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Long-Term Intervention Monitoring of the Ecological Responses to Commonwealth Environmental Water Delivered to the Lower Murray River Selected Area in 2016/17

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

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Cover photos: Golden perch, electrofishing, water quality station and Murray River (SARDI Aquatic Sciences); microinvertebrates (UoA, WRM); matter transport modelling (UoA, UoWA).

Disclaimer

This report should be attributed as ‘Long-term intervention monitoring for the ecological responses to Commonwealth environmental water delivered to the Lower Murray River Selected Area in 2016/17. A report prepared for the Commonwealth Environmental Water Office.’

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EXECUTIVE SUMMARY

This project assesses the ecological responses to Commonwealth environmental water delivered to the Lower Murray River (LMR) Selected Area during year three (2016/17) of the five-year Commonwealth Environmental Water Office (CEWO) Long-Term Intervention Monitoring (LTIM) project. During 2016/17, ~618 GL of Commonwealth environmental water was delivered to the LMR, in conjunction with other environmental flows (i.e. the Murray–Darling Basin Authority (MDBA) The Living Murray Initiative, Victorian Environmental Water Holder and River Murray Increased Flows), coordinated through a series of watering events across the southern connected Basin to achieve multi-site environmental outcomes. High unregulated flows, resulting in flooding (peak flow ~94,600 ML day^{-1} at the South Australian border) during spring/early summer 2016/17 delayed the majority (~96%, excluding South Australian held entitlement flow) of environmental flow delivery until after mid-December 2016. Environmental watering assisted in slowing the flood recession between mid-December 2016 and late January 2017, maintaining river flow in the LMR at 14,700–17,500 ML day^{-1} during mid-January, which otherwise would have been 7,500–14,300 ML day^{-1}. Commonwealth environmental water helped increase river flow in the LMR between February and mid-April 2017 from ~4,100–7,400 ML day^{-1} to 5,800–10,600 ML day^{-1}. Environmental water supplemented freshwater flows to the Lower Lakes and Coorong throughout the year (July 2016–June 2017), which was critical in maintaining barrage releases during a dry autumn.

Seven indicators were used to evaluate the ecological response to Commonwealth environmental water, with a focus on the main channel of the LMR. Category 1 indicators primarily aimed to evaluate Basin-scale objectives and outcomes, and in some instances, also local (Selected Area) objectives, following basin-wide standard protocols, whereas Category 3 indicators aimed to address local evaluation questions, using area specific methods. These indicators were:

- Hydrology (channel) (Category 1)
- Stream Metabolism (Category 1)
- Fish (channel) (Category 1)
- Hydrological Regime (Category 3)
- Matter Transport (Category 3)
- Microinvertebrates (Category 3)

- Fish Spawning and Recruitment (Category 3)

Category 2 indicators primarily aimed to address local evaluation questions, but followed basin-wide standard protocols. There were no Category 2 indicators in the LMR Selected Area.

Key ecological outcomes

Monitoring in 2016/17 identified some ecological responses associated with the delivery of Commonwealth environmental water in the LMR. However, it was particularly challenging to identify environmental water contribution to ecological outcomes for some indicators (i.e. Stream Metabolism, Microinvertebrates and Fish Spawning and Recruitment) because of high unregulated flows, flooding and delivery of relatively small volumes of environmental water during spring and early summer, when most of the field sampling occurred. Therefore, biological responses for these indicators were largely assessed against the overall flow regimes in 2016/17. Key findings, in relation to CEWO short-term evaluation questions, are summarised in Table 1. Results from the empirical monitoring and modelling were evaluated and discussed in the context of our contemporary understanding of flow-related ecology in the LMR.

Table 1. Summary of the key findings from Category 1 and Category 3 indicators relating to the CEWO short-term (one-year) evaluation questions (answers in blue text) associated with environmental water releases to the Lower Murray River (LMR) Selected Area during 2016/17. Key findings for Category 1 Hydrology (channel) are not presented as they do not have specific Selected Area evaluation questions. Evaluation of CEW for Hydrological Regime and Matter Transport indicators is based on modelled data. Objectives and Selected Area-specific hypotheses for each indicator are provided in the LMR LTIM M&E Plan (SARDI *et al.* 2016). CEW = Commonwealth environmental water, TLM = The Living Murray, VEWH = Victorian Environmental Water Holder, RMIF = River Murray Increased Flows.

CEWO SHORT-TERM EVALUATION QUESTIONS AND ANSWERS	KEY FINDINGS
Category 1: Stream Metabolism What did CEW contribute to: • Dissolved oxygen levels? Flooding reduced dissolved oxygen levels to below 50% saturation (~4.5 mg L^{-1}). Environmental water that supplemented releases from Lake Victoria maintained oxygen levels above 4 mg L^{-1} in the Rufus River. • Patterns and rates of primary productivity and decomposition? A marked increase in ecosystem respiration (oxygen consumption) at the site below Lock 6 aligned with an increased delivery of turbid water from the Darling River.	The 2016/17 monitoring was dominated by an extended period of high unregulated flows and flooding that reduced dissolved oxygen levels to below 50% saturation (~4.5 mg L^{-1}). Downstream of Lock 6, dissolved oxygen concentrations fell to 0 mg L^{-1} for a 4-day period in early December 2016. Prolonged exposure to dissolved oxygen concentrations below 2 mg L^{-1} is detrimental to a range of aquatic organisms, including fish, while zero oxygen levels are lethal to many. Lake Victoria releases maintained oxygen levels above 4 mg L^{-1} in the Rufus River from early December 2016. This positive influence extended downstream to the South Australian border (Customs House) from 11 to 17 December, but the effect there was small. Environmental water supplemented continued releases from Lake Victoria from 17 to 31 December, helping to maintain oxygen levels above 4 mg L^{-1} in the Rufus River during that period. Following the flood, flows returned to the channel and varied over a narrow range with environmental water delivered from different sources across the southern Murray–Darling Basin. As a result, it was difficult to identify the effects of environmental flow on metabolism. One notable influence on metabolism was a marked increase in ecosystem respiration (a process that consumes oxygen) at the site below Lock 6. This aligned with delivery of water from the Darling River, a tributary that tends to be naturally turbid. It is likely that suspended sediments temporarily reduced light penetration, thereby increasing oxygen consumption relative to oxygen production (via photosynthesis). Fluctuations in metabolic activity in response to changing flow conditions is part of the natural variability expected in a river reach. However, the accumulative long-term influences of water quality attributes on energy supply and thus food webs need to be assessed to inform environmental water management.

Category 1: Fish (channel) The contribution of CEW to native fish survival and community resilience was evaluated at the Basin-scale level. At the local scale, data from this indicator answered several evaluation questions from South Australia's Long Term Environmental Watering Plan (Appendix I).	For the third consecutive year, small Murray cod (<150 mm total length, likely age 0+) were sampled by electrofishing in the LMR during 2016/17, indicating successful recruitment. Furthermore, cohorts from 2014/15 and 2015/16 persisted in 2017. The mechanisms behind the recruitment of cohorts of Murray cod from the last three years remain unexplored and unclear. Based on electrofishing length frequency data, there was no recruitment (to age 0+) of golden perch, silver perch or freshwater catfish in 2016/17. Overbank flows were conducive to spawning of golden perch and silver perch, but hypoxic (low dissolved oxygen) conditions during spring/summer 2016/17 may have directly (reduced survival of eggs and larvae) or indirectly (reduced food resources) compromised recruitment. In 2017, there was a decrease in the abundance of small-bodied fishes and an increase in the abundance of exotic common carp, relative to the previous two years. This assemblage change is typical of flood years. Reduction of submerged vegetation (by reduced light availability and scouring from high flows) in the main channel of the LMR during 2016/17 likely resulted in the reduction of small-bodied fishes, whilst increased common carp abundance was driven by enhanced recruitment of this species in 2016/17, associated with floodplain inundation.
Category 3: Hydrological Regime **(modelling)** What did CEW contribute to: • Hydraulic diversity within weir pools? CEW slowed the decline in velocity on the flood recession over January 2017. Following this event, environmental water contributed to small increases in weir pool median water velocities (typically by 0.05–0.07 m s^{-1}), with some reaches exceeding 0.17 m s^{-1}. • Variability in water levels within weir pools? Environmental water reduced the fall in water levels on the flood recession by 0.7–0.9 m. Following this event, environmental water increased water levels by up to 0.2–0.4 m in the upper reaches of weir pools during a watering event in March 2017.	Environmental water (CEW, TLM and VEWH) increased the median velocity in Weir Pool 5 in the last week of December by 62% to 0.47 m s^{-1}, maintaining flowing habitat on the flood recession. After this event, environmental water increased weir pool median velocity by a small degree (typically 0.05 – 0.07 m s^{-1}), with some sections of the river greater than 0.17 m s^{-1}. Restoring flowing habitat is critical for the rehabilitation of riverine biota and ecological processes in the lower River Murray. On the flood recession, modelled water levels receded between 2.5 m and 3. 8 m over a two to three-week period. Without environmental water, this water level drop was simulated to be an additional 0.7–0.9 m over the same period. Following the flood, environmental water increased water levels for the remainder of the year, up to 0.2–0.4 m in the upper reaches of weir pools as return flows from a pulse in the Goulburn River from CEW and VEWH, as well as RMIF coincided in March 2017. Periodic increases in water levels could improve the condition of riparian vegetation and increase biofilm diversity, which is a key component of riverine food webs.

Category 3: Matter Transport **(modelling)** What did CEW contribute to: • Salinity levels and transport? CEW reduced salinity concentrations in the Coorong. CEW increased export of salt from the Murray River Channel, Lower Lakes, and Coorong. • Nutrient concentrations and transport? CEW contributed to minor differences in the concentrations of nutrients, but increased transport of all studied nutrients. • Concentrations and transport of phytoplankton? Whilst there was no apparent effect on phytoplankton concentrations, there was an increased transport of phytoplankton through the system, due to CEW. • Water quality to support aquatic biota and normal biogeochemical processes? CEW delivery reduced salinity concentrations in the Coorong, which likely improved habitat for estuarine biota in the region. • Ecosystem function? CEW delivery increased exchange of nutrients and phytoplankton between critical habitats of the lower River Murray, which may have supported primary and secondary productivity in the region and in doing so supported food webs of the LMR, Lower Lakes and Coorong.	Modelling suggests that environmental water generally had a positive impact on the concentrations of dissolved and particulate matter. This was observed through: • A significant reduction in salinity levels in the Coorong, with annual median salinities of 12.97 PSU# with all water compared to 17.46 PSU without CEW. High flows resulted in much lower salinity than in 2015/16 where the median salinity was 27.73 PSU with all water compared to 35.23 PSU without CEW. • Minor differences in the nutrient concentrations, with the most apparent differences being a slight dilution of silica in the Lower Lakes with CEW. The net cumulative load of phosphate decreased downstream which could be due to uptake by phytoplankton and adsorption to sediment or particles. The modelling suggests that environmental water increased the export of dissolved and particulate matter. This was observed through: • Increased salt exports from the Murray River Channel, Lower Lakes, and Coorong. In contrast to 2015/16, when there was a net import of 1,850,028 tonnes of salt to the Coorong with all water, despite CEW contributing to 4,591,269 tonnes of export, the total export in 2016/17 was 3,679,277 tonnes, with CEW contributing to 519,292 tonnes of salt export. • Increased exports of nutrients from the Murray River Channel, Lower Lakes and Coorong. Considerable loads of nutrients were exported in 2016/17 due to high flows and additional water provided by CEW. Nutrients are a resource that increase primary production, which is the base of the food web and fixes the carbon that eventually ends up as higher level organisms. Resourcing primary productivity in rivers and estuaries is critical for food webs. • Increased exports, relative to without CEW, of phytoplankton biomass from the Murray River Channel, Lower Lakes and Coorong. This may have provided benefits for the Lower Lakes, Coorong and near-shore environment by providing energy to support secondary productivity, as phytoplankton are consumed by higher trophic organisms (e.g. zooplankton). Two orders of magnitude more phytoplankton was exported in 2016/17 than in 2015/16, demonstrating the role of high flows in driving productivity, fixing carbon and having food resources available to support the recruitment and maintenance of other communities.

Category 3: Micro-invertebrates What did CEW contribute: • To microinvertebrate diversity? CEW delivery from late December–early January coincided with a decline in diversity, which was likely driven by reduced flows and a recession of water levels post-flood. However, warm-water taxa, likely from Darling sources, appeared in January 2017, following CEW (and TLM water) delivery. • Via upstream connectivity to microinvertebrate communities of the LMR? CEW contributed to longitudinal connectivity and most likely the transport of heleoplanktonic* warm-water taxa, including novel taxa for the LMR or the continent, to the LMR in January 2017. These most likely originated from Darling River flows. • The timing and presence of key species in relation to the diet of large-bodied native fish larvae? Relationship between timing of ambient (present in environment) microinvertebrates, driven by CEW, and their presence in fish diet could not be determined. • To microinvertebrate abundance? CEW delivery from late December–early January coincided with a decline in microinvertebrate abundance, which was proportional to substantially reduced flows following the flood recession.	For all sites in 2016/17, microinvertebrate diversity increased proportionately with increased unregulated flows from late September to mid-December 2016 and declined with reduced flows in January 2017 on the flood recession. The exception to this was early December, when a 'sag' in diversity was attributed to low dissolved oxygen. During spring/early summer 2016/17, a relatively high proportion of taxa that are littoral (along the bank), epiphytic (attached to plants) and epibenthic (on the surface of sediment) in habit were recorded in the main channel where they would not normally occur. This reflected increased lateral connectivity, i.e. water returning from floodplains and littoral margins during overbank flows and the flood recession. There was considerably greater microinvertebrate diversity below Locks 1 and 6 during 2016/17, compared to 2015/16 and 2014/15. This finding suggested increased longitudinal connectivity, i.e. different water sources from high flows above Lock 6, primarily the Murray and its tributaries, indicated by taxa known only from those catchments. Following the flood, warm-water taxa indicative of the Darling River (or by diversion, Lake Victoria) appeared from late December–January, coinciding with environmental water delivery (including CEW) from the lower Darling River. Introduced taxa first collected during 2015/16 were again recorded in the LMR, apparently established there or above Lock 6. Previously unrecorded taxa were also sampled, including the tropical *Brachionus durgae* (new to the continent) and at least two new species of *Brachionus*. Furthermore, dominance of the river zooplankton from late September to early November by the ciliate *Codonaria* is unusual for freshwater/riverine systems. Elsewhere the genus is marine/estuarine. The potential influence of this species on the river ecosystem (e.g. food web impacts) is unknown.

Category 3: Fish Spawning and Recruitment What did CEW contribute to: • Reproduction of golden perch and silver perch? Delivery of CEW to the lower River Murray in 2016/17 coincided with spawning, but negligible recruitment of golden perch (to young-of-year, age 0+).	In spring–summer 2016/17, golden perch (but not silver perch) spawning occurred in the lower River Murray in association with substantial overbank flows. An absence of young-of-year golden perch and silver perch in 2017, however, indicated localised recruitment failure and/or negligible immigration from spatially distinct spawning sources such as the lower Darling and mid-Murray rivers. The mechanisms contributing to localised recruitment failure of golden perch were not explored as a component of this project, but the coincidence of a hypoxic blackwater event with spawning may have contributed to larval mortality. This could include the direct impacts of low dissolved oxygen concentrations on larval survival and/or indirectly through the impacts of hypoxia on food resources.

PSU (practical salinity unit) was used for Matter Transport modelling purposes in the report. PSU is approximately equal to 1 part per thousand (ppt or ‰) or 1 g L^{-1}.
* heleoplankton = plankton derived from billabongs and other floodplain still, generally-vegetated, waters.

Key learnings and management implications

In the LMR, 2016/17 was a high flow year, with overbank flows occurring during spring/early summer. In contrast to previous dry years (2014/15 and 2015/16), high flows provided longitudinal and lateral hydrological connectivity and returned hydraulic complexity to the weir pools of the LMR, which enhanced key ecological processes including stream metabolism and matter transport and export. Flooding in 2016/17, however, was also characterised by an extensive hypoxic (low dissolved oxygen) blackwater event, which may have caused some negative biological impacts. In the LMR, these potentially included reduced microinvertebrate abundance and diversity, and recruitment failure of golden perch.

During this year, most Commonwealth environmental water was delivered to the LMR between December 2016 and June 2017. In December 2016, environmental water was used to supplement flow releases from Lake Victoria, providing localised well-oxygenated refuge areas to mitigate negative impact of low dissolved oxygen on aquatic biota. Environmental water delivery also helped slow the rate of flood recession, and slightly increased in-channel flows in later months in the LMR. While discharge rates remained <12,000 ML day^{-1} between late January and June 2017 (much lower than that during the flood), some hydraulic and ecological outcomes were achieved through the delivery of environmental water (Table 1). Based on insights from this project and our contemporary understanding of ecological response to flow in the LMR, the following points should be considered with regard to environmental water planning and management in the LMR[a]:

- Improving hydraulic conditions (velocity and water levels) is fundamental to restoring ecosystem function of the lower River Murray (downstream of the Darling River junction). Environmental water delivery can increase hydraulic diversity, potentially leading to ecological benefits by improving habitat and restoring riverine ecosystem function.

[a] Management recommendations provided in this report are subject to environmental water availability and operational feasibility. Furthermore, priorities of ecological objectives and trade-offs associated with watering actions must be considered at a local- and Basin-scale. For example, large watering events such as a spring/early summer in-channel pulse may compromise other objectives (e.g. autumn/winter barrage flow) in a given year. Therefore, actions such as these should be planned using a multi-year approach guided by ecological restoration principals.

- Environmental water delivery to contribute to freshes (i.e. in-channel flow pulses) of 20,000–45,000 ML day^{-1} can significantly improve hydraulic conditions, with >50% of a weir pool transforming from lentic (slower flowing water, median velocities <0.3 m s^{-1}) to lotic habitats (faster flowing water, ≥0.3 m s^{-1}). Restoring such hydrodynamic conditions will underpin riverine ecosystem processes and support the rehabilitation of many declined biota that are adapted to a flowing environment in the LMR. In addition, contributing to flows >45,000 ML day^{-1} (approximate bankfull level) will increase inundated area along the LMR, supporting off-channel ecological processes and biota.
- The timing of environmental flow delivery is important, which should continue to align with ecological objectives and consider biological processes and life history requirements (e.g. reproductive season of flow-cued species in spring/summer or spawning migration of diadromous fishes in winter).
- Environmental water can be delivered to mitigate low dissolved oxygen concentrations at a local scale, such as that occurred during flooding in 2016/17 (i.e. Rufus River). Strategic use of environmental water, aligning with other objectives (e.g. improved floodplain vegetation and tree condition), could also be considered to support managed inundation of floodplains at appropriate return intervals, which may reduce the risk of extensive low dissolved oxygen events due to prolonged accumulation of organic materials.
- Environmental flows should continue to be delivered to promote both longitudinal and lateral connectivity, which will increase productivity in the LMR through increased carbon and nutrient input. Connectivity will also facilitate the transport and dispersal of aquatic biota (e.g. microinvertebrates, fish larvae) to and throughout the LMR, leading to increased species diversity and potentially enhanced recruitment.
- Water source (i.e. origin) can alter inputs to the LMR (e.g. water quality, nutrients, plankton composition). These attributes can be further affected by river operations that re-route flow (e.g. floodplain regulators or water storages). Combined, these changes can lead to changes in ecological responses and the structure and function of aquatic food webs.

- In the lower River Murray, continuing to seek opportunities to maintain the hydrological integrity (i.e. magnitude, variability and source) of flow from upstream (e.g. Darling River or mid-Murray) is important to support broad-scale ecological processes and promote positive outcomes (e.g. improved productivity, enhanced spawning and recruitment of flow-dependent fish species at >15,000 ML day^{-1}).
- Although 2016/17 was a year dominated by high flows, consideration should be given to using Commonwealth environmental water in drier years, guided by a multi-year watering strategy, to reinstate key features of the natural hydrograph of the lower River Murray. For example, spring/early summer 'in-channel' increases in discharge (~15,000–20,000 ML day^{-1}) are conspicuously absent from the contemporary flow regime. These pulses of flow increase longitudinal connectivity and contribute to a broad range of ecological outcomes in riverine and estuarine ecosystems (e.g. increased matter transport, lotic habitats and spawning and migratory cues for fishes). To restore these hydrological features, a given volume of Commonwealth environmental water may need to be delivered at a higher magnitude over a short duration (weeks) rather than low magnitude delivery over a long duration (months).

More specific management considerations from indicators are provided in Section 4. These were based on ecological outcomes and findings presented in Section 2.

1 INTRODUCTION

1.1 General background

River regulation and flow modification have severely impacted riverine ecosystems throughout the world (Bunn and Arthington 2002; Nilsson *et al.* 2005), including the Murray–Darling Basin (MDB) (Maheshwari *et al.* 1995; Kingsford 2000). 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 (herein, the lower River Murray) 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 hydrological 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).

Environmental flows have been used to re-establish key components of the natural flow regime for ecological restoration of the MDB (MDBA 2012a; Koehn *et al.* 2014; Webb *et al.* 2017). The main channel of the Murray River, which includes the South Australian section (herein, Lower Murray River, LMR), represents a significant ecological asset to be targeted for environmental watering (MDBC 2006). The LMR is a complex system that includes the main river channel, anabranches, floodplain/wetlands, billabongs, stream tributaries and the Lower Lakes, Coorong and Murray Mouth, which provide a range of water dependent habitats and support significant flora and fauna. Understanding biological and ecological responses to flow regimes in the LMR provides critical knowledge to underpin environmental flow management to achieve ecological outcomes (Walker *et al.* 1995; Arthington *et al.* 2006).

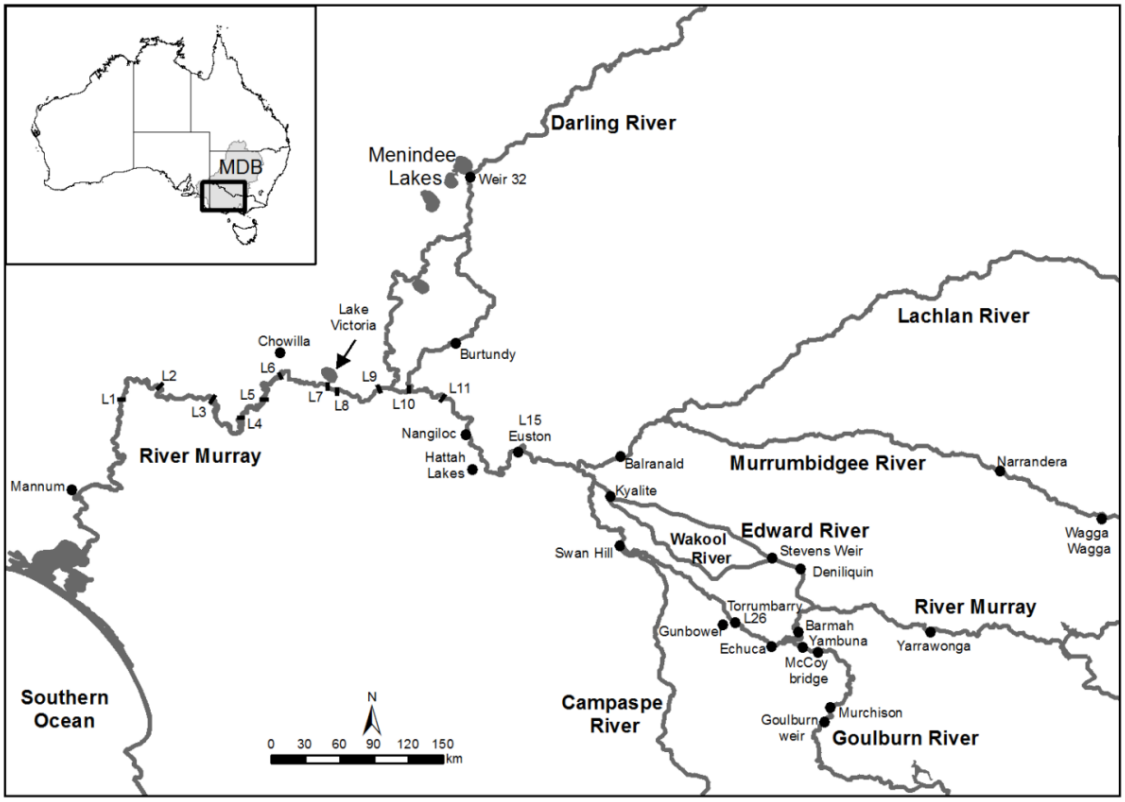

Figure 1. Map showing the location of the Murray–Darling Basin and the major rivers that comprise the southern Murray-Darling Basin, the numbered Locks (L) and Weirs (up to Lock 26, Torrumbarry), the Darling, Lachlan, Murrumbidgee, Edward–Wakool, Campaspe and Goulburn rivers and Lake Victoria, an off-stream storage used to regulate flows in the lower River Murray.

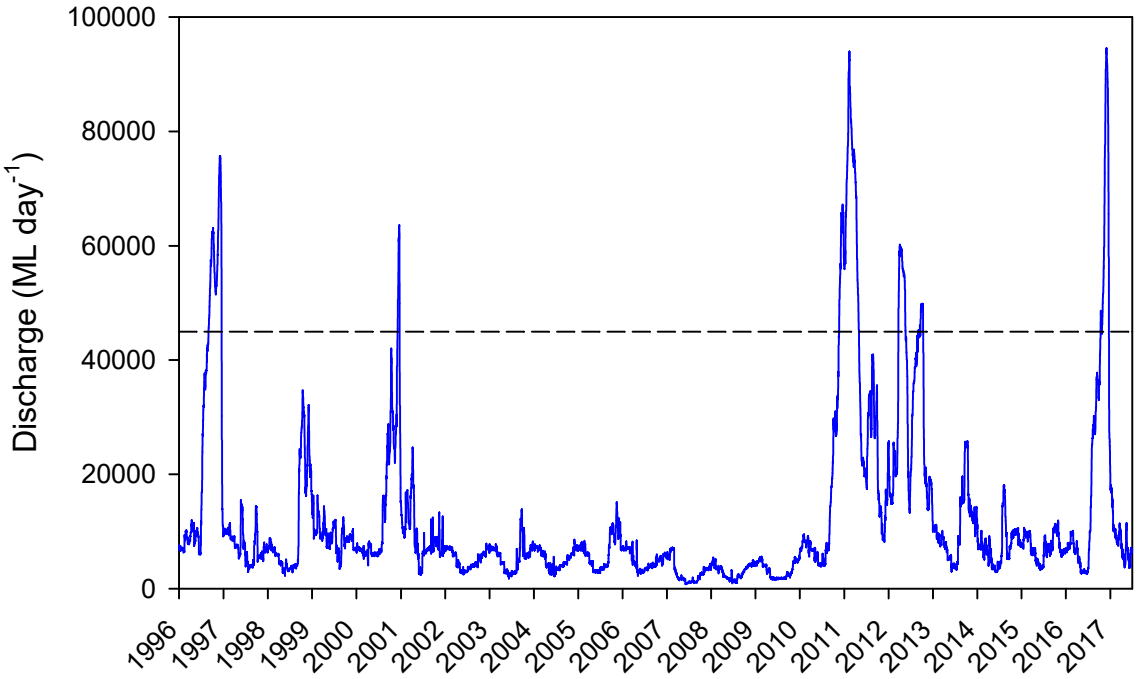

Figure 2. Daily flow (ML day^{-1}) in the LMR at the South Australian border from January 1996 to July 2017. Dotted line represents approximate bankfull flow in the main channel of the LMR.

1.2 Commonwealth environmental water

Since 2011/12, significant volumes of Commonwealth environmental water have been delivered to the LMR, in conjunction with other environmental flows (e.g. flows through the Murray–Darling Basin Authority (MDBA), The Living Murray Initiative and the Victorian Environmental Water Holder), to facilitate ecosystem restoration (Table 2; www.environment.gov.au/water/cewo). Some of these flow deliveries to South Australia have been coordinated through a series of environmental watering events across the southern connected Basin to achieve multi-site environmental outcomes (http://www.environment.gov.au/water/cewo/catchment/lower-murray-darling/history). Intervention monitoring of responses to environmental flows from 2011 to 2016 have demonstrated the ecological benefits in the LMR (Ye *et al.* 2015a; 2015b; 2016a; 2016b; 2017).

Table 2. Total annual volumes (gigalitres) of environmental water, including Commonwealth environmental water (CEW), delivered to the LMR (excludes wetland watering). Volumes are sourced from the CEWO and include the environmental components of the South Australian entitlement. Note that there are differences among data sources depending on whether water delivery by the end of a water year is based on accounted flows or flows physically delivered in real time. TLM = The Living Murray, VEWH = Victorian Environmental Water Holder, RMIF = River Murray Increased Flows.

Water year	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17
CEW	329	786	480	581	798	618
Total	467 (139 TLM)	1,075 (289 TLM)	595 (107 TLM; 7 VEWH)	714 (107 TLM; 26 VEWH)	914 (101 TLM; 15 VEWH)	998 (234 TLM; 43 VEWH; 100 RMIF)

In contrast to 2014/15 and 2015/16 (i.e. the first two years of LTIM), 2016/17 was characterised by high unregulated flows[b] during spring/early summer, resulting in overbank flooding (>45,000 ML day^{-1}) from mid-October to mid-December 2016 (Figure 2). During this year, ~618 GL[c] of Commonwealth environmental water was

[b] 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.

[c] Although the accounting by the MDBA accounts for ~621 GL of Commonwealth environmental water delivered to the SA border, approximately 3 GL of this was used by the CEWO to water off-channel

delivered to the LMR in conjunction with other sources of environmental water (i.e. The Living Murray, Victorian Environmental Water Holder and River Murray Increased Flows) (Table 2). Additional dilution flow of 201 GL was also provided to the LMR from November 2016 to February 2017 subject to the operational rule for the Menindee Lakes. Whilst Commonwealth environmental water was delivered to the LMR from early July to mid-August 2016, and from early November 2016 to late June 2017, the majority (~96%, excluding South Australian held entitlement flow[d]) of Commonwealth environmental water was delivered after mid-December 2016, following the flood recession (Figure 3).

In addition to the Commonwealth held South Australian entitlement flow (not shown in graph), from 1 July to 20 August 2016, low volumes (<300 ML day^{-1}) of Commonwealth environmental water were delivered to South Australia from upstream watering events in the Victorian tributaries during increasing unregulated flows (Figure 3). At the South Australian border, flows became overbank (>45,000 ML day^{-1}) in mid-October and increased to a peak of ~94,600 ML day^{-1} on 30 November 2016, before rapidly receding to <20,000 ML day^{-1} by 1 January 2017. The large rise and steep recession of the hydrograph reflected the high rainfall events across the MDB, and the combined run-off of peak flows from the Murray River and its tributaries over a short time period. On the recession of the hydrograph, from mid- to late December 2016, ~30 GL of Commonwealth environmental water and 30 GL of The Living Murray water were directly released from Lake Victoria to mitigate hypoxic (low dissolved oxygen) effects of blackwater in the Rufus River (Appendix A). During this period, Commonwealth environmental water delivery peaked in the LMR on 22 December 2016 at 8,100 ML day^{-1}(discharge at the South Australian border, QSA), maintaining river flow at 29,100 ML day^{-1} (Figure 3).

[c (continued)] wetlands (source: CEWO). Therefore, this report uses the figure of approximately 618 GL of Commonwealth environmental water used in the Lower River Murray Channel, Lower Lakes, Coorong and Murray Mouth.

[d] It should be noted that in Figure 3, which all flow volumes and percentages are sourced from unless otherwise specified, it is assumed that the full South Australian entitlement flow would have been delivered to the South Australian border in the 'no environmental water' scenarios. As such, the 'no environmental water' flow rates reported at the South Australian border do not represent the full benefit of the 151.1 GL of South Australian entitlement held by the Commonwealth Environmental Water Holder. The benefit of this volume being delivered to South Australia for the environment, as opposed to consumptive use, is included in the Hydrologic Regime and Matter Transport indicators that present results along the length of the LMR, where this water would historically have been diverted.

