • Lamprey and congolli migration.	• A series of unregulated flow pulses from rain in upstream catchments kept QSA above 5,000 ML/d, up to 13,000 ML/d throughout July, August and most of September. • CEW was also delivered to SA during these months, with small return flow volumes from Victorian tributaries complementing the CEW component of SA entitlement. • As the elevated flows reached the lower lakes in mid-July, additional bays were opened alongside fishways at Tauwitchere and Goolwa to attract and encourage fish species including lamprey and congolli to migrate. Releases were generally above 5,000 ML/day from mid-July, with a brief period of lower flows in late August due to barrage closures during reverse head conditions. • Lake levels rapidly increased from 0.65m AHD at the start of July to exceed 0.8m by the start of August.
Spring pulse (October to December 2020) CEW volume: LMR 417,935 ML, barrages 343,556 ML.	• Coorong water quality, • Estuarine habitat, • Fish habitat and condition, • Riverine function and productivity, • Riverine fish spawning, • Lower lakes fish recruitment.	• Return flows from the Murray, Murrumbidgee (TLM) and Goulburn coordinated spring freshes began reaching the SA border at the end of September. QSA increased from 6,600 ML/d on 26 September up to a series of peaks of 11,400 ML/d (5 Oct), 12,000 ML/d (25 Oct) and 17,800 ML/d (26 Nov). • To complement returns flows, maintain the peak and slow recession of the pulse, an additional 40 GL was ordered at the SA border and delivered during early December. This water was originally planned to be delivered later as part of the summer/autumn direct trade but was brought forward to coincide with the back part of the spring pulse. The earlier release specifically aimed to support perch spawning activity in the SA Murray detected by the Flow-MER program. • Barrages releases were maintained between 3,000 and 8,000 ML/d (8-20 gates) until late-December when flows from the river declined. Barrage openings were reduced to two gates to protect Lower Lakes water levels, in order to maintain steady low flows to the Coorong for as long as possible into summer. • The Lower Lakes levels remained above 0.8m throughout this period.
Summer base flows in river, fishway flow and connection through barrages (January to February 2021)	• Protect assets and avoid damage in the river channel, Lower Lakes and Coorong.	• The delivery of 110GL summer/autumn direct trade commenced with 40 GL in January and 30 GL in February. This water allowed fishways and generally 1-2 additional gates to remain open. • The Lower Lakes levels declined from above 0.8m to below 0.75m throughout this period.

Watering action and target	**Expected outcomes**	**Delivery details**
CEW volume: LMR 108,311 ML, barrages 79,820 ML.		- 220 ML of return flows from the Murraylands and Riverland Landscape Board were returned to the site during this quarter.
Base flows and opportunistic pulses through the barrages (March to June 2021) CEW volume: LMR 105,473 ML (incl. 220 ML return flows within SA), barrages 142,638 ML.	- Coorong water quality, - Estuarine habitat, - Fish habitat and condition, - Riverine function and productivity.	- Summer/autumn direct trade continued with 20 GL delivered in March and 20 GL in April. - Efforts were made to provide opportunistic releases to the Coorong (in addition to base releases) from April-June in response to water levels and weather conditions, however conditions were generally unfavourable for pulsing when compared with previous years. Two short (single day) pulsing events occurred during June. Flow through the barrages during this period was otherwise via low steady baseflows through 1-3 gates. - The Lower Lakes levels declined to just below 0.6m in mid-late April before increasing above 0.7m by the end of the water year.
Weir Pool 4 raising (24 July to 29 November 2020) CEW volume: 433 ML, total volume: 1,433 ML (includes water used for Katarapko)	Primary: Maintaining the extent and condition of riparian and in-channel vegetation. Secondary: Contributing to riverine functioning.	- A medium level first operation of the Katarapko floodplain infrastructure in conjunction with raising of Murray River Weir 4 provided a rise in surface water levels at the outflow regulator structures at Carparks Lagoons, The Splash, Piggy Creek and Sawmill Creek equivalent to that which would be generated at approximately 45,000 ML/day. Lock 4 was raised by 0.335 m (up to 13.535 m AHD) to support increased inflows into the Eckerts Creek anabranch. - Additional 320 hectares inundated in the Weir 4-5 River reach due to Weir 4 raising.
Katarapko Floodplain and wetlands (7 September to 24 December 2020) CEW volume: 433 ML, total volume: 1,433 ML (includes water used for Weir Pool 4)	Primary: Maintain viable river red gum, black box, river cooba and lignum populations. Secondary: Provide for carbon and nutrient movement from the floodplain to the creek and river to generate localised fluctuations in productivity.	- Water level was raised upstream of the Katarapko regulators by approximately 2.8m, to the target peak of 12.8m AHD, and was maintained around this level for 6 days from 28 October 2020 (with a recorded maximum of 12.84 m AHD on the 29th of October). - Additional 468 hectares on the Katarapko floodplain.
Weir Pool 5 raising (10 August to 23 December 2020) CEW and total volume: 4,910 ML (includes water used for Pike)	Primary: Maintaining the extent and condition of riparian and in-channel vegetation. Secondary: Contributing to riverine functioning.	- A low level first operation of the Pike floodplain infrastructure in conjunction with raising of Murray River Weir 5 to provide a rise in surface water levels at the outflow regulator structures at Pike and Tanyaca similar to that which would be generated at approximately 55,000 ML/day. - Lock 5 was raised by 0.46 m (up to 16.76 m AHD) to support increased inflows to the anabranch. - Additional inundation of approximately 500 hectares of Pike floodplain
Pike Floodplain and wetlands (17 September to 29 December 2020) CEW and total volume: 4,910 ML (includes water used for Weir Pool 5)	Primary: Maintain viable river red gum, black box, river cooba and lignum populations. Secondary: Provide for carbon and nutrient movement from the floodplain to the creek and river to generate localised fluctuations in productivity.	- Water levels were raised upstream of the Pike regulator by approximately 0.7 m, to the target peak of 15.25 m AHD which was maintained around this level for 19 days from 20 November 2020. - Additional inundation of approximately 1,033 hectares along the Weir 5 to 6 reach.

APPENDIX B: OVERVIEW OF OTHER MANAGEMENT ACTIVITIES DURING 2020-21

In addition to environmental water deliveries to the Lower Murray in 2020-21 (Section 1.4), the following management actions are relevant to the analyses and interpretations in this report.

Other watering and management activities in the Lower Murray

Manipulation of water levels in Weir Pools 1, 2, 4 and 5

In 2020-21, manipulations of Weir Pools 4 and 5 occurred in conjunction with the operation of the Katarapko and Pike Anabranch regulators, respectively. Drawdown of water levels in Weir Pool 4 from peak level (+0.335 m normal pool level (NPL)) occurred in November 2020, and water levels returned to NPL ahead of the flow peak to South Australia in late November (Table A1; Figure B1). Raising of Weir Pool 5 occurred later and peaked at +0.46 m in November and early December, before undergoing a drawdown to NPL in mid-December 2020. During December 2020, minor lowering of water levels below the NPL of Weir Pools 1 (-0.11 m), 2 (-0.09 m) and 4 (-0.06 m) also occurred. Approximately 5,343 ML of Commonwealth environmental water was delivered to account for losses (e.g. evaporation) during the manipulation of weir pools and floodplain regulator operation (Table A1).

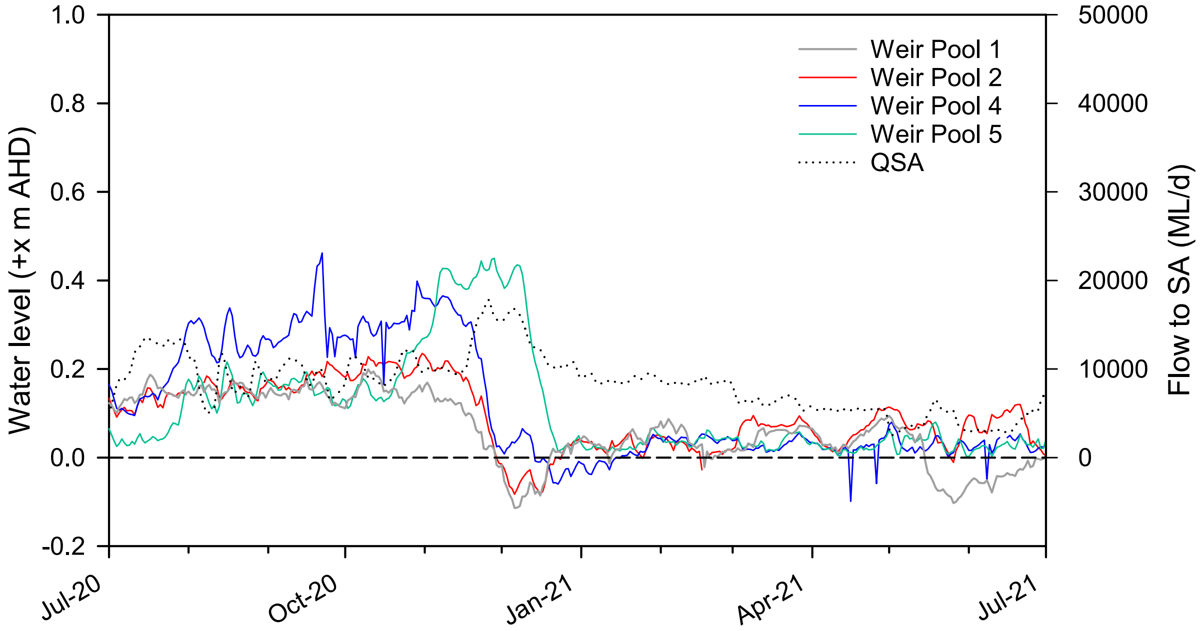

Figure B1. Water levels in the Lock 1, 2, 4 and 5 weir pools in 2020-21, showing weir pool manipulations (DEW). Water levels are measured at Lock 1 US (A4260902) + 3.2 m AHD, Lock 2 US (A4260518) + 6.1 m AHD, Lock 4 US (A4260514) +13.2 m AHD and Lock 5 US (A4260512) + 16.3 m AHD.

Katarapko and Pike floodplain regulator operation

The first operation of the Pike and Katarapko floodplain regulators occurred in 2020-21. Water levels in the Pike anabranch were raised 0.7 m above normal level in conjunction with the raising of Weir Pool 5, which commenced in mid-September 2020 (Figure B2; Table A1). Water levels were held at a maximum for 19 days during late November–early December before returning to normal levels by end December 2020. Similarly, in conjunction with the operation of Lock 4, water levels in Katarapko creek were raised 2.8 m above normal level and held for 6 days during late November–early December, before returning to normal levels by late December 2020 (Figure B2; Table A1).

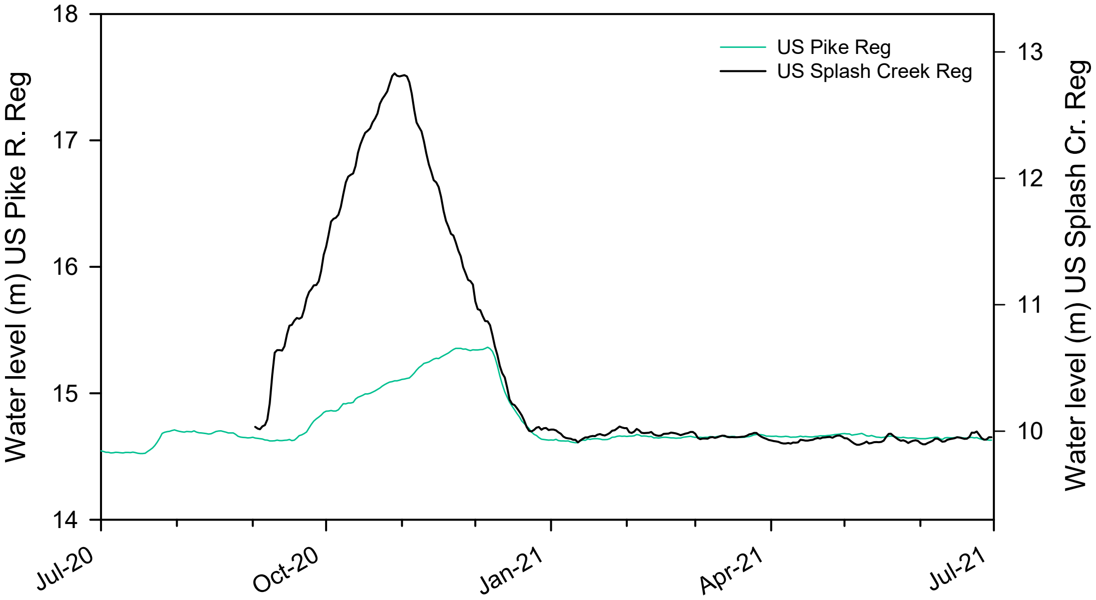

Figure B2. Water levels upstream of the Pike River regulator (A4261053) and the Splash Creek Regulator (A4261790) in 2020-21, showing regulator operations (DEW).

Watering and management activities outside of the Lower Murray

Other watering events and management actions

During 2020-21, environmental water was delivered to the LMR channel, primarily as deliveries from return flows through coordinated watering events across the southern connected Basin, to achieve multi-site environmental outcomes. The major upstream watering events that were supported by environmental water and may be relevant to the evaluation in this report are summarised in Table B1.

Table B1. Details for upstream watering events and management actions supported by environmental water (eWater) in 2020-21 (source: CEWO). CEW = Commonwealth environmental water, IVT = Inter-Valley Transfer, TLM = The Living Murray, VEWH = Victorian Environmental Water Holder, RMIF = River Murray Increased Flows.

Event	Event description and timing	Supporting eWater
'Southern Spring Flow'	• Spring/summer large fresh targeting 12–15,000 ML/d for a minimum of 10 days, for faster flowing fish habitat, improved groundwater and surface water quality, increased golden and silver perch spawning response and aquatic productivity. • Timing of the Murray fresh/overbank pulse (October–December) was coordinated with the Goulburn and Murrumbidgee rivers as they main opportunity to provide for a system-scale pulse. Increased magnitude and duration of this event would provide improved outcomes; however, this would likely only be possible through high unregulated flow contributions from upstream tributaries including the Baaka (Lower-Darling). • A combination of coordinated return flows from the Murray, Murrumbidgee (TLM) and Goulburn an order at the SA border, helped to shape the flows in the Lower Murray. As a result, a flow duration of >15,000 ML/d for 3 weeks was achieved at the SA border with a peak just under 18,000 ML/d. • The peak of the flow at the SA border was reduced by increased operational transfers from Hume and IVT delivery in the Goulburn (both of which reduced the amount of environmental water and subsequent return flows). Expert advice indicates that maximising both the height and duration of the peak flow into SA (to expand the amount of lotic habitat) will likely increase the potential for successful recruitment of native perch. • To complement returns flows, maintain the peak and slow recession of the pulse, an additional 40 GL was ordered at the SA border and delivered during early December. This water was originally planned to be delivered later as part of the summer/autumn direct trade but was brought forward to coincide with the back part of the spring pulse. The earlier release specifically aimed to support perch spawning activity in the SA Murray detected by the Flow-MER program.	CEWO, TLM, VEWH and RMIF

Event	Event description and timing	Supporting eWater
Barmah-Millewa Forest regulator operation	• The higher river levels from the spring watering action inundated around 25% of Barmah-Millewa Forest before returning to the Murray River. These flows were timed to coordinate with flows from the Goulburn, Murrumbidgee and Baaka (Lower-Darling) to create a flow pulse along the Murray River from Yarrawonga the Coorong in South Australia. • Deliveries to the Barmah-Millewa forest (between August and December) were provided via various combinations of translucent regulator delivery, followed by overbank flows, then a return to translucent regulators with a total 31,834 ML delivered. This volume includes a small additional in-channel flow that was delivered to Barmah Forest between December and February to support waterbird breeding. • On 14 August 2020 environmental water holders decided to open regulators on both sides of the Barmah-Millewa forest as part of the planned in-channel open regulator strategy. Regulators were opened before flows exceeded 5,000 ML/d downstream of Yarrawonga to avoid delivery of a potentially damaging surge of water entering the creek network within the forest. • Regulators remained open on both sides of the river till 23 December 2020 to allow native fish to exit the forest and return to the Murray. Millewa Forest (NSW) regulators were closed on 23 December 2020 while some Barmah Forest (Victoria) regulators remained open until 22 February 2021 to allow small, extended deliveries to the Boals Deadwoods wetland to support colonial waterbird breeding – mainly white and straw-necked Ibis.	VEWH, TLM and RMIF

Event	Event description and timing	Supporting eWater
Goulburn spring fresh	- Wet conditions from May 2020 continued into the new water year with high natural flows negating the need for a winter fresh and e-water used to extend a natural flow in October to deliver a spring fresh. - Natural high flows at Murchison continued from June 2020 until 7 September 2020. To slow the recession of the natural high flow, e-water was released from Murchison commencing on 8 Sept 2020. - A series of watering actions in the Goulburn River delivered over 250 GL of environmental water from September to November (at McCoys Bridge). At McCoys the peak of the fresh was 9,768 ML/day on 14 October. Additional environmental water was delivered to maintain increased baseflows for the Goulburn River both before and after the spring flows. - IVT flows commenced on 14 Nov 2020, contributing water to the spring fresh together with CEW, VEWH and TLM. - A combination of flow constraints and the delivery of Goulburn IVT (14 Nov 2020–27 May 2021) displaced environmental water from the hydrograph. Both of these issues resulted in a continued reduction of flows through the Goulburn, but also into the downstream Murray and across the SA border.	CEWO, VEWH, TLM and IVT
Lower Darling/Baaka channel	- Through the first half of 2021, additional inflows (with protection of inflows in the northern NSW basin via new 'first flush' rules) resulted in the Menindee Lakes filling to over 1,000 gigalitres total storage (~63%), including inflows to the bottom lakes (Menindee and Cawndilla) for the first time since 2016-17. The increase in water availability provided an opportunity to deliver elevated baseflows to provide additional food resources and habitat for growing young fish that were spawned during the spring event. - Commonwealth and TLM environmental allocations were used to increase minimum baseflows from 200 ML/day to 500 ML/day from 1 May to 30 June 2021 (and into 2021-22). - As Menindee Lakes storage had risen above 640 GL during autumn and returned to MDBA control, the MDBA was able to call on releases from Menindee Lakes for operational purposes in the Murray River. Environmental releases were paused during late May/mid-June while operational releases were made by MDBA. - Environmental releases resumed once MDBA's order was complete. - The May to June 2021 watering action was expected to: o inundate more of the river channel, providing increased food and habitat for growing young fish. o support the connection between the north and south of the Basin by ensuring some of the increased flows coming down the Barwon-Darling River make it all the way to the Murray River.	CEW and TLM

APPENDIX C: DEW EVALUATION QUESTIONS

Table C1. DEW short-term (one-year) and long-term (five-year) evaluation questions for CEWO LTIM/MER indicators. Evaluation questions are based on ecological targets from the Long-Term Environmental Watering Plan (LTWP) for the South Australian Murray River and Basin Plan Matter 8 report. DEW evaluation questions serve as 'additional' questions as there may be some CEWO questions that are also relevant to DEW's targets from the LTWP and/or the Matter 8 report. CEW = Commonwealth environmental water; eWater = environmental water.

Contribution (to what extent CEW contributed towards the outcome, with the significance of the outcome considered):

⬜ Unknown 🟥 Negative ⬜ None/negligible 🟩 Minor 🟩 Moderate 🟦 Substantial

Hydrology (Channel) and Hydraulic Regime (modelling)

DEW evaluation questions	**Outcomes of CEW delivery (2014-15–2020-21)**						
	2014-15	**2015-16**	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**
To what extent did CEW contribute to meeting the EWRs (all metrics) for the Lower Murray Channel?	**0/0**	**1/0**	**9/9**	**1/0**	**0/0**	**0/0**	**1 EWR met with CEW/0 EWR met without CEW**
	The 10,000 ML/d for 60 days Environmental Watering Requirement (EWR) was met in 2020-21 if the variability component of the EWR is taken into account. Without CEW contributions this EWR would not have been met. Total number of EWRs is 7 for the channel + 5 floodplain = 12 (DEWNR 2015).						
To what extent did CEW contribute to meeting the expected outcome for velocity in the Lower Murray? *The majority of lower third weir pools will have median cross-sectional velocities of >0.3 m/s for at least 60 consecutive days between September–March.*	**0/0**	**0/0**	**1/1**	**0/0**	**0/0**	**0/0**	**0 EWR met with CEW/0 EWR met without CEW**
	Assuming significant weir pool manipulations are not occurring, analysis of velocity results and expert elicitation has identified that this evaluation question is met by the 20,000 ML/d for 60 days EWR. This EWR was not met in 2020-21.						