From January–March 2017, in addition to flows from the upper Murray (Hume), and return flows from the Goulburn–Broken system, the Darling River and Great Darling Anabranch, 80 GL of Commonwealth environmental water (direct trade) was delivered to the South Australian border in January 2017 (40 GL with 10 GL The Living Murray water) and February 2017 (40 GL) for Murray barrage fishways and releases into the Coorong (Figure 4). This delivery maintained river flow at 14,700–17,500 ML day^{-1} during mid-January, which otherwise would have been 7,500–14,300 ML day^{-1}. Flows declined to ~10,000 ML day^{-1} in early February and ~7,800 ML day^{-1} by early March 2017. However, without Commonwealth environmental water, river flow would have been ~5,200–7,400 ML day^{-1} during this period.

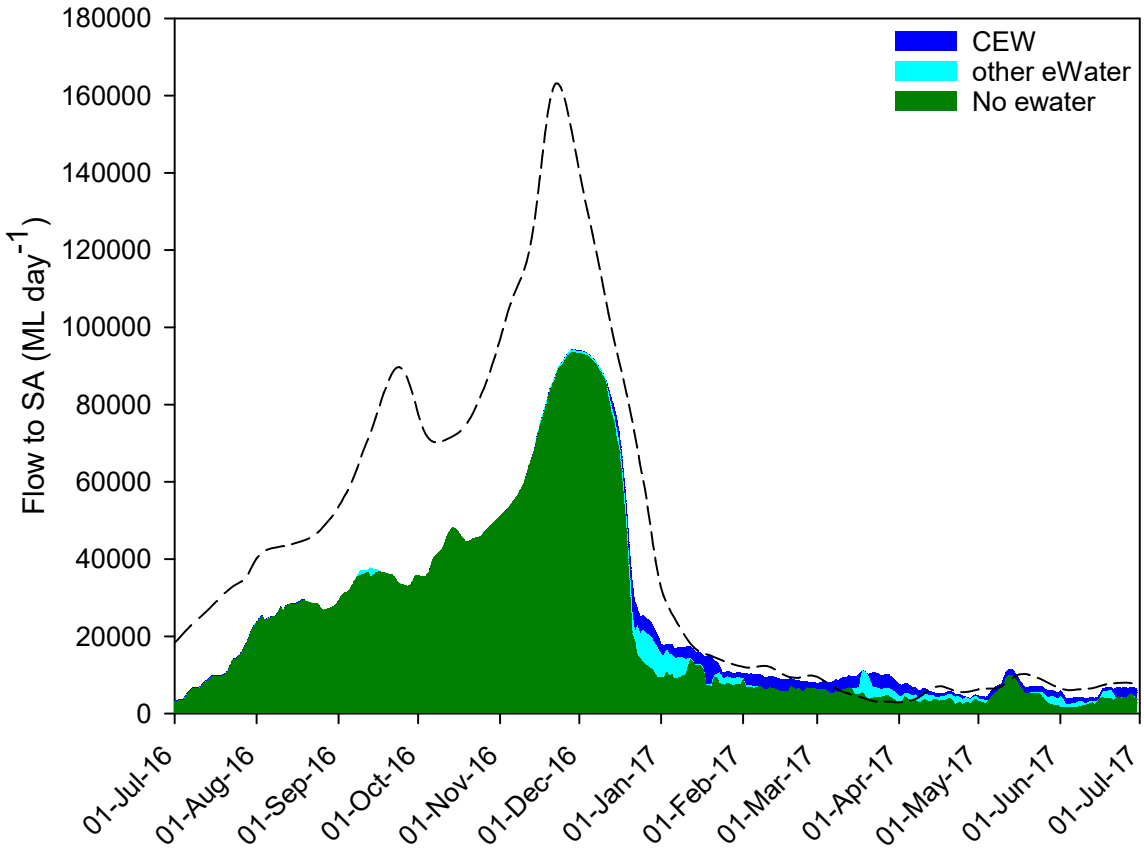

Figure 3. Flow to South Australia from July 2016 to June 2017 (stacked area chart) compared to modelled flow under natural conditions (black dotted line). CEW = Commonwealth environmental water; other eWater = other eWater such as The Living Murray, Victorian Environmental Water Holder and water delivered as part of River Murray Increased Flows (RMIF). The 'no eWater' component includes 151.1 GL of South Australian entitlement held by the Commonwealth Environmental Water Holder and 47.0 GL held by The Living Murray, and 201 GL from Additional Dilution Flows subject to the operation rule for the Menindee Lakes.

From April to end June 2017, Commonwealth environmental water comprised of return flows, primarily from an autumn fresh followed by base flows in the Goulburn River, but also from the lower Darling River and Great Darling Anabranch (Figure 4; Appendix A). In May 2017, a rejection of irrigation orders (known as a rainfall rejection event) resulted in additional operational water (not environmental water) being available for use in the LMR channel. This additional water was used in conjunction with Commonwealth environmental water in-transit from the Goulburn River, to provide releases at the barrages for lamprey attractant flow. This water remained in the Murray River main channel and bypassed Lake Victoria, increasing river flow in the LMR from ~3,300–10,000 ML day^{-1} to 4,900–11,500 ML day^{-1} during May 2017. Outputs from modelling indicated that >600 GL of Commonwealth environmental water contributed to barrage releases throughout the 2016/17 water year (July 2016 to June 2017), which was particularly significant in contributing to barrage releases to the Coorong during a dry autumn.

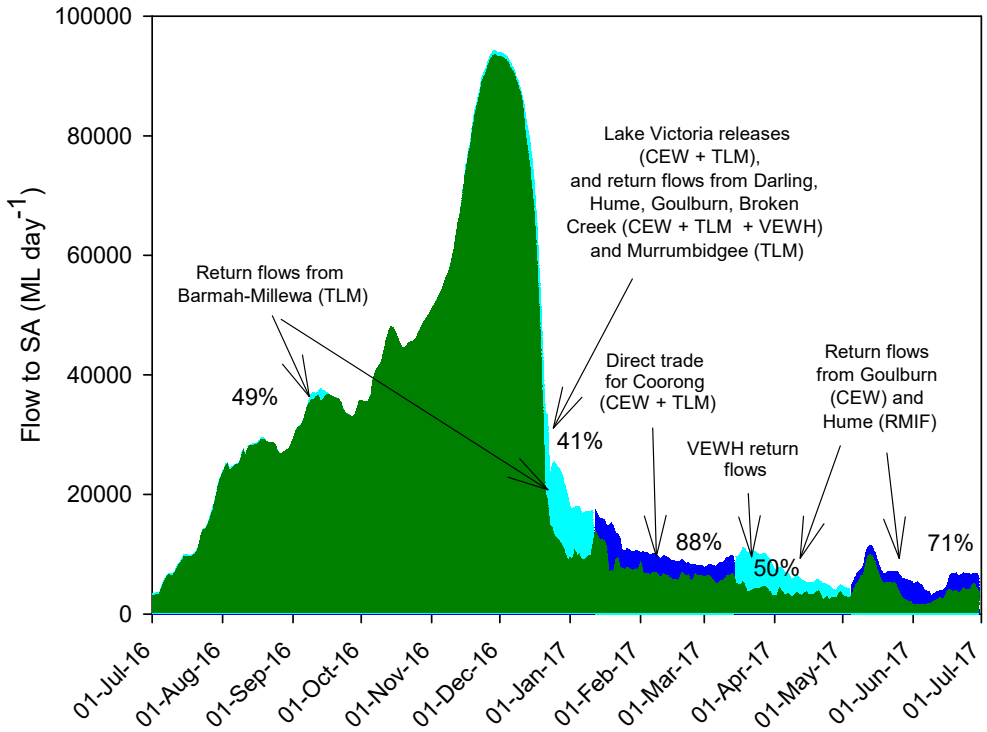

Figure 4. Commonwealth environmental water contribution to main watering events in 2016/17. Shading of the blue environmental water area represents the proportion of Commonwealth environmental water (CEW) of the total environmental water, with darker blue indicating greater proportions of CEW. Timing of major watering actions are indicated. TLM = The Living Murray, RMIF = River Murray Increased Flows. The 'no eWater' component (green) includes 151.1 GL of South Australian entitlement held by the Commonwealth Environmental Water Holder and 47.0 GL held by The Living Murray, and 201 GL from Additional Dilution Flows subject to the operation rule for the Menindee Lakes.

The original source of the water arriving in South Australia can also affect the environmental response. The sources of all flow to South Australia (not just environmental flow) in 2016/17 can be seen in Figure 5[e]. Flow to South Australia was mainly comprised of flow from the upper Murray River, Murrumbidgee River and Victorian tributaries of the Murray River from July to mid-December 2016. However, the proportional flow from the Darling River was greater from mid-December 2016 to mid-May 2017, relative to the previous six months.

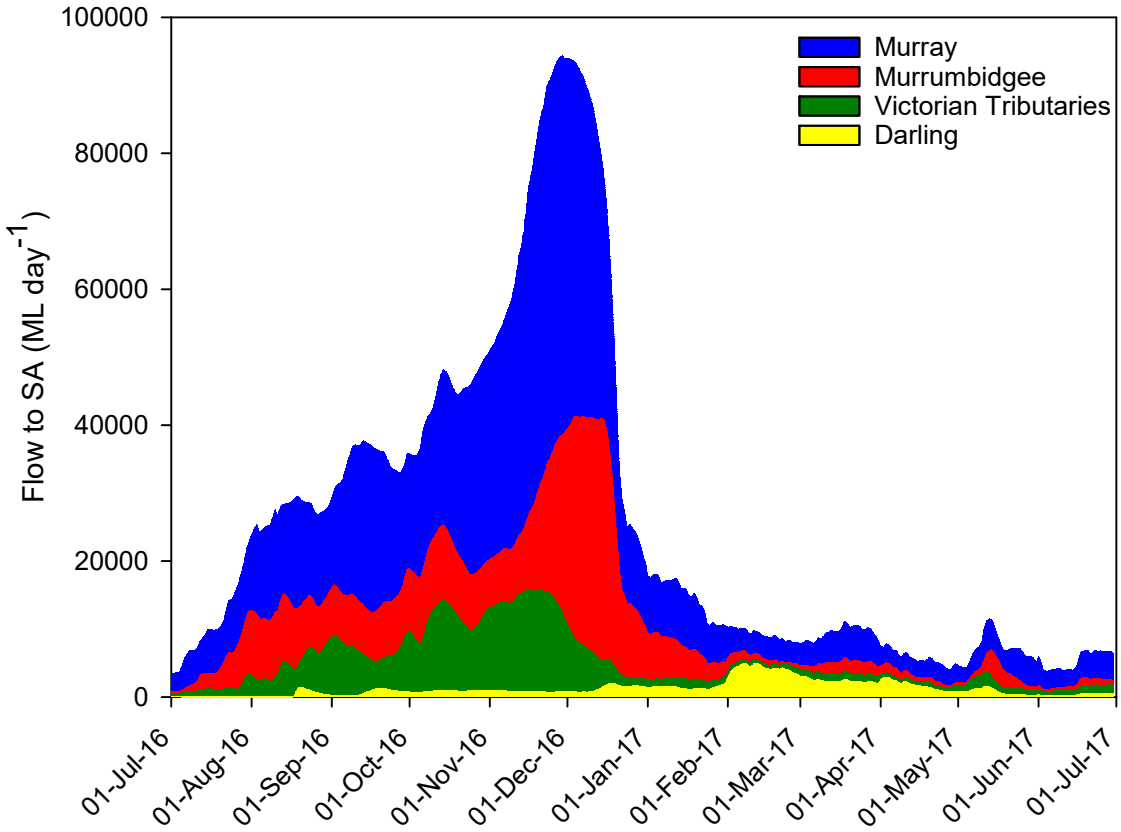

Figure 5. Source of all (environmental and consumptive) water delivered to the South Australian border (MDBA). Caveats for estimated water delivery time are mentioned above. Refer to Figure 1 for location of rivers and tributaries, relative to the LMR.

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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 represents an improvement in estimation of travel times over information used previously in Ye *et al.* (2016a), which was based on observed changes in flow along the main channel.

Concurrently with overbank flows and environmental water deliveries described above, there were other management interventions that occurred within or upstream of the LMR, such as manipulations of Weir Pools 2, 5, 6, 7, 8, 9 and 15, and Chowilla floodplain inundation (refer to Appendix A for more information). These events may also have affected ecological responses in the LMR.

1.3 CEWO LTIM project in the LMR Selected Area

In 2014, a five-year (2014/15 to 2018/19) intervention monitoring project (CEWO LTIM) was established to monitor and evaluate long-term ecological outcomes of Commonwealth environmental water delivery in the MDB. The project was implemented across seven Selected Areas throughout the MDB, including the LMR, to enable Basin-scale evaluation in addition to Selected Area (local) evaluation. The overall aims of the project are to demonstrate the ecological outcomes of Commonwealth environmental water delivery and support adaptive management.

The CEWO LTIM project in the LMR focuses on the main channel of the Murray River between the South Australian border and Wellington, with only one targeted investigation (i.e. Matter Transport) including modelling and evaluation for the Lower Lakes and Coorong (Figure 6). The general region for the CEWO LTIM project herein is referred to as the 'LMR Selected Area'. Targeted investigations (for indicators) were conducted at various sites in the Selected Area, covering three geomorphic zones and the Lower Lakes and Coorong (Wellington to Murray Mouth). The three geomorphic zones were:

- Floodplain (South Australian border to Overland Corner);
- Gorge (Overland Corner to Mannum);
- Swamplands (Mannum to Wellington);

The following indicators were used to assess ecological responses to environmental water delivery in the LMR:

Category 1

- Hydrology (channel);
- Stream Metabolism;
- Fish (channel).

Category 3

- Hydrological Regime;
- Matter Transport;
- Microinvertebrates;
- Fish Spawning and Recruitment.

The above indicators were selected in line with Commonwealth environmental water evaluation questions for the Basin and Selected Area. The details are presented in the Monitoring and Evaluation Plan for the LMR (SARDI *et al.* 2016), which is available at https://www.environment.gov.au/water/cewo/publications/cewo-ltim-lower-murray-2016. Category 1 indicators followed standard protocols to support quantitative Basin-wide and Selected Area evaluation, where applicable (Hale *et al.* 2014). Category 3 indicators were developed to address objectives and test a series of Selected Area-specific hypotheses with respect to biological/ecological response to environmental flows (SARDI *et al.* 2016). There were no Category 2 indicators for the LMR, which aimed for selected area evaluations but followed basin-wide standard protocols.

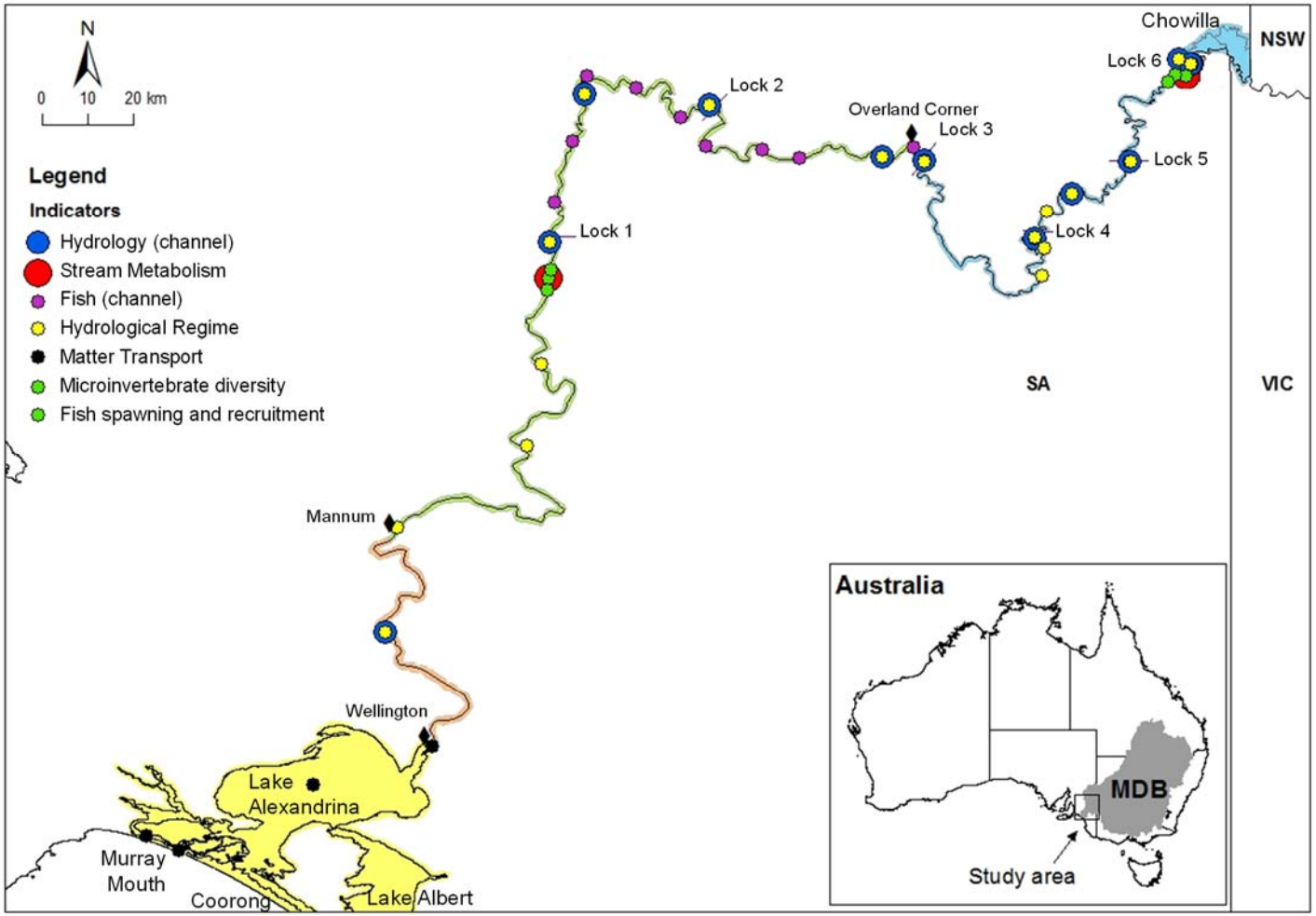

Figure 6. Map of the LMR Selected Area showing the floodplain (blue), gorge (green) and swamplands (orange) geomorphic zones, and the Lower Lakes, Coorong and Murray Mouth (yellow). Sampling sites are indicated by coloured circles. Fish Spawning and Recruitment sites represent larval sampling only. Refer to Figure B1 in Appendix B for a map of the additional weir pool monitoring sites for 2016/17.

1.4 Key findings from the CEWO LTIM project for years 1 (2014/15) and 2 (2015/16)

During Years 1 (2014/15) and 2 (2015/16) of the CEWO LTIM project, which were relatively dry years with flows mostly remaining <12,000 ML day^{-1}, ~581 and ~798 GL of Commonwealth environmental water, respectively, were delivered to the LMR main channel. The delivery of this water, often in conjunction with other sources of environmental water (e.g. MDBA, The Living Murray), helped to maintain river flow in the LMR at ~9,000–11,700 ML day^{-1} during spring and from mid-summer to early autumn. Watering events also supplemented flows to the Lower Lakes and contributed to barrage releases to the Coorong from September to June in both years. In 2015/16, Commonwealth environmental water also supported weir pool raising events in Weir Pools 2 and 5.

Commonwealth environmental water delivery contributed to a number of short-term hydrodynamic and ecological outcomes in the LMR during 2014/15 and 2015/16, which are detailed in the annual evaluation reports for this Selected Area (Ye *et al.* 2016a; Ye *et al.* 2017). In general, outcomes from both years included:

- Increased median velocities in weir pools, which may have increased suitable habitat for fishes with life histories adapted to lotic (flowing water) environments (e.g. golden perch *Macquaria ambigua*, Murray cod *Maccullochella peelii*).
- Increased water levels throughout weir pools, particularly the upper reaches, which would have increased the inundated area of the riparian zone of the river channel.
- Increased transport of nutrients and phytoplankton, which would have likely stimulated primary and secondary productivity in downstream ecosystems.
- Intermittent increases in supplies of organic material, likely from return flows from inundated floodplains (e.g. Chowilla Floodplain), which are deemed important to the food webs of rivers.
- Increased microinvertebrate diversity and abundance, likely triggered by connection with floodplain or riparian habitats, including contributions from upstream.

- Reduced salinity concentrations in the Murray River Channel, Lower Lakes and, in particular, the Coorong; increased salt export from the Murray River Channel and Lower Lakes; and reduced salt import into to the Coorong.

However, there was limited golden and silver perch spawning and recruitment in both years due to the absence of favourable hydrological conditions, such as spring–summer in-channel flow variability or overbank flows.

1.5 Purpose of the CEWO LTIM report for year 3 (2016/17)

This report presents a summary of the third year's (2016/17) key findings of indicators for the LMR (Section 2), and answers CEWO short-term (one-year) evaluation questions[f] (Section 3). The Department of Water and Natural Resources (DEWNR) short-term evaluation questions, which serve as additional questions for the LMR and relate to ecological targets of the South Australian Murray River Long-Term Environmental Watering Plan (LTWP), are discussed in Appendix I. During 2016/17, most Commonwealth environmental water (96%, excluding South Australian held entitlement flow) was delivered to the LMR after mid-December 2016, following flooding. Many findings presented in this year's report are interpreted in context of the high unregulated flows and flooding when spring/summer monitoring took place. General recommendations for environmental flow management in the LMR are provided in Section 4, based on monitoring and evaluation outcomes, and expert knowledge. As stated in SARDI *et al.* (2016), monitoring and evaluation of Commonwealth environmental water delivery in the LMR from 2014/15 to 2018/19 focusses on spring/summer given this was the primary period for biological response of selected indicators in the LMR; therefore, our findings and recommendations on

[f] Category 1 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 1.2. For the Category 1 Fish (channel) indicator, there are no CEWO evaluation questions for this Selected Area; however, fish monitoring data are consolidated to evaluate a number of fish targets of DEWNR's LTWP (Appendix H). The Basin-scale evaluation for fish community responses to Commonwealth environmental water are being undertaken by the Monitoring and Evaluation (M&E) Advisors, i.e. the Murray–Darling Freshwater Research Centre (LMR LTIM M&E Plan, SARDI *et al.* 2016).

environmental water management are most relevant to this period. Nevertheless, considering the annual cycle of flow (beyond spring/summer) is important for maintaining and restoring ecological integrity of riverine ecosystems. More detailed information (e.g. methodology, statistics, etc.) for each indicator in the LMR are provided in the Appendices and SARDI *et al.* (2016).

2 KEY FINDINGS

2.1 Category 1

Stream Metabolism

River metabolism measurements estimate the in-stream rates of photosynthesis and respiration, and provide information on the energy being processed through riverine food webs (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). Metabolism measurements help identify whether the sources of organic materials that provide the food resources have come from within the river (autochthonous) or from the surrounding landscape (allochthonous). They describe the fundamental trophic energy connections that characterise different food web types (e.g. detrital, autotrophic, planktonic), and indicate the size of the food web and so its capacity to support higher trophic levels including fish and water birds (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006).

For estimating stream metabolism, *in situ* logging of the dissolved oxygen concentration, water temperature and incident light were undertaken at single river sites in the gorge (downstream of (below) Lock 1) and floodplain (below Lock 6) geomorphic zones of the LMR in 2016/17 (refer to SARDI *et al.* 2016). Discrete water quality samples were collected approximately every four weeks and analysed for chlorophyll *a*, total nitrogen, nitrate and nitrite combined, ammonium, total phosphorus, dissolved forms of phosphorus, and dissolved organic carbon. The detailed monitoring and analysis protocol described in Hale *et al.* (2014), including collection of samples for water quality, was consistently followed, but with several small modifications (Appendix C).

Extra sites were included in the 2016/17 monitoring period in an effort to provide additional information on changes in metabolism in the LMR and inform on changes associated with the raising of Weir Pools 2 and 5 (Appendix B). In the end, no conclusions could be drawn about the influence of weir pool raising on river metabolism due to overbank flows which masked the management action (detail in Appendix B and Appendix C).

During the 2016/17 monitoring, oxygen concentrations declined quickly between early November and the end of December 2016 across the sites, falling below the 50% saturation level (~4.5 mg L^{-1}) considered acceptable by DEWNR and targeted in the Basin Plan (S9.14) (Figure C1 Appendix C). Downstream of Lock 6, dissolved oxygen concentrations fell to 0 mg L^{-1} for a 4-day period in early December. Prolonged exposure to dissolved oxygen concentrations below 2 mg L^{-1} is detrimental to a range of aquatic organisms, including fish, while zero oxygen levels are lethal to many. These deleterious oxygen concentrations were the result of extensive flooding throughout the Murray River (CEWO *In prep*) 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), with oxygen depletion exacerbated by the reduced frequency of floodplain inundation due to river regulation which has increased the time for accumulation of organic debris and detritus on the floodplains (Howitt *et al.* 2007). This material stimulates increased biological activity on flooding, causing rapid oxygen decline. Flows in the Murray River declined rapidly through December 2016 and, by 22 December, were in-channel and disconnected from the floodplains (~45,000 ML day^{-1}). At this point dissolved oxygen concentrations rapidly increased, particularly at Lock 6, but then progressively downstream through the sites (Figure C1 in Appendix C). The substantial increase in the oxygen concentration at Lock 6 (~15–25 December 2016) was mostly due to improved water quality arriving from upstream in the Murray River or the Murrumbidgee River. The source(s) of this water, which potentially included environmental water, was difficult to determine and not explored in this report. The timing of rapid increase in oxygen concentrations also coincided with water returning in-channel, following overbank flows.

During the low oxygen period, flow releases from Lake Victoria were made to maintain oxygen levels in the Rufus River and provide a refuge habitat for aquatic fauna (Appendix A). During these releases, from mid-November 2016, the oxygen concentrations in the Murray River, downstream of the Lake Victoria outflow, were consistently higher than those upstream (Figure C6 in Appendix C). The size of the difference varied from zero up to 1 mg L^{-1} on 25 November 2016, during peak Lake Victoria releases (5,560 ML day^{-1}) (Figure A3 in Appendix A), but concentrations had generally declined again by the time the flow reached Lock 6. However, for a short period from 11 to 17 December 2016, the positive influence of the releases on oxygen

concentrations appeared to extend to Customs House (Figure C6 in Appendix C), downstream of the South Australian border (Figure A5 in Appendix A). These results suggest that the release of oxygenated waters from Lake Victoria had a wider impact than just the local environment of the Rufus River and, although the influence was small, it occasionally extended downstream for a substantial distance. Why the oxygen increase at this time was sustained so far downstream is not apparent and a more dynamic analysis accounting for oxygen metabolism and exchange at the water surface is required to address this question. Commonwealth environmental water and The Living Murray water supplemented Lake Victoria releases from 17 to 31 December 2016 and maintained dissolved oxygen levels above 4 mg L^{-1} in the Rufus River (Figure A4 in Appendix A).

Following overbank flows, from January until the end of monitoring in early March 2017, a significant proportion of the water to South Australia was provided through environmental flows (Figure 3), which included contributions by Commonwealth environmental water, as well as other sources (e.g. The Living Murray water). Over this period, flows reduced gradually from 25,000 to 10,000 ML day^{-1}, with environmental flows contributing between 25 and 50% of the water. A marked increase in ecosystem respiration (oxygen consumption) at the site below Lock 6 occurred during this period and aligned with an increased delivery of water from the Darling River (Figure C7 and C8 in Appendix C). It is likely that increased turbidity due to flows from the Darling River, which is naturally highly turbid, reduced the light available to the phytoplankton, affecting their metabolism. Fluctuations in metabolic activity in response to changing flow conditions is part of the natural variability expected in a river reach, but as with other environmental characteristics influenced by river operation, the timing, frequency and magnitude of these changes is expected to be important, which may lead to long-term effects in the food web.

Fish (channel)

The main channel of the LMR supports a diverse fish assemblage, which is comprised of small- and large-bodied species that have various life history requirements (e.g. reproduction and habitat use). Variation in flow influences riverine hydraulics and in turn structural habitat (e.g. submerged vegetation), which may influence fish assemblage structure (Bice *et al.* 2014).

During March/April 2017, small- and large-bodied fish assemblages were sampled from the gorge geomorphic zone of the LMR (Figure 6) using fyke nets and electrofishing, respectively. Sampling of half the electrofishing sites was delayed to August 2017 due to equipment malfunction. Prescribed methods outlined in Hale *et al.* (2014) were used and population structure data were obtained for seven target species (Appendix D). The Category 1 Fish (channel) data were collected to inform Basin-scale evaluation of fish community responses to Commonwealth environmental water, which are being undertaken by the Monitoring and Evaluation (M&E) Advisors, i.e. the Murray–Darling Freshwater Research Centre (SARDI *et al.* 2016). While there is no CEWO local (Selected Area) evaluation questions for this indicator, we analysed autumn monitoring data from the LMR to investigate temporal variation in fish assemblage and population structure between years 1 (2015), 2 (2016) and 3 (2017) (Appendix D).

Relatively low (<15,000 ML day^{-1}), stable flows predominated in the LMR during 2014/15 and 2015/16. Consequently, small-bodied fish abundance and diversity were high in 2015 and 2016 (Figure 7b; Table D2 in Appendix D), and there was no significant change in small-bodied fish assemblage structure from 2015 to 2016. Abundances of flow-cued spawning species (i.e. golden perch and silver perch (*Bidyanus bidyanus*)) remained similar in both years; however, there was a significant change in the large-bodied fish assemblage, driven primarily by a decrease in bony herring (*Nematalosa erebi*) in 2016 (Figure 7a; Table D1 in Appendix D). Following the flood (>45,000 ML day^{-1}) in spring/summer 2016 (Figure 2), there was a significant change to the small- and large-bodied fish assemblages with an overall decrease in the abundances of small-bodied species (e.g. carp gudgeon (*Hypseleotris* spp.), gambusia (*Gambusia holbrooki*) and Murray rainbowfish (*Melanotaenia fluviatilis*)) and an increase in the abundance of common carp (*Cyprinus carpio*) in 2017 (Figure 7). 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 the decreased abundance of small-bodied fishes. Increased abundance of common carp in 2017 appeared to be driven by a large recruitment event in 2016/17 associated with flooding (Figure D7 in Appendix D). Enhanced recruitment of common carp has been previously observed in the LMR (Bice *et al.* 2014) and elsewhere in the MDB (King *et al.* 2003; Stuart and

Jones 2006), during floodplain inundation. Following a recession in water levels in summer 2017 (Figure 2), large numbers of age 0+ common carp likely entered the main channel from off-channel floodplain and wetland habitats (their typical spawning habitat) and were captured during sampling in autumn and winter 2017.

Based on length frequency data from electrofishing, there was no recruitment (to age 0+) of golden perch and silver perch in 2014/15 and 2015/16 (Figure D4 in Appendix D). This is consistent with our contemporary understanding of the influence of flow on the life histories of these flow-cued spawners (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b) (also see Section 0 Category 3 Fish Spawning and Recruitment). During higher flows in 2016/17, golden perch eggs and larvae were collected (see Appendix H Fish Spawning and Recruitment), but there was no recruitment (to age 0+) in 2016/17, based on electrofishing sampling (Figure D4 in Appendix D). The mechanisms contributing to recruitment failure were not explored as a component of this project, but the coincidence of a hypoxic blackwater event with spawning may have contributed to larval mortality (see Section 0 Fish Spawning and Recruitment).

Based on length frequency data from electrofishing, there has been no recruitment (to age 0+) of freshwater catfish (*Tandanus tandanus*) from 2014/15–2016/17 (Figure D4 in Appendix D). Freshwater catfish spawn independent of flows (Davis 1977); however, their recruitment dynamics in the lower River Murray are poorly understood and their current spawning biomass in this region is historically low (Ye *et al.* 2015c). For the third consecutive year, small Murray cod (<150 mm TL, likely age 0+) were sampled in the LMR during 2017 (Figure D4 in Appendix D), indicating successful recruitment. Furthermore, the cohorts from 2014/15 and 2015/16 seemed to have persisted in 2016/17. In the main channel of the lower River Murray, Murray cod recruitment has been poor in association with periods of low flow and positively associated with years of elevated flow (in-channel and overbank) (Ye *et al.* 2000; Ye and Zampatti 2007; Zampatti *et al.* 2014). The mechanisms facilitating the recruitment of cohorts of Murray cod from 2014/15 and 2015/16, both low flow years, remain unclear.

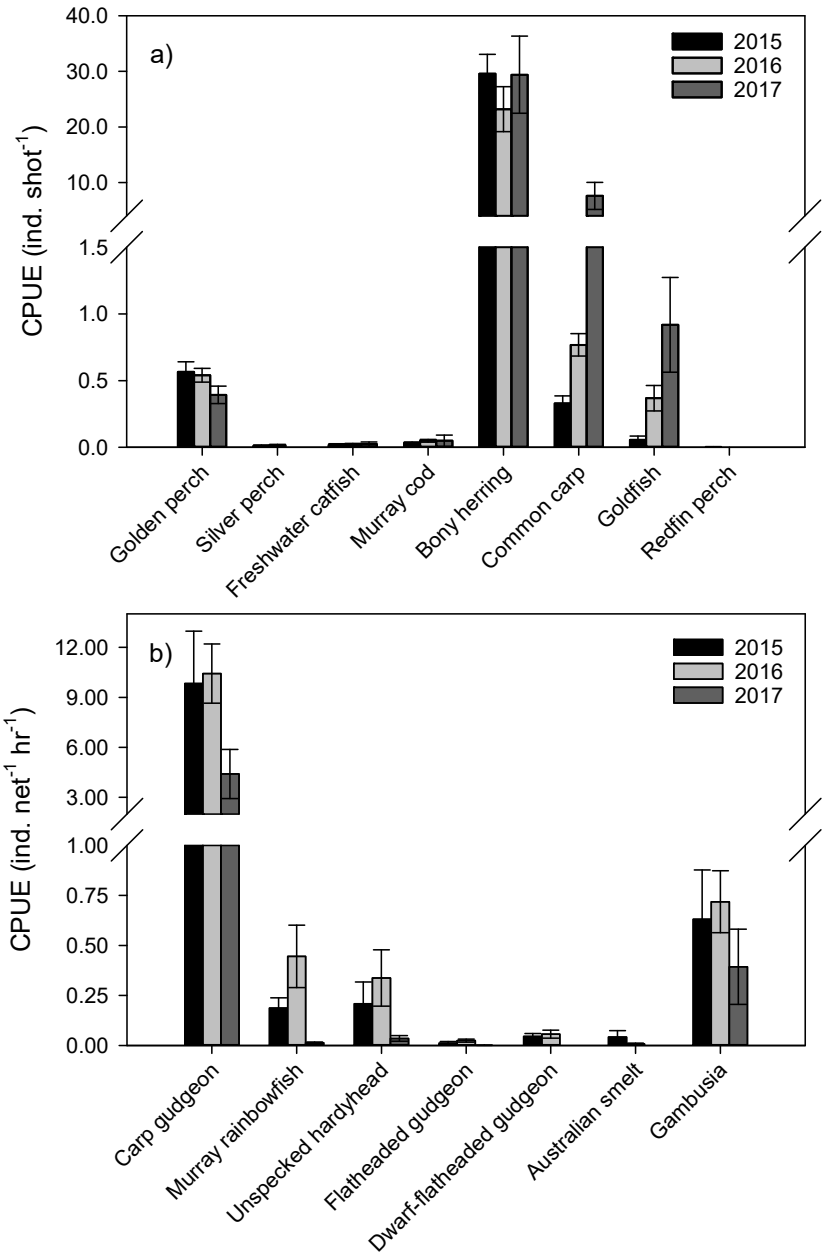

Figure 7. Mean catch-per-unit-effort (CPUE) ± standard error of (a) large-bodied fish species captured using electrofishing (individuals per 90 second shot) and (b) small-bodied fish species captured using fine-mesh fyke nets (individuals per net per hour) in the gorge geomorphic zone (10 sites) of the LMR in Autumn from 2015–2017. Electrofishing CPUE data from five sites are presented for 2017 as other sites were sampled during winter (Appendix C).

2.2 Category 3

Hydrological Regime

Regulation of the lower River Murray, through the construction of weirs, has resulted in significant changes to the hydraulic nature (e.g. water velocity and water level) of the main river channel. Pre-regulation, the lower River Murray was a lotic (flowing) riverine environment characterised by water velocities ranging ~0.2–0.5 m s^{-1}, even at discharges <10,000 ML day^{-1} (Bice *et al.* 2017). After the construction of serial weirs, main channel water velocities have been reduced to ~0.05–0.3 m s^{-1} and riverine habitats have been converted to predominantly lentic weir pools at discharges <10,000 ML day^{-1} (Mallen-Cooper and Zampatti 2015). Lotic habitats are important for ecological and life history processes for many native biota that are adapted to flowing riverine environments. For example, they provide stimuli for spawning of flow-cued species (e.g. golden perch) (King *et al.* 2016), facilitate downstream drift and transportation of plankton, macroinvertebrates and fish larvae, and provide diverse hydraulic habitats that are suitable for a range of species (e.g. Murray cod) (Zampatti *et al.* 2014). Conversely, lentic habitats provide spawning and nursery areas suitable for generalist species (e.g. carp gudgeons), particularly at low flows when aquatic macrophytes are abundant (Bice *et al.* 2014). The reduction in the abundance and distribution of lotic biota (e.g. Macquarie perch *Macquaria australasica* and Murray crayfish *Euastacus armatus*) throughout the MDB (Lintermans 2007) highlights the importance of restoring hydraulic conditions (e.g. lotic habitats), which is particularly needed in the heavily regulated lower River Murray.

The Hydrological Regime indicator used models (see Appendix E) to convert the discharge delivered to the LMR in 2016/17 to water levels and velocities, as such all results presented are based on modelling. These variables were calculated for the observed (with all water, including environmental water) conditions, as well as the without environmental water cases. The models were calibrated to observed discharge, water level and velocity measurements, to ensure they provide an accurate representation of reality. Details of the model calibration are presented in Appendix E.

Figure 8 presents changes in water levels and velocity for Weir Pool 3, with results for all weir pools presented in Appendix E. In each plot, the changes due to

Commonwealth environmental water can be seen between the 'with all water' case (blue) and the 'no CEW' case (green), and the change due to all environmental water by comparing the 'with all water' case to the 'no eWater' case (orange). The water level at the upper end of the weir pool (e.g. directly below Lock 4 for the Weir Pool 3 case) has been presented, as the upper end of the weir pool is the least influenced by the weir and hence most responsive to changes in discharge when the weirs are controlling water levels (below 54,000 ML day^{-1}–67,000 ML day^{-1} depending on the weir). For velocity, the median modelled velocity in each weir pool is presented as the solid line, and the range in velocities within the weir pool shown as the shaded band (as the 10th and 90th percentiles).

As seen in Figure 3, high unregulated flow conditions in the first half of the year resulted in little environmental water delivery, beyond some small return flows from tributaries. As such, there was little difference between the scenarios over this period (Figure 8). Environmental water delivered in late December 2016 and January 2017 helped slow the recession in water levels and velocities once flows returned to within channel (<45,000 ML day^{-1}). With environmental water, the water level decreased between 2.5 m at Weir Pool 3, 4 and 5 and 3. 8 m at Weir Pool 1 over a two to three-week period. However, without environmental water, modelling showed that the water level drop would have been an additional 0.7–0.9 m over the same two to three-week period (Figure 8).

Following overbank flows, the environmental water delivered from January to June 2017 led to increased water levels, potentially re-wetting a small proportion of the littoral zone that would have been inundated in spring/early summer. The substantial increases occurred in late March 2017, by 0.2–0.4 m downstream of each Lock (see in Figure 8 for Weir Pool 3, for example), during return flows from a pulse in the Goulburn River from the Commonwealth and Victorian Environmental Water Holders, as well as delivery of River Murray Increased Flows (Figure 5).

Commonwealth environmental water, and water from The Living Murray and Victorian Environmental Water Holder increased the median velocity in Weir Pool 5 in the last week of December by 62% to 0.47 m s^{-1}, maintaining lotic habitat on the flood recession. Following this event, median velocities tended to be less than 0.1 m s^{-1} (considered to represent lentic habitat) across the weir pools without environmental water, with the exception of during an unregulated flow event in May 2017 (Figure 8).

For the case with environmental water in the second half of the year, weir pool median velocity increased to a small degree (0.05–0.07 m s^{-1}), with some sections of river modelled to have greater than 0.17 m s^{-1}, particularly during the event in March 2017 when environmental water increased the flow to South Australia to 10,000 ML day^{-1}.

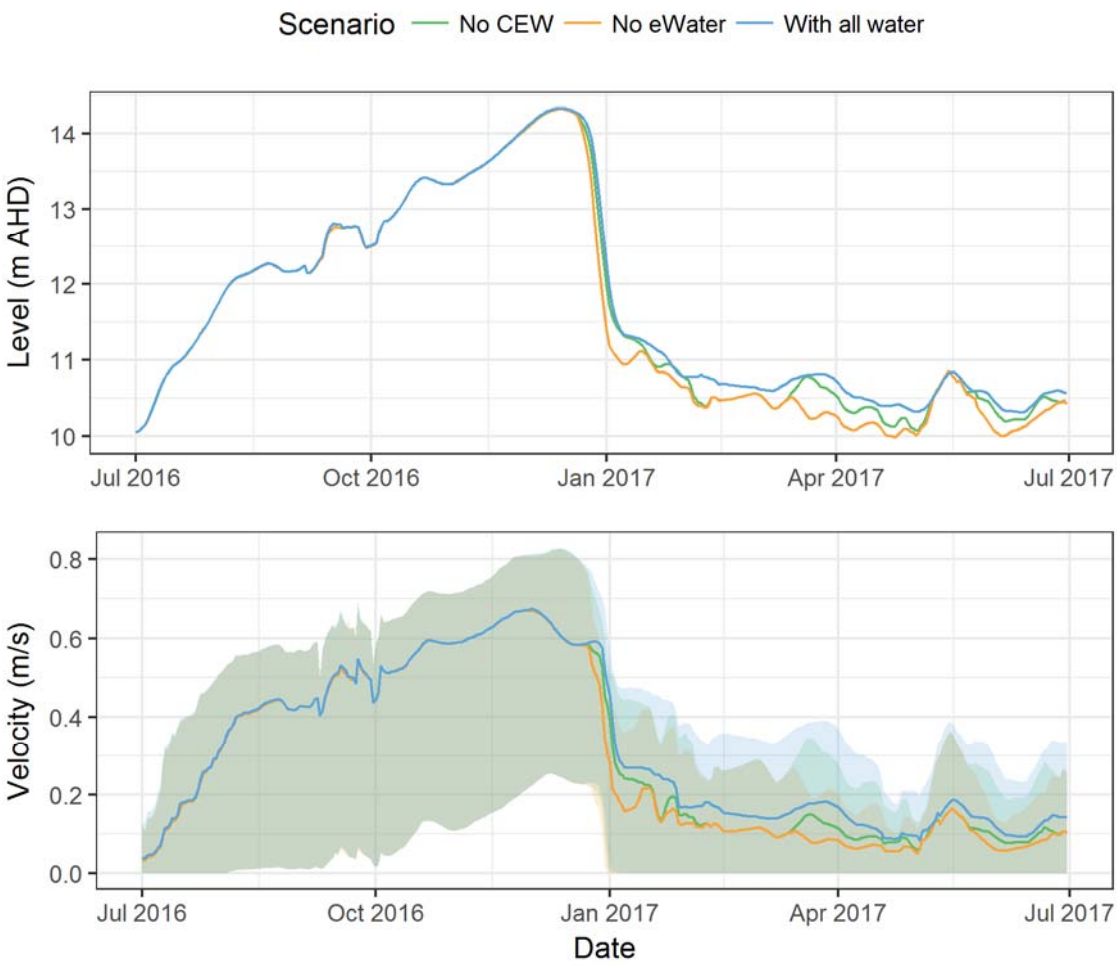

Figure 8. Modelled water level at the upstream end of Weir Pool 3 (above), and median modelled velocity (line), with the shaded band representing the range within the weir pool (as the10th and 90th percentiles) (below). The range in velocities represented by the shaded area overlaps for the majority of the time, and when this is the case the green, yellow and blue shading combines accordingly.

Matter Transport

Modification to the flow regime can alter the biogeochemistry of rivers and the adjacent floodplain system. For example, reduced flow may increase the intrusion of salt into the system and decrease the export of salt from the system. Additionally, a change in the flow regime will alter the mobilisation of nutrients from the floodplain

and so change the primary productivity with the river. Environmental flows can be used to reinstate some of the natural processes, or the magnitude of the processes that control the availability and transport of dissolved and particulate matter. Environmental flows can, therefore, be manipulated to derive ecological benefits through the provision of habitat, resourcing food webs and flushing contaminants from the system.

The contribution of environmental water to the transport of salt, nutrients and phytoplankton was assessed with a coupled hydrodynamic-biogeochemical model for the reach below Lock 1 to the Murray Mouth. Salt, nutrient and phytoplankton transport was predicted for three different flow scenarios: with all water (i.e. the observed flow), flow without the Commonwealth environmental water, and flow without any environmental water

When modelling, it is necessary to make assumptions on the relationships between flow and nutrients or salt, nutrient dynamics in sediments and floodplain habitats, and the utilisation of nutrients by phytoplankton. This leads to a degree of uncertainty in model outputs; however, it is considered that this uncertainty is within reasonable bounds (Aldridge *et al.* 2013) and the results can be used to assess the general response to environmental water.

Salinity

Although environmental water had little impact on the salinity (concentration of salt) in the Murray River Channel or Lake Alexandrina during 2016/17 (Table 3), the salinity in the Coorong was much lower because of environmental water. The model predicted that if there was no Commonwealth environmental water, the salinity would have been 17.46 practical salinity units (PSU)[g]; however, with environmental water, the median concentration only reached 12.97 PSU (Table 3).

Flow at the South Australian border in 2016/17 peaked at approximately 94,600 ML day^{-1}, which was considerably higher than in 2015/16, where maximum flow was approximately 11,600 ML day^{-1}. The higher flow meant much more material was

[g] PSU was used for modelling purposes in the report. PSU is approximately equal to 1 part per thousand (ppt or ‰) or 1 g L^{-1}.

transported through the reach between Lock 1 and the Southern Ocean. Total export of salt through the Murray Mouth in 2016/17 was 3,679,277 tonnes (Figure 9; Table 4), whereas in 2015/16 there was a net import of salt into the Murray Mouth/Coorong of 1,850,028 tonnes (Ye *et al.* 2017). Commonwealth environmental water contributed substantially to the export of salt from the Coorong/Murray Mouth in 2016/17, with 519,292 tonnes of salt export attributable to Commonwealth environmental water. Further upstream at Wellington, export of 114,463 tonnes of salt was achieved with Commonwealth environmental water and export over the barrages of 120,866 tonnes.

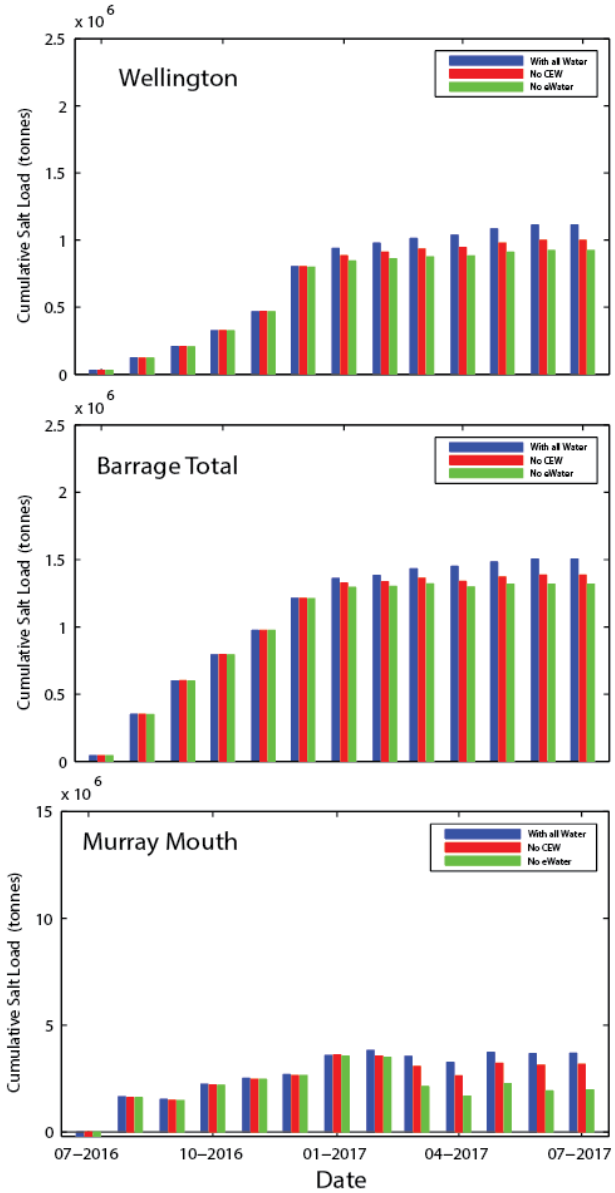

Figure 9. Modelled cumulative salt exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

Dissolved nutrients

Ammonium concentrations were similar for each of the flow scenarios, with little impact by environmental water (Table 3). The net transport of ammonium did not vary greatly between the three scenarios for the Murray River Channel and Lake Alexandrina, but export of ammonium through the Murray Mouth would have been approximately 4.4 tonnes less if no environmental water was delivered.

The mean phosphate concentration was slightly diluted by environmental water in the Murray River Channel and Lake Alexandrina, but was higher in the Coorong than would be expected with environmental water. Interestingly, the net cumulative load of phosphate exported from each site decreased downstream. This could be due to either uptake of the bioavailable form of phosphorus by phytoplankton (i.e. the transformation of inorganic phosphorus to particulate organic phosphorus) or absorption to particles. The particulate phosphorus could then be transported downstream or lost to sediment as the particles settle. Cook *et al.* (2010) found that on average 92% of filterable reactive phosphorus entering Lake Alexandrina was retained in the lake. This processing and retention in the Lower Lakes significantly reduces nutrient export and modifies the nutrient available to drive estuarine productivity.

Environmental water diluted silica concentrations in the Murray River Channel at Wellington and in Lake Alexandrina, but led to higher silica concentrations in the Coorong relative to the scenarios with no environmental water (Table 3). During 2016/17, the higher flows relative to 2015/16 changed the dynamics of silica transport. The total export of silica from Wellington (Murray River Channel) was 49,964 tonnes in 2016/17, whereas it was 1,760 tonnes in 2015/16. In 2016/17, Commonwealth environmental water contributed to 2 and 11% of total silica export from the Murray River Channel and Lower Lakes, respectively. In contrast, in 2015/16, Commonwealth environmental water contributed 41 and 95% of total silica export from the Murray River Channel and Lower Lakes, respectively. Silica is an important nutrient for the growth of diatoms, a group of phytoplankton that constitute an important food source in riverine and estuarine environments. The difference in silica export between the two years highlights the importance of environmental water to deliver nutrient resources, particularly in dry years.

Particulate organic nutrients

The particulate organic nitrogen concentrations were similar for all flow scenarios (Table 3). The particulate organic phosphorus showed slightly more variability at Wellington at the different flow scenarios. Although the 'with all water' scenario had the lowest particulate organic phosphorus concentration, the export from this site was greatest with this flow scenario owing to the additional volume of environmental water (Table 4).

The particulate organic nitrogen loads were 3,949 tonnes exported from Wellington and 3,819 tonnes through the Murray Mouth in the 'with all water' scenario. This suggests most of the nitrogen originated from upstream and there was minimal transformation or input in the lower reaches of the LMR under the high flows observed in 2016/17. For the same scenario, there was less particulate organic phosphorus as the water flows downstream suggesting transformation and retention within the Lower Lakes.

The nitrogen to phosphorus ratio (N:P) can provide insight into which nutrient may become limiting and can provide some insight into the transport of nitrogen and phosphate. In 2016/17, the N:P ratio (particulate organic nitrogen: particulate organic phosphorus) ranged between 3.5–4.3:1, whereas in 2015/16 the N:P ratio ranged between 12.2–14.1:1 when there was net export of nitrogen and phosphorus. This suggests a difference in the transport of nitrogen and phosphate at low and high flow. The Redfield ratio is the average ratio of carbon:nitrogen:phosphorus found in phytoplankton but this can be modified by nutrient limitation. By mass, the Redfield N:P ratio is 7:1. The ratio of 3.5–4.3:1 in 2016/17 suggests there was abundant phosphorus and if concentrations became low enough to become limiting, then nitrogen would become limiting first. This differs from 2015/16 where the N:P ratio was 12.2-14.1:1, which suggests phosphorus was the nutrient most likely to become limiting. This also demonstrates a difference in the capacity of the river to transport nitrogen and phosphorus with phosphorus transport reduced in drier years.

Chlorophyll a

The high nutrient loads in 2016/17 supported reasonably high abundances of phytoplankton. Chlorophyll *a* concentrations were 29.8 mg L^{-1} in the Murray River Channel (Wellington), 33.0 mg L^{-1} in the Lower Lakes, and 20.2 mg L^{-1} in the Coorong.

The concentrations and loads of chlorophyll *a* were similar for each of the flow scenarios (Table 4). This contrasts sharply with 2015/16 when Commonwealth environmental water contributed 44, 92 and 93% of the total export of phytoplankton biomass from the Murray River Channel, Lower Lakes and Coorong/Murray Mouth, respectively (Ye *et al.* 2017). However, due to high flows in 2016/17, the total phytoplankton loads were two orders of magnitude higher than 2015/16.

Table 3. Median concentration of salinity, nutrients and chlorophyll *a* during 2016/17 for the modelled scenarios at three selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

Site	Scenario	Salinity (PSU)	Ammonium (mg L^{-1})	Phosphate (mg L^{-1})	Silica (mg L^{-1})	Particulate organic nitrogen (mg L^{-1})	Particulate organic phosphorus (mg L^{-1})	Chlorophyll *a* (mg L^{-1})
Wellington	With all water	0.17	0.001	0.009	3.05	0.49	0.11	29.8
	No CEW	0.17	0.001	0.011	3.86	0.50	0.14	32.6
	No eWater	0.16	0.001	0.012	6.04	0.50	0.15	34.8
Lake Alexandrina Middle	With all water	0.23	0.001	0.016	6.99	0.54	0.13	33.0
	No CEW	0.22	0.001	0.018	9.20	0.54	0.14	33.0
	No eWater	0.21	0.001	0.020	10.01	0.54	0.14	32.8
Murray Mouth	With all water	12.97	0.017	0.006	4.10	0.51	0.09	20.2
	No CEW	17.46	0.018	0.003	3.03	0.49	0.08	18.0
	No eWater	18.73	0.019	0.002	1.92	0.49	0.08	17.2

Table 4. Net cumulative load (tonnes) of salt, nutrients and chlorophyll *a* during 2016/17 for the modelled scenarios at three selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

Site	Scenario	Salt	Ammonium	Phosphate	Silica	Particulate organic nitrogen	Particulate organic phosphorus	Phytoplankton (as carbon)
Wellington	With all water	1,112,077	28.3	116.5	49,964	3,949	1,084	3,650
	No CEW	997,614	27.9	111.9	48,658	3,705	1,031	3,405
	No eWater	921,483	27.6	108.5	47,410	3,542	990	3,221
Barrage	With all water	1,504,541	39.8	62.6	40,829	3,894	917	2,939
	No CEW	1,383,674	39.3	52.1	36,295	3,627	847	2,748
	No eWater	1,317,791	38.9	45.1	33,003	3,458	801	2,629
Murray Mouth	With all water	3,679,277	30.8	51.9	39,901	3,819	897	2,881
	No CEW	3,159,985	28.4	43.0	35,345	3,559	829	2,708
	No eWater	1,958,989	26.4	37.9	32,392	3,400	787	2,601

Microinvertebrates

Aquatic microinvertebrates (microcrustaceans, rotifers and protists) are a major food source for larger organisms in freshwater systems (Schmid-Araya and Schmid 2000; Pernthaler and Posch 2009), and important for early life stages of fish (i.e. larvae) (Arumugam and Geddes 1987; Tonkin *et al.* 2006). The aquatic microinvertebrates of the MDB have short generation times and are rapid responders to environmental changes (Tan and Shiel 1993). To assess the responses of microinvertebrates to Commonwealth environmental water delivery in the LMR, mid-channel microinvertebrate assemblages were sampled during spring/summer 2016/17 using a Haney trap at sites below Lock 1 and Lock 6, in the gorge and floodplain geomorphic zones, respectively (Appendix F; SARDI *et al.* 2016). In 2016/17, three extra monitoring sites in and below Weir Pool 2 (gorge geomorphic zone) were included to complement existing LTIM monitoring and evaluation, and to investigate the influence of weir pool raising at Lock 2 on microinvertebrate diversity and density (Appendix B).

Over the 2016/17 sampling period (late September 2016 to early January 2017), 289 microinvertebrate taxa (rotifer/protist dominated) were discriminated from 264 trap samples (Appendix F). Not recorded in 2014/15 or 2015/16, 94 taxa (33%) comprised protists (47, mostly rhizopods), rotifers (38), cladocerans (5) and copepods (4).

Microinvertebrate taxa richness (indicating diversity) and assemblage structure were similar among the first four sampling trips from late September to early November 2016 (Figure 10; Appendix F) when flows largely remained in-channel. During this time, the in-channel assemblage was dominated by the ciliate *Codonaria* and a suite of rhizopods, with rotifers and microcrustaceans (e.g. copepods and cladocerans) notably low in abundance and diversity in comparison to previous years (Ye *et al.* 2016a; 2017). Unlike diversities and assemblage structure, which stayed similar over the first four sampling trips, microinvertebrate densities peaked (2,067–3,133 ind L^{-1}) in late October. This peak was driven primarily by *Codonaria* (Figure G2 in Appendix G) and aligned with the timing of the recession of water levels in Weir Pools 2 and 5 (Figure B2 in Appendix B; Figure G6 in Appendix G), and the lowering of water levels in Chowilla Creek following regulator operation (Appendix A). Nevertheless, this response was unlikely caused by any of these management actions. Instead, increased densities of bacteriovores/algivores (*Codonaria* particularly) at all locations

from late September to late October suggests a trophic response to increased bacterial decomposition or algal abundance (Dolan *et al.* 2013), likely associated with a diatom (*Aulacoseira*) bloom evident in the channel at the time (University of Adelaide unpublished data). A marked decline in density at all sites in early November (Figure 10) may have been a dilution effect by increased flows as no other causes were apparent.

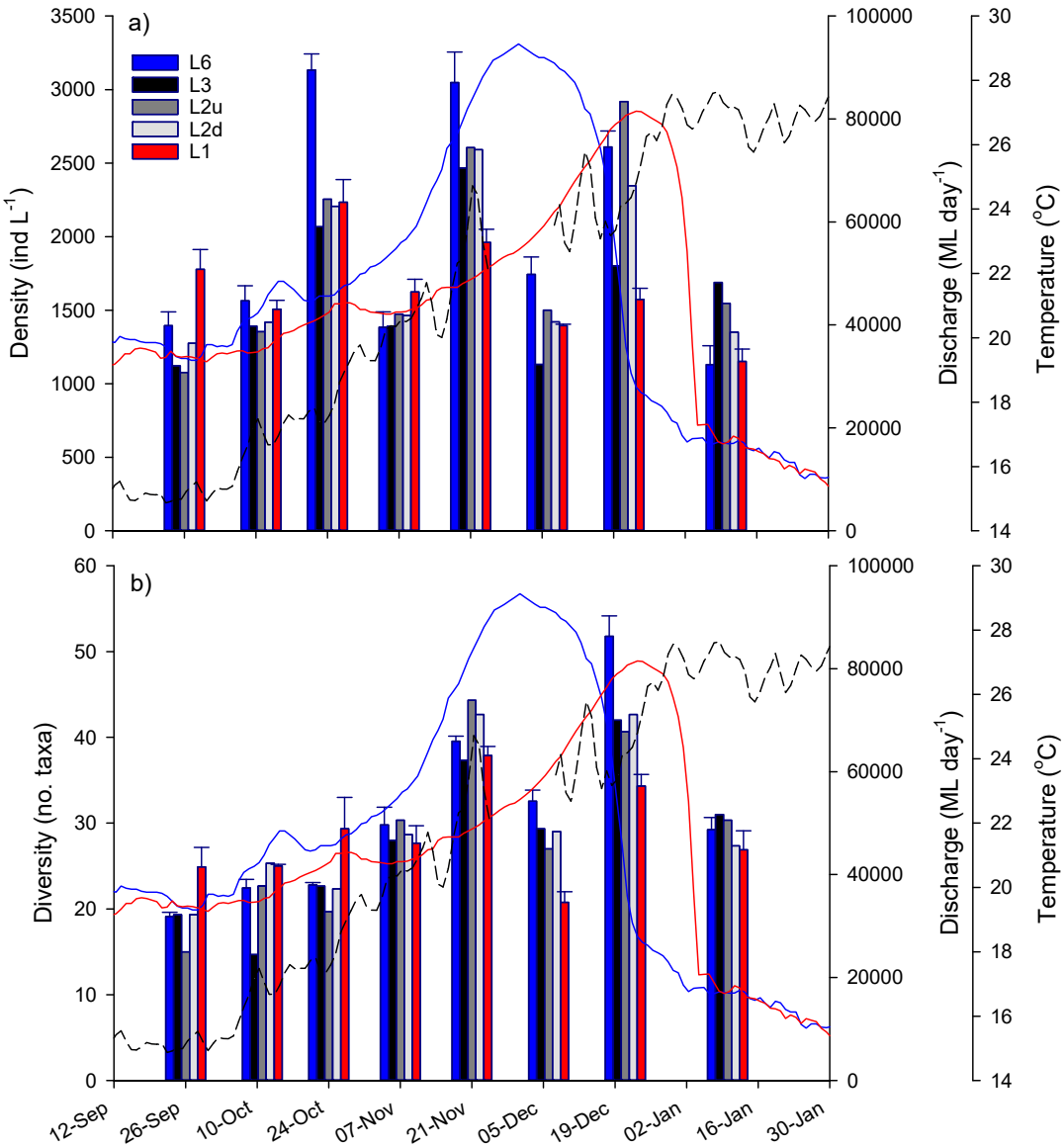

Figure 10. Mean (±S.E.) (a) density and (b) taxa richness of microinvertebrates collected in the LMR at core LTIM sites below Lock 1 (L1) and Lock 6 (L6), and at additional weir pool monitoring sites below Lock 3 (L3) and Lock 2 (L2d), and above Lock 2 (L2u), in 2016/17. Data are plotted against discharge (ML day^{-1}) in the LMR at the South Australian border (solid blue line) and below Lock 1 (solid red line), and against water temperature (°C) (dashed black line). Sampling was undertaken approximately fortnightly from 26 September 2016 to 11 January 2017.

Following the commencement of overbank flows across the LMR after early November (Figure 3), along with the influence of increasing water temperature, there was a significant change in assemblage structure in late November and early December (Appendix F). A diverse rotifer assemblage, with brachionids (*Anuraeopsis, Brachionus* and *Keratella* spp.), synchaetids (*Polyarthra* and *Synchaeta* spp., and trochosphaerids (*Filinia* species) was recorded. *Codonaria* and diverse riparian rhizopods were still present, but in reduced numbers (Figure G2 in Appendix G). The differences in microinvertebrate assemblages between trips, for below Locks 1 and 6, were primarily driven by abundance differences of a few taxa. These included lower abundances of rotifers *Brachionus angularis bidens* and *Polyarthra dolichoptera* from late September to late October; and higher abundance of the rotifer *Pompholyx complanata* in early November, *Keratella* spp. (e.g. *K. cochlearis*) in November, *Anuraeopsis fissa* in late November, and *Asplanchna priodonta* and *Brachionus budapestinensis* in late December. Species found only in upper Murray waters (e.g. *Brachionus angularis bidens*) were likely transported to the LMR during high flows from an upstream Murray River source, when flow from the Murray River was proportionally dominant (Figure 5).