Stream Metabolism and Water Quality

DEW evaluation questions	**Outcomes of CEW delivery (2014-15–2020-21)**						
	2014-15	**2015-16**	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**
To what extent did CEW contribute to managing the concentrations of cyanobacteria?	As the concentrations of cyanobacteria were not measured in the MER Project, the extent to which CEW contributed to managing them is unknown.						
To what extent did CEW contribute to open-water productivity in the Lower Murray?*	1	2	0	2	1.5	1	2.5
	Increased flows generally reduced the volumetric rate of primary production but increased the cross-sectional rate. This increased the overall "carrying capacity" of the river, although the implications of changes in the ratios of these two measures are unknown. At the LMR sites, the percentage increases in cross-sectional GPP due to eWater were negligible due to the largely stable water levels set by weirs. A substantial contribution was considered an increase in cross-sectional GPP of 20% or greater, moderate 11–19%, minor 5–10%, negligible <5%.						
To what extent did CEW contribute to maintaining dissolved oxygen levels above 50% saturation throughout the water column at all times in the Lower Murray?*	0	52	22	51	26	30	79
	CEW decreased the likelihood of low DO by increasing water mixing and oxygen exchange at the surface. This was assessed as the extra days per year with water velocities > 0.18 m/s due to CEW. A substantial contribution was considered greater than 30 days, moderate 15–30 days, minor 7–14 days and negligible < 7days.						

*Refer to the evaluation in Section 2.2 for details.

Littoral Vegetation Diversity and Productivity

DEW evaluation questions	Outcomes of CEW delivery	
	2019-20	**2020-21**
To what extent did CEW (and other environmental water) contribute to littoral understorey vegetation diversity and productivity?	CEW delivery increased native plant species diversity by 40–100% across all reaches in the LMR. This was evidenced by consistently higher native species richness in inundated zones in each reach compared to non-inundated areas; higher native species richness (albeit lower than inundated zones) on the edge of the inundation footprint, where there was increased soil moisture due to capillary action, compared to non-inundated zones (where soil moisture was not increased) was also observed. Environmental water delivery in the form of a spring pulse produced conditions suitable for the recruitment of specialised riparian species that are adapted to fluctuating water levels and do not inhabit terrestrial or truly aquatic habitats, increasing plant functional diversity. There were no clear patterns of above ground biomass with regards to environmental water delivery; therefore, the influence of environmental water delivery on understorey productivity is inconclusive.	CEW delivery increased native plant species diversity by 42–82% across all reaches in the LMR. This was evidenced by consistently higher native species richness in inundated zones in each reach compared to non-inundated areas. Environmental water delivery in the form of a spring pulse produced conditions suitable for the recruitment of specialised riparian species that are adapted to fluctuating water levels and do not inhabit terrestrial or truly aquatic habitats, increasing plant functional diversity. Environmental water delivery increased above-ground biomass by delivery of increased soil moisture in the root zone at the edge of the inundation footprint. Increased above-ground biomass was also observed in inundated areas across all reaches and at pool level downstream of Locks 1 and 4, compared to areas not inundated.

Micro-invertebrate Assemblage

DEW evaluation questions	Outcomes of CEW delivery (2014-15–2020-21)						
	2014-15	**2015-16**	**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**
To what extent did CEW contribute to increased dispersal of organisms between river and wetlands in the Lower Murray?	12	42	-1	49	NA	26	-27
	eWater increased the density of taxa dependent upon lateral connectivity by an average of 26% across all years (with CEW accounting for 97% of this). The significance of the contribution was classified as none-negligible if the contribution was 0–9%, minor = 10–19%, moderate =20–29% and substantial = ≥30%. Refer to the evaluation in Section 2.5 for details.						

Fish (all indicators)

Fish data have been consolidated to evaluate a number of fish targets of DEW's LTWP. These questions and answers do not relate to evaluation of flow or Commonwealth environmental water. Furthermore, the LTIM Fish monitoring program is not designed to determine what is facilitating changes in population dynamics of fish species for DEW's LTWP evaluation questions, e.g. spawning and recruitment of freshwater catfish or common carp. NA = not applicable.

Outcome: 🟥 = negative; 🟩 = positive; ▨ = unable to be detected.

DEW evaluation questions	**Answers to evaluation questions (2015 to 2021)**						
	15	**16**	**17**	**18**	**19**	**20**	**21**
Did the population age structure of Murray cod include recent recruits (i.e. <300 mm TL), sub-adults (300–600 mm) and adults (>600 mm) in the Lower Murray?	X	√	√	√	√	√	√ (Figure 57)
Did the length-frequency distribution for Murray cod indicate a large recruitment event, demonstrated by a YOY cohort representing >50% of the population from the Lower Murray?	X	X	X	X	√	√	X (7%, Figure 57)
Did the abundance of Murray cod in the Gorge zone increase by ≥20% over a 5-year period?	NA	NA	NA	NA	√	√	√ (56%, Figure 60)
Did the population age structure of golden perch include adults (age ≥4+) and sub-adults (age 1+–3+)?	√	√	√	√	√	√	√ (Figure 49)
Was there a large recruitment event of golden perch, demonstrated by a YOY cohort representing >30% of the population from the Lower Murray.	X	X	X	X	X	X	X (Figure 49)
Did the abundance of golden perch in the Gorge zone increase by >30% over a 5-year period?	NA	NA	NA	NA	X	X	X (9%, Figure 60)
Did the abundance of silver perch in the Gorge zone increase by >30% over a 5-year period?*	NA	NA	NA	NA	X	X	√ (100%, Figure 60)
Did the abundance of freshwater catfish in the Gorge zone increase by ≥30% over a 5-year period?*	NA	NA	NA	NA	X	X	X (-67%, Figure 60)

DEW evaluation questions	Answers to evaluation questions (2015 to 2021)						
	15	16	17	18	19	20	21
Did the length-frequency distribution for bony herring** in the Gorge zone include size classes representing YOY?	NA	√	√	√	√	√	√
Did the length-frequency distribution for Murray rainbowfish and carp gudgeon, include size classes representing YOY in the Gorge zone?	√	√	√	√	√	√	√
Did the relative abundance of common carp in the Gorge zone increase during the current year, relative to the previous year, in the absence of an increase in the relative abundances of flow-dependent native species decreased?***	NA	√	√	X	X	X	X (Figure 60)
Did the estimated biomass of common carp in the Gorge zone increase during the current year, relative to the previous year, in the absence of an increase in the estimated biomass of flow-dependent native species decreased?***	NA	√	X	X	X	X	X

* These results should be interpreted with caution, given the large error estimate.

** Bony herring were not assessed as a target species during 2014.

*** Since the 2019-20 report, the calculations used to answer the evaluation question were changed from an approach using ratios to better reflect the ecological target of the LTWP. To remove sampling season bias, only sites sampled during autumn 2017 were used in comparisons against 2018. Common carp were not weighed as part of the Fish (channel) sampling, so biomass was estimated by converting fork lengths to weights based on a FL–mass equation in Vilizzi and Walker (1999).

APPENDIX D: SUPPLEMENTARY INFORMATION FOR INDICATORS

Matter Transport and Coorong Habitat

The barrage flow and salinity models have been updated during the Healthy Coorong Healthy Basin (HCHB) project in 2021. This produced the changes in the flow and salt fluxes through the barrages. The plots comparing the barrage flow and salt fluxes in the previous report (Ye *et al.* 2021) and this report are below (Figures D1–D4). It is evident that the new model has higher barrage flow rates and salinity. In simulations in this report, we used the final compiled barrage flow data (sent by SA DEW in July 2021) which included the Coorong Hydrodynamic Model (CHM) barrage data and Aquarius Web Portal (AQWP, i.e. Water Data SA) barrage calculations. Given that the AQWP barrage calculator data has been thoroughly validated and reported and is our "point of truth" now for all barrages data from January 2011 onwards, in this report we have agreed to adopt this data as the barrage input data from January 2011 onwards.

The salinity boundary conditions at the barrages also have been improved with more data received during the HCHB project in 2021. For example, DEW adopted a new equation to convert the conductivity to salinity therefore there is a slight change in the salinity at Ewe, Goolwa and Tauwitchere barrages; also, the Mundoo and Boundary barrages shared the same salinity as Ewe barrage in the report of 2019-20 simulations; while in this report they have been set with a new salinity at closer sites which became available in 2021.

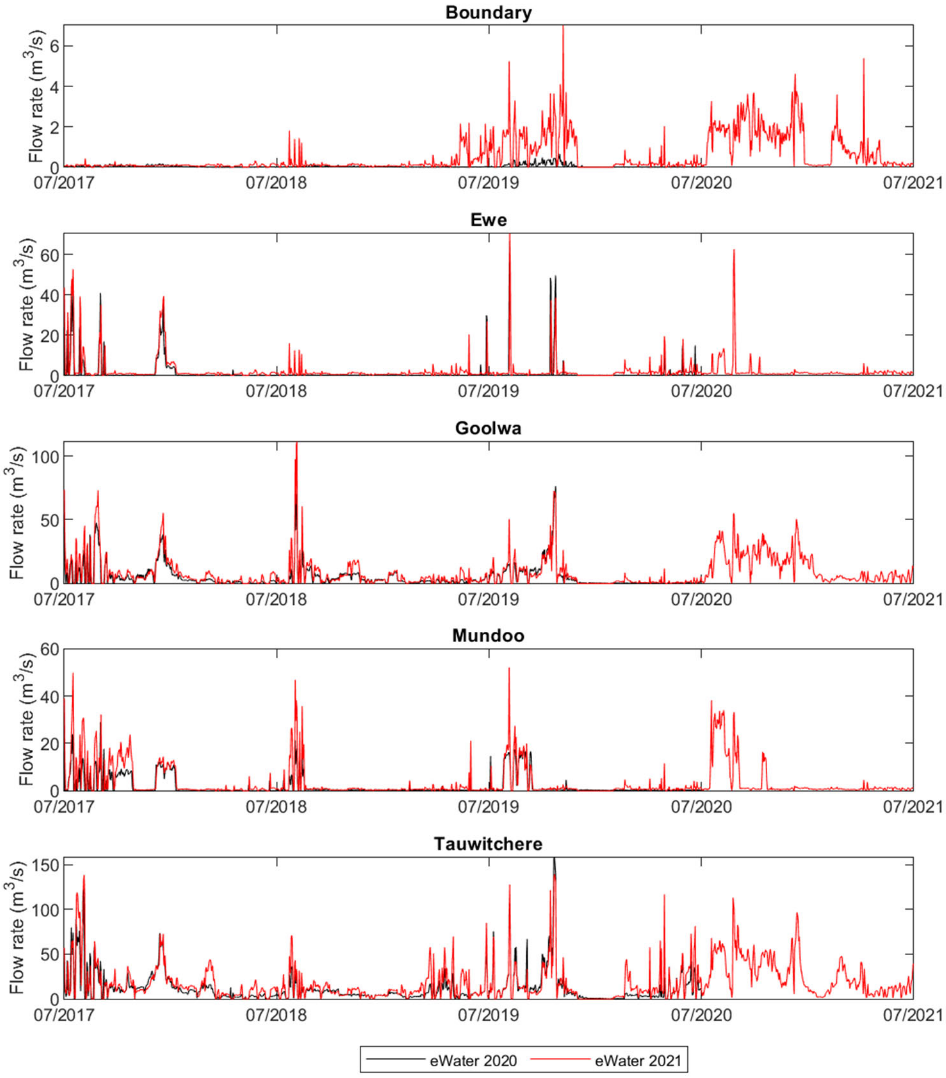

Figure D1. Barrage flow rates over each barrage with data used in previous report (Ye *et al.* 2021) and this report.

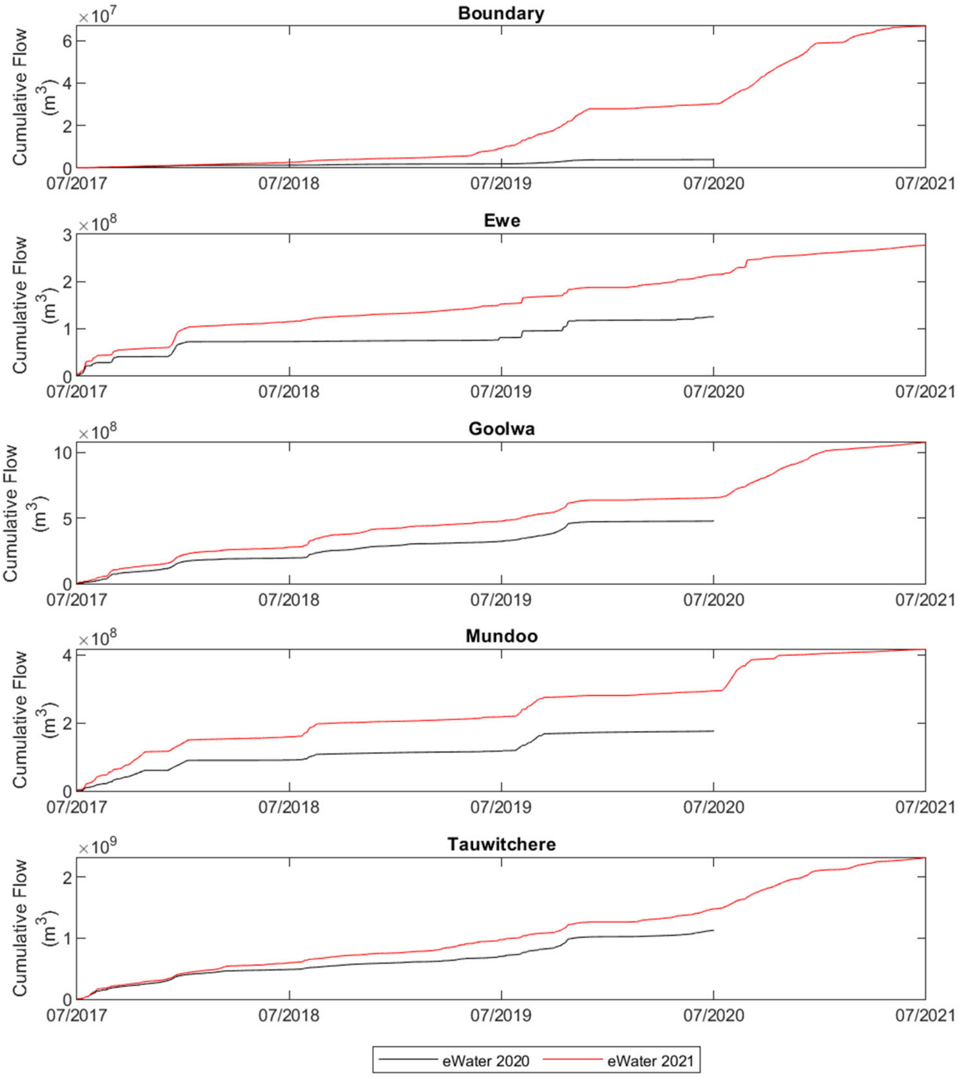

Figure D2. Cumulative flow over each barrage with data used in previous report (Ye *et al.* 2021) and this report.

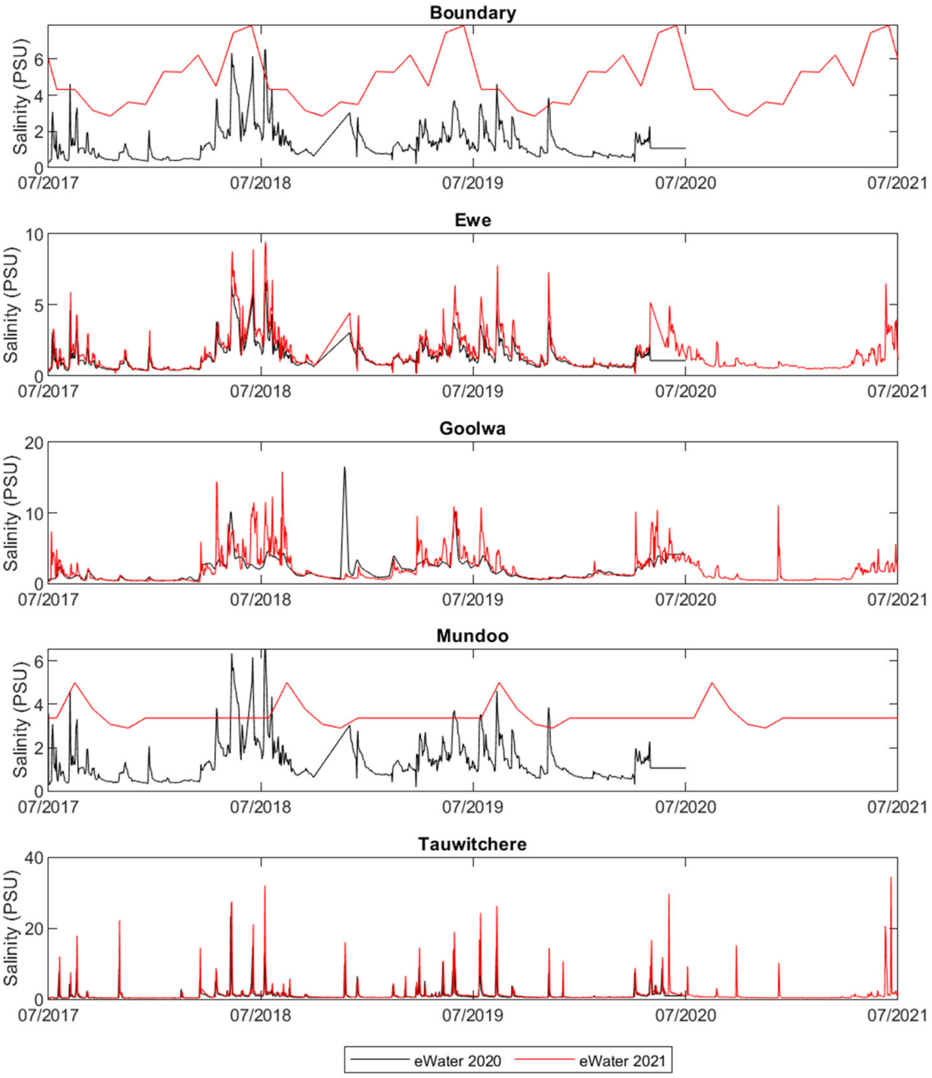

Figure D3. Salinity at each barrage with data used in previous report (Ye *et al.* 2021) and this report.