During peak flooding (>70,000 ML day^{-1}) from late November to late December (Figure 3; Figure 10), density and diversity peaked at most sites, with the exception of early December, when densities and diversities at all sites fell below 1,750 ind. L^{-1} and 33 spp., respectively (Figure 10). This decrease in density and diversity likely occurred as a result of low dissolved oxygen levels, which fell below 2 mg L^{-1} at all sites (Figure C1 in Appendix C).

Following the reduction in river flow and water levels in early January 2017, there was a decline in density and diversity at all sites (Figure 10), despite the delivery of Commonwealth environmental water to slow the rate of flood recession. Physiological stressors and parasite infestation during maximum population densities in late December were evident (Figure G5 in Appendix G) and potentially contributed to the subsequent decline in January. The microinvertebrate assemblages also changed significantly below Lock 1 and Lock 6, compared to that present during rising unregulated and overbank flows (Appendix F). The changes were primarily driven by higher abundance of the rotifer *Trichocerca* cf. *agnatha* (not recorded previously in South Australia) in late December and January for below Locks 1 and 6;

higher abundance of the warm-water rotifers *Brachionus bennini*, *B. caudatus personatus* and *Trichocerca similis grandis*, and protist *Stentor* sp. in January for Lock 6; and higher abundance of the protist *Coleps* sp. in January for Lock 1. It is likely that the ciliates (e.g. *Coleps* sp. and *Stentor* sp.) were responding to higher bacterial levels resulting from in-stream decomposition.

Dominant taxa in the late December and January assemblages at all locations were a mix of Murray and Darling River species. *Trichocerca* cf. *agnatha*, for example, known only from two Murray River records (R. Shiel, unpublished data; D. Furst, pers. comm), may have come from an upstream Murray River source (e.g. Barmah-Millewa releases in late December 2016). However, other taxa during the late December and January sampling period (e.g. *Brachionus caudatus personatus, B. durgae*) were warm-stenotherms or 'tropical' species, most likely sourced from the Darling River or a northern tributary of it. The presence and increased abundances of these warm-water species aligns with a proportional increase in Darling River discharge to the LMR after mid-December, driven by environmental water delivery that included Commonwealth environmental water and The Living Murray water (Figure 5; Appendix A).

Fish Spawning and Recruitment

Spawning and recruitment of golden perch in the southern MDB corresponds with increases in water temperature and discharge, either in-channel or overbank (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b). Similarly, abundant year classes of silver perch in the southern MDB correspond to in-channel increases in discharge (Mallen-Cooper and Stuart 2003). As such, golden perch and silver perch are considered candidate flow-dependent species for measuring ecological response to environmental water allocations. Understanding the influence of hydrology on the population dynamics of golden perch and silver perch, however, is reliant on accurately determining the hydrological conditions at the time and place of crucial life history processes. For example, to be able to accurately associate river flow with spawning, including environmental water delivery, the time and place of spawning must be known.

To evaluate the contribution of Commonwealth environmental water to the spawning and recruitment of golden perch and silver perch in the LMR in 2016/17, we: (1)

sampled larval and young-of-year (YOY) fish (Figure 11) at sites in the gorge and floodplain geomorphic zones of the LMR (Figure 6); (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 collect a representative subsample of the golden perch and silver perch populations in the LMR to enable determination of population demographics.

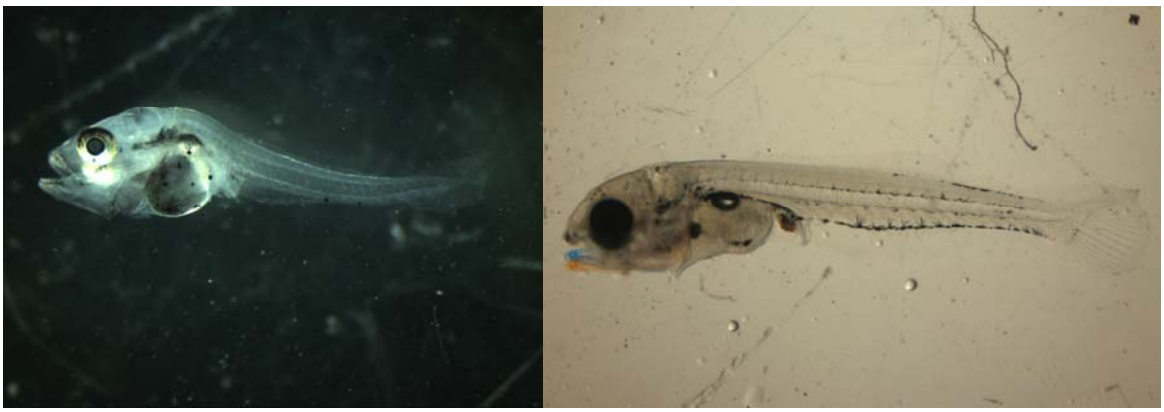

Figure 11. Larval golden perch (left) and silver perch (right) were sampled as an indicator for spawning, while young-of-year were sampled as an indicator for recruitment.

In 2016/17, golden perch eggs and larvae were collected from the gorge and floodplain geomorphic zones of the LMR between October 2016 and January 2017, with the majority of larvae (*n* = 14) collected downstream of Lock 6 on 10 January 2017. The age of these larvae (predominantly 9–17 days) and otolith $^{87}Sr/^{86}Sr$ indicate these fish were spawned from 24 December–1 January, in the lower River Murray between the Darling River junction and Lock 6. One 27-day old golden perch larvae was also collected at Lock 6 on 10 January 2017, and otolith $^{87}Sr/^{86}Sr$ indicated this fish was spawned in the Darling River.

Overall, the presence of eggs and larvae with a lower River Murray provenance indicates that in 2016/17, golden perch spawning in the lower River Murray extended from October to early January and occurred in association with the ascending and descending limbs of a peak flow of ~94,600 ML day^{-1}. As such, some spawning coincided with the period when Commonwealth environmental water was used to augment flow in the LMR during the flood recession (Section 1.2).

In 2017, the golden perch population in the floodplain and gorge geomorphic zones of the LMR was dominated by age 7+, 6+ and 5+ fish. No age 0+ golden perch were collected by electrofishing, although two YOY golden perch were collected

incidentally in fyke nets. There was, however, a general absence of age 0+ golden perch in the LMR in 2017 indicating negligible recruitment from spawning in spring–summer 2016/17. The mechanisms contributing to recruitment failure were not explored as a component of this project, but the coincidence of a hypoxic blackwater event with spawning may have contributed to larval mortality. This could include the direct impacts of low dissolved oxygen concentrations on larval survival and/or indirectly through the impacts of hypoxia on food resources (Gehrke 1991; Section 4 Microinvertebrates).

Also in 2017, the silver perch population sampled in the LMR was comprised of age 3+–7+ fish spawned from 2009/10–2013/14 in association with in-channel and overbank increases in flow in the lower and mid-Murray River, and the Darling River. No age 0+–2+ silver perch were collected, indicating negligible recruitment from 2015–2017.

These findings augment contemporary conceptual models of the flow-related ecology of golden perch and silver perch in the Murray River. Previous investigations indicate that golden perch and silver perch recruitment in the LMR is promoted by spawning associated with spring–summer increases in flow (in-channel and overbank) in the lower and mid-Murray River, and lower Darling River (Zampatti and Leigh 2013a; Zampatti *et al*. 2015; Ye *et al*. 2017). As well as local spawning, immigration of age 0+ or 1+ fish can substantially enhance populations, particularly during years of high flow (Zampatti and Leigh 2013b; Zampatti *et al*. 2015).

3 SYNTHESIS AND EVALUATION

The aim of Commonwealth environmental water delivery is to restore aspects of the flow regime that have been impacted by flow regulation in order to protect and rehabilitate the ecological assets of the MDB, and the flora and fauna that depend on them (Gawne *et al.* 2014). In South Australia, this may involve contributing to base flows, increasing the magnitude, duration and/or frequency of natural freshes and contributing to overbank flows. Over the long-term, this is expected to make a significant contribution to achieving ecological outcomes in the LMR, through restoring ecological processes and improving habitat for biota in the main channel and floodplain/wetlands. To assess the ecological response to Commonwealth environmental water, a series of evaluation questions were investigated for CEWO. These questions were adapted from Basin-scale questions (SARDI *et al.* 2016) to be relevant for the LMR. In this third year's report of the five-year project, the focus is to evaluate the ecological outcomes of Commonwealth environmental water delivery during 2016/17 and answer CEWO short-term (one-year) evaluation questions (Table 5). DEWNR short-term questions, which serve as additional questions for the LMR and relate to ecological targets of the South Australian Murray River LTWP, are discussed in Appendix I.

In the LMR, 2016/17 was characterised by high unregulated flows and flooding in spring/early summer, with a flow peak of 94,600 ML day^{-1} at the South Australian border in late November 2016. High flows this year were of particular hydrological and ecological significance given that 2013/14–2015/16 comprised three consecutive dry years in the LMR (Figure 2). Flooding is an integral part of the natural flow regime, which plays an important role in maintaining the ecological integrity of floodplain rivers (Junk *et al.* 1989). Overbank flows during spring/early summer 2016 provided longitudinal and lateral hydrological connectivity and returned hydraulic complexity to the weir pools of the LMR. This facilitated key ecological processes including enhanced stream metabolism and increased transport and export of dissolved and particulate matter. Flooding in 2016/17, however, was accompanied by an extensive hypoxic (low dissolved oxygen) blackwater event throughout the Murray River (CEWO 2018) 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), with

oxygen concentrations falling below 50% saturation (~4.5 mg L^{-1}) across the LMR between early November and the end of December 2016, and to zero mg L^{-1} for a 4-day period below Lock 6 in early December. This rapid oxygen decline was caused by the biological breakdown of accumulated organic debris and detritus on the floodplains (Howitt *et al.* 2007), exacerbated by reduced frequencies of floodplain inundation due to river regulation. Prolonged exposure to dissolved oxygen concentrations below 2 mg L^{-1} is detrimental to a range of aquatic organisms, including fish, while zero oxygen levels are lethal to many.

During 2016/17, ~618 GL of Commonwealth environmental water was delivered to the LMR, with the majority (96%, excluding South Australian held entitlement flow) occurring after mid-December 2016, following the flood recession. Environmental flow was delivered to this region through a series of targeted watering events, along with return flows from the Murrumbidgee River, Victorian tributaries, the Lower Darling River and the Great Darling Anabranch. Quantifying the ecological benefits of Commonwealth environmental water in 2016/17 was particularly challenging, as may be expected given the volume and timing of environmental water delivery in relation to substantial flooding during spring/early summer, which was during the main field sampling period in the LMR. Nevertheless, some short-term hydrodynamic and ecological outcomes associated with environmental water deliveries were identified in the LMR during this year (Table 5). Key outcomes are summarised below.

Environmental water (including Commonwealth environmental water) provided on the flood recession helped slow the rapid reduction in velocity and water levels in the LMR during January 2017. For example, environmental water maintained lotic habitats (median velocities ≥0.3 m s^{-1}) for an additional 4–7 days in Weir Pools 1–4, and an additional 19 days in Weir Pool 5. Similarly, water levels would have fallen an additional 0.7–0.9 m over a two to three-week period in the LMR without environmental water.

After the flood recession (e.g. March 2017), environmental water contributed to an increase in median velocity in weir pools in the range of 0.05–0.07 m s^{-1}, with some sections of the river becoming greater than 0.17 m s^{-1}. Improving hydraulic conditions (e.g. flowing habitat) is critical for ecological restoration in the lower River Murray. Pre-regulation, this region was characterised by lotic, riverine habitats, with water velocities ranging ~0.2–0.5 m s^{-1}, even at discharges <10,000 ML day^{-1} (Bice *et al.* 2017). Commonwealth environmental water also contributed to increased water levels (e.g.

up to 0.2–0.4 m in the upper reaches of each weir pool in March 2017) in the LMR. Periodic increases in water levels also help improve the condition of riparian vegetation and increase biofilm diversity. However, it should be noted that these increases in velocity and water levels post flood were relatively minor compared to those achieved through the high unregulated flows in this year.

Additional environmental/ecological outcomes in the LMR, associated with Commonwealth environmental water delivery in 2016/17, included:

- Maintaining oxygen levels in the Rufus River during an extensive hypoxic blackwater event associated with flooding, which potentially provided refuge areas for aquatic organisms.
- Increased transport of nutrients and phytoplankton, which would likely stimulate primary and secondary productivity in downstream ecosystems, providing potential benefit to food webs of the LMR, Lower Lakes, Coorong and Southern Ocean, adjacent to the Murray Mouth.
- Transfer of warm-water taxa of microinvertebrates to the LMR from upstream sources (e.g. Darling River or a northern tributary of it), suggesting improved longitudinal connectivity and enhanced microinvertebrate dispersion.
- Reduced salinity concentrations in the Coorong, which would have improved habitat for estuarine biota in the region.
- Increased salt export from the Murray River Channel, Lower Lakes and Coorong/Murray Mouth. The total export was 3,679,277 tonnes in this high flow year (2016/17), with 14% attributed to Commonwealth environmental water.

However, there was negligible recruitment (to YOY, age 0+) of golden perch and silver perch during 2016/17. Contemporary conceptual models of the flow-related ecology of golden perch and silver perch suggest that spawning and recruitment of these species in the lower River Murray are associated with spring/summer in-channel flow variability and overbank flows in this region (nominally greater than 15,000 ML day^{-1}) or substantial flow pulses (e.g. 2,000–3,000 ML day^{-1}) in the lower Darling River) (Zampatti and Leigh 2013a; Zampatti *et al*. 2015). Such hydrological characteristics were present in 2016/17, and golden perch eggs and larvae were sampled from early October to late December 2016. Recruitment to YOY, however, was negligible. The

mechanisms leading to recruitment failure of golden perch in 2017 remain unexplored, but may in part be associated with the hypoxic blackwater, which may have impacted directly on egg and larval development, or indirectly via the effect of food resources in the LMR.

Table 5. CEWO short-term (one-year) evaluation questions by Category 1 and 3 indicators. Evaluation questions are sourced or adapted from Gawne *et al.* (2014). Category 1 Hydrology (channel) and Category 1 Fish (channel) did not directly address specific CEWO evaluation questions thus are not presented, but Category 1 Hydrology (channel) provided fundamental information for analysis and evaluation of monitoring outcomes against hydrological conditions and environmental water delivery for all indicators. Evaluation of CEW for Hydrological Regime and Matter Transport indicators is based on modelled data. CEW = Commonwealth environmental water, VEWH = Victorian Environmental Water Holder, RMIF = River Murray Increased Flows.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 1. Stream Metabolism	What did CEW contribute to dissolved oxygen levels?	Flooding reduced dissolved oxygen levels to below 50% saturation (~4.5 mg L^{-1}). Environmental water that supplemented releases from Lake Victoria maintained oxygen levels above 4 mg L^{-1} in the Rufus River.
	What did CEW contribute to patterns and rates of primary productivity and decomposition?	A marked increase in ecosystem respiration (oxygen consumption) at the site below Lock 6 aligned with an increased delivery of turbid water from the Darling River.
Category 3. Hydrological Regime (modelling)	What did CEW contribute to hydraulic diversity within weir pools?	CEW slowed the decline in velocity on the flood recession over January 2017. Following this event, environmental water contributed to small increases in weir pool median water velocities (typically by 0.05–0.07 m s^{-1}), with some reaches exceeding 0.17 m s^{-1}.
	What did CEW contribute to variability in water levels within weir pools?	Environmental water reduced the fall in water levels on the flood recession by 0.7–0.9 m. Following this event, environmental water increased water levels by up to 0.2–0.4 m in the upper reaches of weir pools during a watering event in March 2017.
Category 3. Matter Transport (modelling)	What did CEW contribute to salinity levels and transport?	CEW reduced salinity concentrations in the Coorong. CEW increased export of salt from the Murray River Channel, Lower Lakes, and Coorong.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 3. Matter Transport (modelling)	What did CEW contribute to nutrient concentrations and transport?	CEW contributed to minor differences in the concentrations of nutrients, but increased transport of all studied nutrients.
	What did CEW contribute to concentrations and transport of phytoplankton?	Whilst there was no apparent effect on phytoplankton concentrations, there was an increased transport of phytoplankton through the system, due to CEW.
	What did CEW contribute to water quality to support aquatic biota and normal biogeochemical processes?	CEW delivery reduced salinity concentrations in the Coorong, which likely improved habitat for estuarine biota in the region.
	What did CEW contribute to ecosystem function?	CEW delivery increased exchange of nutrients and phytoplankton between critical habitats of the lower River Murray, which may have supported primary and secondary productivity in the region and in doing so supported food webs of the LMR, Lower Lakes and Coorong.
Category 3. Micro-invertebrates	What did CEW contribute to microinvertebrate diversity?	CEW delivery from late December–early January coincided with a decline in diversity, which was likely driven by reduced flows and a recession of water levels post-flood. However, warm-water taxa, likely from Darling sources, appeared in January 2017, following CEW (and TLM water) delivery.
	What did CEW contribute via upstream connectivity to microinvertebrate communities of the LMR?	CEW contributed to longitudinal connectivity and most likely the transport of heleoplanktonic* warm-water taxa, including novel taxa for the LMR or the continent, to the LMR in January 2017. These most likely originated from Darling River flows.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 3. Micro-invertebrates	What did CEW contribute to the timing and presence of key species in relation to diet of large-bodied native fish larvae (e.g. golden perch)?	Relationship between timing of ambient (present in environment) microinvertebrates, driven by CEW, and their presence in fish diet could not be determined.
	What did CEW contribute to microinvertebrate abundance (density)?	CEW delivery from late December–early January coincided with a decline in microinvertebrate abundance, which was proportional to substantially reduced flows following the flood recession.
Category 3. Fish Spawning and Recruitment	What did CEW contribute to reproduction of golden perch and silver perch?	Delivery of CEW to the lower River Murray in 2016/17 coincided with spawning, but negligible recruitment of golden perch (to young-of-year, age 0+).

* heleoplankton = plankton derived from billabongs and other floodplain still, generally-vegetated, waters.

4 MANAGEMENT IMPLICATIONS AND RECOMMENDATIONS

Monitoring outcomes from the CEWO LTIM Project, in conjunction with our contemporary understanding of flow related ecology in the LMR, underpin the adaptive management of Commonwealth environmental water and river operations, aiming to maximise ecological benefits from available water. During this high flow year, the broad range of river discharge allowed the establishment of their relationship with velocity and inundation area in the LMR. Such information can be used to guide hydrological/hydraulic restoration in the LMR, supported by environmental flow deliveries. For example, the use of environmental water to increase flows to 20,000–45,000 ML day^{-1} can improve hydraulic conditions significantly, with >50% of a weir pool transforming from lentic (median velocities <0.3 m s^{-1}) to lotic habitats (≥0.3 m s^{-1}) in the LMR (Bice *et al.* 2017). Such hydraulic restoration is fundamental to underpin riverine ecosystem processes and support rehabilitation of biota that have life histories adapted to flowing environment in this region. In addition, environmental water contributing to flows >45,000 ML day^{-1} at the South Australian border (approximate bankfull level) will increase inundated area along the LMR, supporting off-channel processes and floodplain biota (e.g. floodplain vegetation and tree health). Overbank flow is also an integral part of the natural flow regime in maintaining ecosystem health of floodplain rivers. When the timing of flow delivery aligns with biological requirements (e.g. reproductive season of flow-cued species in spring/summer or spawning migration of diadromous fishes in winter), significant ecological outcomes can be achieved in the absence of mitigating factors, such as the extensive hypoxic blackwater event that occurred in 2016/17.

Although floods drive significant ecological processes in floodplain rivers (Junk *et al.* 1989), water oxygen reduction can result from the biological breakdown of accumulated organic debris and detritus on the floodplains. In the contemporary world, river regulation has exacerbated oxygen depletion by reducing flood frequency and thus increasing the accumulation time for materials on the floodplains (Howitt *et al.* 2007). Environmental water can be delivered to mitigate low oxygen concentrations to provide refuge habitat in some circumstances, such as augmented flows in the Rufus River in December 2016. Furthermore, strategic use of environmental

water, aligning with other objectives (e.g. improved floodplain vegetation and tree condition), could support managed inundation of floodplains at appropriate return intervals, which may reduce the risk of prolonged accumulation of organic materials. This may also help with intermittent increases in the carbon/energy supplies to support riverine food webs.

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. Connectivity will also facilitate the transport and dispersal of aquatic biota (e.g. microinvertebrates, fish larvae) to and throughout the LMR. Also important is the source of water (i.e. origin), which can influence water quality, biological responses and ecological processes. For instance, this study found that flows from the Darling River, a naturally turbid water source, were associated with a temporary increase in ecosystem respiration in the LMR; however, they also facilitated dispersal of warm-water microinvertebrate taxa to this region, contributing to species diversity in the LMR. Historically, Darling River flow was an integral source for the lower River Murray during summer; whereas Murray flow often peaked during winter/spring (MDBA 2012b). The spawning of golden perch from the Darling River has contributed to a significant proportion of the contemporary golden perch population in the LMR (Appendix H), with Darling flows facilitating the downstream transport of larvae and YOY (Ye *et al.* 2015b).

Furthermore, maintaining flow integrity from upstream (e.g. Darling River or mid-Murray) to the lower River Murray is important to support broad-scale ecological processes and promote positive outcomes (e.g. improved productivity, enhanced spawning and recruitment of flow-dependent fishes). In this regard, consideration 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.

Moreover, we acknowledge that the quantifiable ecological benefits of Commonwealth environmental water were limited during this high flow year, when substantial overbank flows (peaked of ~94,600 ML day^{-1} at the South Australian

border) occurred in the LMR. While the use of environmental water to slow the flood recession was likely important (Kilsby and Steggles 2015), under the same hydrological scenario, consideration could be made to reallocate environmental water to the following year, in the context of a multi-year strategy for ecosystem restoration. Environmental water could be used to reinstate key features of the natural hydrograph of the lower River Murray in subsequent dry years, which may be more beneficial from an ecological perspective. For example, spring/early summer in-channel increases of discharge (~15,000–20,000 ML day^{-1}) are conspicuously absent from the contemporary flow regime in the lower River Murray. These pulses of flow increase longitudinal connectivity and contribute to a broad range of ecological outcomes in riverine and estuarine ecosystems (e.g. increased matter transport, lotic habitats and spawning and migratory cues for fishes). Commonwealth environmental water could be used to restore these hydrological features in the LMR, which could lead to tangible ecological outcomes particularly in dry years. The following sections provide specific management considerations[h] based monitoring outcomes from LMR indicators, which relate to the key findings in Section 2.

Hydrology and Hydrological Regime

The large range in discharge over 2016/17 provides an opportunity to investigate the relationship between discharge and velocity within each weir pool. Figure 12 presents the modelled range in velocities in each weir pool for a given discharge. The discharge presented is into the weir pool (i.e. discharge at Lock 2 for the Weir Pool 1 results), and the results have been aggregated into 5,000 ML day^{-1} increments; for example, the 5,000 ML day^{-1} results are based on discharges from 2,500–7,500 ML day^{-1}. Velocities greater than 0.3 m s^{-1} are considered indicative of lotic conditions (Bice *et al.* 2017). At flows of approximately 20,000 ML day^{-1}, approximately 50% of a weir pool has lotic conditions; whereas these conditions are present in the

[h] It should be noted that management recommendations provided in this report are subject to environmental water availability and operational feasibility. Furthermore, priorities of ecological objectives and trade-offs associated with watering actions must be considered at a local- and Basin-scale. A multi-year approach should be adopted, guided by ecological restoration principals.

majority of the lower River Murray channel at flows exceeding 40,000 ML day^{-1} (Figure 12).

Increases in discharge can be expected to result in increases in velocity up to bankfull level at approximately 45,000 ML day^{-1} (Figure 12). For discharges greater than this, the velocity ranges remain constant or decrease slightly, as broad overbank flow starts to occur. This increase in inundated area along the lower River Murray as discharges exceed 45,000 ML day^{-1} can be seen in Figure 13, where the area of vegetation inundated for increasing flow to South Australia is presented.

This information is intended to provide a simple summary of the hydraulic changes that can be expected for a given change in discharge in the LMR. For example, providing environmental water to increase discharge from 5,000 ML day^{-1} to 10,000 ML day^{-1} does increase velocity, but to only a limited degree and does not significantly change the proportion of the reach expected to be experiencing lotic conditions. However, an increase from 10,000 ML day^{-1} to 15,000 ML day^{-1}, and again to 20,000 ML day^{-1}, could be expected to have a more substantial improvement on the proportion of the LMR characterised by lotic conditions.

It should be noted that these results have been derived from the scenario with environmental water (i.e. simulated actual conditions) for the 2016/17 flow regime. Some variation may result from the changes in discharge over time; for example, if different velocity distributions occur on the rising or falling limb of a flow event.

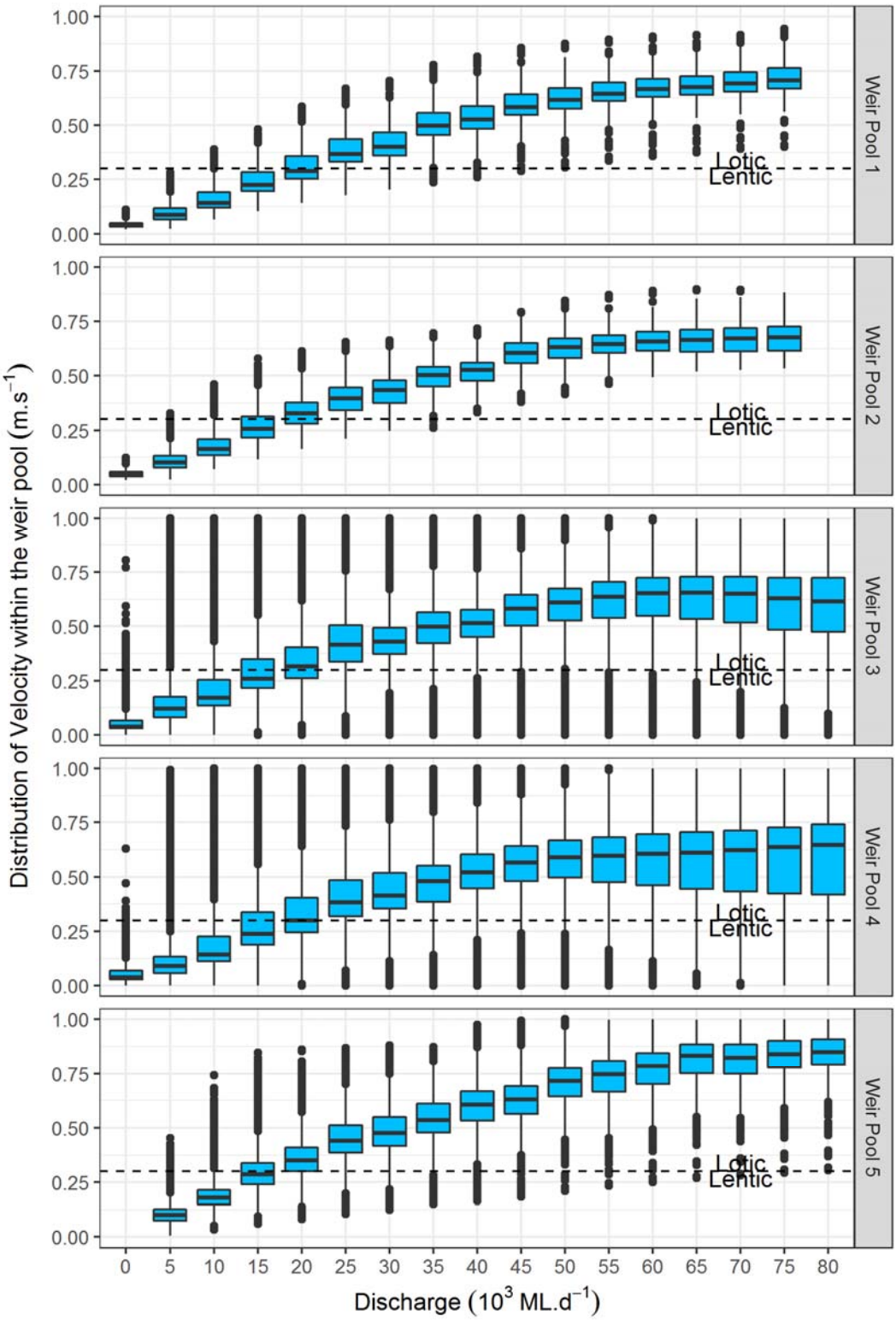

Figure 12. Relationship between discharge and distribution of velocity within a weir pool. The boxplot for a given discharge represents the range in velocity within the weir pool occurring for that discharge. The models for weir pools 1, 2 and 5 are 1D models only, and therefore the velocities are cross section averages. Weir pools 3 and 4 are modelled a small 2D elements, and as such the spatial scale represented is smaller and range in velocities are greater.

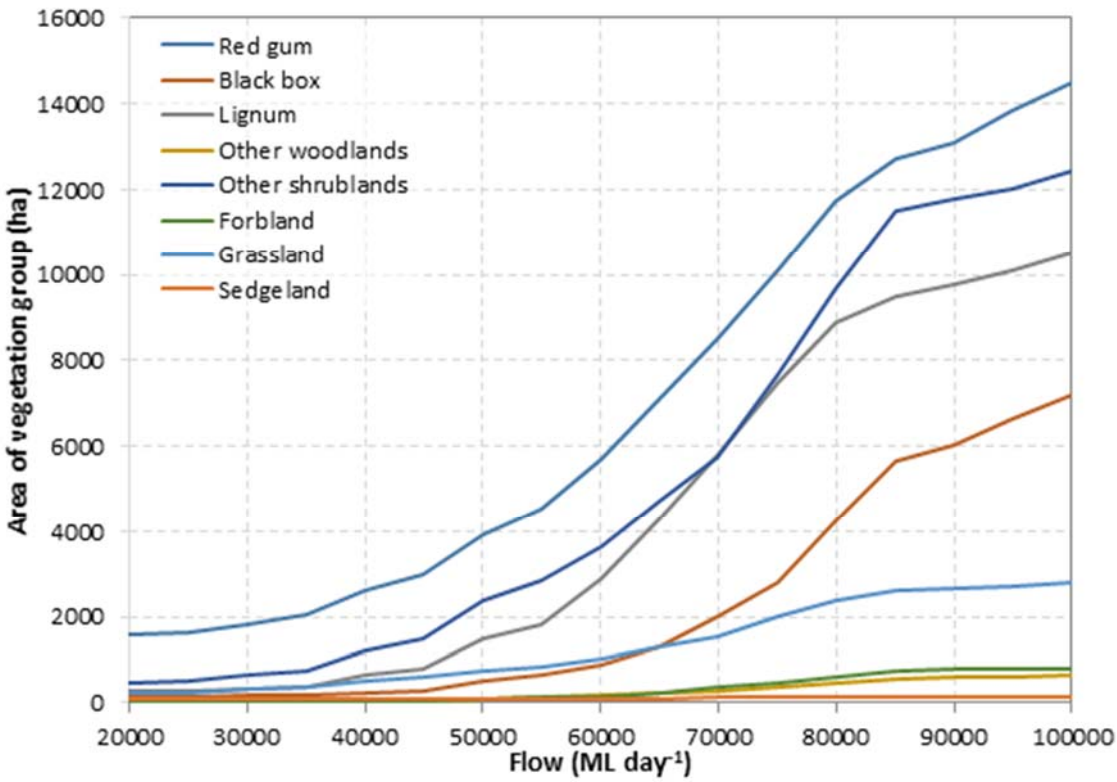

Figure 13. Area of vegetation inundated for increasing flow to South Australia (adapted from Gibbs *et al.* 2012).

Stream Metabolism

Deleterious oxygen concentrations (i.e. less than 50% saturation or ~4.5 mg L^{-1}) from early November to late December 2016 were the result of flooding, with oxygen depletion exacerbated by the reduced frequency of flooding under river regulation, which has increased the time for accumulation of organic debris and detritus on the floodplains (Howitt *et al.* 2007). Consideration of return times for managed flooding of low lying floodplains may help reduce this problem, as river regulation has impacted most on small and moderate floods and environmental flows may be sufficient to partially redress the issue at this scale.

Environmental water supplemented releases from Lake Victoria to maintain oxygen levels in the Rufus River during the low oxygen period (Appendix A). The oxygen monitoring results suggest that the release of oxygenated waters from Lake Victoria had a wider impact than just the local environment of the Rufus River and although the influence was small, it extended a substantial distance downstream to the South Australia border for a short period. Why the oxygen increase at this time was sustained so far downstream is not apparent and a more dynamic analysis accounting for

oxygen metabolism and exchange at the water surface is required to address this question. This would be useful as reducing the time and extent of extremely low oxygen levels is an important management strategy for ecological health in the lower River Murray.

From January to March 2017, enhanced ecosystem respiration below Lock 6 was associated with the increased turbidity of the Darling River water that reduced the light available to the phytoplankton, altering their metabolism. Management activities that alter the ratio of the photic (illuminated) to euphotic (unilluminated) zone through alterations in turbidity, or changes in water depths, will influence the balance between the relative rates of photosynthesis and respiration. Also, changes in flow velocity that reduces mixing intensity so that some phytoplankton can maintain themselves in the photic zone, can also have an effect on the metabolic balance. Fluctuations in metabolic activity in response to changing flow conditions is part of the natural variability expected in a river reach, but as with other environmental characteristics impacted by river operation, the timing, frequency and magnitude of these changes is expected to be important. During in-channel flows, management actions can significantly impact phytoplankton production and its availability to the food web, but the effect of these on time-integrated production is currently poorly understood. The picture becomes more complex when the river is flooding and supplies of organic carbon are transferred into the river from the floodplain, either as plankton growing in the shallow waters or detritus as this may counteract reductions in channel metabolism.

Matter Transport

The contributions of environmental water appear to have significantly increased the exchange of dissolved and particulate matter through the LMR to the Southern Ocean. General recommendations about optimal use of environmental water for the transport of dissolved and particulate matter in a hydrologically complex system, such as the LMR, are difficult to reach without a broader assessment. Based on insights provided by this study and previous studies over the past five years, including Aldridge *et al*. (2013), Ye *et al*. (2015b), Ye *et al*. (2016a) and Ye *et al.* (2017) the following points could be used to help guide future environmental water use:

- Environmental flow delivery can reduce salinity concentrations in the Lower Murray Channel and Lower Lakes and, in particular, can considerably reduce salinity concentrations within the Murray Mouth and Northern Coorong; the comparison of salt import/export through the Murray Mouth during low and high flow periods highlights this. Total salt export through the Murray Mouth in 2016/17 (high flow year) was 3,679,277 tonnes, with Commonwealth environmental water contributing to 14% of this. In 2015/16 (low flow year), there was a net modelled import of salt to the Coorong of 1,850,028 tonnes with all water, but without Commonwealth environmental water the modelling suggests this would have been 6,441,297 tonnes;
- Environmental flow deliveries appear to have capacity to only have a minor impact on nutrient and phytoplankton concentrations, although may have a greater impact during extended low flow periods when water levels in the LMR would otherwise fall with concentrations driven by internal processes, such as wind-driven resuspension;
- Environmental flow deliveries during periods where there would otherwise be negligible water exchange between the Lower Lakes and Coorong, can provide for the exchange of matter between these two water-bodies that would otherwise not occur;
- Environmental water use that results in floodplain inundation will likely result in increased nutrient concentrations (mobilisation) and export. This may be achieved by moderate to large floods (e.g. >45,000 ML day^{-1}) that inundate previously dry floodplain and wetland habitats. This may also partially be achieved through weir pool manipulation and the operation of floodplain infrastructure, although large areas of inundation and appropriate water exchange would be required to result in significant downstream ecological benefits;
- Environmental water delivery during low to moderate flow periods (e.g. 10,000–40,000 ML day^{-1}) will increase the transport and export of dissolved and particulate matter and can reduce the import of material from the Southern Ocean;

- Maximum exports of dissolved and particulate matter from the Coorong/Murray Mouth are likely to be achieved by delivering environmental water during periods of low oceanic water levels (e.g. summer). However, this may reduce water availability at other times, increasing the import of matter from the Southern Ocean during those times. In contrast, delivery of environmental water to the Murray River Channel at times of high oceanic water levels is likely to increase the exchange of water and associated nutrients and salt through the Coorong, rather than predominately through the Murray Mouth. This may decrease salinities and increase productivity within the Coorong more than what would occur if water is delivered at times of low oceanic water levels;
- Flows during winter may result in limited assimilation of nutrients by biota (slower growth rates), whilst deliveries during late summer could increase the risk of blackwater events and cyanobacterial blooms, depending on hydrological conditions. Flows during late winter to early summer are likely to minimise these risks, but also maximise the benefits of nutrient inputs (e.g. stimulate productivity to support microinvertebrate and larval fish survival).

Microinvertebrates

In years of high flows, as for 2016/17, microinvertebrate assemblages are transferred onto and over riparian margins and floodplains, allowing resting eggs and other dormant life stages to be deposited, rebuilding floodplain propagule storages. This replenishment of 'egg banks' maintains diversity of the planktonic and littoral (near bank) microfaunal assemblage: hatching is cued in subsequent inundations, whether by floods, river management (e.g. weir pool raising) or environmental watering. During the recession of the flood, transported and newly hatched microinvertebrates are returned to the main channel of the LMR and delivered to downstream areas. A significant increase in littoral/riparian taxa from all sites below Lock 6 (60% of identified plankters) during 2016/17 sampling is evidence of the importance of overbank flows in maintaining in-channel assemblage diversity.

The extended period of dissolved oxygen depletion recorded during spring/early summer 2016 potentially mitigated against microinvertebrate taxa with higher biological oxygen demands such as copepods and cladocerans. Protists with lower

oxygen demands were predominant during this period. Increases in dissolved oxygen levels above 4 mg L^{-1} in the main channel of the LMR in late December/early January likely permitted an increase in microcrustaceans that are deemed an important food resource for large-bodied larval fish (King 2005; Kaminskas and Humphries 2009).

Fish Spawning and Recruitment

In 2016/17, golden perch spawning in the lower River Murray was associated with spring–summer overbank flows, but recruitment was negligible. The mechanisms leading to recruitment failure are unclear, but may include the direct and indirect impacts of the hypoxic backwater event associated with flooding.

There has been no substantial recruitment of golden perch in the lower River Murray since 2012/13, leading to a population dominated by only a few distinct cohorts. To improve the resilience of golden perch populations in the lower River Murray, it would be pertinent in the coming years to provide flows in the lower Murray that facilitate golden perch spawning and recruitment. Specifically, Commonwealth environmental water could contribute to spring/early summer in-channel flow pulses (~15,000–20,000 ML day^{-1}). This key feature of the natural hydrograph of the lower River Murray, which is now predominantly absent from the regulated flow regime, has been associated with spawning and conspicuous recruitment of golden perch (Zampatti and Leigh 2013b).

5 CONCLUSION

2016/17 was a wet year, with high unregulated flows and flooding during spring/early summer, peaking at ~94,600 ML day^{-1} at the South Australian border in late November 2016. During this year, ~618 GL of Commonwealth environmental water was delivered to the LMR, in conjunction with other environmental water, with the majority (~96%, excluding South Australian held entitlement flow) of Commonwealth environmental water delivered after mid-December 2016.

Quantifying the ecological benefits of Commonwealth environmental water in the LMR during 2016/17 was particularly challenging, as may be expected given the volume and timing of environmental water delivery in relation to substantial flooding during spring/early summer, which was during the main field sampling period. Nevertheless, some hydrodynamic and ecological outcomes achieved by environmental watering in the LMR were observed. These included maintained or increased hydraulic diversity (velocity and water levels) in the river channel (weir pools); increased transport of nutrients and phytoplankton, likely stimulating primary productivity in downstream ecosystems; increased connectivity and microinvertebrate dispersion; reduced salinities in the Coorong and increased salt export through the Murray Mouth. However, the extensive hypoxic blackwater event associated with flooding potentially compromised biological outcomes. Whilst not specifically explored in this project, this may have included impacts on microinvertebrate abundance and diversity during early December, and golden perch and silver perch recruitment in the LMR.

Hydrodynamic restoration is fundamental to maintaining or reinstating ecosystem function of the lower River Murray. Environmental water delivery can increase hydraulic diversity, particularly when contributing to freshes of 20,000–45,000 ML day^{-1}, potentially leading to ecological benefits by improving habitat and restoring riverine ecosystem processes. 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 and facilitate the transport and dispersal of aquatic biota (e.g. microinvertebrates, fish larvae) to and throughout the LMR. The timing of environmental flow delivery is also important, which should continue to align with ecological objectives and consider biological processes and

life history requirements (e.g. reproductive season of flow-cued species in spring/summer or spawning migration of diadromous fishes in winter). Lastly, for high unregulated flow years, consideration could be made to reallocate environmental water to the following year, guided by a multi-year watering strategy for ecosystem restoration. Environmental water could be used to reinstate key features of the natural hydrograph of the lower River Murray; for example, restore distinct spring/early summer flow pulses (~15,000–20,000 ML day^{-1}) in subsequent dry years, which may provide significant ecological benefits in the LMR.

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7 APPENDICES

APPENDIX A: OVERVIEW OF OTHER WATERING AND MANAGEMENT ACTIVITIES DURING 2016/17

In addition to environmental water deliveries to the LMR in 2016/17 (Figure 3), the following management actions are relevant to the analyses and interpretations in this report. Wetland water delivery by the Nature Foundation South Australia and the South Australian Natural Resources Management Board was not considered to influence any of the main channel indicators in the LMR.

Other watering and management activities in the LMR

Raising of water levels in Weir Pools 2 and 5

Raising of Weir Pool 2 (between Locks 2 and 3, gorge geomorphic zone) and Weir Pool 5 (between Locks 5 and 6, floodplain geomorphic zone) in the LMR occurred between early July and early October 2016. Water levels within Weir Pools 2 and 5 were raised to a maximum of 0.75 and 0.48 m above the normal pool level (NPL), respectively, before undergoing a rapid recession in mid-October 2016 to allow for increasing flows and avoid any threat to the structural integrity of the weirs (Figure A1). Flows continued to increase to ~94,600 ML day^{-1} at the South Australian border in late November 2016, resulting in water levels increasing beyond the heights achieved during weir pool raising (Figures A1). Although no Commonwealth environmental water was delivered directly, as the weir pools were re-filled by unregulated flow, the outcomes from this action can still be attributed to Commonwealth environmental water, as it was the commitment of Commonwealth environmental water underwriting the requirement of the weir-pools refilling that enabled the river operators to enact the infrastructure manipulation (source, CEWO). The weir pool raising event is described in Hanisch *et al.* (2017).

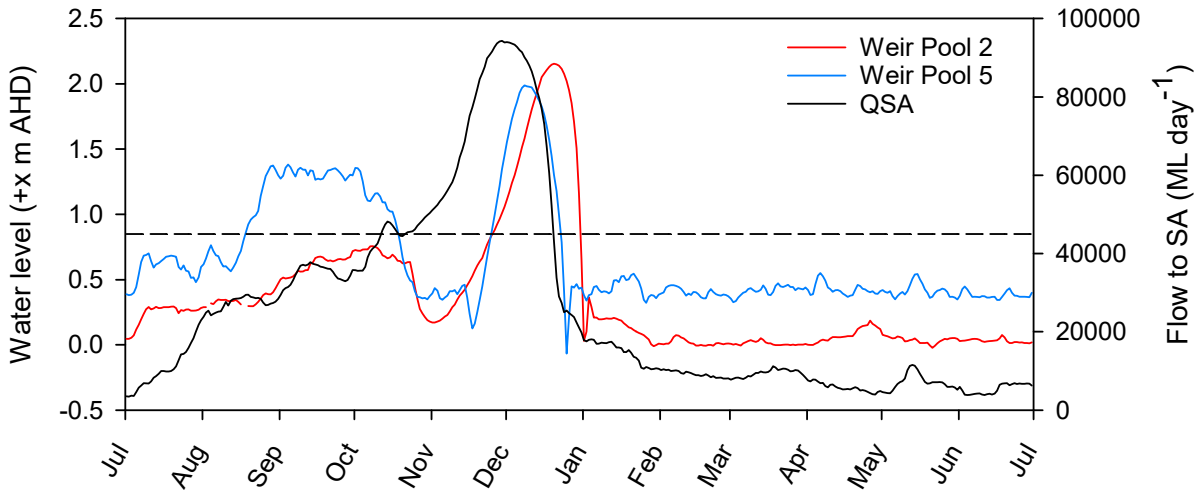


Figure A1. Water levels in the Lock 2 (US Lock 2) and Lock 5 (US Lock 5) weir pools between July 2016 and March 2017, showing weir pool raising between July and November 2016 (DEWNR). Flow to South Australia (QSA) and the approximate bankfull flow are represented by solid and dotted black lines, respectively. Water level is measured at Lock 2 US (A4260518) and Lock 5 US (A4260512) sites.

Chowilla regulator high-level testing event and raising of Weir Pool 6

Elevated flows in spring 2016 (>30,000 ML day^{-1}) provided opportunity for higher level testing of the operation of the Chowilla regulator, building upon first testing in spring 2014. Commencing in early August 2016, water levels within Chowilla Creek were raised (3.45 m above NPL to a maximum of 19.75 m AHD on 28 September) and held at ~19.6 m AHD from mid-September to mid-October 2016 (Figure A2). In conjunction with the regulator operation, the water level directly upstream of Lock 6 was raised to ~19.8 m AHD (+0.59 m above NPL) to achieve Chowilla Floodplain targets. Water level recession at Chowilla Creek and above Lock 6 began shortly after peak levels were reached, in mid-October 2016. The Chowilla regulator (floodplain inundation) event was achieved primarily using The Living Murray water. A detailed description of the Chowilla Floodplain inundation event can be found at https://www.environment.sa.gov.au/files/sharedassets/public/water/chowilla-floodplain-icon-site-event-summary-2016.pdf.

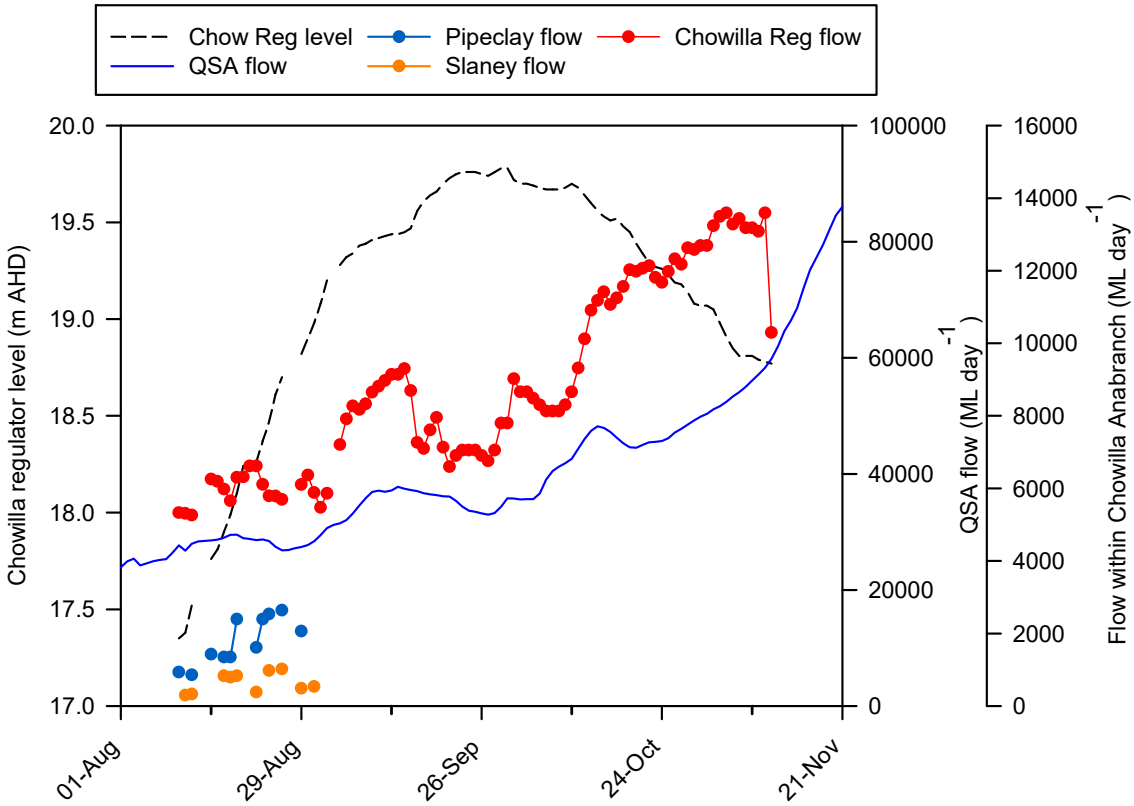


Figure A2. Water level (m AHD) and flow (ML day^{-1}) upstream of the Chowilla regulator, and flow for creeks within the Chowilla Anabranch and at the South Australian border (QSA) (source, DEWNR).

Watering and management activities above the LMR

Rufus River refuge habitat

During November 2016, dissolved oxygen in the lower River Murray fell to lethal levels (<2 mg L^{-1}) as a result of widespread flooding in the Murray–Darling Basin, resulting from high unregulated flows. Releases were made from Lake Victoria, which had adequate dissolved oxygen levels, to provide refuge habitat for aquatic fauna downstream of the Darling junction. Releases increased from minimum flows of 500 ML day^{-1} on 10 November to ~5,500 ML day^{-1} on 24 and 25 November and were maintained at ~3,500 ML day^{-1} from 2 to 16 December. From 17 to 31 December, Lake Victoria outflow into the Rufus River was supplemented by environmental water (59 GL: 50% Commonwealth environmental water, 50% The Living Murray), increasing flows above 3,500 ML day^{-1} by adding ≥3,000 ML day^{-1} to minimum flows of

500 ML day^{-1} (Figure A3). As a result of these flows, dissolved oxygen levels at the Lake Victoria outlet and buoy line, downstream of the outlet regulator, remained above 4 mg L^{-1} throughout December 2016 (Figure A4).

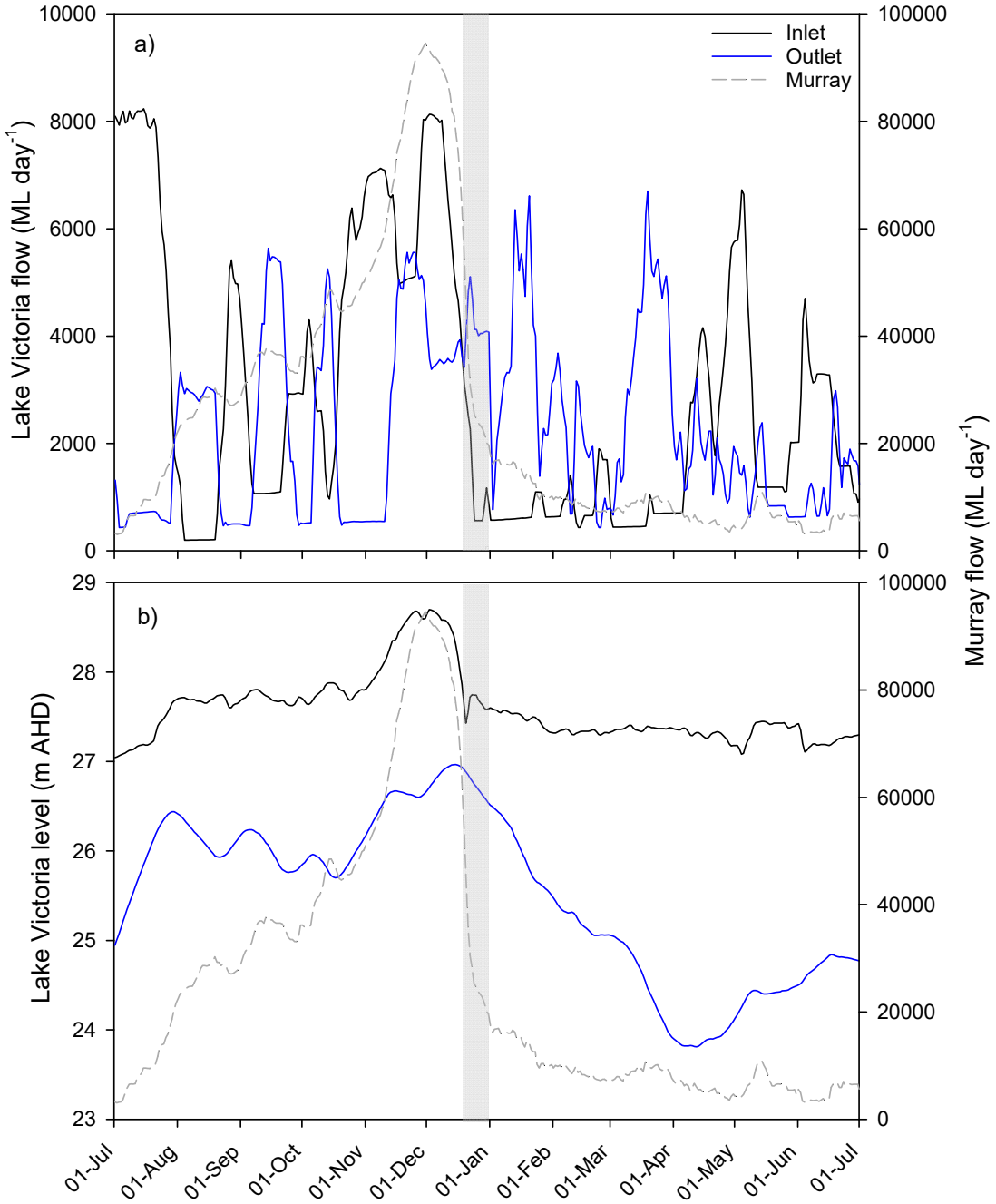


Figure A3. Water level (m AHD) and flow (ML day^{-1}) at Frenchman's Creek (Lake Victoria inlet) and Rufus River (downstream of the Lake Victoria outlet) from July 2016 to July 2017. Water levels and flow are plotted against flow in the main channel of the Murray, immediately downstream of the Rufus River (source, MDBA and WaterConnect). The shaded grey bar indicates the period of water releases into the Rufus River.

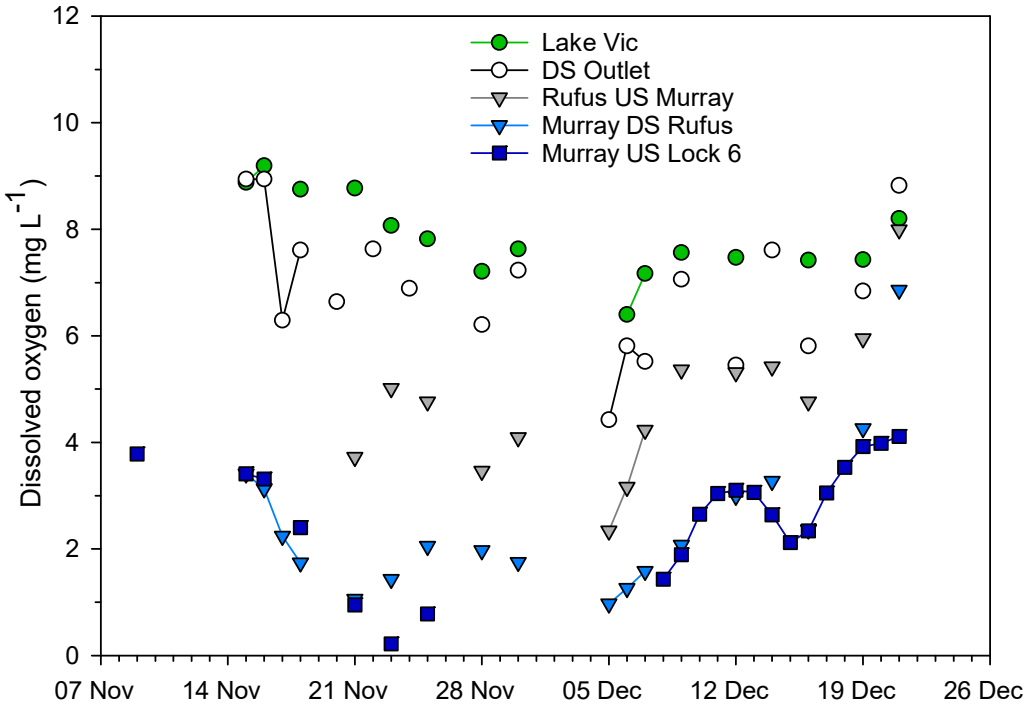


Figure A4. Dissolved oxygen (mg L^{-1}) at Lake Victoria pontoon (Lake Vic), in the Rufus River downstream (DS) of the Lake Victoria outlet regulator, in the Rufus River 300 m upstream (US) of the Murray, in the Murray River 200 m downstream (DS) of the Rufus River and in the Murray River at Customs house upstream (US) of Lock 6, from 9 November to 21 December 2016 (source: SA Water).

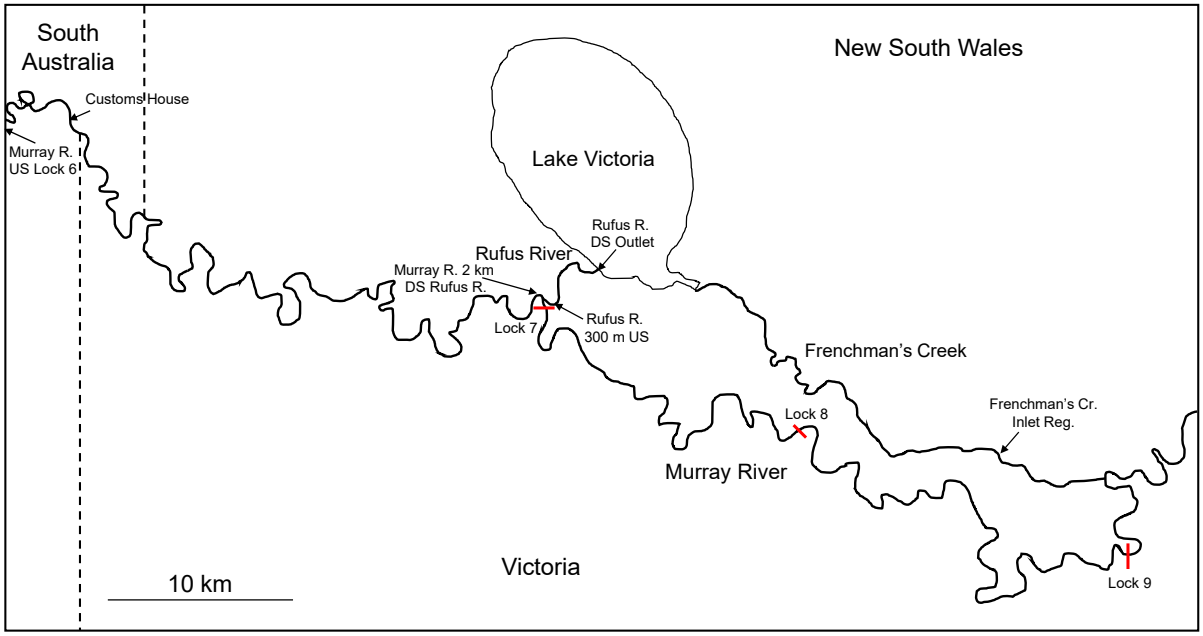


Figure A5. Map of Lake Victoria in the lower River Murray and sites where dissolved oxygen measurements were taken (see Figure A4 and Figure C6 in Appendix C). Red bars represent locks and weirs.

Manipulation of water levels in Weir Pools 7, 8, 9 and 15

In 2016/17, water levels in Weir Pools 7, 8, 9 and 15 were manipulated (raised and/or lowered), within operational limits, to introduce a more natural wetting and drying cycle for the benefit of the riverine environment by increasing variability in river levels (Table A1; Figure A5). No environmental water was used to raise weir pools above Normal Pool Level (NPL) because the initial managed raisings were achieved by unregulated flows. However, it was Commonwealth environmental water underwriting the requirement of the weir-pools re-filling that enabled the river operators to undertake the manipulations (source, CEWO). Following flooding in late 2016 where weirs were removed, weir pools were adjusted to suit the conditions and, where possible, align with modelled natural flows (Figure A5).

Table A1. Operational ranges for the weir pools upstream of the LMR during 2016/17 (source, MDBA). NPL = normal pool level.

Weir pool	Operational range in 2016-17	Watering information
7	+0.55 m above to -0.9m below NPL	Lowering currently limited to -0.9 m below NPL to maintain minimum flow target of 400 ML day^{-1} diversion to Mullaroo Creek.
8	+0.85 m above to -1.0 m below NPL	Disconnection of Potterwalkagee Creek occurs before the pool level drops to -1.0 m NPL. The ecological benefits and risks of periods of disconnection are continuing to be investigated and results may influence future operating range.
9*	+0.24 m above to -0.1 m below NPL	Lowering currently limited due to water access by bulk water supply pump on Lock 9 weir pool, which supply's Lake Cullulleraine.
15	+0.6 m above to -0.45 m below NPL	Lowering pool is currently limited during irrigation season as level impacts access to water in adjacent Bonyaricall Creek. Advice was received to also avoid lowering during significant recreational events.

* The operating range at Lock 9 is currently very restricted. The weir pool is generally raised or lowered a small amount when Lock 7 and 8 are altered so it follows the same pattern.

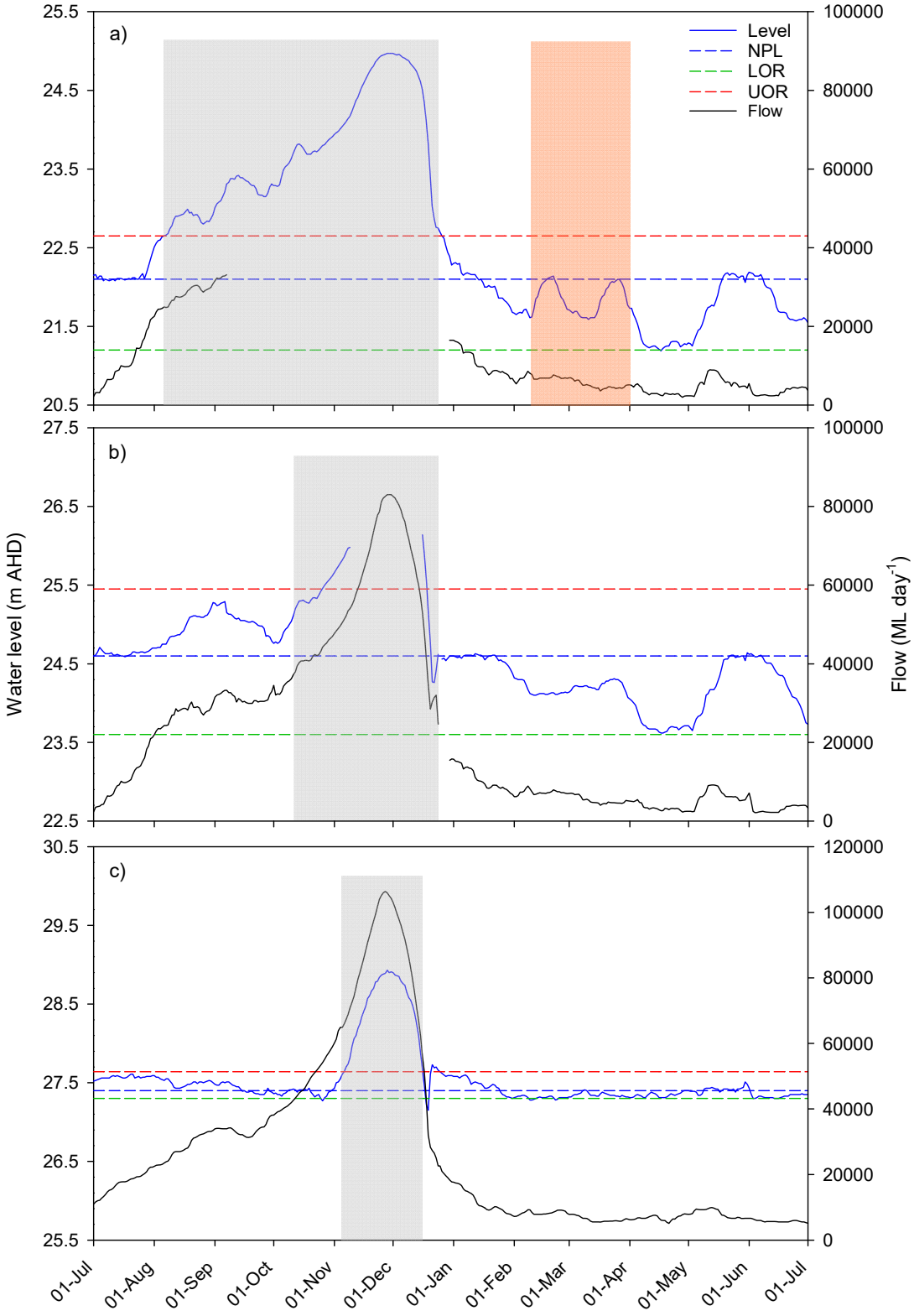


Figure A5. Operations of Weir Pools (a) 7, (b) 8 and (c) 9 in 2016/17. Water levels are taken from the downstream end of the weir pool, while flow is taken from the upstream end. Water levels are given in context of normal pool level (NPL), lower operating range (LOR) and upper operational range (UOR) (source, MDBA). Grey bars indicate periods of weir removal during overbank flows, while the orange bar indicates operations facilitating boat passage.