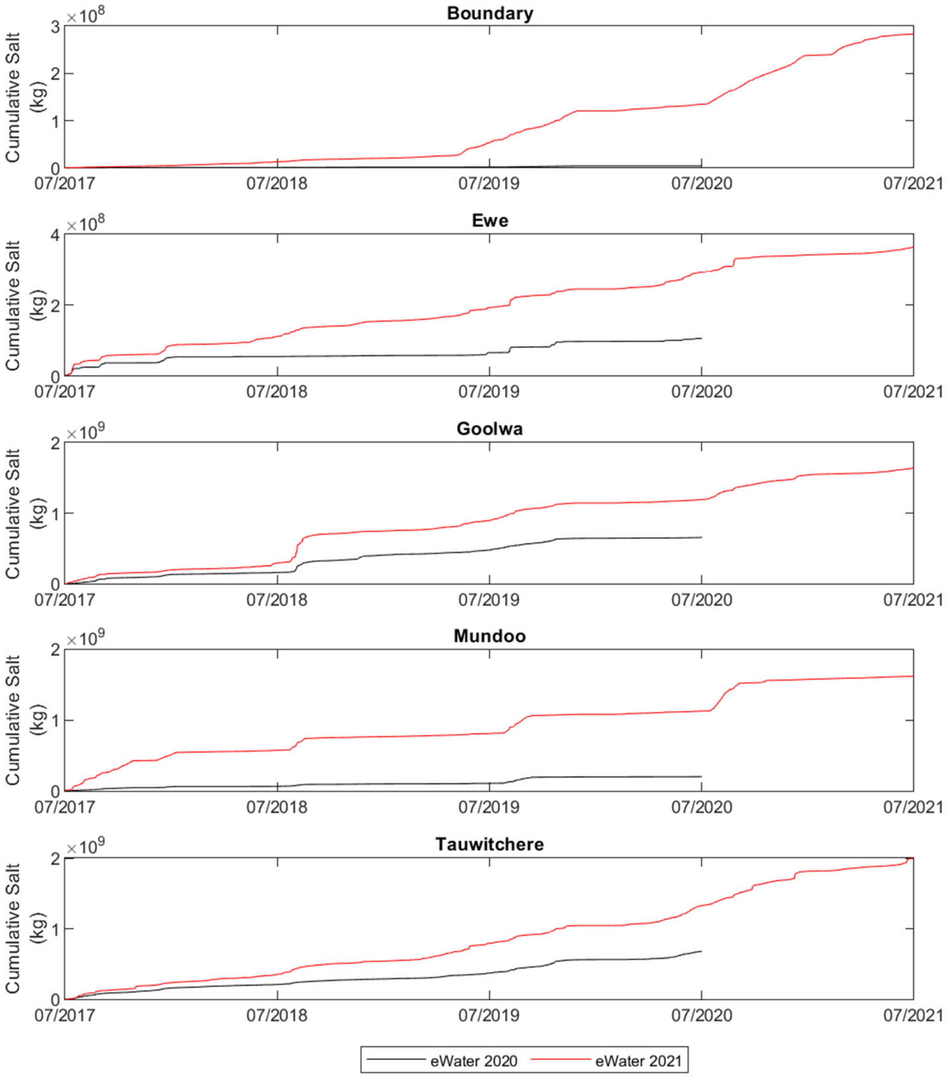

Figure D4. Cumulative salt over each barrage with data used in previous report (Ye *et al.* 2021) and this report.

Details of all salt and nutrient fluxes in each of the modelling year are included below. Predicted salinity changes in all scenarios at selected sites along the Coorong are presented below in Figure D5.

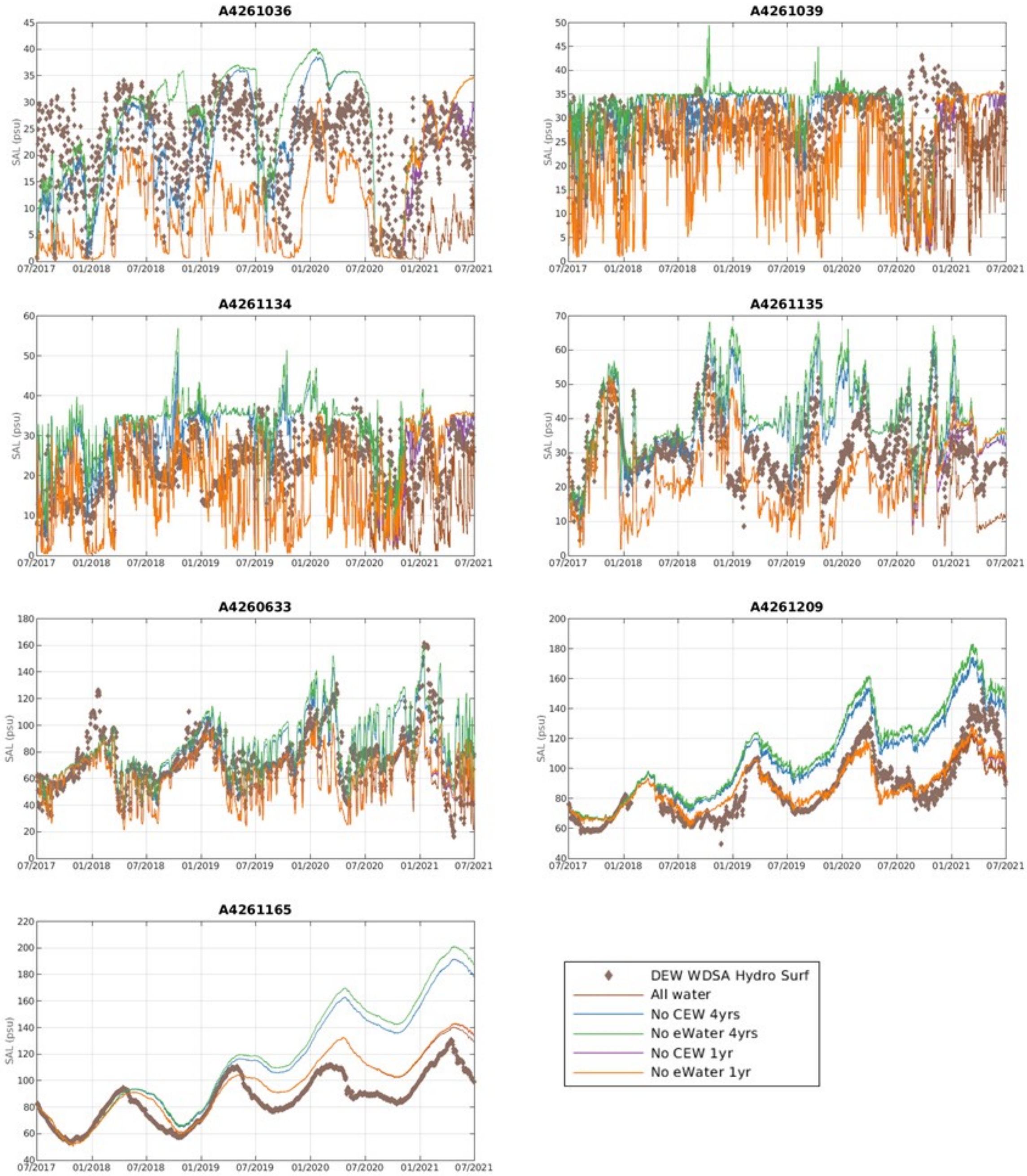

Figure D5. Comparison of measured and simulated salinity at key monitoring points within the Coorong lagoon, moving from the Murray Mouth into the South Lagoon.

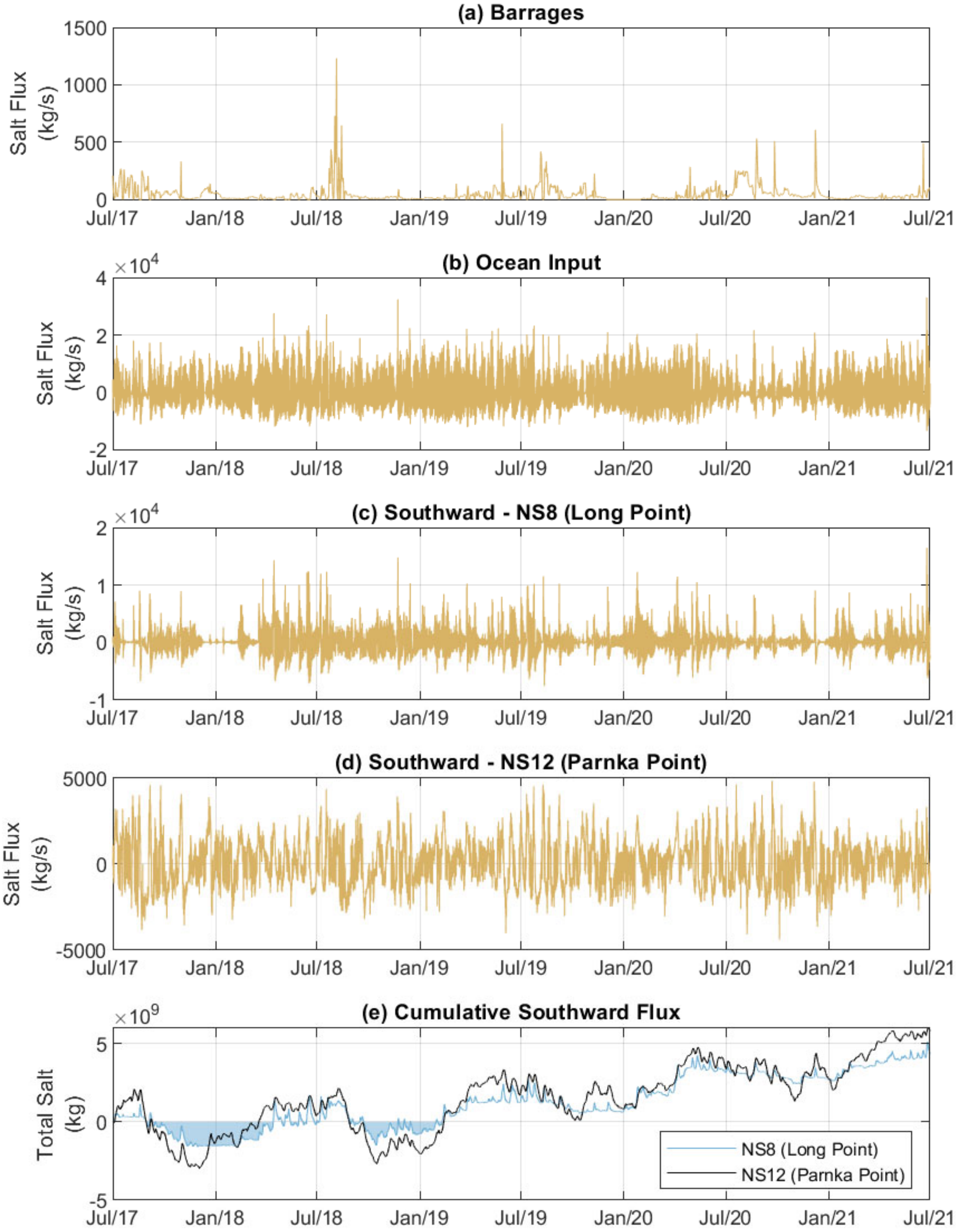

Figure D6. Detailed salt flux analysis at 4 locations in the Coorong (a-d) in the base-case scenario, which has environmental water flow through the barrages each year. Panel (e) shows the cumulative salt flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point); the shaded blue area shows salt export from the Coorong towards the Murray Mouth.

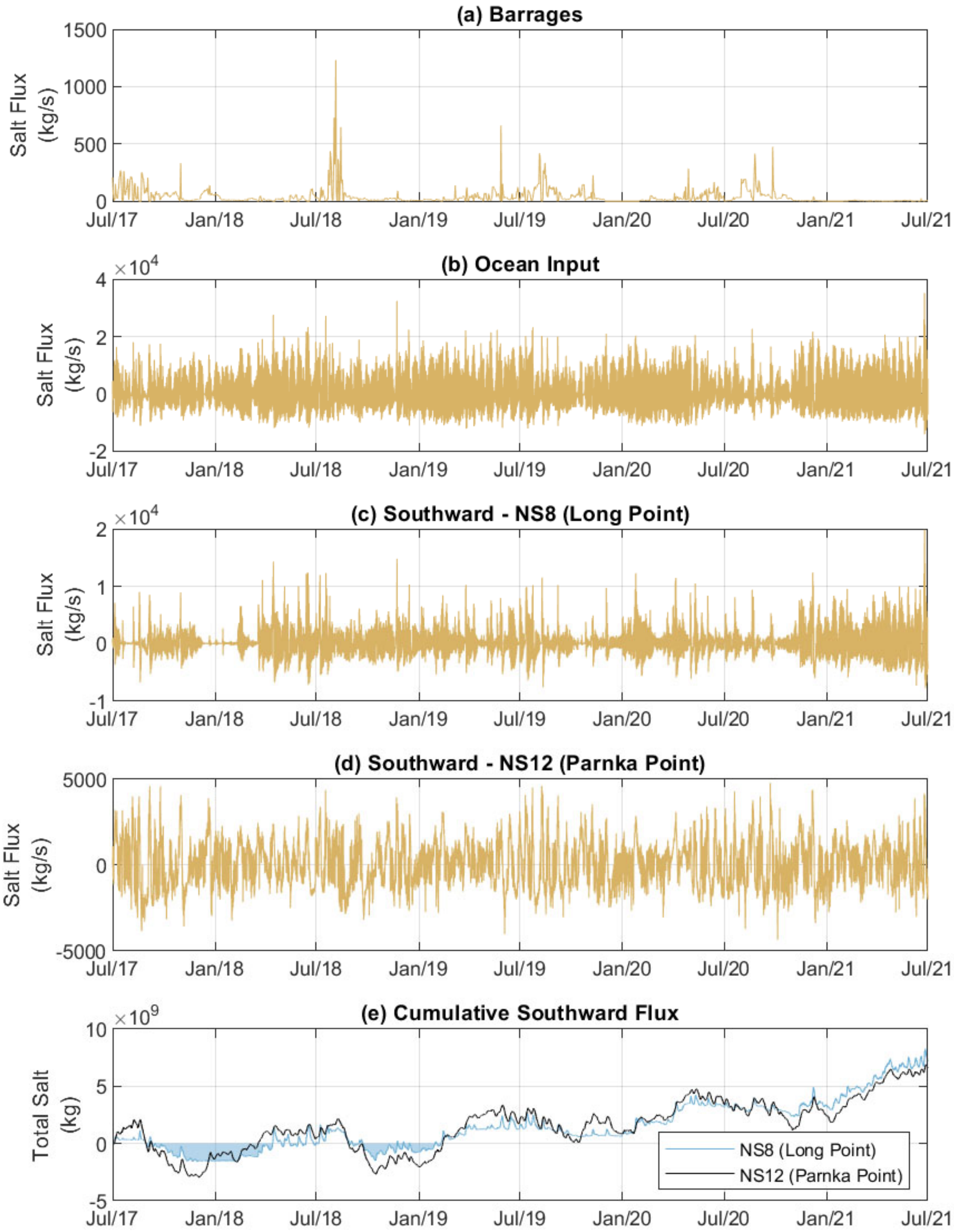

Figure D7. Detailed salt flux analysis at 4 locations in the Coorong (a-d) in the 'no CEW 1 year' scenario. Panel (e) shows the cumulative salt flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point); the shaded blue area shows salt export from the Coorong towards the Murray Mouth.

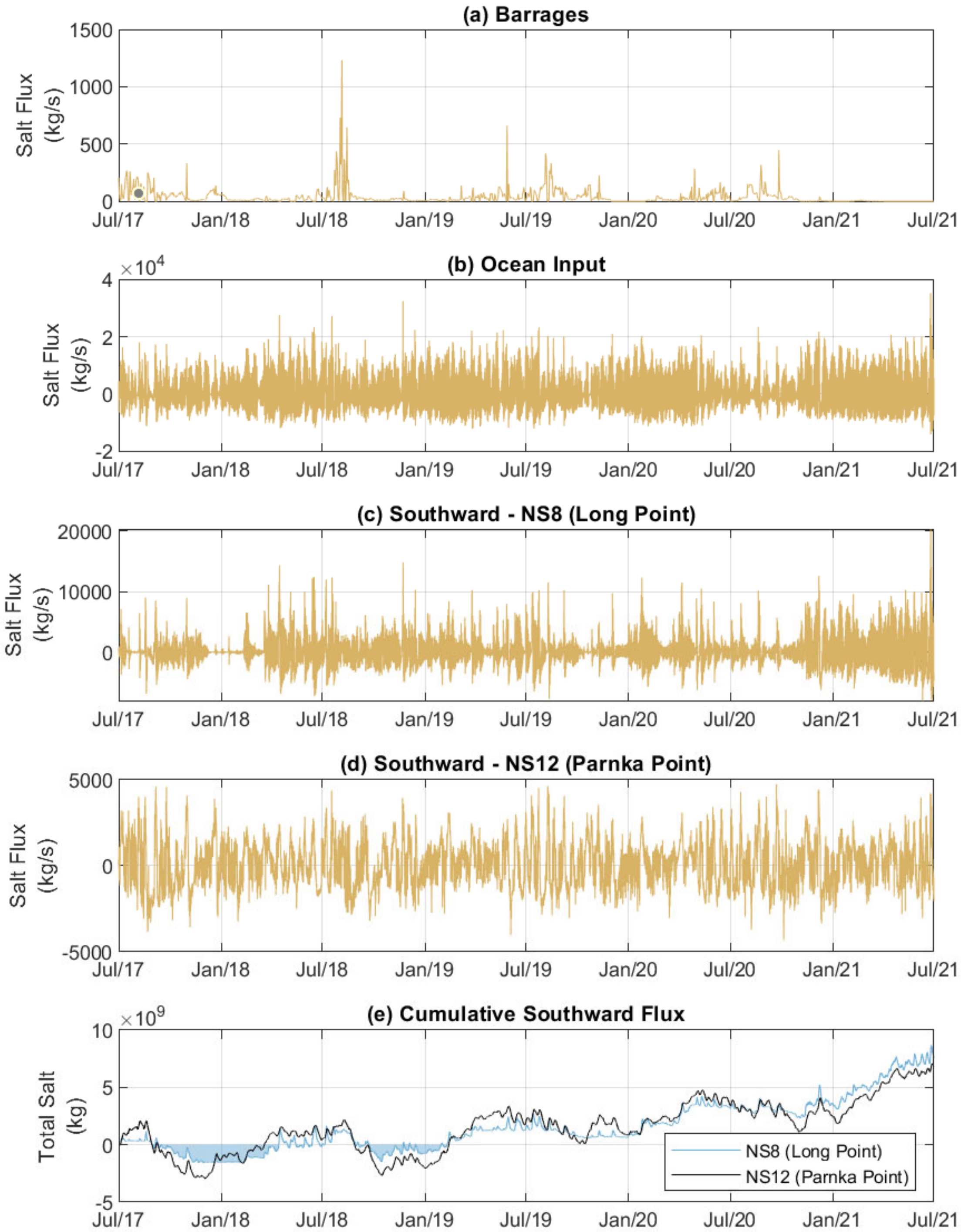

Figure D8. Detailed salt flux analysis at 4 locations in the Coorong (a-d) in the 'no eWater 1 year' scenario. Panel (e) shows the cumulative salt flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point); the shaded blue area shows salt export from the Coorong towards the Murray Mouth.