Yarrawonga autumn flow pulse

Between June and December 2016, ~39 GL of Commonwealth environmental water was delivered from Hume (solely in November and December) to the Murray River main channel (source, CEWO). From 19–30 November 2016, Commonwealth environmental water was released from Hume to maintain the flow downstream of Yarrawonga Weir at 15,000 ML day^{-1} (Figure A6). The Commonwealth environmental water component varied between 0–3,000 ML day^{-1} during this period. From January onwards, the CEWO maintained water levels in the River to enable water flow into the creeks of Barmah–Millewa, but the actual water accounted for this action was by The Living Murray.

From 12 February to 5 March 2017, the CEWO provided 30 GL of water to create a fish pulse (followed by pulses in the Goulburn and Campaspe Rivers), targeting 9,500 ML day^{-1} downstream of Yarrawonga (although around the 17 February, operational releases pushed this up to 10,000 ML day^{-1} for a short period) (source, CEWO) (Figure A6). Daily Commonwealth environmental water flow component varied from 0–3,000 ML day^{-1}.

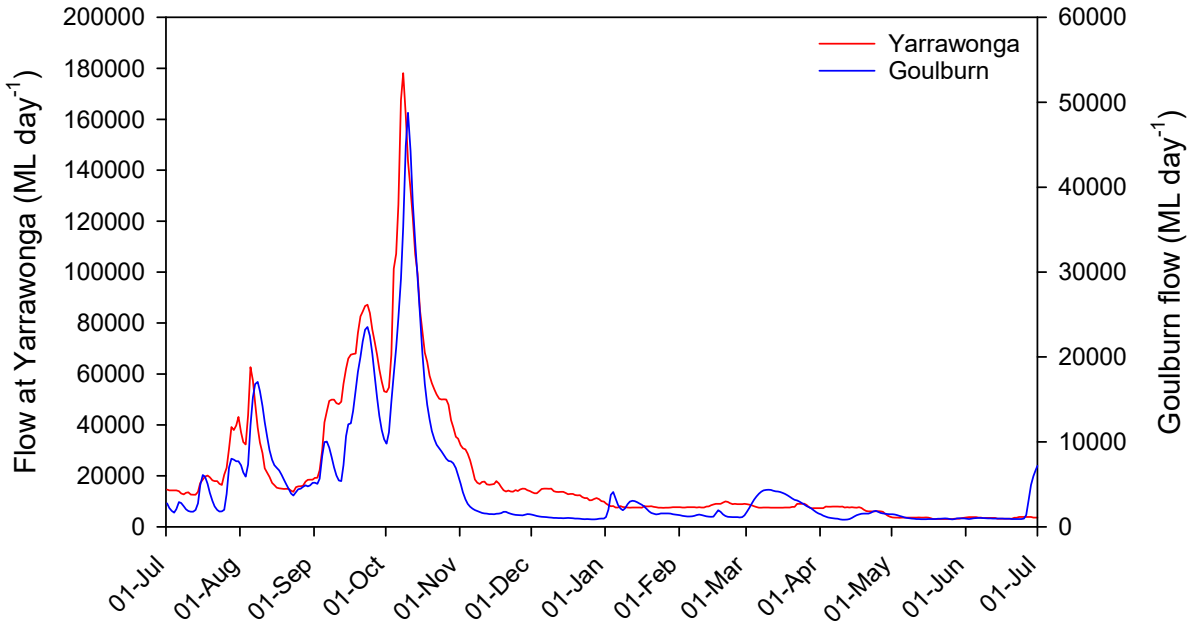


Figure A6. Flow (ML day^{-1}) in the Murray River, downstream of Yarrawonga weir and at McCoys bridge in the Goulburn River.

Goulburn autumn fresh

Commonwealth environmental water (182 GL) and environmental water from The Living Murray (27.5 GL) and Victorian Environmental Water Holder (20 GL) were delivered to the Goulburn River during 2016/17 (source, CEWO). An autumn fresh occurred in the Goulburn River between late February and early April 2017 (Figure A6). Autumn flow peaked at ~4,400 ML day^{-1}, which consisted of a combination of Commonwealth environmental water (~70 GL) and water from the Victorian Environmental Water Holder (~16 GL) and Inter Valley Transfer (~10 GL) (source, CEWO).

Darling River flow events

In 2016/17, 71 GL of Commonwealth environmental water and 48 GL of The Living Murray water was delivered to the Lower Darling River, with flows reaching ~6,500 ML day^{-1} at Weir 32 in early January during operational releases (Figure A7). Commonwealth environmental water was delivered from early December 2016 to early January 2017, and from late April to late June 2017, while The Living Murray water was delivered from mid-September to late November 2016 (Figure A7).

Also in 2016/17, Commonwealth environmental water (89 GL) and environmental water from the New South Wales Office of Environment and Heritage (11 GL) were delivered down the Great Darling Anabranch between February and June 2017 (Figure A8). Commonwealth environmental water delivery occurred between mid-February and late May 2017, while water from the New South Wales Office of Environment and Heritage was delivered from late May and end June 2017.

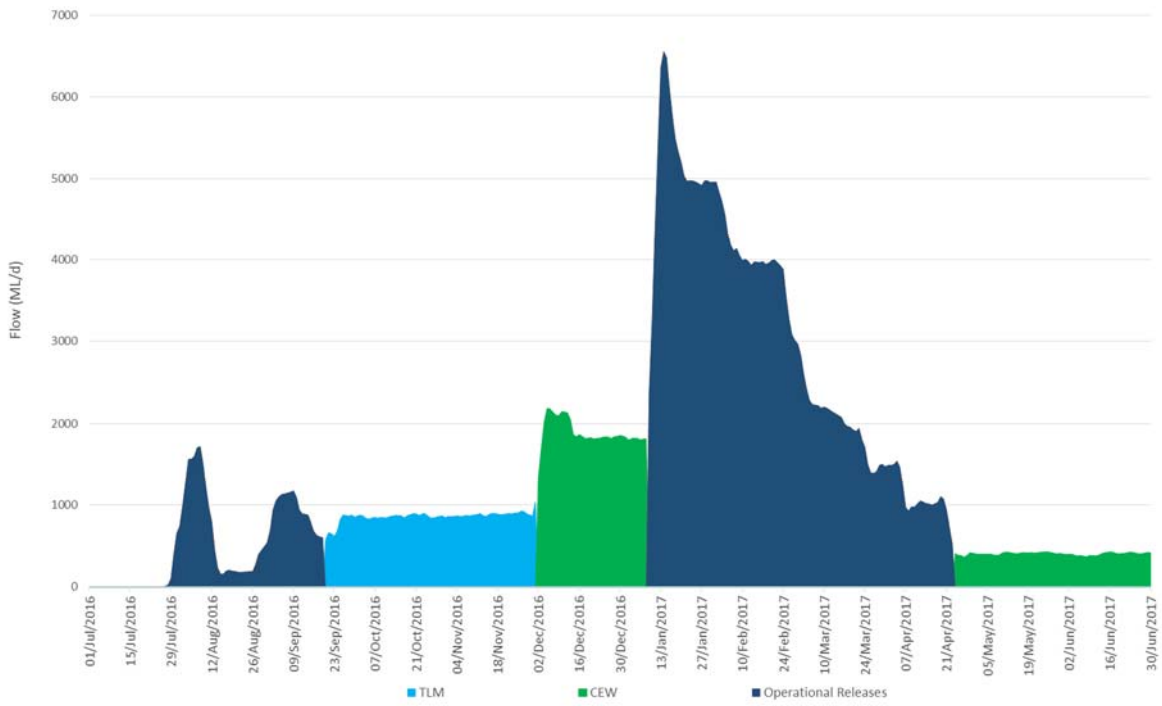


Figure A7. Flow (ML day^{-1}) at Weir 32 (lower Darling) in 2016/17, showing the contributions to flow by water holder (CEW and TLM) (source, CEWO). MDBA = Murray–Darling Basin Authority, TLM = The Living Murray, CEW = Commonwealth environmental water. Total volume of CEW delivered in main-stem Lower Darling River was 71 248.6 ML.

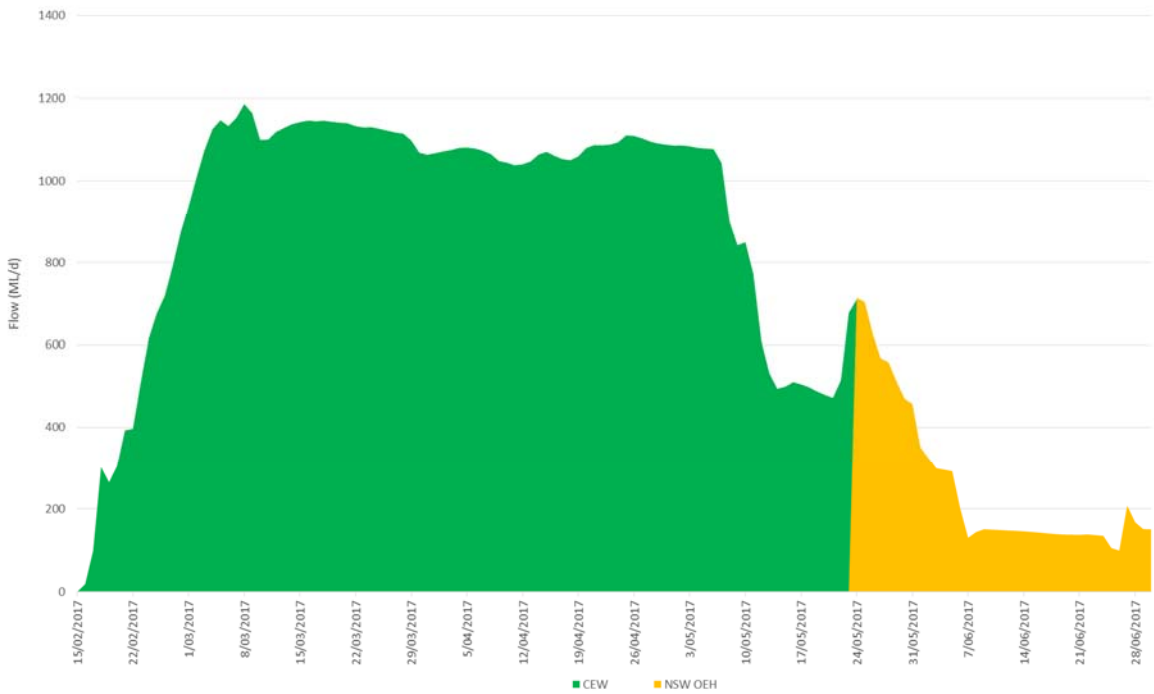


Figure A8. Delivery of environmental water (CEW and NSW OEH) at Packer's crossing on Redbank Creek (Darling Anabranch), 2017. Total volume of CEW delivered in the Great Darling Anabranch was 89 204 ML (source: CEWO).

APPENDIX B: ADDITONAL SAMPLING FOR THE RAISING OF WEIR POOLS 2 AND 5

In 2016/17, additional monitoring activities for Category 1 Stream Metabolism and Category 3 Microinvertebrates were undertaken to: (1) gain insights into the effects of weir pool raising (Appendix A) on indicators of interest; and (2) complement existing LTIM sampling and evaluation. In addition to sites below Lock 1 and Lock 6 for LTIM (Figure 6), Stream Metabolism sampling occurred at two sites in Weir Pool 2 (above Lock 2 and below Lock 3) and one site in Weir Pool 5 (above Lock 5) (Figure B1; Table B1). Additional Microinvertebrate sampling occurred in Weir Pool 2 (two sites, one below Lock 3 and one above Lock 2) and Weir Pool 1 (one site below Lock 2) (Figure B1; Table B1).

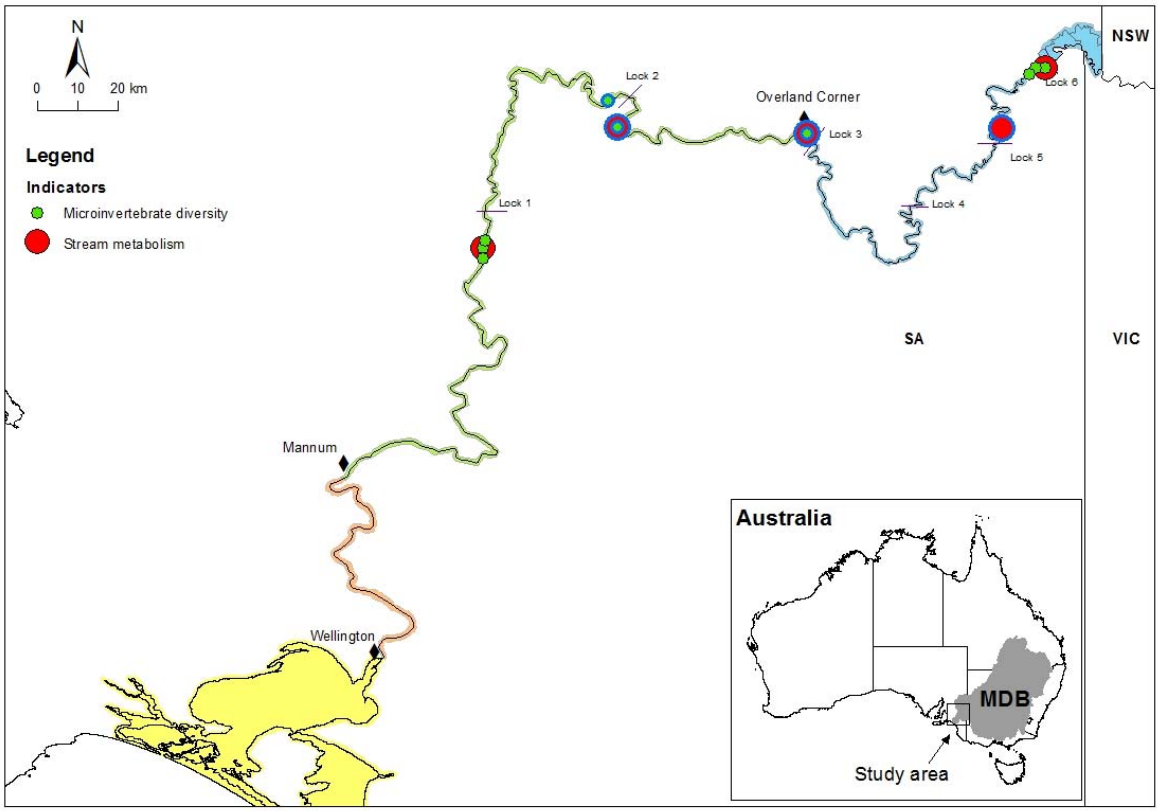


Figure B1. Additional monitoring sites (blue outline) for Stream Metabolism and Microinvertebrates associated with weir pool raising at Weir Pools 2 and 5 during 2016/17, in conjunction with the CEWO LTIM project in the LMR Selected Area. Core LTIM sites (thin black outline) from Figure 6 are also presented. For Stream Metabolism, water quality measurements were taken at sites below Lock 6, below Lock 3 and above Lock 2, while temperature sensors were deployed at sites below Lock 6, above Lock 5 and above Lock 2.

Table B1. Details of additional weir pool monitoring sites for Stream Metabolism (SM) and Microinvertebrate (Mic) indicators during 2016/17.

Zone	Site location	Indicator(s)	Latitude	Longitude
Floodplain	7 km US Lock 5	SM	S34.154258	E140.779379
Gorge	5 km DS Lock 3	SM, Mic	S34.165447	E140.340925
Gorge	10 km US Lock 2	SM, Mic	S34.15036	E139.915016
Gorge	5 km DS Lock 2	Mic	S34.092559	E139.893181

At all Stream Metabolism sites, dissolved oxygen was recorded by loggers set at 50 cm below the surface from 26 September 2016 to 7 February 2017, while temperature loggers recorded temperature at sites at the downstream end of the weir pools throughout the same period. Monthly water samples were also collected at Weir Pool 2 sites (Figure B2). Sampling for Microinvertebrates was conducted fortnightly from 26 September 2016 to 10 January 2017 (Figure B2), with three Haney trap samples performed at each site. Methods for the additional weir pool sampling were consistent with LTIM sampling methods to ensure the data were comparable (Appendix C and G).

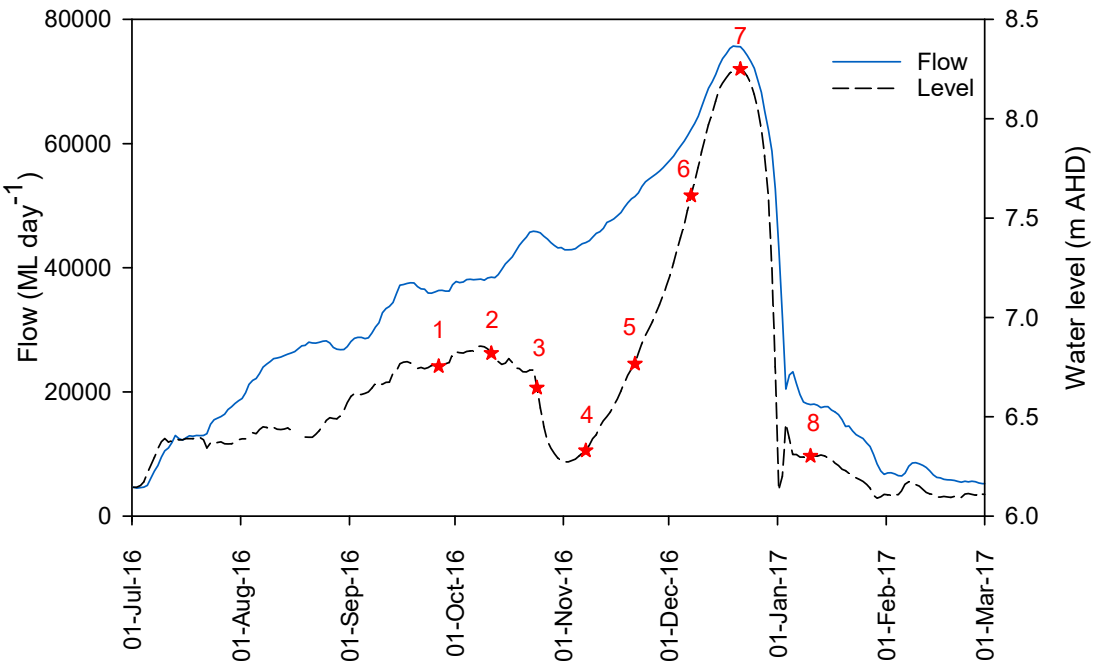


Figure B2. Timing of additional Category 3 Microinvertebrate sampling trips in 2016/17 in relation to water level (above Lock 2) and discharge (Overland Corner A4260528, below Lock 3) in Weir Pool 2 of the LMR. Monthly water samples for Category 1 Stream Metabolism were collected on trips 1, 3, 5, 7 and 8.

At the phase of project design, it was acknowledged that the sampling did not allow for the explicit evaluation of the effects of weir pool raising events on Stream Metabolism and Microinvertebrates in Weir Pool 2 and 5 as the project would miss the 'before', but capture the 'during' and 'after' raising events (Figure B2). Therefore, the intention of data collection was to allow for qualitative inferences to be made regarding the effects of weir pool raising and, most importantly, to contribute to the broader evaluation of Commonwealth environmental water delivery to the LMR, i.e.:

- What did Commonwealth environmental water contribute to patterns and rates of primary productivity?
- What did Commonwealth environmental water contribute to patterns and rates of decomposition?
- What did Commonwealth environmental water contribute to patterns and rates of dissolved oxygen levels?
- What did Commonwealth environmental water contribute to microinvertebrate diversity?

Increasing unregulated flows in spring 2016 led to the early termination of weir pool raising (Appendix A) and did not allow for the appropriate capture of the 'after' sampling period (Figure B2). This made any inferences on the influence of weir pool raising on ecological indicators difficult; however, the data complemented analyses at core LTIM sites (Figure 6), contributing to the broader evaluation of Commonwealth environmental water delivery to the LMR. Refer to Appendix C (Stream Metabolism) and G (Microinvertebrates) for data report from the additional weir pool monitoring and incorporation of findings into the evaluation of Commonwealth environmental water for each indicator.

APPENDIX C: STREAM METABOLISM

Background

River metabolism measurements estimate the in-stream rates of photosynthesis and respiration, and provide information on the energy being processed through riverine food webs (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). Metabolism measurements help identify whether the sources of organic materials that provide the food resources have come from within the river (autochthonous) or from the surrounding landscape (allochthonous). They describe the fundamental trophic energy connections that characterise different food web types (e.g. detrital, autotrophic, planktonic), and indicate the size of the food web and so its capacity to support higher trophic levels including fish and water birds (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006).

Methods

Stream metabolism is measured by monitoring the rates of change in dissolved oxygen concentration over day and night cycles. These diel changes are caused by the balance between photosynthetic oxygen production which occurs in the light, oxygen depletion by respiration which occurs continuously, and oxygen exchange at the air-water interface. The surface oxygen exchange is driven by the difference between the actual water oxygen concentration and the expected saturation concentration when in balance with the atmosphere. The monitoring of oxygen levels also informs directly on whether dissolved concentrations are suitable for the survival of aquatic organisms and in the current context identifies concentration changes associated with environmental flows.

Monitoring involved the continuous *in situ* logging of dissolved oxygen concentration, water temperature, barometric pressure, and photosynthetically active incident solar radiation (PAR), from which the daily rates of river metabolism were calculated (Oliver and Merrick 2006; Oliver and Lorenz 2010; Grace and Imberger 2006; Grace *et al.* 2015). The detailed monitoring and analysis protocol described in Hale *et al.* (2014) was followed with some small modifications. Instead of measuring barometric pressure independently, data were obtained from two nearby meteorological stations operated by the Bureau of Meteorology (BOM), one at Nuriootpa and one at

Renmark. At these sites, barometric pressure is measured every 30 minutes, and the 10-minute data required for metabolism analyses were determined by interpolation.

Two sampling sites form the core of the LMR monitoring program, one downstream of Lock 6 and one downstream of Lock 1 (Figure 6). These were selected to represent the floodplain and gorge geomorphic zones, respectively. During the 2016/17 monitoring period, three extra monitoring sites were included to enhance the data on dissolved oxygen changes associated with environmental flows, and to describe the influence of weir pool raising at Locks 2 and 5 on river metabolism (Appendix B). The extra monitoring sites were situated upstream of Lock 2 and downstream of Lock 3, so monitoring the upper and lower reaches of Weir Pool 2, and at a site upstream of Lock 5, that in conjunction with the LTIM site downstream of Lock 6 monitored the upper and lower reaches of Weir Pool (Figure B1 in Appendix B). As weir pool raising has been observed to influence river metabolism (Ye *et al.* 2017), it is necessary to account for these effects, both for their local influence, and because the manipulated weir pools have the potential to affect measurements at the two core monitoring sites.

Hydrological characteristics at the sampling sites including water level, water velocity and average depth were determined from established gauging stations and hydrological modelling. Discrete water quality samples were collected approximately every 4 weeks during field trips for oxygen probe maintenance and analysed by the Australian Water Quality Centre, a registered laboratory with the National Association of Testing Authorities. Samples were analysed for chlorophyll a, total nitrogen (TN, the sum of all forms of nitrogen), nitrate and nitrite the oxides of nitrogen (NO_X), ammonium (NH_4), total phosphorus (TP, the sum of all forms of phosphorus), dissolved forms of phosphorus (PO_4), and dissolved organic carbon (DOC).

Oxygen concentrations were monitored continuously from 14 September 2016 to 15 March 2017 at the core sites downstream of Lock 1 and downstream of Lock 6, with only a few interruptions of several hours duration during probe maintenance. Similar measurements commenced at sites upstream of Lock 2 and downstream of Lock 3 on 27 September, and upstream of Lock 5 on 5 October, continuing until early March with few interruptions to the series. Probes were deployed from buoys or floating platforms at a depth of 500 mm, with probe housings facing downstream to minimise fouling by debris. Metabolic rates for gross photosynthesis (GPP), ecosystem

respiration (ER) and net ecosystem production (NEP) were estimated using the BASE program (Grace *et al.* 2015). This fits a widely used mass balance model describing the daily fluctuations in water column dissolved oxygen concentrations (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006) to the measured changes in oxygen concentration using Bayesian regression routines. The acceptability of the fitted models was assessed from a set of statistical indicators of the goodness of fit using the following criteria; coefficients of determination (r^2) are greater than 0.9; coefficients of variation for GPP, ER and the gas exchange coefficient (K) are less than 0.5; and the posterior predictive p-value (PPP) lies between 0.1 and 0.9.

Refer to the "Category 1 Stream Metabolism" section of the LMR Selected Area SOP for more information on the sampling protocol including sites, timing and equipment, and on data analysis and evaluation, data management and quality assurance/quality control measures. Refer to Section 5 in SARDI *et al.* 2016 for timing of monitoring activities and more information on sampling sites and zones.

Results

Oxygen concentration time series

The time series of dissolved oxygen concentrations showed similar trends at all sites (Figure C1). Notable was the rapid decline in dissolved oxygen concentration, progressively downstream, that was associated with flooding into South Australia. Upstream flooding had increased loads of organic material causing enhanced microbial respiration rates that reduced dissolved oxygen concentrations (Figure C2). The calculated saturation concentration of dissolved oxygen (International Oceanographic Tables 1973) was similar across sites and is represented in Figure C1 by data from Lock 6. This shows that before and after the flood dissolved oxygen concentrations were similar to saturation levels. In contrast, during the flow peak between early November and the end of December 2016, dissolved oxygen concentrations declined below 50% saturation (~4.5 mg L^{-1}) at all sites. At the peak of the flow, between mid-November and mid-December, oxygen concentrations fell below 2 mg L^{-1} for a period of time at all sites, decreasing in extent with distance downstream from Lock 6. At Lock 6 the dissolved oxygen concentration decline was greatest, reaching a minimum of 0 mg L^{-1} for a 4-day period. Prolonged exposure to

dissolved oxygen concentrations below 2mg L^{-1} is detrimental to a range of aquatic organisms, including fish, while zero oxygen levels are lethal to many.

Flows declined rapidly through December 2016 and by 22 December flows were in-channel and disconnected from the floodplains (~40,000 ML day^{-1}). At this point dissolved oxygen concentrations rapidly increased, particularly at Lock 6, but then progressively downstream through the sites, until all returned to near saturation levels (Figure C1). A period of falling oxygen concentrations during February 2017, particularly at Lock 1, was attributed to biofouling of the oxygen probe (Figure C1).

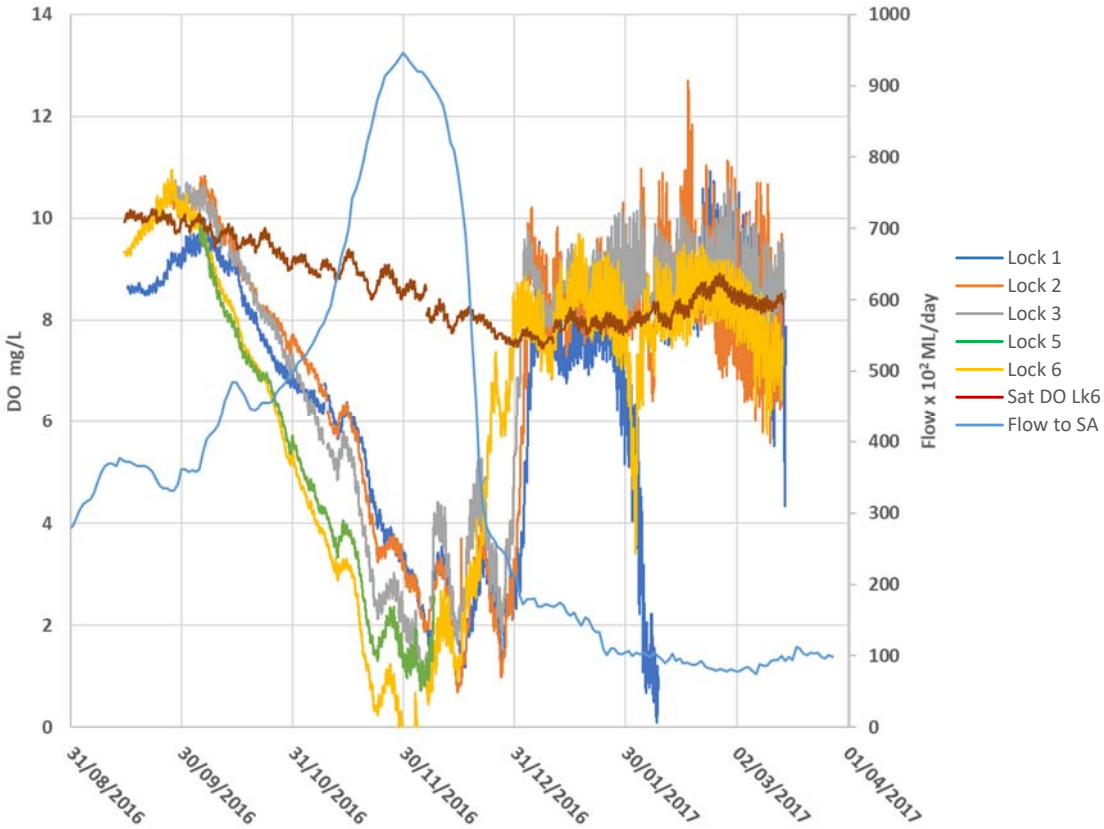


Figure C1. Dissolved oxygen concentrations at major LMR monitoring sites compared with the typical saturation oxygen concentration typified by Lock 6 data. The flow to South Australia is shown for reference. Data from the upstream Lock 5 site (maintained by DEWNR) was unavailable for the period 9 December 2016 to 7 March 2017.