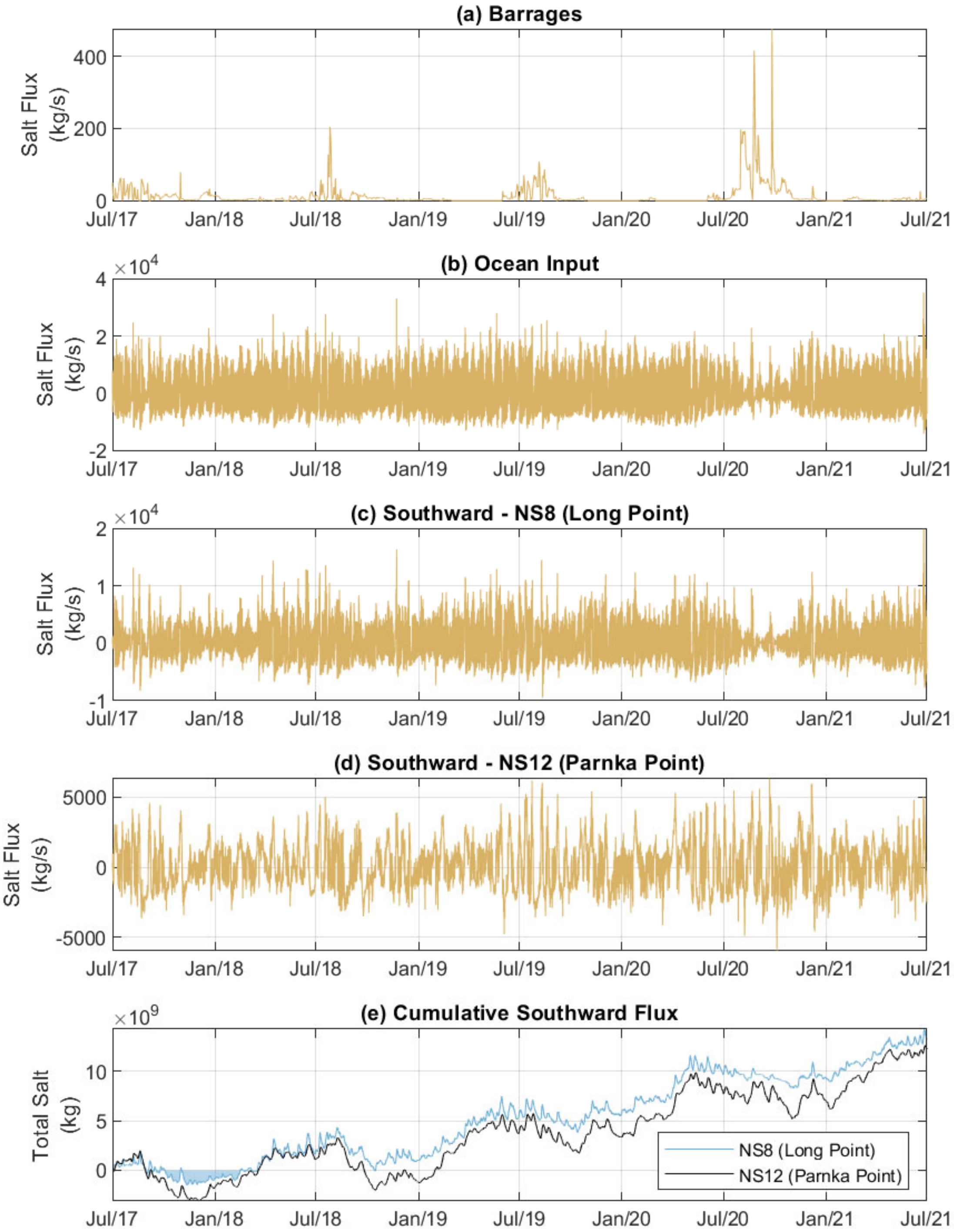

Figure D9. Detailed salt flux analysis at 4 locations in the Coorong (a-d) in the 'no CEW 4 year' scenario. Panel (e) shows the cumulative salt flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point); the shaded blue area shows salt export from the Coorong towards the Murray Mouth.

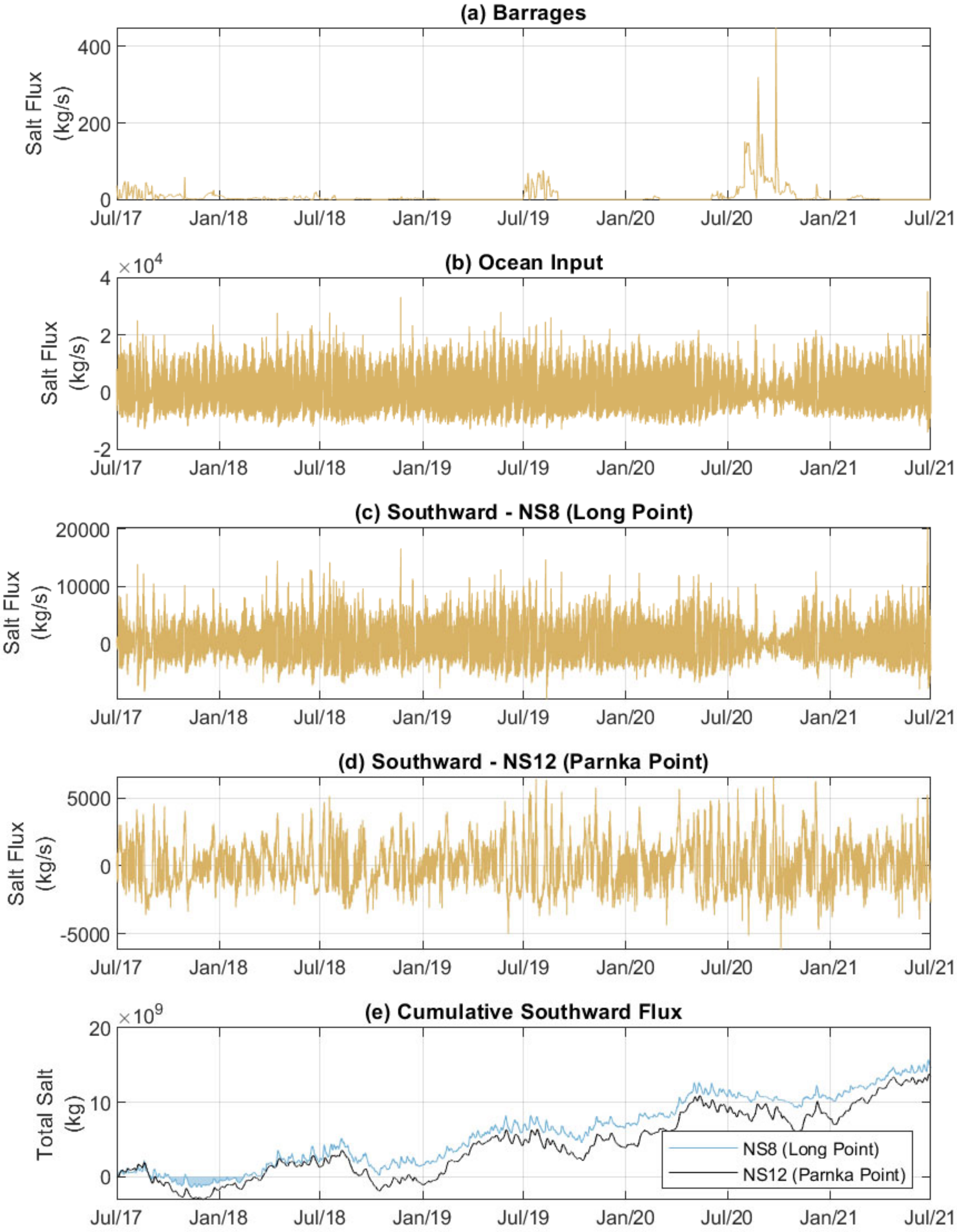

Figure D10. Detailed salt flux analysis at 4 locations in the Coorong (a-d) in the 'no eWater 4 year' scenario. Panel (e) shows the cumulative salt flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point); the shaded blue area shows salt export from the Coorong towards the Murray Mouth.

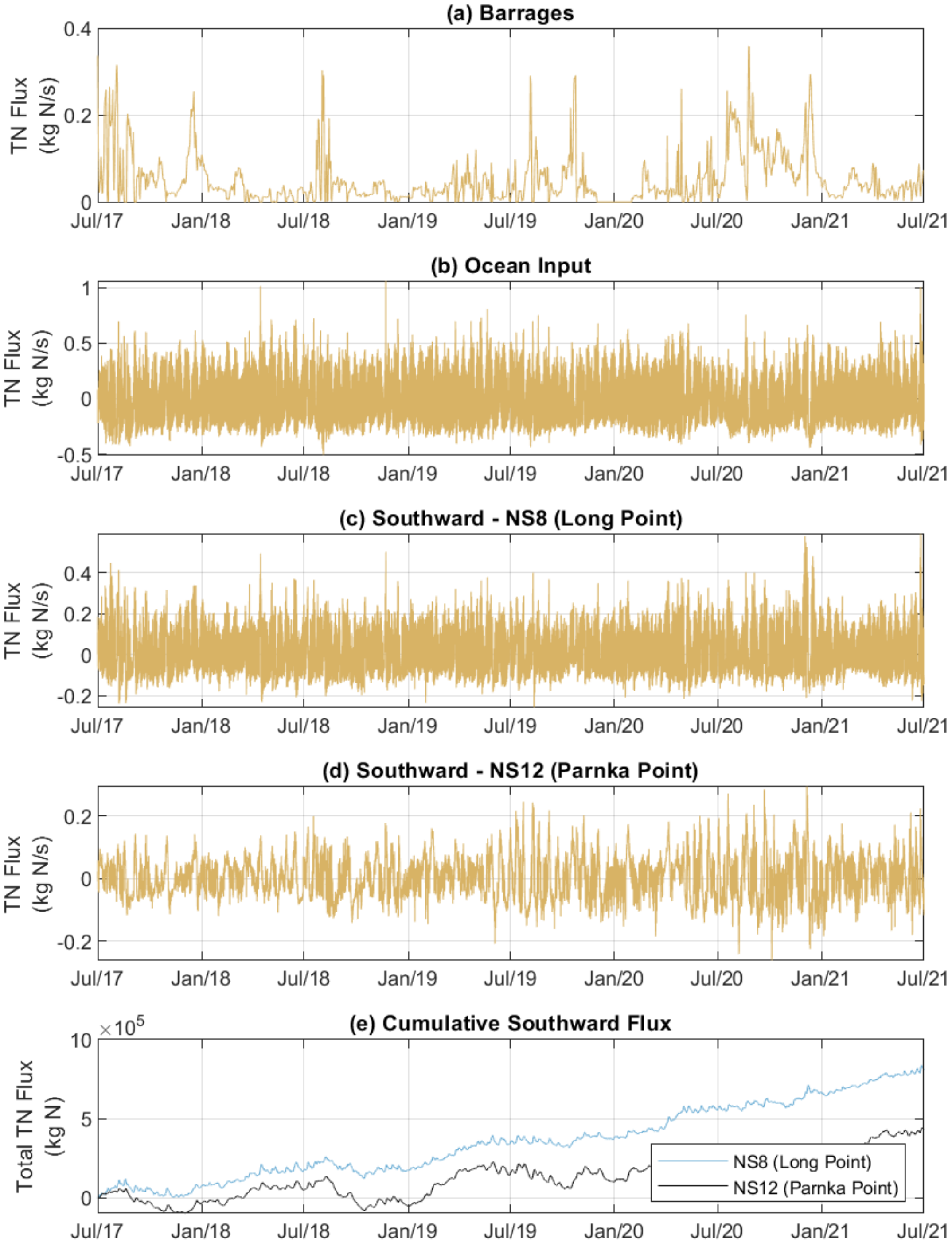

Figure D11. Detailed TN flux analysis at 4 locations in the Coorong (a-d) in the base-case scenario, which has environmental water flow through the barrages each year. Panel (e) shows the cumulative TN flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point).

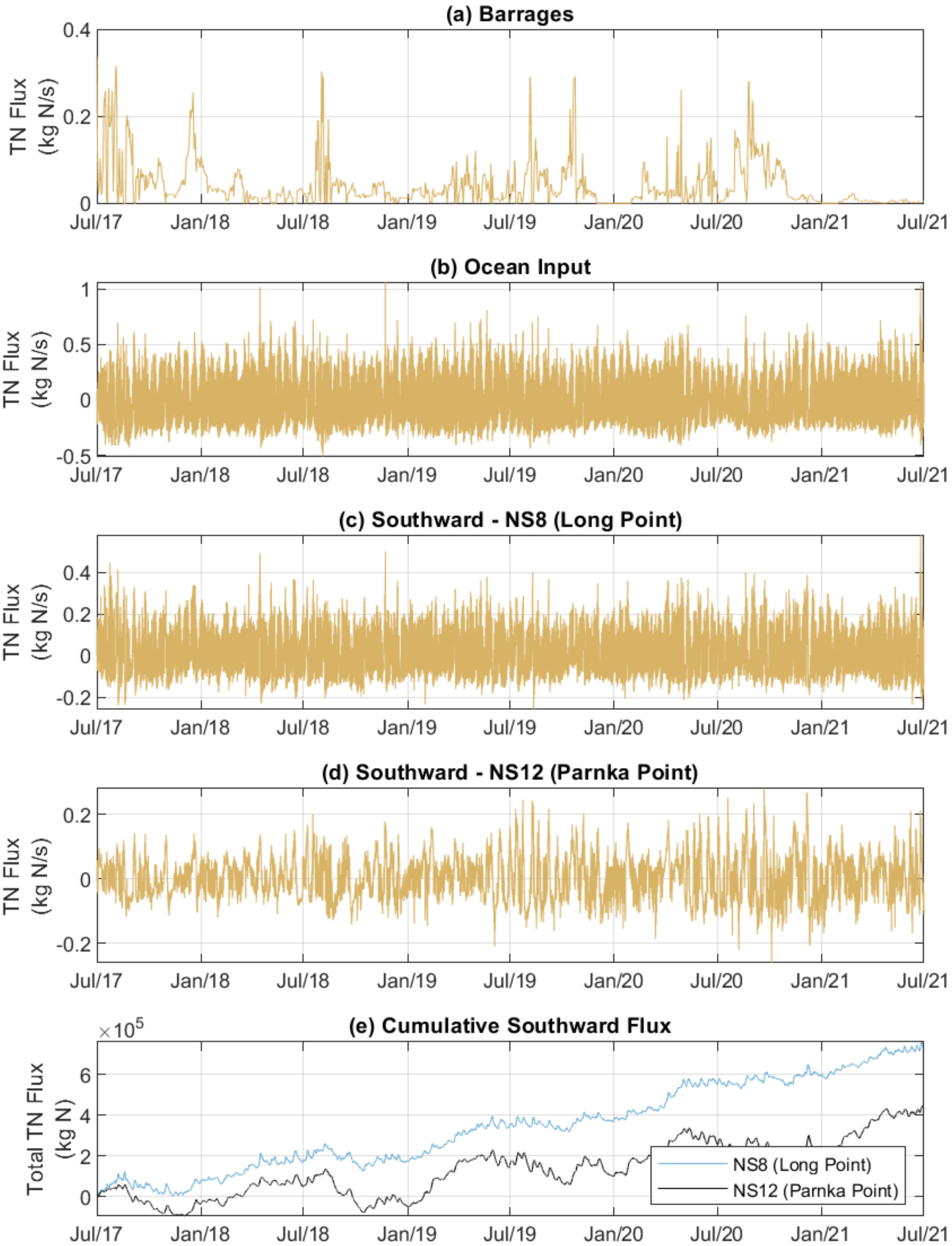

Figure D12. Detailed TN flux analysis at 4 locations in the Coorong (a-d) in the 'no CEW 1 year' scenario. Panel (e) shows the cumulative TN flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point).

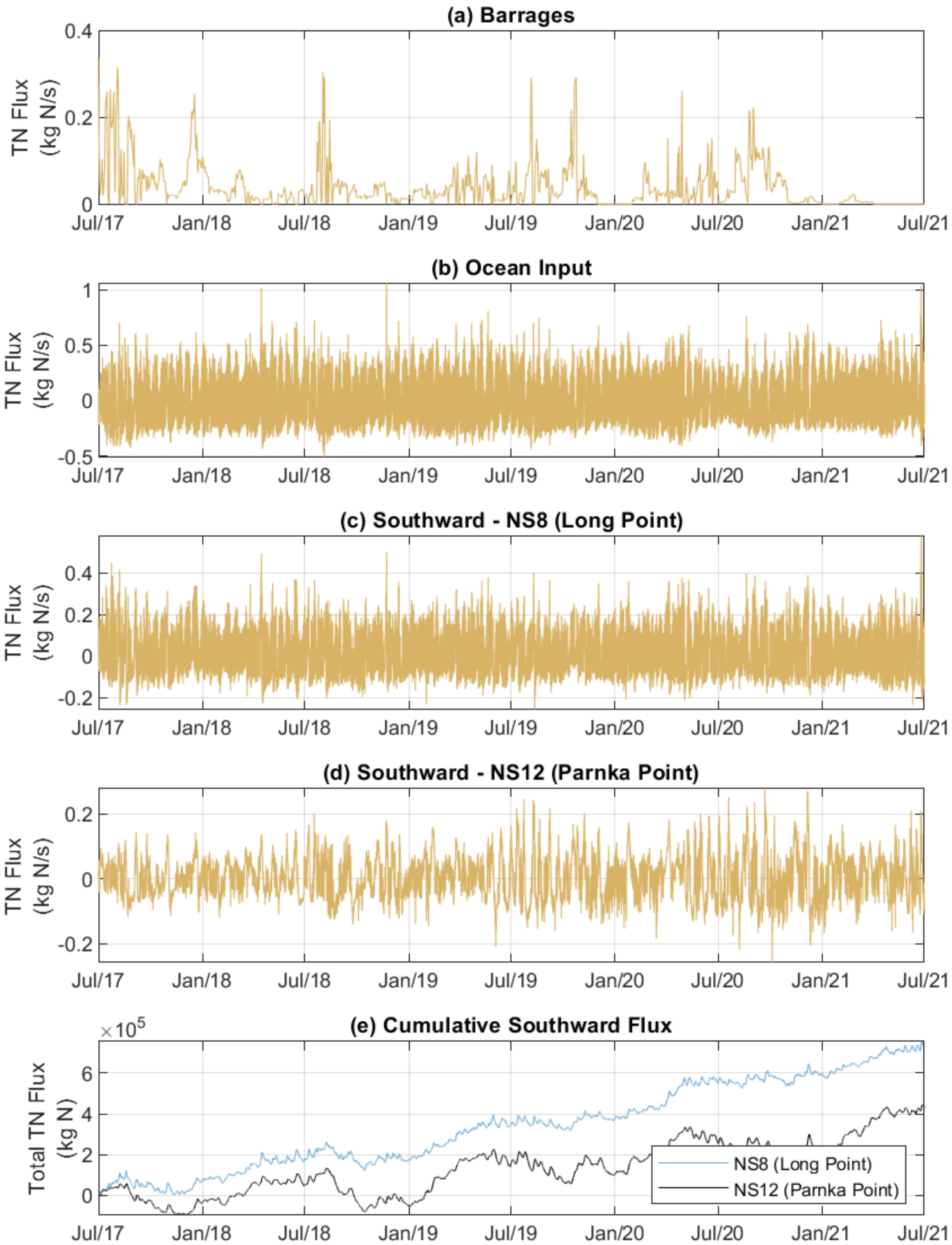

Figure D13. Detailed TN flux analysis at 4 locations in the Coorong (a-d) in the 'no eWater 1 year' scenario. Panel (e) shows the cumulative TN flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point).

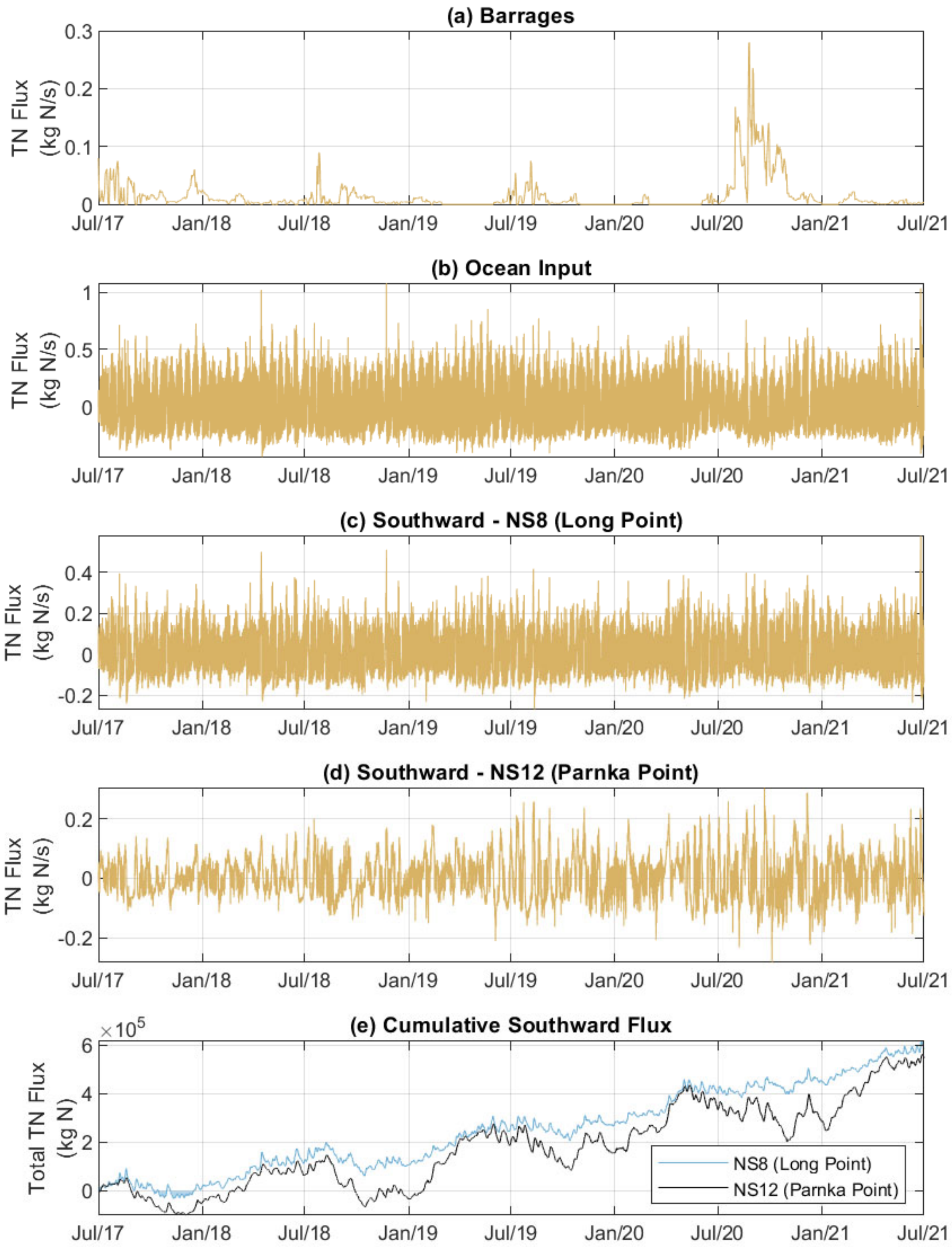

Figure D14. Detailed TN flux analysis at 4 locations in the Coorong (a-d) in the 'no CEW 4 years' scenario. Panel (e) shows the cumulative TN flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point).

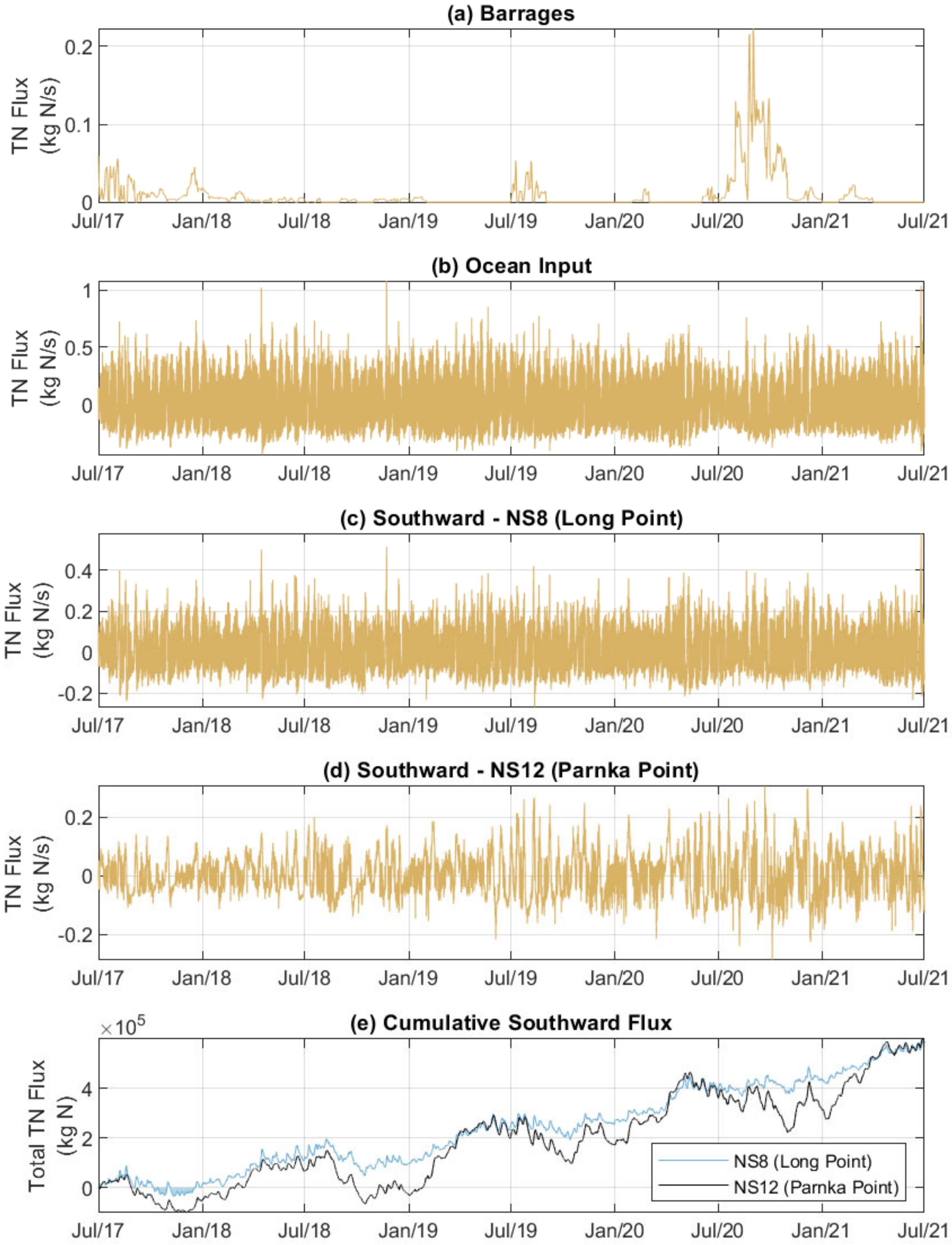

Figure D15. Detailed TN flux analysis at 4 locations in the Coorong (a-d) in the 'no eWater 4 years' scenario. Panel (e) shows the cumulative TN flux into the North Lagoon (past Long Point) and into the South Lagoon (past Parnka Point).

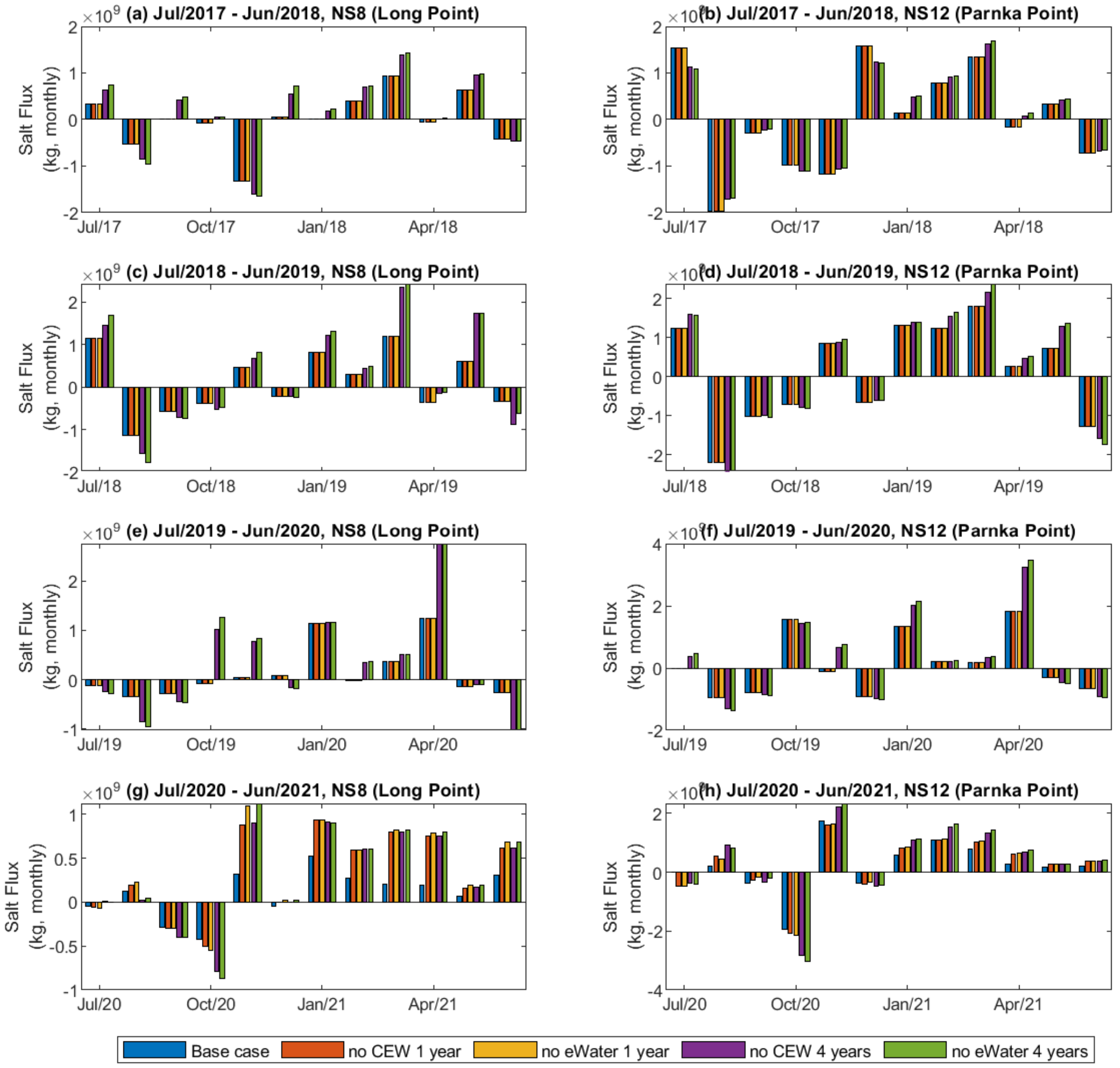

Figure D16. Monthly salt flux with and without environmental water delivery for July 2017–June 2021.

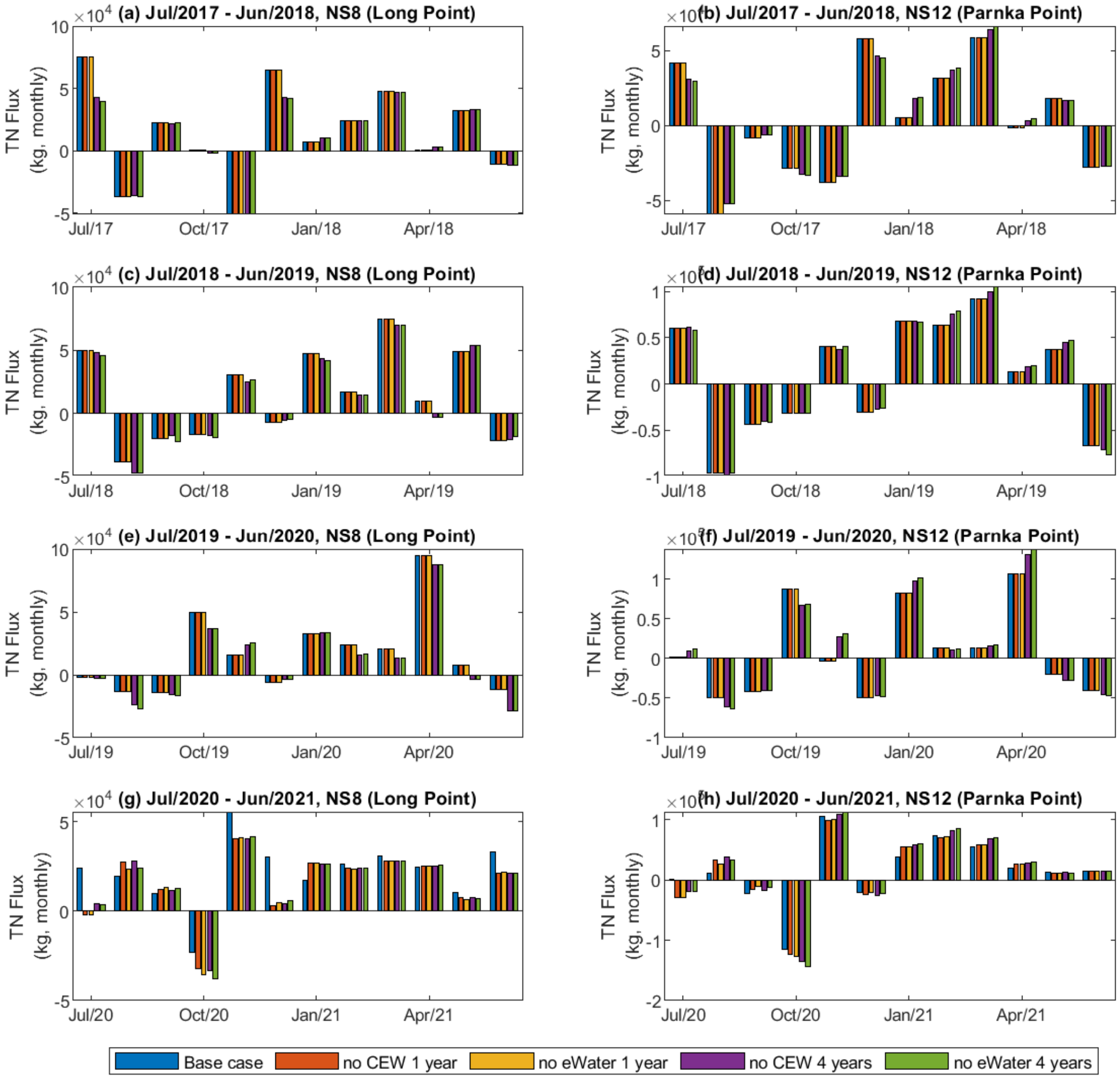

Figure D17. Monthly TN flux with and without environmental water delivery for July 2017–June 2021.

Table D1. Summary of mean salinity, nutrients, and chlorophyll *a* concentrations at the Murray Mouth, North Lagoon, and South Lagoon between July 2020 – June 2021.

Site	Scenario	Salinity (PSU)	Ammonium (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)	Organic nitrogen (mg/L)	Organic phosphorus (mg/L)	Chlorophyll *a* (ug/L)
Murray Mouth	With all water	12.6	0.035	0.020	0.005	1.163	0.974	0.073	21.2
	No CEW 4 years (07/2017 - 07/2021)	24.3	0.037	0.025	0.0021	1.253	0.981	0.063	17.7
	No eWater 4 years (07/2017 - 07/2021)	25.8	0.0362	0.024	0.0018	1.252	0.9851	0.063	17.51
	No CEW 1 year (07/2020 - 07/2021)	23.1	0.038	0.028	0.0021	1.241	0.975	0.062	17.92
	No eWater 1 year (07/2020 - 07/2021)	24.5	0.0374	0.027	0.0018	1.239	0.978	0.062	17.7
North Lagoon	With all water	18.3	0.036	0.020	0.0008	1.265	1.304	0.168	5.7
	No CEW 4 years (07/2017 - 07/2021)	38.5	0.036	0.015	0.0003	1.875	1.452	0.124	3.0
	No eWater 4 years (07/2017 - 07/2021)	41.1	0.038	0.019	0.0003	1.958	1.475	0.125	2.9
	No CEW 1 year (07/2020 - 07/2021)	30.31	0.034	0.014	0.0004	1.727	1.424	0.117	3.2
	No eWater 1 year (07/2020 - 07/2021)	32.11	0.035	0.015	0.0003	1.787	1.438	0.116	3.0
South Coorong	With all water	95.41	0.008	0.0003	0.0057	8.803	5.883	0.518	47.5
	No CEW 4 years (07/2017 - 07/2021)	135.2	0.120	0.305	0.0016	9.897	6.374	0.583	55.5
	No eWater 4 years (07/2017 - 07/2021)	142.4	0.124	0.413	0.0016	10.090	6.453	0.595	56.7
	No CEW 1 year (07/2020 - 07/2021)	98.67	0.019	0.002	0.0051	9.020	5.990	0.526	48.4
	No eWater 1 year (07/2020 - 07/2021)	99.2	0.022	0.003	0.005	9.049	6.002	0.527	48.5

Table D2. Summary of median salinity, nutrients, and chlorophyll *a* concentrations at the Murray mouth, north Coorong, and south Coorong in year July 2020 – June 2021.

Site	Scenario	Salinity (PSU)	Ammonium (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Silica (mg/L)	Organic nitrogen (mg/L)	Organic phosphorus (mg/L)	Chlorophyll *a* (µg/L)
Murray Mouth	With all water	12.7	0.027	0.019	0.003	1.116	0.963	0.061	21.2
	No CEW 4 years (07/2017 - 07/2021)	29.6	0.034	0.022	0.001	1.205	0.987	0.064	17.7
	No eWater 4 years (07/2017 - 07/2021)	30.5	0.033	0.021	0.001	1.200	0.996	0.066	17.3
	No CEW 1 year (07/2020 - 07/2021)	27.7	0.035	0.023	0.001	1.200	0.985	0.064	17.78
	No eWater 1 year (07/2020 - 07/2021)	29.5	0.033	0.023	0.001	1.193	0.994	0.066	17.5
North Coorong	With all water	18.1	0.038	0.018	0.0005	1.135	1.205	0.128	4.4
	No CEW 4 years (07/2017 - 07/2021)	37.7	0.034	0.013	0.0003	1.650	1.336	0.118	2.5
	No eWater 4 years (07/2017 - 07/2021)	39.4	0.035	0.014	0.0002	1.715	1.349	0.118	2.5
	No CEW 1 year (07/2020 - 07/2021)	32.0	0.034	0.013	0.0003	1.533	1.301	0.113	2.6
	No eWater 1 year (07/2020 - 07/2021)	33.7	0.034	0.013	0.0003	1.575	1.314	0.112	2.5
South Coorong	With all water	95.0	0.006	0.0001	0.006	8.697	5.977	0.536	44.9
	No CEW 4 years (07/2017 - 07/2021)	136.6	0.118	0.255	0.001	9.786	6.475	0.602	52.4
	No eWater 4 years (07/2017 - 07/2021)	143.9	0.122	0.362	0.001	9.994	6.555	0.615	53.5
	No CEW 1 year (07/2020 - 07/2021)	100.1	0.009	0.0002	0.005	8.926	6.107	0.543	45.5
	No eWater 1 year (07/2020 - 07/2021)	101.4	0.009	0.0002	0.005	8.9676	6.112	0.5436	45.6

Littoral Vegetation Diversity and Productivity

Table D3. GPS coordinates for the lowest elevation of each transect.

Reach	Transect	Latitude	Longitude
Lock 1	1	-34.479019	139.596311
Lock 1	2	-34.477575	139.598798
Lock 1	3	-34.446681	139.609714
Lock 1	4	-34.419635	139.613482
Lock 1	5	-34.41855	139.61395
Lock 1	6	-34.391968	139.619028
Lock 4	1	-34.361848	140.565262
Lock 4	2	-34.355854	140.576385
Lock 4	3	-34.355763	140.568069
Lock 4	4	-34.350657	140.562584
Lock 4	5	-34.343043	140.553056
Lock 4	6	-34.342492	140.554519
Lock 6	1	-34.021533	140.867416
Lock 6	2	-34.01918	140.876627
Lock 6	3	-34.016288	140.88713
Lock 6	4	-33.997601	140.879789
Lock 6	5	-33.995581	140.880611
Lock 6	6	-33.994623	140.882183

Table D4. Species list, functional classification, life history strategy, conservation status recorded in the February 2021 monitoring (state conservation status from listings in Barker *et al.* (2005) (*denotes exotic species, **denotes proclaimed pest plant in South Australia).