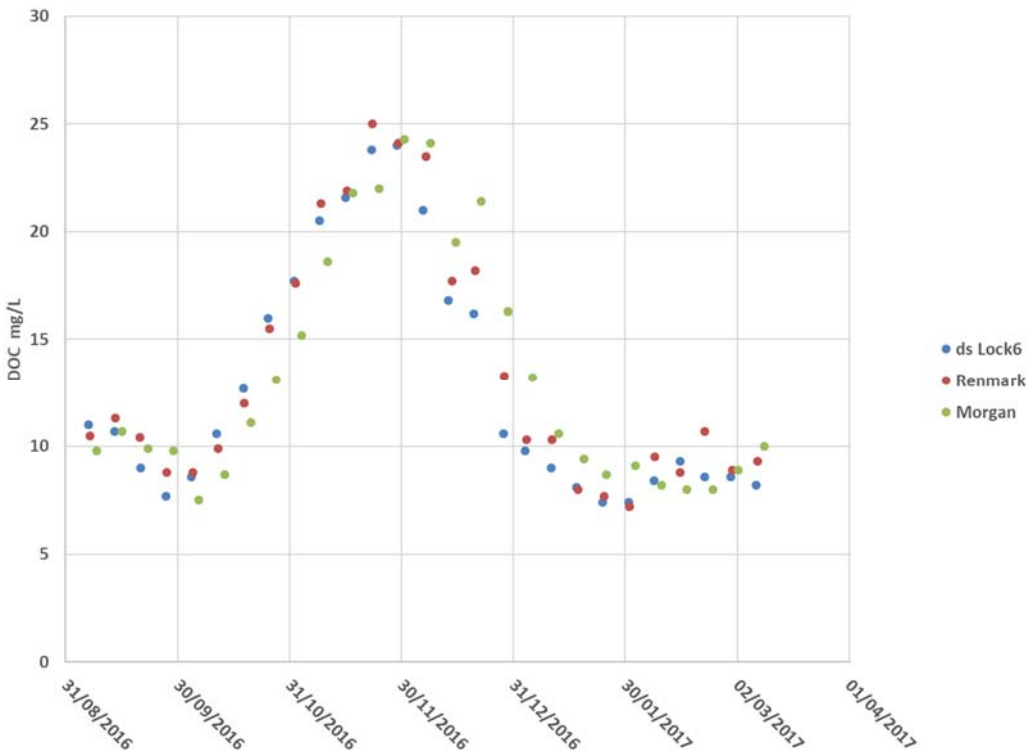


Figure C2. Dissolved organic carbon concentrations at three sites across the LMR.

Metabolism

Estimates of metabolic rates were made using comparable data from probes deployed at a depth of 500 mm. This included the two core LTIM sites, that is the sites downstream of Lock 1 and downstream of Lock 6, and also sites associated with the manipulation of Weir Pool 2, that is the sites upstream of Lock 2 and downstream of Lock 3 (Appendix B). The BASE model was applied and the results assessed against the standard acceptability criteria. These analyses showed that the model and fitting routines were not able to describe the daily changes in dissolved oxygen concentrations during almost all of the flood, and also for some periods before and after the flood. The effect of this is displayed in Figure C3 which shows for Lock 6, the site where modelling was most successful, the full set of metabolism estimates, and the sub-set that passed the acceptability criteria. Across the four sites the total number of days monitored for dissolved oxygen ranged from 159 to 175 with acceptable data comprising between 22 and 44% of the datasets and occurring largely before and after the flood (Figure C3). This result is not dissimilar to the return on data achieved across all three years of monitoring at the core sites (Table C1). This is an unsatisfactory outcome and there is a need to resolve the problems associated with fitting the model

of the dissolved oxygen balance to the monitoring data to ensure confidence in the results. Improvements are clearly required for the data devoid 2016/17 flood to understand the effects of floods on river metabolism. However, the intermittent loss of daily data before and after the flood also needs to be addressed as the reduced dataset made it difficult to assess the influence on metabolism of Commonwealth environmental water flows, including those associated with weir pool raising before the flood, and those maintaining channel flows following the flood.

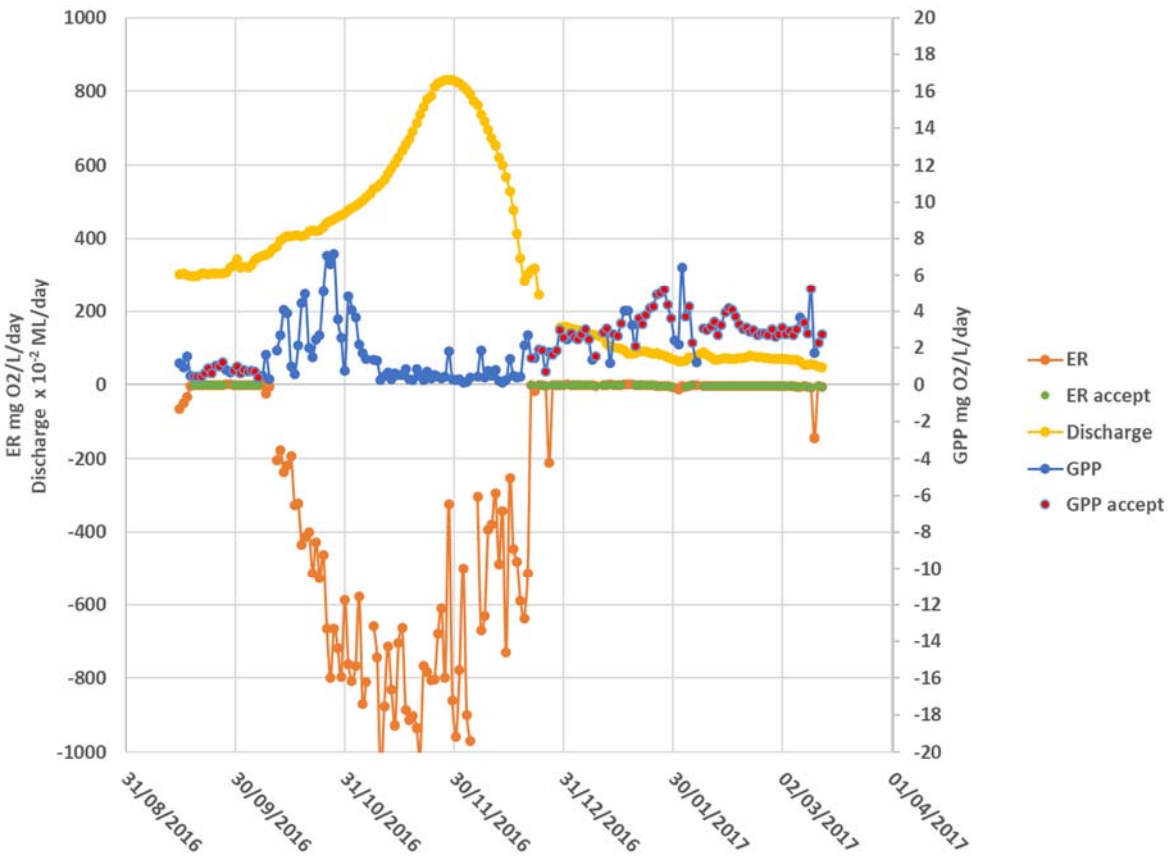


Figure C3. Modelled rates of gross photosynthesis (GPP), ecosystem respiration (ER) and net ecosystem production (NEP) at Lock 6, depicting metabolic estimates considered acceptable according to the statistical selection criteria. The flow to South Australia (discharge) is shown for reference.

Table C1. Three years of monitoring the two core LMR stream metabolism sites, downstream of Lock 1 (LK1DS) and Lock 6 (LK6DS) indicating the start and finish of the annual monitoring periods, the number of total days with data, the numbers of days that metabolism analysis was acceptable, and the percent return on collected data.

Site	Start monitoring	Finish monitoring	Total days monitored	Days analysis accepted	% success
LK1DS_265km	05/11/2014	24/02/2015	104	89	86
LK6DS_616km	05/11/2014	23/02/2015	105	49	47
LK1DS_265km	24/09/2015	02/03/2016	154	117	76
LK6DS_616km	23/09/2015	01/03/2016	133	92	69
LK1DS_265km	16/09/2016	14/03/2017	174	72	41
LK6DS_616km	15/09/2016	13/03/2017	175	83	47

The metabolic data considered reliable for each of the four sites are shown in Figure C4 a–c progressing downstream from Lock 6 to Lock 1. The data points are not connected because of the many breaks in the data series where daily results could not be modelled. The flow to South Australia is shown on each set to provide a reference to major flow conditions.

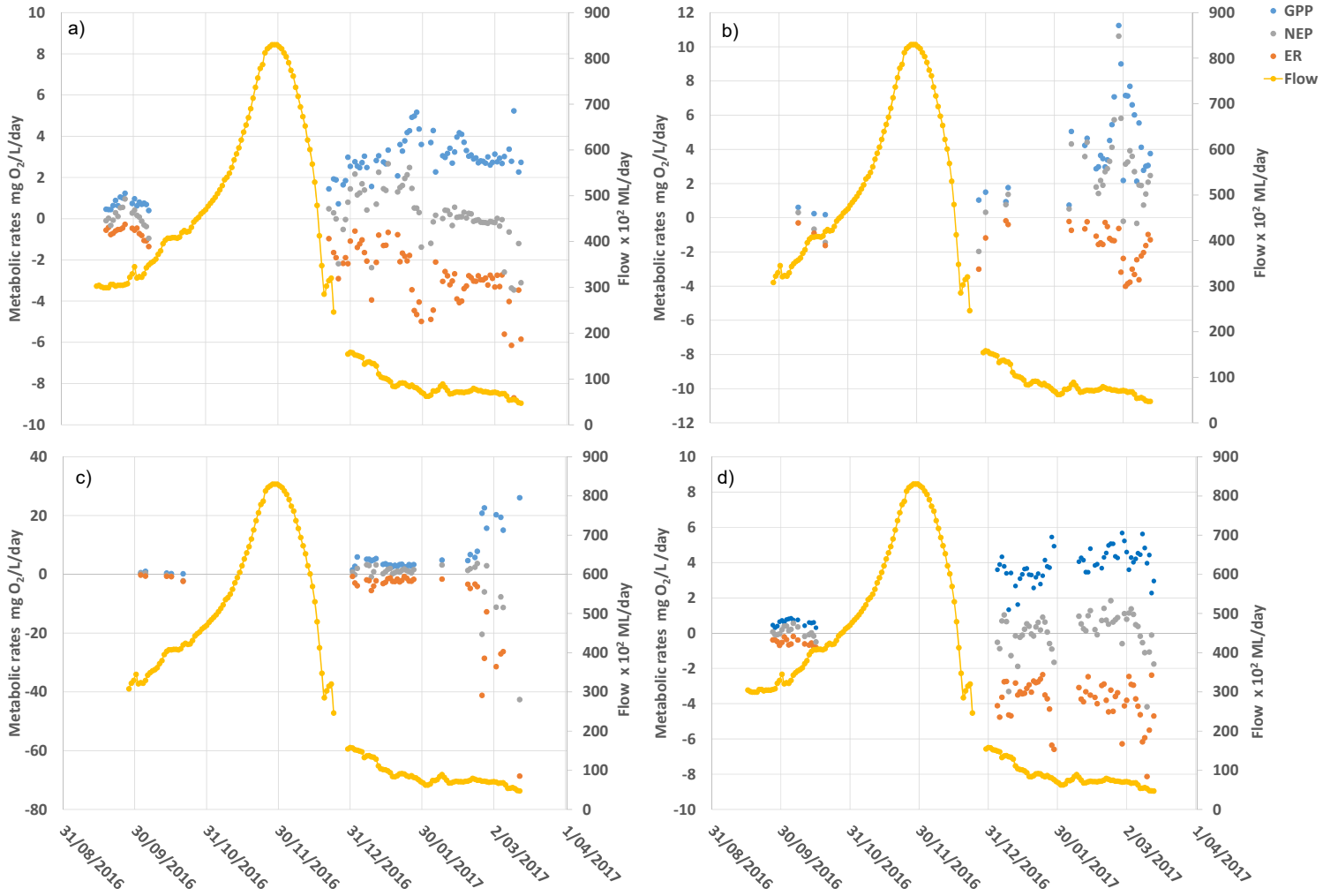


Figure C4. Estimates of gross photosynthesis (GPP), ecosystem respiration (ER) and net ecosystem production (NEP) accepted as reliable at the four sites: a) downstream of Lock 6, b) downstream of Lock 3, c) upstream of Lock 2 (note scale), d) downstream of Lock 1. Flow to South Australia (discharge) is shown for reference.

In general, the metabolic rates (GPP, ER and NEP) were within the range of ± 10 $mgO_2\ L^{-1}\ day^{-1}$, except at the site upstream of Lock 2 where late in the season both GPP and ER increased greatly. GPP and ER reached values of 26 $mgO_2\ L^{-1}\ day^{-1}$ and -68 $mgO_2\ L^{-1}\ day^{-1}$, respectively, with the more rapid increase in ER resulting in large negative NEP values (Figure C4). Although the analyses yielding these high metabolic rates met the acceptance criteria for the BASE model, and visually the model fitted the data reasonably well, these high rates are unlikely to be reliable. The site immediately upstream in the same weir pool (Lock 3 downstream site) also showed slightly increased GPP and NEP at this time, but not to the extent of the downstream site. Such differences in metabolic rates across sites have been observed previously, with lower and more balanced rates of metabolism generally occurring at actively flowing sites downstream of weirs, and increased metabolism in the deeper, more slowly flowing pools just upstream of weirs where phytoplankton growth can be enhanced (Oliver and Lorenz 2010). This may account in part for the increased metabolic rates upstream of Lock 2 compared with the other sites that are all downstream of weirs, but further analysis is required to assess the unusually high rates. Despite the different patterns in metabolism observed in the middle sites, the patterns at the end sites, downstream of Lock 6 and Lock 1, were similar to each other and showed typical increased metabolic rates later in the season when temperatures were substantially higher.

During the monitoring period, a series of environmental water management events occurred across the LMR that had the potential to impact on river metabolism measurements and therefore needed to be considered (Appendix A). The raising of water levels in Weir Pools 2 and 5 occurred between July and early October 2016. Monitoring of the dissolved oxygen concentrations at sites associated with the weir pools commenced in late September, with reliable BASE results collected at intervals from 19 September to 7 October above Lock 5, and 3 October to 21 October above Lock 2 (Figure C4). These data series were collected after the weir pool raising commenced in July, and also during and post maximum water levels. In Weir Pool 2, modelled rates of GPP and ER were relatively stable during the period prior to the termination of the weir pool raising on 24 October due to increasing unregulated flow, making it difficult to assess any change associated with the raising (Figure C5a). Metabolism then became increasingly varied with the arrival of overbank flows.

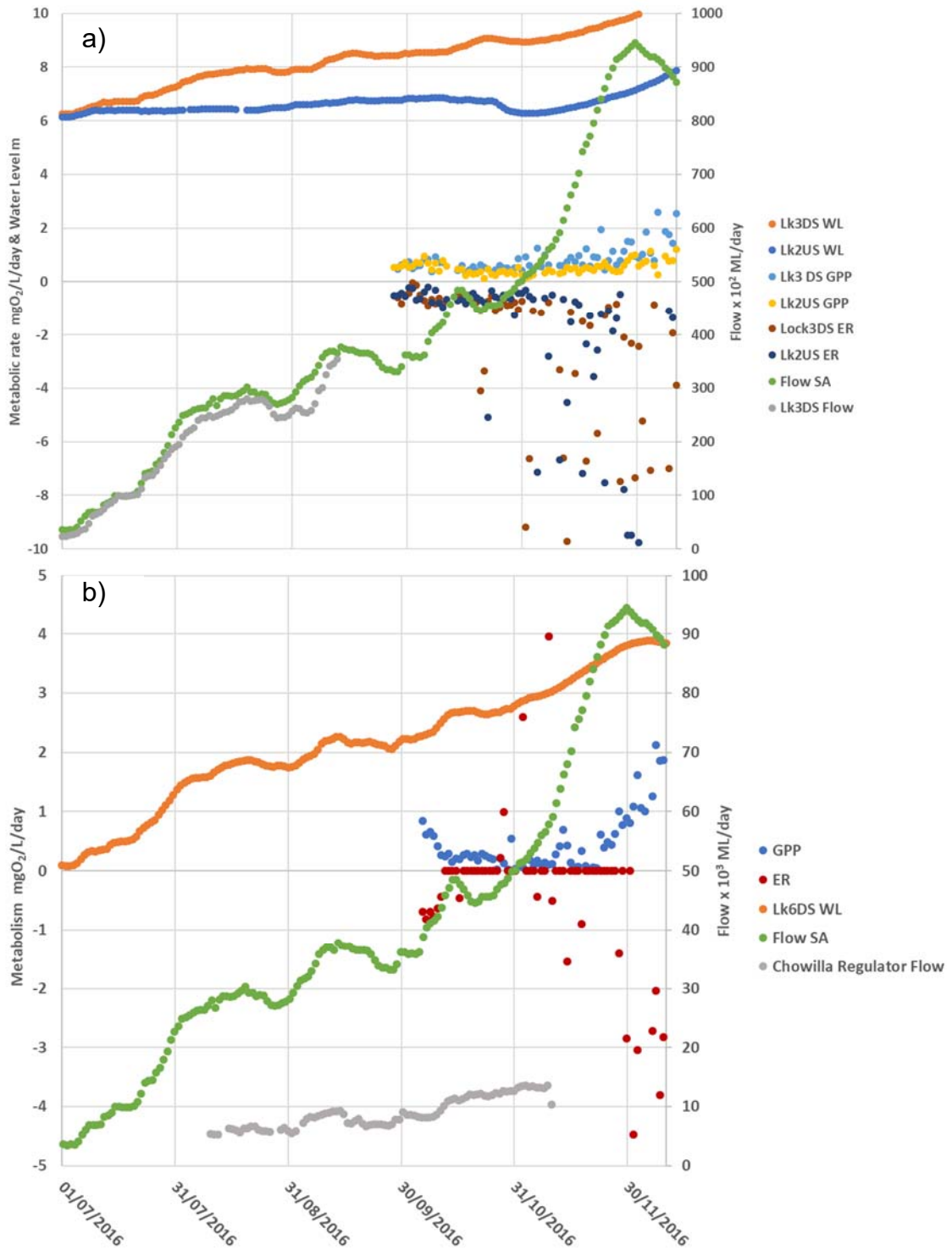


Figure C5. Water level changes and estimates of gross photosynthesis (GPP) and ecosystem respiration (ER) for (a) Weir Pool 2 and (b) Weir Pool 5. Flow to South Australia (for both weir pools), below Lock 3 (for Weir Pool 2) and in Chowilla Creek (for Weir Pool 5) are shown for reference.

The timing of the monitoring was also an issue above Lock 5 with only a small overlap of monitoring data before water level raising was terminated on 17 October (Figure

C5b). Interpretation of this data was particularly difficult as the weir pool raising coincided with the testing of the Chowilla regulator that occurred between early August and mid-November (Appendix A). Return water from the Chowilla Creek enters just upstream of the monitoring site above Lock 5 making it difficult to separate the effects from weir pool raising and Chowilla floodplain inundation. During the short period that monitoring data was available prior to the floods, it appeared that there was enhanced metabolic activity upstream of Lock 5. Whether this enhanced activity was associated with the returning Chowilla waters, the raising of Weir Pool 5, or with transport of material from Lock 6, which was also raised as a function of the Chowilla regulator testing, is being investigated using oxygen measurements provided by other agencies. In order to identify the effects of weir pool raising, and other management activities on metabolism, the dissolved oxygen monitoring needs to better align with each activity, preferably starting earlier to provide pre-treatment data and to capture responses to the initial changes.

Another intervention that utilised environmental water was the release of flows from Lake Victoria to maintain oxygen levels in the Rufus River and provide a refuge habitat for aquatic fauna (Appendix A). During the period of increased releases from Lake Victoria, from about 16 November 2016 onwards, the oxygen concentrations in the Murray River downstream of the inflow were consistently higher than those upstream. The size of the difference varied from zero up to 1 mg L^{-1} on 25 November 2016, during peak Lake Victoria releases (5,560 ML day^{-1}) (Figure A3 in Appendix A), but concentrations had generally declined by the time the flow reached Lock 6 (Figure C6). However, for a short period from 11 to 17 December 2016, the influence of the releases on oxygen appeared to reach to Customs House (Figure C6), downstream of the South Australian border (Figure A5 in Appendix A). These results suggest that the release of oxygenated waters from Lake Victoria had a wider impact than just the local environment of the Rufus River and although the influence was small, it occasionally extended downstream for a substantial distance. Why the oxygen increase at this time was sustained so far downstream is not apparent and a more dynamic analysis accounting for metabolism and gas exchange at the water surface is required to address this question.

Environmental water supplemented Lake Victoria releases from 17 to 31 December and maintained dissolved oxygen levels above 4 mg L^{-1} in the Rufus River (Figure A4

in Appendix A). During this period, the oxygen concentration increased rapidly at the site downstream of Lock 6 to over 6 mg L^{-1}. The timing suggests that the Lake Victoria flows may have had an influence further downstream (Figure C1). However, a detailed analyses of oxygen data collected by SA Water from the Rufus River outlet of Lake Victoria, and from the Murray River upstream and downstream of the Rufus River confluence indicated that the outflow played only a minor role in the improved oxygen concentrations at Lock 6 (Figure C6) and that the substantial increase in the oxygen concentration at Lock 6 (~15–25 December 2016) was mostly due to improved water quality arriving from upstream in the Murray River or the Murrumbidgee River. The source(s) of this water, which potentially included environmental water, was difficult to determine and not explored in this report. The timing of rapid increase in oxygen concentrations also coincided with water returning in-channel, following overbank flows.

An instantaneous calculation of the oxygen concentration resulting from mixing of different waters gives an idea of the potential magnitude of influence. These calculations need to be interpreted carefully as oxygen concentrations change rapidly in response to the biota and physicochemical conditions and are not a conservative tracer of water mixing. Based on average flows and average oxygen concentrations in the Rufus River from 17 to 24 December 2016, inflows to the Murray River could have increased the oxygen concentration by ~0.8 mg L^{-1}. During this period, there was also an increase in flow from the Darling River, and using a similar calculation this potentially could have increased the Murray River oxygen concentration by ~0.3 mg L^{-1}. At Lock 6, the oxygen concentration over this time period increased from 1.9 to 6.9 mg L^{-1}. If oxygen was acting conservatively, the inflows from Lake Victoria and the Darling River contributed 20% to the change in oxygen. Sites further downstream also showed an initial rise in oxygen at this time, but it was not sustained, and their oxygen concentrations fell back to previous levels until the end of the month (Figure C1). Oxygen concentrations then rapidly increased to near saturation levels at all sites indicating the return of normally oxygenated water from upstream.

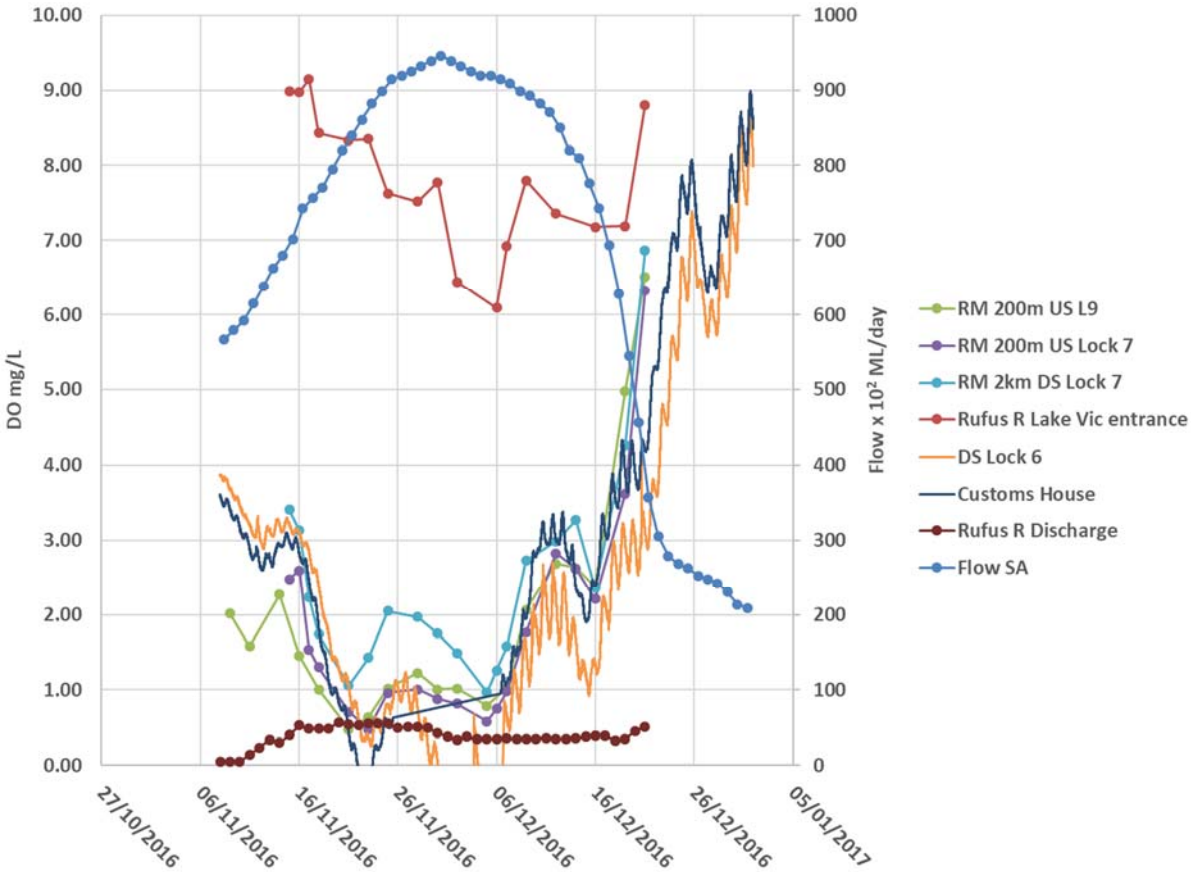


Figure C6. Dissolved oxygen concentrations at two sites in the Murray River above the influence of Lake Victoria (US Lock 9 and US Lock 7); in the Rufus River outflow from Lake Victoria; at a site immediately downstream of the Rufus River influence (DS Lock 7); and two sites in South Australia (Customs House and DS Lock 6). The Rufus River discharge and flow to South Australia are shown for comparison. Refer to Figure A5 in Appendix A for locations of sampling sites.

On return to operational flows in January 2017, a significant proportion of the water to South Australia was provided through environmental flows, including contributions by Commonwealth environmental water, as well as from a number of other sources (e.g. The Living Murray, Figure 3). During the monitoring period from January until early March 2017, flows were in a moderate range reducing gradually from 25,000 to 10,000 ML day^{-1} with environmental flows contributing between 25 and 50% of the water. At these discharge rates, flow is constrained within the channel, and flow velocities are relatively high, producing a well-mixed water column. Although the rates of metabolism will be influenced by associated changes in physical characteristics over this period, such as decreasing water depths and velocities resulting from the reduced flows, these changes are likely to be relatively small in this flow range. In contrast, changes in water quality that might result from alterations in the mix of waters being delivered from different sources are likely to be more

influential. Such an effect is evident in the metabolic data, with a significant increase in ER at Lock 6 during February compared with January (Figure C7). This change aligns with a step increase in the contribution of flow from the Darling River, resulting in it providing nearly 50% of the total flow (Figure 5). There is a brief increase in GPP during the step change in flow from the Darling River, but it quickly returns to levels similar to those before the flow change.

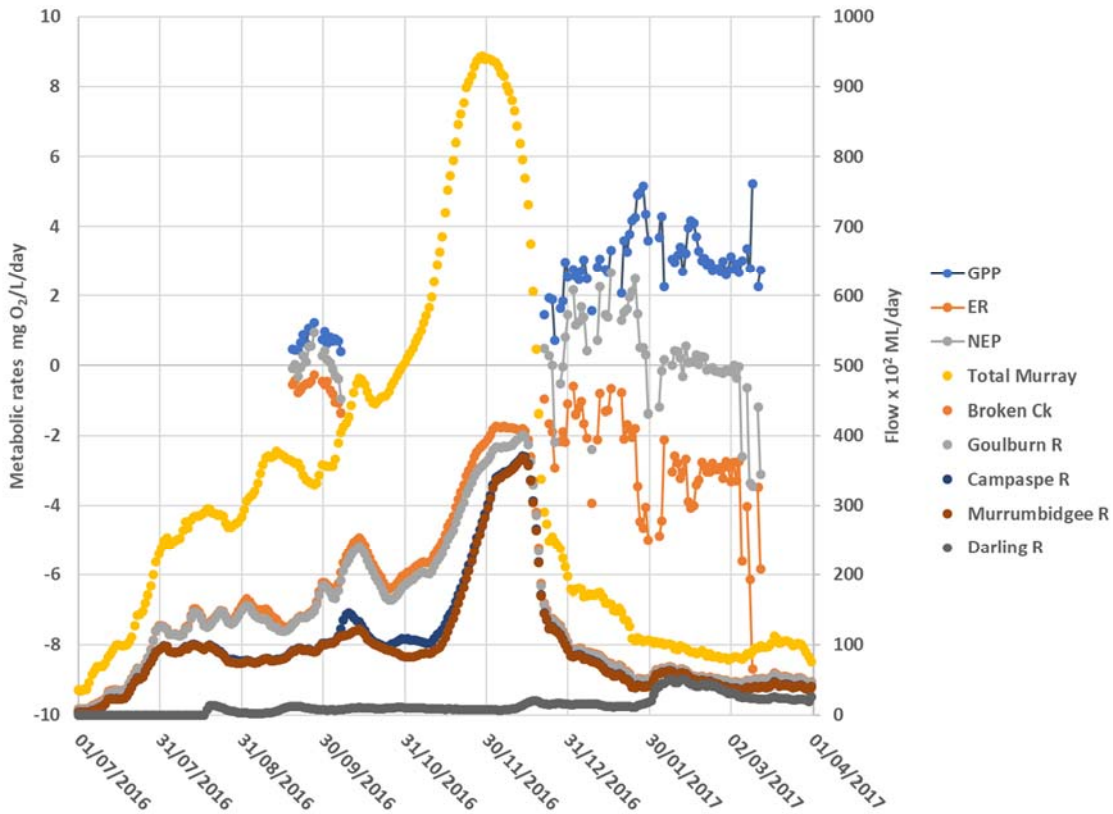


Figure C7. Metabolic data from Lock 6 compared with a stacked area chart of flows to South Australia from July 2016 to April 2017.

These results suggest an increase in the respiratory metabolism of food resources in the river, and this may be due to increased supplies of organic material from the Darling River, although this is not reflected in the measurements of DOC (Figure C2). Turbidity did increase in conjunction with the flow change (Figure C8), perhaps indicating an increased supply of particulate organic materials. However, despite little change in GPP, the step increases in respiration rate resulted in NEP approaching zero, whereas prior to this it had been largely positive (Figure C4). This "balance" suggests an alternative explanation of the results. The increased turbidity associated with the enhanced contribution of flow from the Darling River (Figure C8), which is naturally

highly turbid, may have altered the light conditions in the river such that the phytoplankton spent proportionately longer periods in the dark. This causes phytoplankton to respire more of their organic carbon reserves for cellular maintenance, rather than accumulating them. The negligible change in GPP indicates that photosynthetic production by the phytoplankton remained light saturated despite the change in light conditions. Management activities that alter the ratio of the photic (illuminated) to euphotic (unilluminated) zone through alterations in turbidity, or changes in water depths, will influence this balance.