Species	Family	Status	Life history strategy/growth form	Functional Group
Alternanthera denticulata	Amaranthaceae	Native	Annual herb	Flood dependent
Ammannia multiflora	Lythraceae	Native	Annual herb	Flood dependent
*Apium graveolens**	Apiaceae	Exotic, Naturalised	Annual herb	Terrestrial
Atriplex	Chenoppodiaceae	Native	Perennial sub-shrub	Terrestrial
Atriplex suberecta	Chenoppodiaceae	Native	Annual herb	Flood dependent
Bolboschoenus caldwellii	Cyperaceae	Native	Perennial sedge	Emergent
Brachyscome paludicola	Asteraceae	Native	Annual herb	Flood dependent
Calotis hispidula	Asteraceae	Native	Annual herb	Flood dependent
*Centaurea calcitrapa**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
Centipeda minima	Asteraceae	Native	Annual herb	Flood dependent
Chenopodium nitrariaceum	Chenoppodiaceae	Native	Perennial shrub	Terrestrial
*Cuscuta campestris***	Convolvulaceae	Exotic, Declared Pest Plant in South Australia	Perennial parasite	Terrestrial
Cyperus difformis	Cyperaceae	Native	Perennial sedge	Amphibious
Cyperus gymnocaulos	Cyperaceae	Native	Perennial sedge	Amphibious
Disphyma crassifolium	Aizoaceae	Native	Annual herb	Terrestrial
*Dittrichia graveolens**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
Duma florulenta	Polygonaceae	Native	Perennial shrub	Amphibious
Dysphania pumilio	Chenoppodiaceae	Native	Annual herb	Flood dependent
Einadia nutans	Chenoppodiaceae	Native	Perennial sub-shrub	Terrestrial
Eleocharis acuta	Cyperaceae	Native	Perennial sedge	Emergent
Enchylaena tomentosa	Chenoppodiaceae	Native	Perennial sub-shrub	Terrestrial
*Erigeron bonariensis**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
*Erodium botrys**	Geraniaceae	Exotic, Naturalised	Annual herb	Terrestrial
Eucalyptus camaldulensis	Myrtaceae	Native	Perennial tree	Amphibious
Euchiton involucratus	Asteraceae	Native	Perennial herb	Flood dependent

Species	Family	Status	Life history strategy/growth form	Functional Group
Euphorbia drummondii	Euphorbiaceae	Native	Annual herb	Flood dependent
*Gazania rigens***	Asteraceae	Exotic, Declared Pest Plant in South Australia	Perrennial herb	Terrestrial
Glinus lotoides	Aizoaceae	Native	Annual herb	Flood dependent
Goodenia heteromera	Goodeniaceae	Native	Perennial herb	Flood dependent
*Heliotropium curassavicum**	Boraginaceae	Exotic, Naturalised	Annual herb	Terrestrial
*Heliotropium europaeum**	Boraginaceae	Exotic, Naturalised	Annual herb	Terrestrial
*Hypochaeris glabra**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
Isoetopsis graminifolia	Asteraceae	Native	Annual herb	Flood dependent
Juncus usitatus	Juncaeae	Native	Perrennial rush	Amphibious
Lachnagrostis filiformis	Poaceae	Native	Annual grass	Flood dependent
*Lactuca serriola**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
Limosella australis	Scrophulariaceae	Native	Perrennial herb	Flood dependent
Ludwigia peploides	Onagraceae	Native	Perrennial herb	Amphibious
Lythrum hyssopifolia	Lythraceae	Native	Annual herb	Flood dependent
*Medicago**	Fabaceae	Exotic, Naturalised	Annual herb	Terrestrial
Melaleuca lanceolata	Myrtaceae	Native	Perennial tree	Terrestrial
*Melilotus indicus**	Fabaceae	Exotic, Naturalised	Annual herb	Terrestrial
*Mesembryanthemum crystallinum**	Aizoaceae	Exotic, Naturalised	Annual herb	Terrestrial
Mollugo cerviana	Aizoaceae	Native	Annual herb	Flood dependent
Mukia maderaspatana	Cucurbitaceae	Native	Annual herb	Terrestrial
Myoporum montanum	Myoporaceae	Native	Perennial tree	Terrestrial
Myriophyllum verrucosum	Haloragaceae	Native	Perrennial herb	Amphibious
Nicotiana velutina	Solanaceae	Native	Perrennial herb	Terrestrial
Paspalidium jubiflorum	Poaceae	Native	Perrennial grass	Flood dependent
Paspalum distichum	Poaceae	Native	Perrennial grass	Amphibious
Persicaria lapathifolia	Polygonaceae	Native	Perrennial herb	Amphibious
Phragmites australis	Poaceae	Native	Perrennial grass	Emergent

Species	Family	Status	Life history strategy/growth form	Functional Group
Phyllanthus lacunarius	Euphorbiaceae	Native	Annual herb	Flood dependent
Polygonum plebeium	Polygonaceae	Native	Annual herb	Flood dependent
Pseudognaphalium luteoalbum	Asteraceae	Native	Annual herb	Flood dependent
*Reichardia tingitana**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
Rhagodia spinescens	Chenoppodiaceae	Native	Perennial sub-shrub	Terrestrial
Schoenoplectus tabernaemontani	Cyperaceae	Native	Perennial sedge	Emergent
Sclerolaena tricuspis	Chenoppodiaceae	Native	Perennial sub-shrub	Terrestrial
Senecio cunninghamii	Asteraceae	Native	Perennial shrub	Flood dependent
Senecio runcinifolius	Asteraceae	Native	Perrennial herb	Flood dependent
*Sonchus oleraceus**	Asteraceae	Exotic, Naturalised	Annual herb	Terrestrial
Sphaeromorphaea australis	Asteraceae	Native	Annual herb	Flood dependent
Sporobolus mitchellii	Poaceae	Native	Perrennial grass	Flood dependent
Stemodia florulenta	Scrophulariaceae	Native	Perrennial herb	Flood dependent
*Symphyotrichum subulatum**	Asteraceae	Exotic, Naturalised	Annual herb	Flood dependent
Tetragonia tetragonoides	Aizoaceae	Native	Annual herb	Terrestrial
Teucrium racemosum	Lamiaceae	Native	Perrennial herb	Flood dependent
Wahlenbergia fluminalis	Campanulaceae	Native	Annual herb	Flood dependent
*Xanthium occidentale***	Asteraceae	Exotic, Declared Pest Plant in South Australia	Annual herb	Amphibious

Table D5. Response to inundation for each functional group.

Functional Group	Inundation Response
Terrestrial	Intolerant of either partial or complete inundation
Flood dependent	Intolerant of complete inundation as mature or juvenile plants but germinate on newly exposed soil after inundation but not in response to rainfall. Many of these species are short-lived annuals that take advantage of the bare soil (lack of competition) and high soil moisture after inundation and can complete their life cycle in a matter of weeks (Nicol 2004)
Amphibious	Requires fluctuating water levels and are adapted to survive inundation and exposure as juvenile or adult plants. Brock and Casanova (1997) split this group into five different groups based on their anatomical responses to inundation; however, they all have similar water regime requirements
Emergent	Have a requirement for permanent shallow water or continuous high soil moisture in the root zone to survive. Unlike amphibious species these species are well adapted to static water levels and can displace amphibious species under these conditions

Table D6. Indicator Species Analysis results comparing plant community in each inundation zone downstream of a. Lock 1, b. Lock 4 and c. Lock 6 in February 2021, * denotes exotic species, ** denotes declared pest plant in South Australia, yellow highlighting denotes significant indicator; blue highlighting denotes not significant but exclusive to the Pool level and Inundated zones.

a.

Taxon	Zone	P
Alternanthera denticulata	Inundated	0.0168
Ammannia multiflora	Inundated	0.5415
*Apium graveolens**	Inundation extent	0.2855
Atriplex	Not inundated	0.5575
Brachyscome paludicola	Inundated	0.7832
Calotis hispidula	Inundated	0.5577
*Centaurea calcitrapa**	Inundation extent	0.7465
Centipeda minima	Inundated	0.3277
*Cuscuta campestris***	Inundated	1
Cyperus gymnocaulos	Pool level	0.3589
*Dittrichia graveolens**	Not inundated	0.5313
Duma florulenta	Inundation extent	0.3609
Eleocharis acuta	Pool level	0.003
Enchylaena tomentosa	Not inundated	0.2212
*Erigeron bonariensis**	Inundated	0.3479
Eucalyptus camaldulensis	Inundation extent	0.8944
Euphorbia drummondii	Not inundated	0.1098
*Heliotropium curassavicum**	Inundation extent	0.1892
*Hypochaeris glabra**	Inundated	1

Taxon	**Zone**	**P**
Juncus usitatus	Pool level	0.0004
Lachnagrostis filiformis	Inundated	0.5577
*Lactuca serriola**	Inundation extent	0.9164
Limosella australis	Pool level	0.0002
Ludwigia peploides	Pool level	0.4387
Lythrum hyssopifolia	Pool level	0.1346
*Medicago**	Inundated	0.1966
*Melilotus indicus**	Inundation extent	0.8464
Myoporum montanum	Not inundated	0.6527
Paspalidium jubiflorum	Pool level	0.6993
Paspalum distichum	Pool level	0.0242
Phragmites australis	Pool level	0.2018
Phyllanthus lacunarius	Not inundated	0.5487
Pseudognaphalium luteoalbum	Not inundated	0.5515
Schoenoplectus tabernaemontani	Pool level	0.022
Senecio runcinifolius	Inundated	0.7137
Sphaeromorphaea australis	Inundated	0.0028
Sporobolus mitchellii	Not inundated	0.0542
Stemodia florulenta	Inundation extent	0.8586
*Symphyotrichum subulatum**	Pool level	0.2054
Teucrium racemosum	Inundation extent	0.3165
Wahlenbergia fluminalis	Inundated	0.4577
*Xanthium occidentale***	Inundated	0.18

b.

Taxon	**Zone**	**P**
Alternanthera denticulata	Inundated	0.1938
Ammannia multiflora	Inundated	1
Atriplex	Inundated	1
Bare soil	Not inundated	0.096
Bolboschoenus caldwellii	Pool level	0.5887
Brachyscome paludicola	Not inundated	0.0058
Centipeda minima	Inundated	0.5459
Cyperus difformis	Inundated	0.2863
Cyperus gymnocaulos	Inundation extent	0.3475
Disphyma crassifolium	Inundation extent	0.1326
Duma florulenta	Not inundated	0.5497
Einadia nutans	Not inundated	0.5329
Enchylaena tomentosa	Inundation extent	0.0186
*Erigeron bonariensis**	Not inundated	0.6637
*Erodium botrys**	Inundation extent	0.1326
Eucalyptus camaldulensis	Inundated	0.5059

Taxon	**Zone**	**P**
Euchiton involucratus	Inundated	0.5459
*Gazania rigens***	Not inundated	0.5397
*Heliotropium curassavicum**	Inundation extent	0.2749
Isoetopsis graminifolia	Inundated	0.5429
Juncus usitatus	Pool level	0.2859
Lachnagrostis filiformis	Inundated	1
Ludwigia peploides	Pool level	0.1522
Lythrum hyssopifolia	Inundated	1
Melaleuca lanceolata	Not inundated	1
Myriophyllum verrucosum	Pool level	0.2961
Nicotiana velutina	Not inundated	0.0912
Paspalidium jubiflorum	Inundated	1
Paspalum distichum	Pool level	0.6899
Persicaria lapathifolia	Pool level	0.2
Pseudognaphalium luteoalbum	Inundated	1
Senecio cunninghamii	Inundation extent	0.6465
*Sonchus oleraceus**	Not inundated	0.5329
Sphaeromorphaea australis	Inundated	0.1214
Sporobolus mitchellii	Inundation extent	0.1188
Stemodia florulenta	Pool level	0.0254
*Symphyotrichum subulatum**	Pool level	0.1952
Tetragonia tetragonoides	Not inundated	0.5397
Teucrium racemosum	Not inundated	1
Wahlenbergia fluminalis	Inundation extent	0.124
*Xanthium occidentale***	Inundated	0.1174

c.

Taxon	**Zone**	**P**
Alternanthera denticulata	Pool level	0.0672
Ammannia multiflora	Pool level	0.0098
Atriplex	Not inundated	0.0474
Atriplex suberecta	Not inundated	0.5309
Bolboschoenus caldwellii	Pool level	0.0302
Brachyscome paludicola	Not inundated	0.4301
Centipeda minima	Inundated	0.0086
Chenopodium nitrariaceum	Not inundated	1
*Cuscuta campestris***	Inundated	0.2643
Cyperus difformis	Inundated	0.2256
Cyperus gymnocaulos	Inundation extent	0.9606
*Dittrichia graveolens**	Inundation extent	0.0328
Dysphania pumilio	Inundated	0.5917
Einadia nutans	Not inundated	0.1176

Taxon	Zone	P
Enchylaena tomentosa	Inundation extent	0.208
*Erigeron bonariensis**	Inundation extent	0.1648
Eucalyptus camaldulensis	Inundated	0.3379
Euphorbia drummondii	Not inundated	0.2222
Glinus lotoides	Inundated	0.1702
Goodenia heteromera	Inundated	1
*Heliotropium curassavicum**	Inundation extent	0.5771
*Heliotropium europaeum**	Inundated	0.189
Isoetopsis graminifolia	Pool level	0.2757
*Lactuca serriola**	Not inundated	0.5827
Ludwigia peploides	Pool level	0.0054
Lythrum hyssopifolia	Pool level	0.3627
*Mesembryanthemum crystallinum**	Not inundated	0.5707
Mollugo cerviana	Inundated	1
Mukia maderaspatana	Inundated	1
Paspalidium jubiflorum	Not inundated	0.5755
Paspalum distichum	Pool level	0.229
Persicaria lapathifolia	Inundated	0.167
Polygonum plebeium	Inundated	0.1818
Pseudognaphalium luteoalbum	Not inundated	0.7922
*Reichardia tingitana**	Not inundated	0.5755
Rhagodia spinescens	Inundation extent	0.2999
Schoenoplectus tabernaemontani	Pool level	0.2885
Sclerolaena tricuspis	Not inundated	1
Senecio cunninghamii	Inundation extent	0.5979
Senecio runcinifolius	Inundated	0.7161
Sphaeromorphaea australis	Inundated	0.0042
Sporobolus mitchellii	Not inundated	0.7267
Stemodia florulenta	Pool level	0.7568
*Symphyotrichum subulatum**	Pool level	0.3981
Tetragonia tetragonoides	Inundated	1
Teucrium racemosum	Inundation extent	0.4405
Wahlenbergia fluminalis	Not inundated	0.3873
*Xanthium occidentale***	Inundated	0.0628

Microinvertebrate assemblage – statistical outputs

Table D7. Results of negative binomial generalised linear model for microinvertebrate density (individuals per litre) showing selected predictors, estimated coefficients (Estimate), standard errors in estimation (std.Error) and Type I Error probability (*p*-value). Flow data were log transformed prior to modelling. FPA60 quantifies the area of floodplain inundated over the last 60 days (see Methods for full description of predictor calculation). Negative binomial distribution dispersion parameter (theta) = 4.14, standard error = 0.41.

Predictor	Estimate	Std. Error	*p*-value
(Intercept)	5.94	0.34	< 0.001
Month [Oct]	0.67	0.28	0.017
Month [Nov]	0.93	0.34	0.006
Month [Dec]	1.10	0.37	0.003
Month [Jan]	1.42	0.42	0.001
Mean daily flow (ML/d)	0.49	0.10	< 0.001
Water year [2015]	-0.04	0.13	0.775
Water year [2016]	-0.63	0.24	0.009
Water year [2017]	-0.15	0.15	0.313
Water year [2019]	0.45	0.21	0.034
Water year [2020]	0.30	0.17	0.082
Temperature	0.35	0.10	0.001
Electrical conductivity (EC)	0.12	0.10	0.237
10-day flow trend (dQ10)	0.08	0.05	0.112
FPA60	-1.8e-04	3.5e-05	< 0.001
Temperature:EC	0.10	0.06	0.073
EC:FPA60	-1.1e-04	3.2e-05	0.001
dQ10:FPA60	-6.4e-05	3.6e-05	0.077

Table D8. Results of microinvertebrate taxonomic richness Poisson generalised linear model, showing selected predictors, estimated coefficients (Estimate), standard error in the coefficient estimate (std. Error) and Type I error probability (p-value). Explained deviance for the model was 0.61, residual standard error: 0.007. Abundance was predicted for environmental flow scenarios using the model in Table D8.

Predictor	**Estimate**	**Std. Error**	***p-value***
(Intercept)	1.13	0.34	0.001
Abundance	2.0e-04	2.6e-05	< 0.001
Month [Oct]	0.02	0.11	0.824
Month [Nov]	0.17	0.13	0.171
Month [Dec]	0.17	0.14	0.221
Month [Jan]	0.13	0.15	0.380
Mean long-term daily flow	0.11	0.02	< 0.001
Temperature	0.03	0.01	0.002
Electrical conductivity (EC)	4.0e-04	6.9e-04	0.562
10-day flow trend (dQ10)	-0.46	0.35	0.187
FPA28	3.1e-04	1.2e-04	0.009
Temp:dQ10	0.03	0.02	0.057
EC:FPA28	-1.8e-06	6.2e-07	0.004

Table D9. Results of negative binomial generalised linear model for microinvertebrate abundance (individuals per litre) of taxa dependent upon lateral connectivity showing selected predictors, estimated coefficients (Estimate), standard errors in estimation (std.Error) and Type I Error probability (p-value). Flow data were log transformed prior to modelling. Length of lotic was log transformed. FPA60 quantifies the area of floodplain inundated over the last 60 days (see Methods for full description of predictor calculation). Negative binomial distribution dispersion parameter (theta) = 2.41, standard error = 0.26.

Predictor	Estimate	Std. Error	*p*-value
(Intercept)	-13.80	4.22	0.001
Month [Oct]	-0.40	0.39	0.316
Month [Nov]	-2.51	0.487	< 0.001
Month [Dec]	-2.46	0.54	< 0.001
Month [Jan]	-2.96	0.60	< 0.001
Mean daily flow (ML/d)	-0.50	0.27	0.061
Mean long-term daily flow	1.46	0.39	< 0.001
Lock 1 7km	0.11	0.18	0.541
Lock 1 9km	0.08	0.18	0.672
Lock 4 5km	-0.29	0.26	0.262
Lock 6 5km	-1.14	0.50	0.024
Lock 6 7km	-1.27	0.52	0.014
Lock 6 9km	-1.16	0.52	0.024
Water year [2015]	-1.02	0.21	< 0.001
Water year [2016]	-1.80	0.71	0.012
Water year [2017]	-0.51	0.21	0.017
Water year [2019]	-0.13	0.35	0.718
Water year [2020]	-0.66	0.31	0.034
Length of lotic	-0.04	0.19	0.811
Temperature	0.53	0.15	< 0.001
Electrical conductivity (EC)	0.06	0.02	< 0.001
10-day flow trend (dQ10)	-4.43	1.89	0.019
FPA60	-7.1e-04	4.6e-04	0.122
Temperature:EC	-2.3e-03	7.6e-04	0.002
Temperature:dQ10	0.22	0.07	0.002
Temperature:FPA60	2.3e-05	1.5-e05	0.129
EC:dQ10	2.3e-03	4.1e-03	0.570
EC:FPA60	-2.1e-06	1.4e-06	0.138
dQ10:FPA60	-2.1e-04	1.6e-04	0.183

Table D10. Results of negative binomial generalised linear model for microinvertebrate abundance (individuals per litre) of taxa dependent upon longitudinal connectivity showing selected predictors, estimated coefficients (Estimate), standard errors in estimation (std.Error) and Type I Error probability (p-value). Flow data were log transformed prior to modelling. FPA60 quantifies the area of floodplain inundated over the last 60 days (see Methods for full description of predictor calculation). Negative binomial distribution dispersion parameter (theta) = 2.41, standard error = 0.26.

Predictor	Estimate	Std. Error	*p*-value
(Intercept)	1.38	2.98	0.642
Month [Oct]	0.96	0.34	0.004
Month [Nov]	1.52	0.41	< 0.001
Month [Dec]	1.34	0.45	0.003
Month [Jan]	1.98	0.51	< 0.001
Mean daily flow (ML/d)	1.32	0.22	< 0.001
Lock 1 7km	-5.0e-03	0.16	0.975
Lock 1 9km	0.13	0.16	0.409
Lock 4 5km	0.39	0.22	0.081
Lock 6 5km	1.49	0.42	< 0.001
Lock 6 7km	1.42	0.43	0.001
Lock 6 9km	1.35	0.43	0.002
Water year [2015]	-0.46	0.17	0.008
Water year [2016]	-2.43	0.41	< 0.001
Water year [2017]	-0.63	0.19	0.001
Water year [2019]	-7.1e-03	0.28	0.980
Water year [2020]	-0.31	0.25	0.221
Length of lotic	-0.38	0.15	0.010
Temperature	-0.39	0.12	0.002
Electrical conductivity (EC)	-0.05	0.01	0.001
10-day flow trend (dQ10)	0.26	0.16	0.107
FPA60	9.5e-04	3.1e-04	0.002
Temperature:EC	2.3e-03	6.2e-04	< 0.001
EC:FPA60	-3.9e-06	1.2e-06	0.001
dQ10:FPA60	-1.8e-04	1.1e-04	0.079

Table D11. Results of negative binomial generalised linear model for spring microinvertebrate prey-species density (individuals per litre) showing selected predictors, estimated coefficients (Estimate), standard errors in estimation (std.Error) and Type I Error probability (*p*-value). Flow data were log transformed prior to modelling. FPA28 quantifies the area of floodplain inundated over the last 28 days (see Methods for full description of predictor calculation). Negative binomial distribution dispersion parameter (theta) = 3.07, standard error = 0.30.

Predictor	**Estimate**	**Std. Error**	***p*-value**
(Intercept)	-8.64	3.14	0.006
Month [Oct]	-0.39	0.35	0.258
Month [Nov]	-2.18	0.42	< 0.001
Month [Dec]	-1.84	0.47	< 0.001
Month [Jan]	-2.13	0.53	< 0.001
Water year [2015]	-0.97	0.18	< 0.001
Water year [2016]	-2.20	0.53	< 0.001
Water year [2017]	-0.49	0.18	0.006
Water year [2019]	-0.81	0.28	0.004
Water year [2020]	-1.29	0.25	< 0.001
Mean long term flow	0.96	0.29	0.001
Length of lotic	-0.39	0.09	< 0.001
Temperature	0.35	0.13	0.006
Electrical conductivity (EC)	0.03	0.01	0.035
10-day flow trend (dQ10)	-1.75	1.12	0.119
FPA28	-2.1e-04	1.3e-04	0.104
Temperature:EC	-1.3e-03	6.5e-04	0.053
Temperature:dQ10	0.10	0.06	0.059
dQ10:FPA28	-4.4e-04	2.7e-04	0.099

Fish indicators – statistical outputs

Table D12. PERMANOVA pairwise comparison test results for golden perch electrofishing abundance (individuals per 90 second shot) in the gorge geomorphic zone (10 sites) of the Lower Murray River in autumn from 2015–2021. *P*-values presented in bold are significant comparisons, using Bonferroni corrected α = 0.0024 (Narum 2006) for comparisons between years (fifteen comparisons).

Comparison	*t*	*P (perm)*
2015 vs. 2016	0.27262	0.8147
2015 vs. 2017	1.4563	0.1741
2015 vs. 2018	0.82529	0.4445
2015 vs. 2019	3.6323	**0.0019**
2015 vs. 2020	3.5222	**0.0022**
2015 vs. 2021	2.0927	0.0555
2016 vs. 2017	1.6998	0.1103
2016 vs. 2018	0.69301	0.5202
2016 vs. 2019	4.7217	**0.0004**
2016 vs. 2020	4.3351	**0.0006**
2016 vs. 2021	2.3178	0.036
2017 vs. 2018	0.8086	0.449
2017 vs. 2019	2.1526	0.0605
2017 vs. 2020	1.8277	0.1121
2017 vs. 2021	0.19128	0.8588
2018 vs. 2019	2.8114	0.0137
2018 vs. 2020	2.729	0.017
2018 vs. 2021	1.2362	0.2508
2019 vs. 2020	0.23709	0.8767
2019 vs. 2021	1.887	0.0882
2020 vs. 2021	1.8059	0.1007

Table D13. Results of the model selection procedure for linear regression models fitted to Murray cod length–weight reference data (2004–2021).

Model	AICc	Npar	ΔAICc	Likelihood
Exponential	65.7032	2	465.1442	<0.001
Linear	1342.2360	3	1741.677	0
Cubic	-399.4410	5	0	1
Quadratic	-38.5092	6	360.9318	<0.001

Table D14. PERMANOVA pairwise comparisons of the relative condition index (K*rel*) of YOY Murray cod between years (weir pools pooled). P-values presented in bold are significant comparisons (α = 0.05).

Comparison	*t*	*p* value
2015, 2016	0.394	0.700
2015, 2017	2.961	**0.011**
2015, 2018	1.695	0.108
2015, 2019	1.100	0.278
2015, 2020	0.346	0.735
2015, 2021	1.350	0.198
2016, 2017	4.084	**0.001**
2016, 2018	1.661	0.107
2016, 2019	1.643	0.106
2016, 2020	0.005	0.997
2016, 2021	2.110	**0.042**
2017, 2018	4.763	**0.000**
2017, 2019	1.756	0.088
2017, 2020	3.039	**0.005**
2017, 2021	2.049	0.057
2018, 2019	2.710	**0.011**
2018, 2020	1.348	0.183
2018, 2021	3.286	**0.004**
2019, 2020	2.014	**0.043**
2019, 2021	0.156	0.878
2020, 2021	1.600	0.117

Table D15. Results of the model selection procedure for linear regression models fitted to 2017–2021 Murray cod age–length data.

Model	AICc	Npar	∆AICc	Likelihood
Linear	588.302	1	16.576	<0.001
Gompertz	571.726	3	0	1.000
Von Bertalanffy	590.061	2	18.336	<0.001

Table D16. PERMANOVA main test comparisons of Murray cod catch-per-unit-effort (CPUE) between years and weir pools for non-targeted electrofishing. P-values presented in bold are significant comparisons (α = 0.05).

Factor	**df**	***Pseudo-F***	***p* value**
Year	6	2.359	**0.041**
Residuals	63		

Pairwise comparisons (between years)	***t***	***p* value**
2015, 2016	0.735	0.539
2015, 2017	2.734	**0.021**
2015, 2018	0.942	0.379
2015, 2019	0.635	0.549
2015, 2020	0.792	0.438
2015, 2021	1.548	0.124
2016, 2017	1.680	0.139
2016, 2018	0.127	0.777
2016, 2019	1.235	0.183
2016, 2020	1.266	0.203
2016, 2021	0.665	0.415
2017, 2018	1.654	0.149
2017, 2019	3.168	**0.011**
2017, 2020	2.741	**0.017**
2017, 2021	1.056	0.345
2018, 2019	1.467	0.145
2018, 2020	1.428	0.158
2018, 2021	0.575	0.443
2019, 2020	0.324	0.765
2019, 2021	2.035	0.060
2020, 2021	1.871	0.082

Table D17. PERMANOVA main test and pairwise comparisons of Murray cod catch-per-unit-effort (CPUE) between years and weir pools for targeted electrofishing. P-values presented in bold are significant comparisons (α = 0.05). * = demoninator is zero, i.e. CPUE was nil.

Factor	**df**	***Pseudo-F***	***p* value**
Year	7	7.318	**<0.001**
Residuals	22		

Pairwise comparisons (between years)	***t***	***p* value**
2013, 2014	*	
2013, 2015	0.845	1.000
2013, 2016	1.454	0.430
2013, 2017	*	
2013, 2019	2.000	0.393
2013, 2020	8.407	**0.028**
2013, 2021	*	
2014, 2015	1.000	1.000
2014, 2016	1.720	0.423
2014, 2017	*	
2014, 2019	2.390	0.142
2014, 2020	9.948	**0.025**
2014, 2021	*	
2015, 2016	0.720	0.714
2015, 2017	0.845	1.000
2015, 2019	1.198	0.257
2015, 2020	4.076	**0.029**
2015, 2021	1.139	0.441
2016, 2017	1.454	0.429
2016, 2019	0.482	0.653
2016, 2020	2.571	0.057
2016, 2021	1.958	0.167
2017, 2019	2.000	0.397
2017, 2020	8.407	**0.030**
2017, 2021	*	
2019, 2020	1.769	0.115
2019, 2021	2.738	0.103
2020, 2021	11.326	**0.008**

Table D18. PERMANOVA pairwise comparison test results for large- and small-bodied fish assemblages in the gorge geomorphic zone of the Lower Murray River from autumn 2015–2021. *P*-values presented in bold are significant comparisons, using Bonferroni corrected α = 0.0024 (Narum 2006) for comparisons between years (twenty-one comparisons).

Comparison	Large-bodied		Small-bodied	
	t	*P (perm)*	*t*	*P (perm)*
2015 vs. 2016	2.0305	0.0063	1.1526	0.2411
2015 vs. 2017	3.5839	**0.0003**	2.0765	0.0144
2015 vs. 2018	1.7638	0.0368	1.207	0.2226
2015 vs. 2019	1.2874	0.1828	1.0302	0.3376
2015 vs. 2020	1.4718	0.1007	1.2146	0.2115
2015 vs. 2021	3.4391	**0.0001**	1.2215	0.2054
2016 vs. 2017	2.2942	0.0039	3.7883	**0.0002**
2016 vs. 2018	1.7945	0.0287	0.81273	0.5826
2016 vs. 2019	1.6992	0.0435	1.7025	0.0453
2016 vs. 2020	2.6474	**0.0022**	2.2132	0.0101
2016 vs. 2021	1.4695	0.0886	1.732	0.0433
2017 vs. 2018	2.6821	**0.0011**	3.1583	**0.0010**
2017 vs. 2019	3.1843	**0.0006**	1.9555	0.0206
2017 vs. 2020	3.218	**0.0002**	2.8131	**0.0002**
2017 vs. 2021	3.0598	0.0025	3.2642	**0.0003**
2018 vs. 2019	1.7619	0.0439	1.4932	0.0969
2018 vs. 2020	1.9635	0.0225	2.1635	0.0121
2018 vs. 2021	2.3149	0.0088	1.7722	0.0401
2019 vs. 2020	1.6608	0.0714	2.0557	0.0131
2019 vs. 2021	2.2504	0.0075	2.057	0.0141
2020 vs. 2021	3.6386	**0.0007**	1.1674	0.2428

APPENDIX E: CONTINGENCY MONITORING: SEED BANK ASSESSMENT

Background

The soil seed bank is the reserves of viable seeds found in and on the surface of the soil (Roberts 1981) including the associated litter (Simpson *et al.* 1989). It is recognised as an important component of the vegetation in wetland and floodplain ecosystems (Leck 1989), providing a refuge and mechanism for vegetation regeneration after disturbance (usually a drought or flooding event) (Brock *et al.* 2003). However, only some species form a soil seed bank. Some species are serotinous (including the two dominant floodplain tree species on the Lower Murray River (LMR) Floodplain), where seeds are retained in a canopy seed bank (Jensen *et al.* 2008) and released in response to an environmental trigger instead of spontaneously at seed maturation (Cruz *et al.* 2019). Serotinous plant species include some *Melaleuca* (e.g. Holliday 2004, Hamilton-Brown *et al.* 2019) and *Eucalyptus* species (e.g. Colloff 2014). Seeds from aquatic and amphibious species are also dispersed by different mechanisms, including anemochory (wind dispersal) (e.g. Finlayson *et al.* 1983, Hocking *et al.* 1983; Soomers *et al.* 2013), hydrochory (water dispersal) (e.g. Nilsson *et al.* 1991; Nilsson *et al.* 2002; Chambert and James 2009; Favre-Bac *et al.* 2017) and zoochory (animal dispersal) (e.g. Pollux 2011; Raulings *et al.* 2011; Flaherty *et al.* 2018). Nevertheless, the soil seed bank remains a crucial regeneration mechanism for species to survive unfavourable conditions despite the evolution of serotiny and various dispersal mechanisms.

Conditions on floodplains and riparian zones of arid systems are typically unfavourable for survival of vegetative propagules, often prevalent in many wetland species (e.g. Grace 1993), and are not a reliable means of persistence (Thompson 1992). Therefore, a soil seed bank acts as the dominant resident source of propagules, allowing species to persist through unfavourable conditions by regenerating after disturbance when conditions become favourable (Brock and Casanova 1997; Casanova and Brock 1999).

Unlike systems that have predictable flooding regimes (e.g. tropical and temperate rivers and wetlands), arid and semi-arid systems have unpredictable wetting and drying events (Baskin & Baskin 1998; Leck & Brock 2000; Brock *et al.* 2003). The high variability of hydrological regime in Australian arid rivers results in extended periods of low or no flow, hence long periods without inundation of the riparian zone and floodplain (Puckridge *et al.* 1998; Puckridge *et al.* 2000). This is further exacerbated by anthropogenic pressures in the Murray River downstream of the Darling River junction, where ten low level (approximately 3 m head differential) weirs and tidal barrages regulate water levels over 90% of the time (e.g. Walker 1985). Weirs and barrages, coupled with extraction for irrigated agriculture and stock and domestic consumption, has resulted in water levels along the LMR being largely stable, and has caused significantly lower annual flows (Maheshwari *et al.* 1995; Gippel and Blackham 2002). It has also resulted in reduced flooding frequency, duration and magnitude compared to the natural flow regime (Maheshwari *et al.* 1995), causing the floodplain and riparian zones of arid rivers to be unfavourable for the majority of species most of the time (Nicol *et al.* 2018b).

Numerous studies have shown that the reintroduction of the variable water levels lost as a result of river regulation leads to an increase in regional biodiversity by providing opportunities for species adapted to fluctuating water levels to recruit (e.g. Brock and Casanova 1997; Nielsen and Chick 1997; Brock *et al.* 2000; Nicol *et al.* 2003; Siebentritt 2003). Therefore, restoration efforts in the LMR system have focused on reinstating wetting and drying cycles by constructing small regulators to reinstate drying cycles in permanent wetlands, large regulators to temporarily raise the water level in anabranch systems that bypass a main channel weir, weir level manipulations (raising and lowering) and providing environmental flows.

This contingency monitoring component aims to evaluate the effect of Commonwealth environmental water on the resilience of the littoral plant community in the Lower Murray Selected Area as part of the three-year CEWO MER Project. Resilience of plant communities is generally assessed by the composition of soil seed bank as it is the primary source of propagules for regeneration post-disturbance.

Objectives

This project had two objectives:

- To assess the littoral zone soil seed bank of the three vegetation monitoring sites in the Lower Murray Selected Area.
- To provide a quantitative baseline of the soil seed bank that can be compared to future seed bank assessments to evaluate the benefit of Commonwealth environmental water and the long-term evaluation question: What did Commonwealth environmental water contribute to the resilience of littoral plant communities?

Methods

Study sites

Soil samples were collected from the littoral zone in tailwaters downstream of Locks 1, 4 and 6 (Figure 1) in December 2019. Resampling will occur at the end of the MER Project to enable comparison of the seed bank and assessment of the long-term evaluation question.

Sediment sampling protocol

Six transects extending from normal pool level (NPL) to 2 m above NPL were established on the riverbank at each site ($n = 6$ transects per site, Table E1). Soil samples to a depth of 5 cm at 20 cm vertical intervals from NPL to 2 m above NPL were taken with a spade along each transect. Seed bank samples were transported to SARDI, dried at 40°C to a constant weight and stored in sealed plastic containers.

Table E1. GPS Coordinates of each transect sampled in tailwaters downstream of Locks 1, 4 and 6.

Site	Location	Transect	Latitude	Longitude
L1-1	Lock 1	1	-34.479019	139.596311
L1-2		2	-34.477575	139.598798
L1-3		3	-34.446681	139.609714
L1-4		4	-34.419635	139.613482
L1-5		5	-34.41855	139.61395
L1-6		6	-34.391968	139.619028
L4-1	Lock 4	1	-34.361848	140.565262
L4-2		2	-34.355854	140.576385
L4-3		3	-34.355763	140.568069
L4-4		4	-34.350657	140.562584
L4-5		5	-34.343043	140.553056
L4-6		6	-34.342492	140.554519
L6-1	Lock 6	1	-34.021533	140.867416
L6-2		2	-34.01918	140.876627
L6-3		3	-34.016288	140.88713
L6-4		4	-33.997601	140.879789
L6-5		5	-33.995581	140.880611
L6-6		6	-33.994623	140.882183

A sub-sample of 250 g of dried sediment from the seed bank samples were spread onto a base of 15 cm deep sandy loam (80% sand, 20% clay) contained in 20 cm diameter potting bags. In cases where a sub-sample of 250 g of dried sediment could not be obtained, the weight of seed bank samples used were recorded. Osmocote Plus®, a slow-release fertiliser was added to each pot to give a nitrogen loading of 100 g N m^{-2} $year^{-1}$. Samples were subjected to continuous damp conditions for 16 weeks (4 January to 25 April 2021). To take local seed input and contamination of the sandy loam soil into consideration, ten blanks (pots containing only the sandy loam) were randomly placed amongst the samples.

Seed bank assessment

The germinable seed bank was assessed using the seedling emergence technique (sensu Brown 1992; Gross 1990). Seedlings were removed at regular intervals when they could be identified. Seedlings were able to be identified from week 3 onwards (18th January 2021). After 16 weeks, the number of germinants and species were collated, and converted to germinants m^{-2} using the following formula (Nicol *et al.* 2003):

No. germinants m^{-2} = (No. germinants x mass of soil to a depth of 5 cm m^{-2})/sample mass

Plant identification and nomenclature

Plants were identified using keys in Jessop and Tolken (1986), Cunningham *et al.* (1992) and Jessop *et al.* (2006). In some cases, due to immature individuals or lack of floral structures, plants were identified to genus only. Nomenclature follows the Centre for Australian National Biodiversity Research and Council of Heads of Australasian Herbaria (2021).

Data analysis

Germinant density between sites and elevations was compared using two factor univariate PERMANOVA (Anderson and Ter Braak 2003). Floristic composition of the germinable seed bank between sites and elevations was compared with two factor multivariate PERMANOVA (Anderson and Ter Braak 2003) and nMDS ordination (McCune *et al.* 2002).

Results

Germinable seed bank abundance

Overall Lock 1 had the highest seedling density (5,489 germinants m^{-2}), followed by Lock 6 (3,703 germinants m^{-2}) and then Lock 4 (1,128 germinants m^{-2}) (Figure E1) but Lock 6 recorded the highest of exotic germinant density (predominantly *Heliotropium curassavicum*) followed by Lock 1 then Lock 4. However, PERMANOVA detected no significant difference between sites, elevations, or a significant interaction (Table E2).

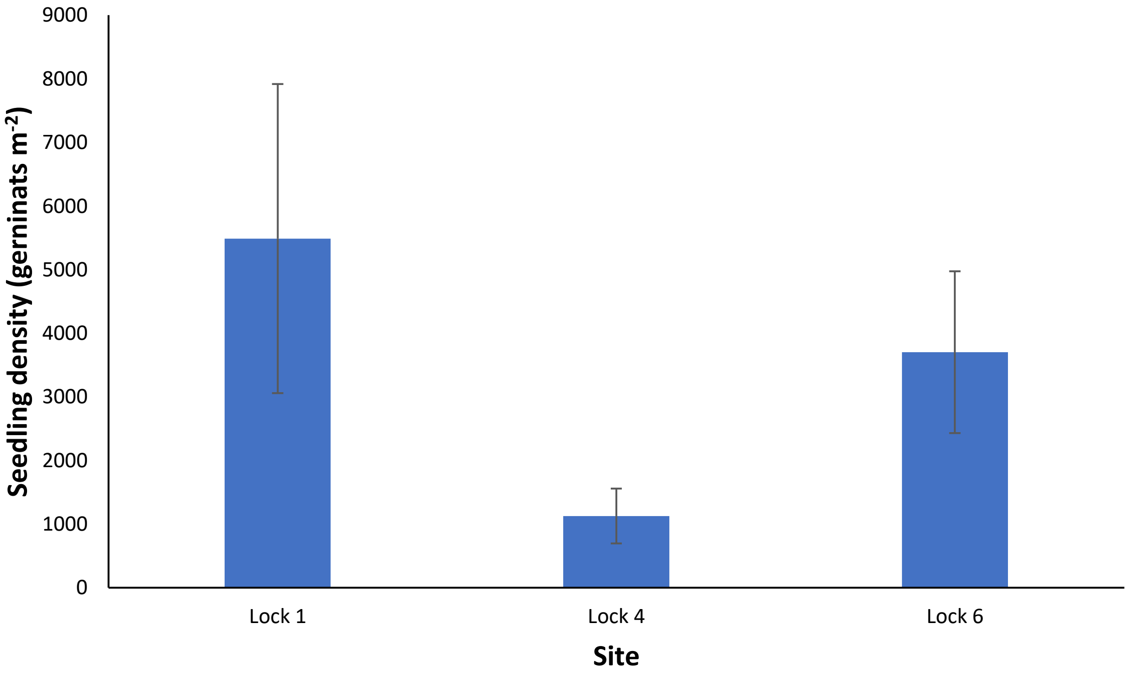

Figure E1. Mean seedling density in the littoral zones downstream of Locks 1, 4 and 6 (error bars ± 1 standard error).

Table E2. PERMANOVA results comparing germinant at different elevations in the littoral zone at sites below Locks 1, 4 and 6 on the Lower Murray River.

Factor	***df***	***Pseudo-F***	***P***
Lock	2, 197	1.88	0.131
Elevation	10, 197	0.90	0.501
Lock x Elevation	20, 197	1.11	0.284

Germinable seed bank species richness

A total of 26 taxa (including nine exotic species) were recorded across all sites and elevations (Table E3). Downstream of Lock 1 had the highest species richness with 18 taxa recorded (including five exotics), 16 taxa (including 4 exotics) were recorded downstream of Lock 6 and 13 taxa (including 6 exotics) were recorded downstream of Lock 4 (Figure E2). Native taxa recorded at all sites were *Sphaeromorphaea littoralis*, *Stemodia florulenta*, *Sporobolus mitchelli, Einadia nutans, Centipeda minima*, *Cyperus gymnocaulos* and *Alternanthera denticulata.* The nine exotic taxa recorded were *Symphyotrichum subulatum*, *Heliotropium curassavicum*, *Medicago*, *Spergularia marina*, *Cotula coronopifolia*, *Sonchus, Lolium*, *Verbena supina*, and *Xanthium strumarium* (Table E3).

Table E3. List of species recorded in the germinable seed bank, including family and life history strategy and conservation status in South Australia (*denotes exotic species, ** denotes proclaimed pest plant in South Australia).

Taxon	Family	Life History Strategy	Conservation Status	Lock 1	Lock 4	Lock 6
Alternanthera denticulata	Amarathaceae	Annual	Least Concern	🟩	🟩	🟩
Atriplex lindleyi	Chenopodiaceae	Perennial	Least Concern			🟩
Brachyscome paludicola	Asteraceae	Annual or perennial	Least Concern	🟩		
Centipeda minima	Asteraceae	Annual	Least Concern	🟩	🟩	🟩
*Cotula coropifolia**	Asteraceae	Perennial		🟩		
Crassula helmsii	Crassulaceae	Perennial	Least Concern		🟩	
Cyperus gymnocaulos	Cyperaceae	Perennial	Least Concern	🟩	🟩	🟩
Dysphania pumilio	Chenopodiaceae	Annual	Least Concern			🟩
Einadia nutans	Chenopodiaceae	Perennial	Least Concern	🟩		🟩
Eucalyptus camaldulensis	Myrtaceae	Perennial	Least Concern			🟩
Euphorbia drummondii	Euphorbiaceae	Perennial	Least Concern	🟩		🟩
*Heliotropium curassavicum**	Boraginaceae	Annual		🟩	🟩	🟩
Limosella australis	Scrophulariaceae	Perennial	Least Concern	🟩		
*Lolium**	Poaceae	Annual		🟩	🟩	
*Medicago**	Fabaceae	Annual		🟩		🟩
Persicaria lapathifolia	Polygonaceae	Perennial	Least Concern	🟩		
Rumex bidens	Polygonaceae	Perennial	Least Concern	🟩		
*Sonchus oleraceus**	Asteraceae	Annual	Least Concern		🟩	🟩
Sphaeromorphaea littoralis	Asteraceae	Annual or perennial	Least Concern	🟩	🟩	🟩
*Spergularia marina**	Caryophyllaceae	Annual or perennial		🟩	🟩	🟩
Sporobolus mitchellii	Poaceae	Perennial	Least Concern	🟩	🟩	🟩
Stemodia florulenta	Scrophulariaceae	Annual or perennial	Least Concern	🟩	🟩	🟩
Symphyotrichum subulatum *	Asteraceae	Perennial		🟩		
Typha domingensis	Typhaceae	Perennial	Least concern			🟩
*Verbena supina**	Verbenaceae	Perennial			🟩	
*Xanthium occidentale***	Asteraceae	Annual			🟩	

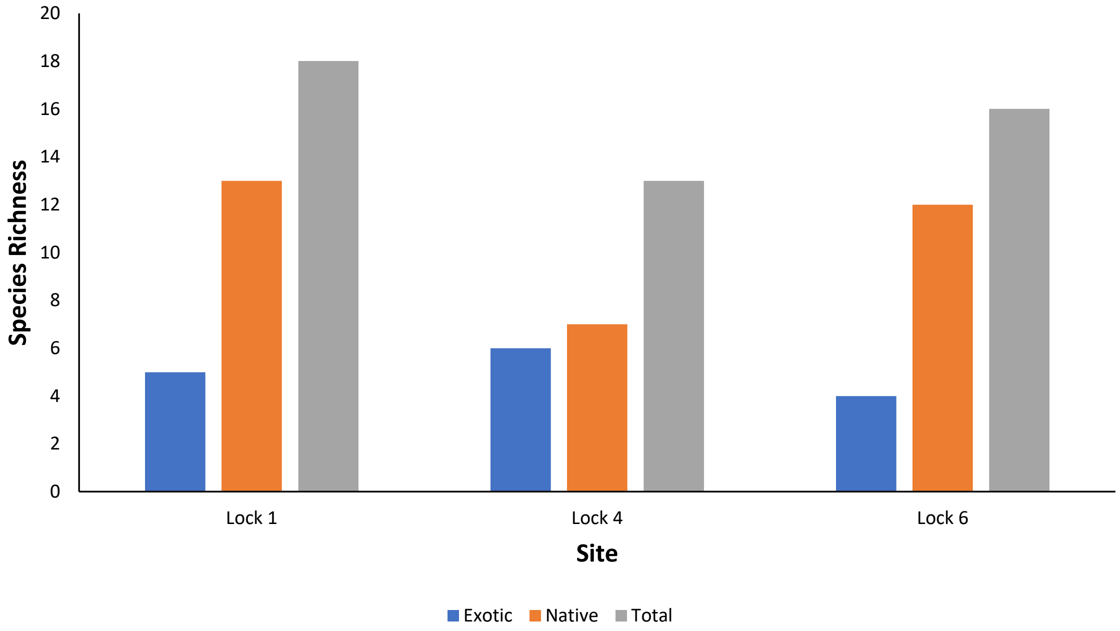

Figure E2. Native, exotic and total species richness of the germinable soil seed banks in the littoral zones downstream Locks 1, 4 and 6.

Germinable seed bank composition

Sphaeromorphaea littoralis had the highest abundance of the native species recorded, followed by *Cyperus gymnocaulos* then *Persicaria lapathifolia*. Lock 1 recorded the highest germinant density of the aforementioned species with *Persicaria lapathifolia* only recorded below Lock 1.

Among all exotics, *Heliotropium curassavicum* followed by *Medicago* were recorded the most across all sites, with *Heliotropium curassavicum* most abundant below Lock 6 and *Medicago* below Lock 1.

Cyperus gymnocaulos was most abundant below Lock 1 but also found below Locks 4 and 6. *Sphaeromorphaea littoralis* was more abundant below Lock 1 and 4 but also present below Lock 6. *Spergularia marina* was widespread across all sites and elevations. *Stemodia florulenta* was recorded across all sites but more abundant below Lock 4.

PERMANOVA comparing the germinable soil seed bank between sites and elevations detected significant differences between sites, elevations and a significant interaction indicating the differences were not consistent across elevations at each site (Table E4).

Table E4. PERMANOVA results comparing the germinable soil seed bank composition at different elevations at sites below Locks 1, 4 and 6 on the Lower Murray River.

Factor	***df***	***Pseudo-F***	***P***
Lock	2, 197	3.51	0.002
Elevation	10, 197	2.19	0.001
Lock x Elevation	20, 197	1.30	0.032

Despite the significant differences between sites and elevations, nMDS ordinations comparing the germinable soil seed bank composition between elevations at each site showed no clear patterns with respect to elevation. Hence, there was no zonation of the littoral zone soil seed bank with respect to elevation downstream of Locks 1, 4 and 6 (Figure E3).

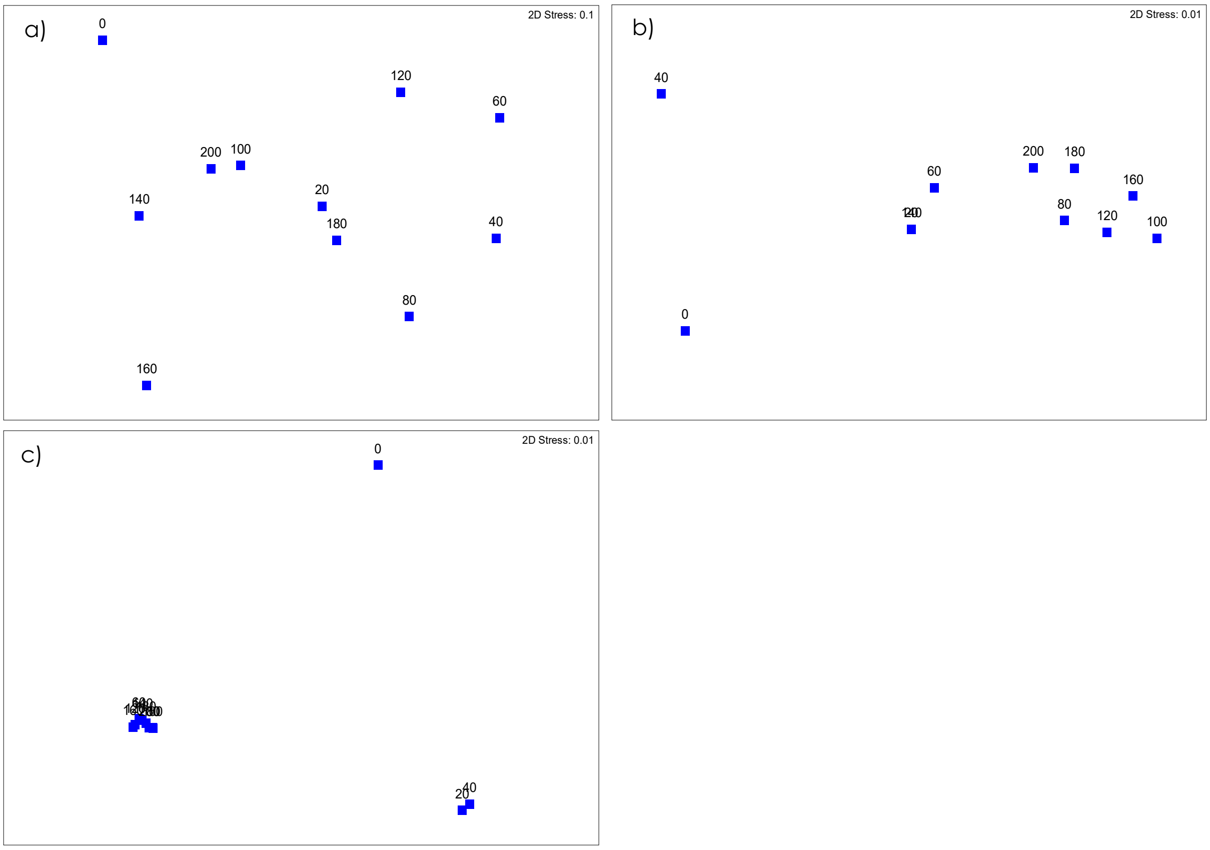

Figure E3. NMS ordination comparing germinable seed bank composition at all sampled elevations downstream a) Lock 1 b) Lock 4 and c) Lock 6 sites.

Discussion and management implications

Results showed that there was a viable soil seed bank in the littoral zone capable of regenerating after disturbance, but several pest species were also present. However, the seed bank of the littoral zone of Lower Murray River is depauperate compared to similar systems such as the Chowilla Floodplain or the Menindee Lakes where seed densities of 50,000 to 100,000 seeds m^{-2} and 30 to 60 native species have been commonly recorded (Table E5).

Table E5. Maximum seed density (to 5 cm depth) and species richness reported for arid/semi-arid wetlands in eastern Australia (*wetlands sampled from the Lower Lakes were historically permanent but dry when sampled due to low water levels between 2007 and 2010).

Wetland	Region	Hydrological regime	Maximum recorded seed density (seeds m^{-2})	Species Richness	Reference
Menindee Lakes	Lower Darling River	Temporary	75,000	59	Nicol 2004
Thegoa Lagoon	Lower Murray River	Temporary	37,683	21	Nicol *et al.* 2007
Goolwa Channel, lower Finniss River and lower Currency Creek	Lower Lakes	Permanent*	14,182	57	Nicol and Ward 2010
Chowilla Floodplain	Lower Murray River	Ephemeral	102,000	61	Kelly 2017; Skinner 2017; Gibbs *et al.* 2020
Bool Lagoon	South East of South Australia	Temporary	78,000	31	Nicol *et al.* 2003
Channel Country, Cooper Creek	Lake Eyre Basin	Temporary	23,000	56	Capon and Brock 2006
Narran Lakes	Lower Balonne Floodplain	Temporary	16,000	77	James *et al.* 2007
Goulburn River Tributaries	Goulburn Catchment	Temporary/ Permanent	27,000	55	Williams *et al.* 2008
Wannon River	Victorian Volcanic Plain	Temporary	Not recorded	69	Casanova 2015
Lower Murray River littoral zone	Lower Murray River	Temporary	5,489	26	Current study

Results also showed the soil seed bank was highly patchy with variable distribution across sites and elevations and no zonation with respect to elevation. This contrasts with the extant vegetation, which showed distinct zonation with respect to elevation at each site (Ye *et al.* 2021). It is unclear why the zonation in the extant vegetation has not translated to the soil seed bank; however, hydrology provides an environmental filter (sensu van der Valk 1981) that spatially and temporally promotes or restricts recruitment of species and has probably caused the zonation observed in the extant vegetation (sensu Nicol *et al.* 2003).

These data have provided a quantitative baseline data on seed banks of the littoral vegetation for future evaluation of the contribution of environmental water to vegetation resilience in the Lower Murray River. This could be achieved by comparison of the soil seed bank across the elevation gradient in the littoral zone in future monitoring.

Conclusions

This study provides baseline data on seed banks and the resilience of the littoral plant community in the Lower Murray River. It has shown that the existing seed bank is depauperate and that there is no zonation of the germinable seed bank across elevations. This study can be used as a comparison for future seed bank assessments to evaluate the benefits of environmental water and to answer the long-term evaluation question: What did Commonwealth environmental water contribute to the resilience of littoral plant communities?

ACRONYMS

AHD	Australian Height Datum
CEW	Commonwealth environmental water
CEWO	Commonwealth Environmental Water Office
CLLMM	Coorong, Lower Lakes and Murray Mouth
DEW	Department for Environment and Water
DOC	Dissolved organic carbon
ENP	Ecosystem net production
ER	Ecosystem respiration
GPP	Gross primary production
LMR	Lower Murray River (South Australian section of the Murray River).
LTIM	Long-Term Intervention Monitoring
M&E	Monitoring and Evaluation
MDB	Murray–Darling Basin
MDBA	Murray–Darling Basin Authority
MER	Monitoring, Evaluation and Research
NPL	Normal pool level
NSW DPIE	New South Wales Department of Planning, Industry and Environment
PSU	Practical salinity units
RMIF	River Murray Increased Flows
TL	Total length
TLM	The Living Murray
VEWH	Victorian Environmental Water Holder
YOY	Young-of-year

GLOSSARY

Allochthonous	Refers to foreign or outside sources. For example, organic matter of an allochthonous source is that which has been produced outside of the river channel, e.g. terrestrial or floodplain material.
Autochthonous	Refers to local sources. For example, organic matter of an autochthonous source is that which has been produced within the river channel.
Base flow	Flows that are confined to the low flow part within the river channel.
Biofilm	A collection of microorganisms (e.g. bacteria) attached as a 'film' on living (e.g. tree root) and non-living (e.g. wooden pylon) surfaces.
Direct trade	"Direct trade" refers to an order for a specified volume of environmental water to be delivered at the South Australian border. Typically a timing and profile for the delivery is specified and river operators can meet the order by providing water from any available source.
Flood or flooding	Refers to flows that are overbank. In South Australia, this is deemed to be above bankfull flow (45,000 ML/d).
Freshes (flow)	Flows greater than base flow but below bank level.
Epibenthic	Organisms living on the surface of sediment.
Epiphytic	Organisms that are attached to plants.
Hatch date	The date at which fish emerge from their eggs, which is one day prior to a fish laying its first daily growth increment on an otolith.
Heleoplankton	Plankton derived from billabongs and other floodplain still, generally-vegetated, waters.
In situ	Used to describe monitoring *in* the field.
Lentic	Refers to slower water velocities associated with 'pool water' habitat in highly regulated systems, typically median velocities of approximately ≤0.3 m/s.
Littoral	The margin along the bank of the river.
Lower Murray	Term used to describe the Selected Area of monitoring, which includes the Lower Murray River (see below) and the Coorong, Lower Lakes and Murray Mouth (CLLMM).
Lower Murray River (LMR)	Defined as the main channel of the Murray River between Wellington and the South Australian border, unless otherwise specified in text (e.g. Murray River downstream of the Darling River junction).
Lotic	Refers to flowing water, typically with median velocities of approximately >0.3 m/s.
Pulse (flow)	A description given to the shape of a hydrograph that is characterised by an increase in discharge, followed by a decrease in discharge, often of similar slope.
Recruitment (reproduction)	Refers to individuals passing the critical stages of early life (e.g. larval) and becoming juveniles in a population, described here as age 0+ years.
Respiration (ecosystem)	Ecosystem respiration is the measure of oxygen depletion in water by respiring animals.
RMIF	River Murray Increased Flows: a type of environmental water. Water entitlements recovered under the Snowy Water Initiative (established in 2002) via infrastructure upgrades and water purchase, which receive annual allocations and are used to supply environmental water to the Snowy River (Snowy River Increased Flows, SRIF) and River Murray (RMIF).
Primary productivity	The rate at which energy is converted to organic substances by autotrophs (e.g. algae and plants) during photosynthesis.
Salt flux	The measure (mass/time) of the movement of salt over a given area or point.

Southern connected Basin	The southern connected Basin is a network of the Murray River and all tributaries that flow into it between the Hume Dam and the sea. The Lower Darling (below Menindee Lakes) is considered part of the Southern Connected Basin, whilst all rivers upstream of Menindee Lakes are considered as the Northern Basin.
QSA	Murray River discharge (Q) to South Australia at the SA-NSW border.
Unregulated flows	Unregulated flows occur when water in the system exceeds demands and are declared to be unregulated by the appropriate authority (source: http://www.bom.gov.au/water/awid/id-1026.shtml). They can be driven by substantial rainfall from upper tributaries, spills from headwork storages and rainfall rejection events.
Weir pool	The area of water upstream of a weir that is influenced by the weir. In this report, a weir pool is often referred to as the stretch of river between two weirs and includes tailwater habitat. For example, Weir Pool 1 is the stretch of river between Weir 1 and 2.