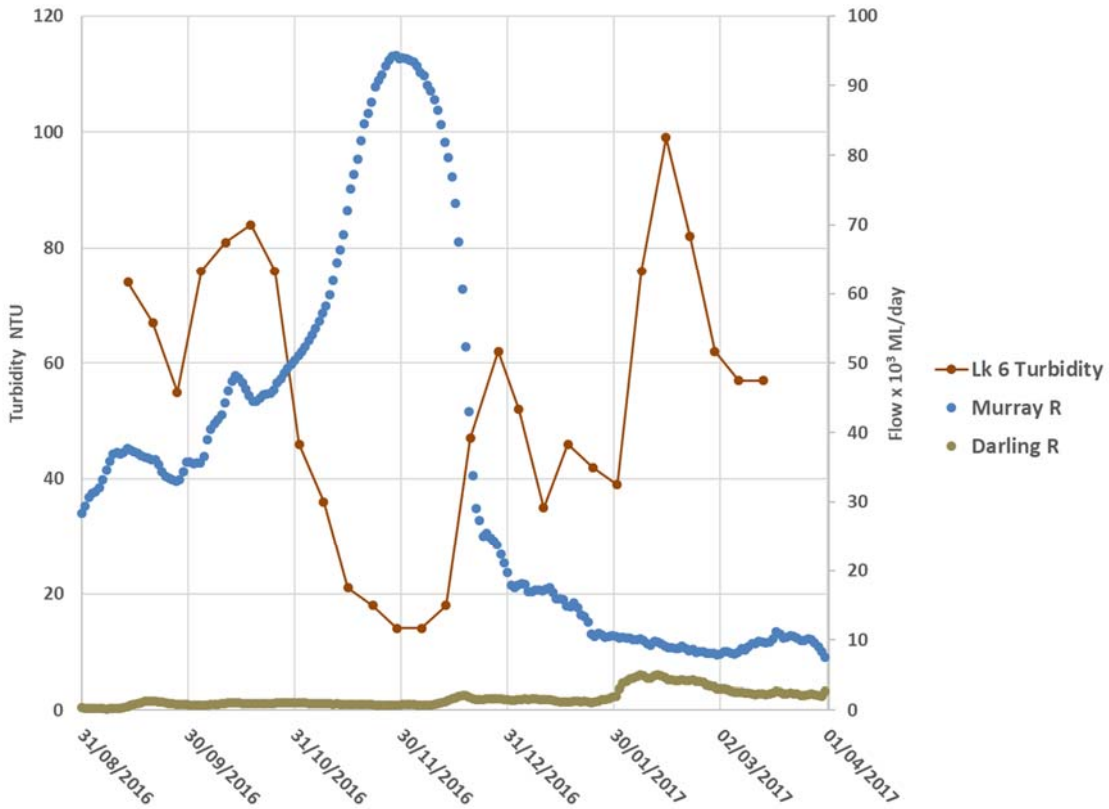


Figure C8. Increases in turbidity below Lock 6 in comparison to the contribution of flow to South Australia from the Murray and Darling River sources.

Fluctuations in metabolic activity in response to changing flow conditions is part of the natural variability expected in a river reach, but as with other environmental characteristics impacted by river operation, the timing, frequency and magnitude of these changes is expected to be important. Assessing the interplay of these factors and their long-term influence on food resources requires detailed measurements across different combinations of environmental conditions. Teasing apart these various scenarios is an important objective of the ongoing analyses of the

Commonwealth environmental water dataset in order to improve the metabolic modelling.

A synopsis of the metabolism data meeting the modelling selection criteria that has been collected over the 3 years of the project is displayed in Figure C9 for Locks 1 and 6, along with matching flow data. Metabolic measurements downstream of Lock 6 were in Weir Pool 5 and so flow from Lock 5 has been used. There are marked similarities in the patterns of metabolic rates within each site across the 3 years, but differences between sites are also evident, especially late in the season. The general patterns are most likely due to the seasonal changes in temperature as well as light. In general, flow rates were quite similar between years, except during the flood in 2016/17 when metabolic rates could not be determined. Because of the expectation that flows in the Lower Murray would largely be in-channel, there has been a focus on examining short-term influences of flow pulses, and also the effects of management strategies including weir pool manipulation and the operation of the Chowilla regulator. The 2016/17 flood was a major perturbation to the system so it is critical that data modelling is improved to enable its effects on river metabolism to be assessed.

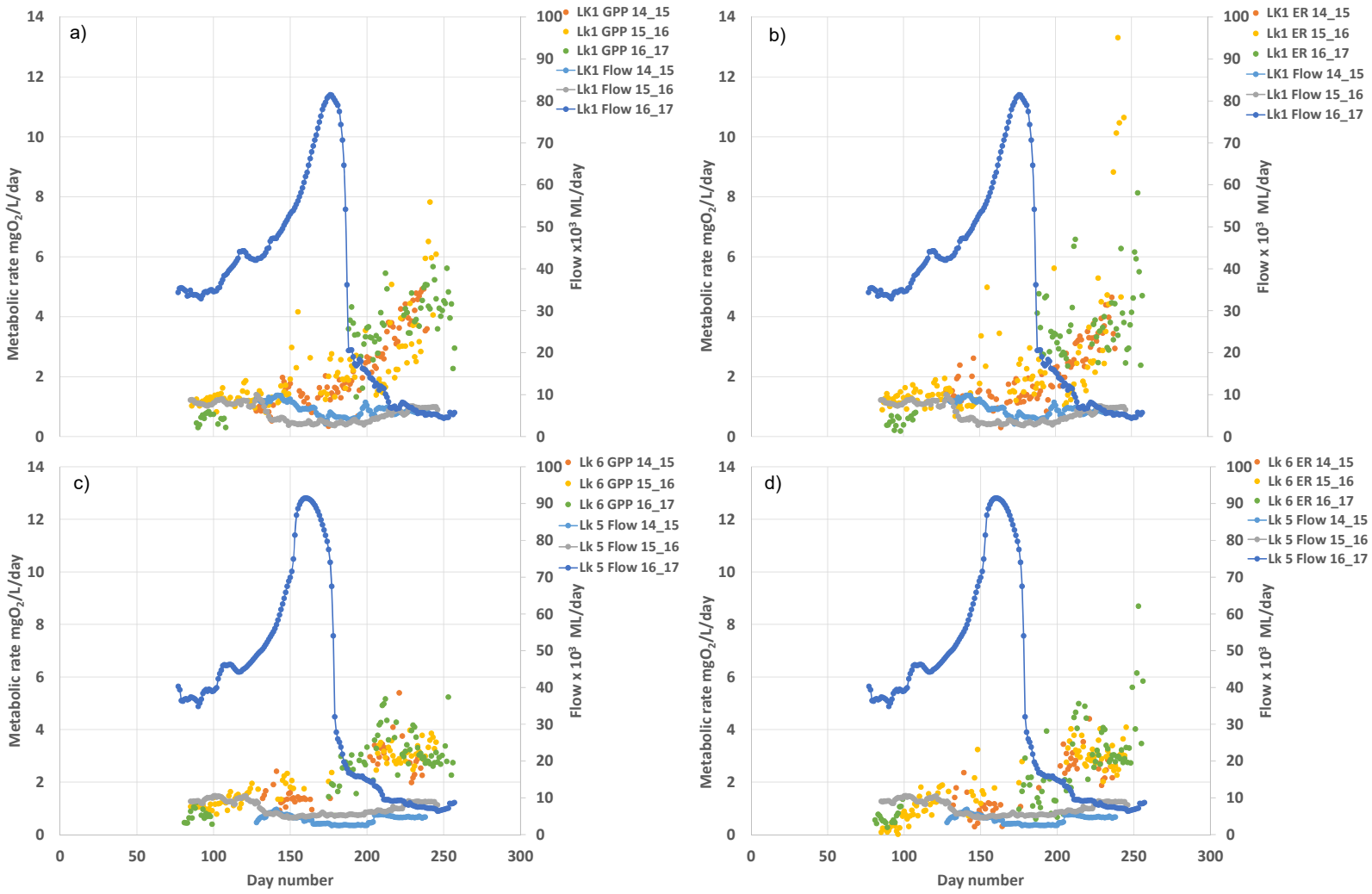


Figure C9. Flow and metabolic rates for 2014/15, 2015/16 and 2016/17 monitoring periods with day number taken from July 1st of each initial year. A = GPP at Lock 1, b = ER at Lock 1, c = GPP at Lock 6 and d = ER at Lock 6.

Conclusions

During the 2016/17 monitoring, oxygen concentrations declined quickly between early November and the end of December 2016 across the sites, falling below the 50% saturation level considered acceptable by DEWNR and targeted in the Basin Plan (S9.14). Downstream of Lock 6, dissolved oxygen concentrations fell to zero mg L^{-1} for a 4-day period. These deleterious oxygen concentrations were the result of overbank flows, with oxygen depletion exacerbated by the reduced frequency of flooding under river regulation which has increased the time for accumulation of organic debris and detritus on the floodplains (Howitt *et al.* 2007). This material stimulates increased biological activity on flooding, causing rapid oxygen decline. Consideration of return times for managed flooding of low lying floodplains may help reduce this problem, as river regulation has impacted most on small and moderate floods, and environmental flows may be sufficient to partially redress the issue at this scale.

The strategy of flow releases from Lake Victoria to maintain dissolved oxygen concentrations in the Rufus River at acceptable levels may have helped improve conditions downstream as far as Customs House, downstream of the South Australian border, although the influence was small. The high unregulated flows, extensive flooding, and changing contributions of upstream river systems to the flows reaching South Australia, made for highly variable conditions in the monitoring region. Although the monitoring equipment recorded data for the whole period from September 2016 to March 2017, the methods for analysing the data were found to be wanting. Only 22–44% of the data were successfully analysed resulting in a significant reduction in the data available to explain the observed changes in metabolic activity. Whether this poor return is because the oxygen balance model does not capture all of the causes of the changes in oxygen concentration, or because the fitting of the model is not sufficiently robust or reflective of the changing environmental conditions, is not yet understood. Investigation of these issues is continuing with the now extensive LMR dataset.

Extra sites were included in the 2016/17 monitoring period in an effort to provide additional information on changes in metabolism in the LMR and inform on changes associated with the raising of Weir Pools 2 and 5 (Appendix B). In the end, no

conclusions could be drawn about the influence of weir pool raising on river metabolism due to overbank flows which masked the management action.

Following overbank flows, there was a return to operational flows in January 2017. From this time until the end of monitoring in early March 2017, a significant proportion of the water to South Australia was provided through environmental flows, which included contributions by Commonwealth environmental water, as well as from other sources (e.g. The Living Murray water). Over this period, flows reduced gradually from 25,000 to 10,000 to ML day^{-1}, with environmental flows contributing between 25 and 50% of the water. A marked increase in ER at the site downstream of Lock 6 occurred during this period and aligned with an increased delivery of water from the Darling River, a naturally turbid water source. It was likely that the increase in turbidity reduced the light available to the phytoplankton. This resulted in the cells spending more time in the dark and having to utilise their carbon reserves for maintenance, rather than accumulating the reserves to enhance growth. More detailed analyses are underway to test this hypothesis, but it highlights the important influence of light on metabolic activity in the river. The incident light is important, but is generally high in the lower River Murray during spring and summer. More relevant is the extent of incident light penetration into the water and the ratio of the photic to euphotic zone of the water column. These factors influence the degree of energy accumulated by the phytoplankton and the proportion made available as a food resource to other organisms. Under less satisfactory light conditions phytoplankton growth will be restricted and more of the captured energy will be respired by the phytoplankton themselves to provide energy for cellular maintenance.

Management activities that influence light penetration through alterations in turbidity, or changes in water depths that alter the ratio of the photic to euphotic zone, or flow changes that alter mixing intensity so that some phytoplankton can maintain themselves in the photic zone, will impact on phytoplankton production. Fluctuations in metabolic activity in response to changing flow conditions is part of the natural variability expected in a river reach, but as with other environmental characteristics impacted by river operation, the timing, frequency and magnitude of these changes is expected to be important. Assessing the interplay of these factors and their long-term influence on food resources requires detailed measurements across different combinations of environmental conditions. The CEWO LTIM program in the LMR is

providing this information and should enable an improved understanding of these factors to be translated into a more reliable understanding of the impacts of environmental flows on river metabolism.

APPENDIX D: FISH (CHANNEL)

Background and aims

The main channel of the LMR supports a diverse fish assemblage, which is comprised of small- and large-bodied species that have various life history requirements (e.g. reproduction and habitat use). Variation in flow influences riverine hydraulics and in turn structural habitat (e.g. submerged vegetation), which may influence fish assemblage structure (Bice *et al.* 2014).

The Category 1 Fish (channel) indicator was designed for the Basin-scale evaluation for fish community responses to Commonwealth environmental water, which are being undertaken by the M&E Advisors (Hale *et al.* 2014). While there is no CEWO local evaluation questions for the Category 1 Fish (channel) indicator in the LMR, in this report we provide commentary on the fish assemblage in the gorge geomorphic zone of the LMR using data collected through this indicator. Our interpretations of the data do not infer association of ecological patterns with Commonwealth environmental water delivery. For this report, our objectives are to:

- Provide basic summary statistics of the catch rates and population demographics for nominated species;
- Describe temporal variation in fish assemblage and population structure between Year 1 (2015), 2 (2016) and 3 (2017); and
- Discuss key findings with some interpretation of the patterns based on published research and our current understanding of fish life histories and population dynamics in the LMR.

Methods

Fish sampling

Small- and large-bodied fish assemblages were sampled from the gorge geomorphic zone of the LMR (Figure 6) using fine-meshed (2 mm mesh) fyke nets and electrofishing, respectively. Sampling followed standard methods prescribed by Hale *et al.* (2014). Half of the sites (sites 2, 5, 8, 9, 10) were sampled by electrofishing in autumn 2017, whilst electrofishing at the remaining sites was delayed to winter due to equipment failure/malfunction. All fyke netting occurred during autumn 2017. Population

structure (i.e. length) data were obtained for seven target species, while age data were also collected for bony herring (*Nematalosa erebi*) (Figure D1). Refer to SARDI *et al.* (2016) for detailed sampling design and methodology.

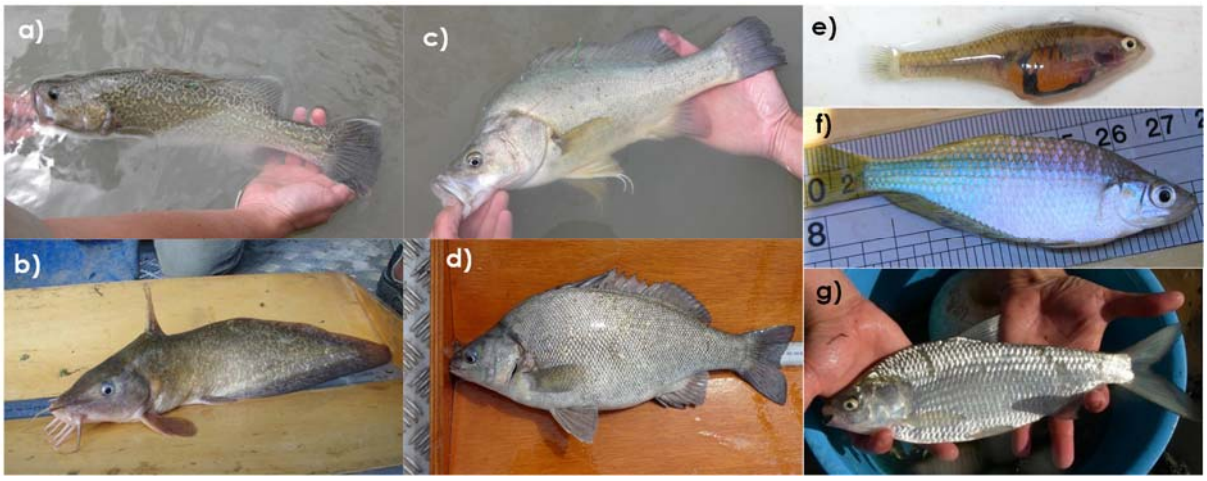


Figure D1. Target species for the LMR: (a) Murray cod and (b) freshwater catfish (equilibrium life history); (c) golden perch and (d) silver perch (periodic life history); and I carp gudgeon, (f) Murray rainbowfish and (g) bony herring (opportunistic life history).

Data analysis

Temporal variation in fish assemblage structure (species composition and abundance), between sampling years (i.e. 2015, 2016 and 2017), was investigated using a one-factor (i.e. year) permutational multivariate analysis of variance (PERMANOVA) in the software package PRIMER v. 6.1.12 (Clarke and Gorley 2006) and PERMANOVA + v.1.02 (Anderson *et al.* 2008). Comparisons were made separately for small- (fyke nets) and large-bodied species (electrofishing). Analyses of large-bodied assemblage data were restricted to autumn as data were significantly different among seasons for 2017 (i.e. winter vs. autumn). Analyses were performed on square-root transformed data from electrofishing (fish. 90 second electrofishing shot^{-1}) and fyke netting (fish. hour^{-1}). PERMANOVA was performed on Bray-Curtis similarity matrices (Bray and Curtis 1957). Non-metric Multi-Dimensional Scaling (MDS), generated from the same matrices, was used to visualise fish assemblages from different years. When differences in fish assemblages occurred between years for PERMANOVA, Similarity Percentages (SIMPER) analysis was used to determine the fish species contributing to these differences, with a 40% cumulative contribution cut-off applied.

To determine temporal variation in population structure, length frequency histograms were qualitatively compared between sampling years.

Results

Catch summary for 2017

A total of 6,349 individuals from six large-bodied species were sampled by electrofishing from ten sites in the gorge geomorphic zone of the LMR (Table D1). Bony herring and common carp (*Cyprinus carpio*) were the most abundant species, representing 75% and 20% of the electrofishing catch composition, respectively (Table D1; Figure D2a).

Fewer small-bodied individuals and species were sampled in 2017, relative to the previous two years (Table D2). A total of 9,661 individuals from five small-bodied species were sampled by fyke nets from ten sites in the gorge geomorphic zone of the LMR (Table D2). Carp gudgeon (*Hypseleotris* spp.) was the most abundant species (4.4 ± 1.5 individuals per net per hour) and dominated fyke net catch composition (92%) (Table D2; Figure D2b). Gambusia (*Gambusia holbrooki*), unspecked hardyhead (*Craterocephalus fulvus*) and Murray rainbowfish (*Melanotaenia fluviatilis*) were the second, third and fourth most abundant species, respectively.

Table D1. Electrofishing catch summary (total catch, 2880 electrofishing seconds per site) for large-bodied fish species in the gorge geomorphic zone of the LMR from 2015–2017. Site numbering increases with distance upstream.* indicates sites where sampling was delayed to winter 2017.

Site No.	1*	2	3*	4*	5	6*	7*	8	9	10	
Site Name	Blanchetown	Scotts Creek	Morgan	Cadell	Qualco	Waikerie	Lowbank B	Lowbank A	Overland Corner B	Overland Corner A	**Total**
2015											
Golden perch	23	14	17	13	6	19	11	33	21	24	**181**
Silver perch							1	2		1	**4**
Freshwater catfish	1	3	1			1					**6**
Murray cod	2	1	1	1	1	1		2		2	**11**
Bony herring	964	916	1,223	978	687	1,816	670	627	820	770	**9,471**
Common carp	10	4	17	4	3	15	11	13	8	20	**105**
Goldfish	3		6			8			1		**18**
Redfin perch							1				**1**
Total	**1,003**	**938**	**1,265**	**996**	**697**	**1,860**	**694**	**677**	**850**	**817**	**9,797**
2016											
Golden perch	21	14	8	18	21	19	14	27	14	17	**173**
Silver perch				1	1				2	1	**5**
Freshwater catfish	1			1		1	2			2	**7**
Murray cod		3	1	2		1	2	3	2	2	**16**
Bony herring	991	820	1,680	536	60	743	700	745	605	547	**7,427**
Common carp	13	39	35	33	21	22	20	15	25	23	**246**
Goldfish	1	8	4	5	9	4	25	16	16	30	**118**
Redfin perch											**0**
Total	**1,027**	**884**	**1,728**	**596**	**112**	**790**	**763**	**806**	**664**	**622**	**7,992**
2017											
Golden perch	8	16	16	11	12	12		18	11	6	**110**
Silver perch											**0**
Freshwater catfish		2				1				2	**5**
Murray cod			2		1			5			**8**
Bony herring	2	1,251	6	1	272	5	58	564	1,190	1,428	**4,777**
Common carp	30	531	18	14	161	11	6	147	279	99	**1,296**
Goldfish		24		2	10	1	3	13	27	73	**153**
Redfin perch											**0**
Total	**40**	**1,824**	**42**	**28**	**456**	**30**	**67**	**747**	**1,507**	**1,608**	**6,349**

Table D2. Fyke net catch summary (total catch, 10 nets per site) for small-bodied fish species in the gorge geomorphic zone of the LMR from autumn 2015–2017. Site numbering increases with distance upstream.

Site No.	1	2	3	4	5	6	7	8	9	10	
Site Name	Blanchetown	Scotts Creek	Morgan	Cadell	Qualco	Waikerie	Lowbank B	Lowbank A	Overland Corner B	Overland Corner A	**Total**
2015											
Carp gudgeon	577	2,003	275	550	480	860	3,080	655	5,649	4,697	**18,826**
Murray rainbowfish	6	59	68	91	29	8	17	37	3	32	**350**
Unspecked hardyhead	18	87	2	23	7	5	2	20	13	248	**425**
Flatheaded gudgeon	15	1		1					1	2	**20**
Dwarf-flatheaded gudgeon	5	4	2	2	11	1	9	5	29	18	**86**
Australian smelt		5		58				7	4	2	**76**
Gambusia	5	206	83	125	8	1	34	36	193	562	**1,253**
Total	**626**	**2,365**	**430**	**850**	**535**	**875**	**3,142**	**760**	**5,892**	**5,561**	**21,036**
2016											
Carp gudgeon	3,575	1,033	692	898	2,959	1,904	1,781	1,597	3,390	1,974	**19,803**
Murray rainbowfish	56	354	47	35	79	87	47	128	14	17	**864**
Unspecked hardyhead	302	64	21	17	56	32	10	35	53	56	**646**
Flatheaded gudgeon	14	10	3	6	7	1			2	3	**46**
Dwarf-flatheaded gudgeon	5	4	2	10	40	11	8	2	12	10	**104**
Australian smelt		1			6	1	4	2			**14**
Gambusia		208	117	227	94	183	63	79	81	324	**1,376**
Total	**3,952**	**1,674**	**882**	**1,193**	**3,241**	**2,219**	**1,913**	**1,843**	**3,552**	**2,384**	**22,853**
2017											
Carp gudgeon	3,580	310	225	268	1,042	977	430	592	796	633	**8,853**
Murray rainbowfish	1	1	6	6	4		4	3			**25**
Unspecked hardyhead	24		1	6	23	4	6	2	1	3	**70**
Flatheaded gudgeon						1				1	**2**
Dwarf-flatheaded gudgeon											**0**
Australian smelt											**0**
Gambusia	32	342	57	75	21	5	18	16	19	126	**711**
Total	**3,637**	**653**	**289**	**355**	**1,090**	**987**	**458**	**613**	**816**	**763**	**9,661**

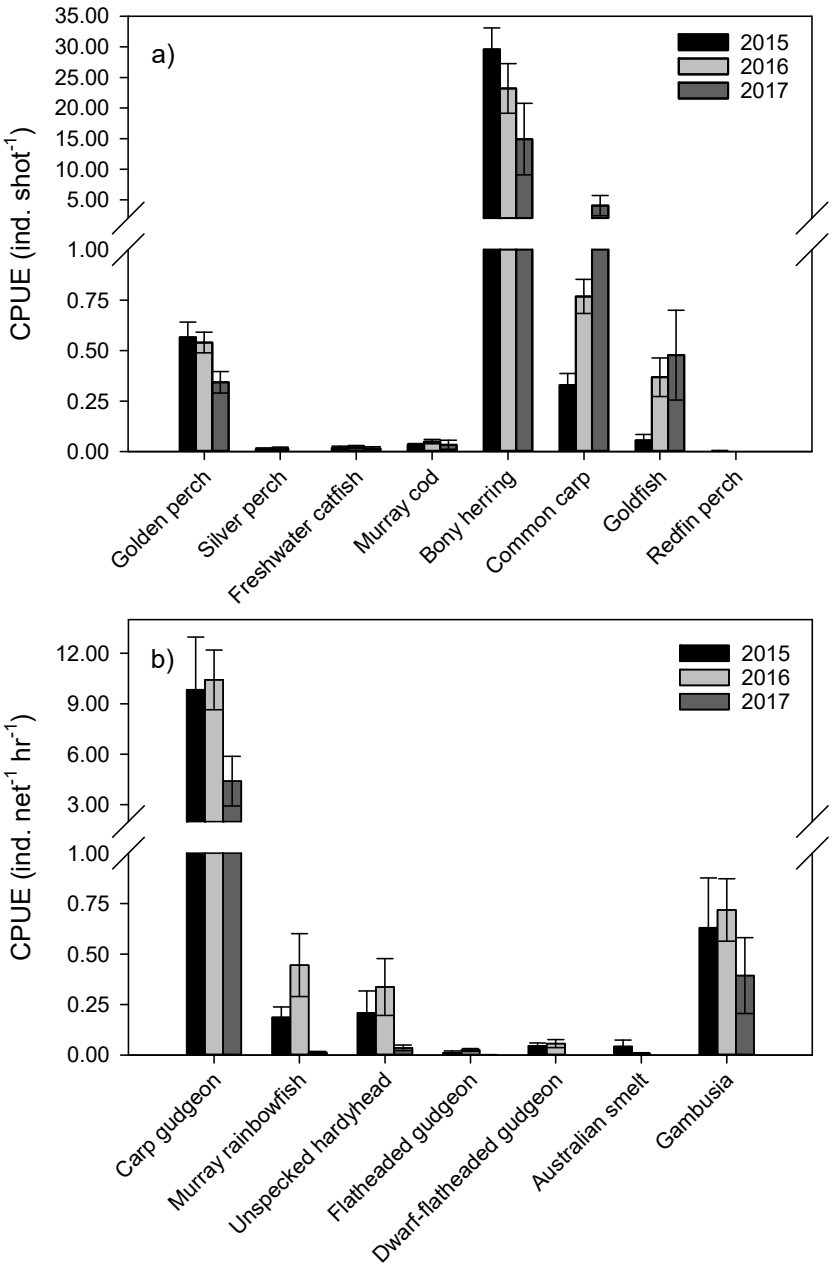


Figure D2. Mean catch-per-unit-effort (CPUE) ± standard error of (a) large-bodied fish species captured using electrofishing (individuals per 90 second shot) and (b) small-bodied fish species captured using fine-mesh fyke nets (individuals per net per hour) in the gorge geomorphic zone (all 10 sites) of the LMR from 2015–2017.

Temporal variability in fish assemblage structure

MDS ordination of electrofishing data showed separation of samples by sampling year, with further separation of 2017 samples by season (Figure D3a). PERMANOVA found that large-bodied fish assemblages were significantly different between season for 2017 (*Pseudo-*$F_{1,9}$ = 25.396, p = 0.0074); therefore, all further analyses of electrofishing data were restricted to autumn. PERMANOVA indicated that large-bodied fish assemblages were significantly different between years (*Pseudo-*$F_{1,24}$ = 6.672, p = 0.0001). Pairwise comparisons revealed significant differences between all years (Table D3).

Table D3. PERMANOVA pairwise comparison test results for large-bodied fish assemblages in the gorge geomorphic zone of the LMR from autumn 2015–2017.

Comparison	***t***	***P (perm)***
2015 vs. 2016	2.0305	0.0076
2015 vs. 2017	3.5839	0.0007
2016 vs. 2017	2.2942	0.0032

There were significant differences between years (*Pseudo-*$F_{1,29}$ = 5.8994, p = 0.0001) for small-bodied fish assemblages. Interspersion of 2015 and 2016 samples and separation of 2017 samples in MDS ordination of fyke netting data (Figure D3b) was supported by PERMANOVA pair-wise comparisons, which revealed significant differences in small-bodied fish assemblages between 2017 and 2015 (t = 2.0746, p = 0.013) and 2016 (t = 3.7835, p = 0.0001), but not between 2015 and 2016 (p > 0.05).

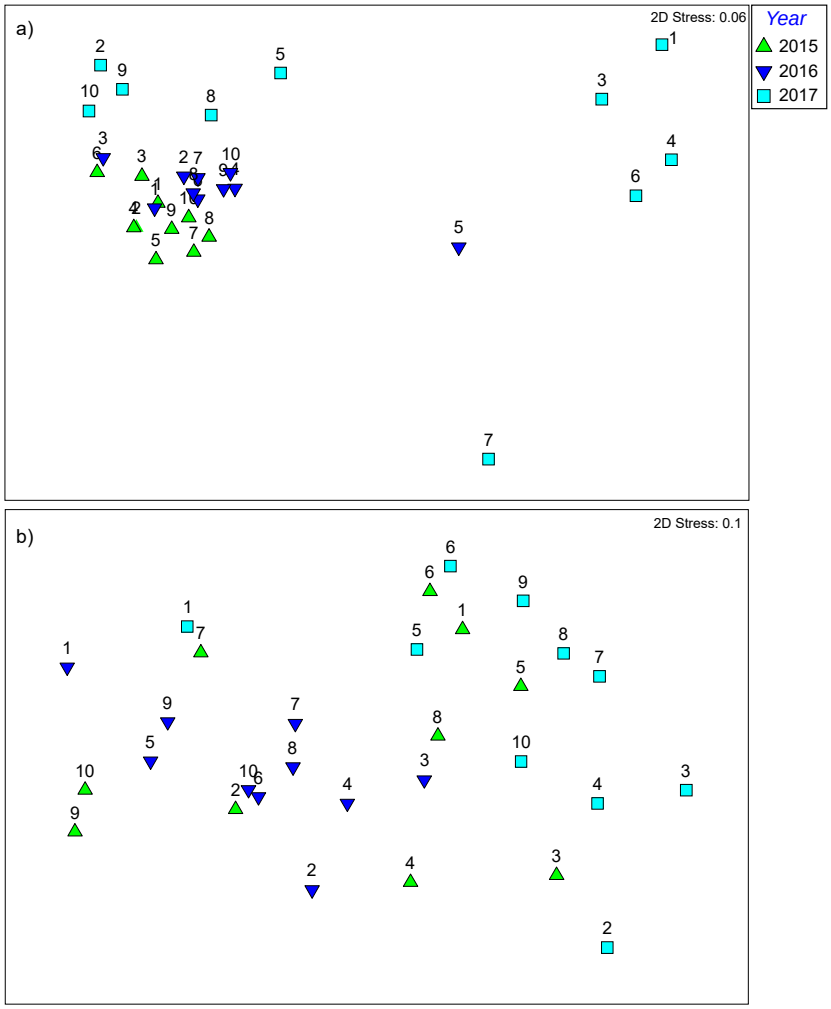


Figure D3. Non-metric multi-dimensional scaling (MDS) plot of (a) large-bodied fish assemblages sampled by electrofishing and (b) small-bodied fish assemblages sampled by fyke netting in the gorge geomorphic zone of the LMR. Numbered sample points represent sampling sites (1–10). Sites 1, 3, 4, 6 and 7 were sampled in winter 2017.

SIMPER indicated that differences between years for large-bodied fish assemblages were driven by higher abundances of common carp in 2017 and lower abundances of bony herring in 2016 (Figure D2; Table D1). SIMPER indicated that differences between 2017 and preceding years for small-bodied fish assemblages were driven by lower relative abundances of carp gudgeon, gambusia, Murray rainbowfish, unspecked hardyhead and dwarf flatheaded gudgeon (*Philypnodon macrostomus*) in 2017 (Figure D2; Table D2).

Temporal variation in population size/age structure

In 2017, golden perch and Murray cod (*Maccullochella peelii*) ranged in total length (TL) from 197–534 mm and 80–384 mm, respectively (Figure D4). For golden perch,

dominant TL modes at 240–260 (10%) and 380–400 mm (13%) in 2016 progressed to 320–400 mm (58%) in 2017. In 2017, the sampled Murray cod population was represented by three TL modes at 80–145 mm (45%), potentially age 0+, 228–232 mm (18%), potentially age 1+, and 338–384 mm (36%), potentially age 2+. Progression of these TL modes from 2015–2017 can be observed in Figure D4.

Population structure data for bony herring in 2017 were similar to the previous year. In 2017, bony herring ranged in fork length (FL) from 35–334 mm and, in age, from 0+ to 6+ years (Figure D5). Age 0+ (89%) and 2+ (4%) cohorts comprised most of the catch. As for 2016, based on length frequencies, there were no age 0+ golden perch, silver perch (*Bidyanus bidyanus*) or freshwater catfish (*Tandanus tandanus*) sampled in 2017 (Figure D4). However, two age 0+ golden perch (47 and 61 mm TL) were captured as part of Category 1 Fish (Channel) fyke netting, intended for small-bodied fish sampling (see Appendix H: Fish Spawning and Recruitment). Length frequencies of Murray rainbowfish and carp gudgeon (Figure D6) indicate that the sampled populations were dominated by individuals that were age 0+, based on length-at-age data from 2015 (Ye *et al.* 2016a).

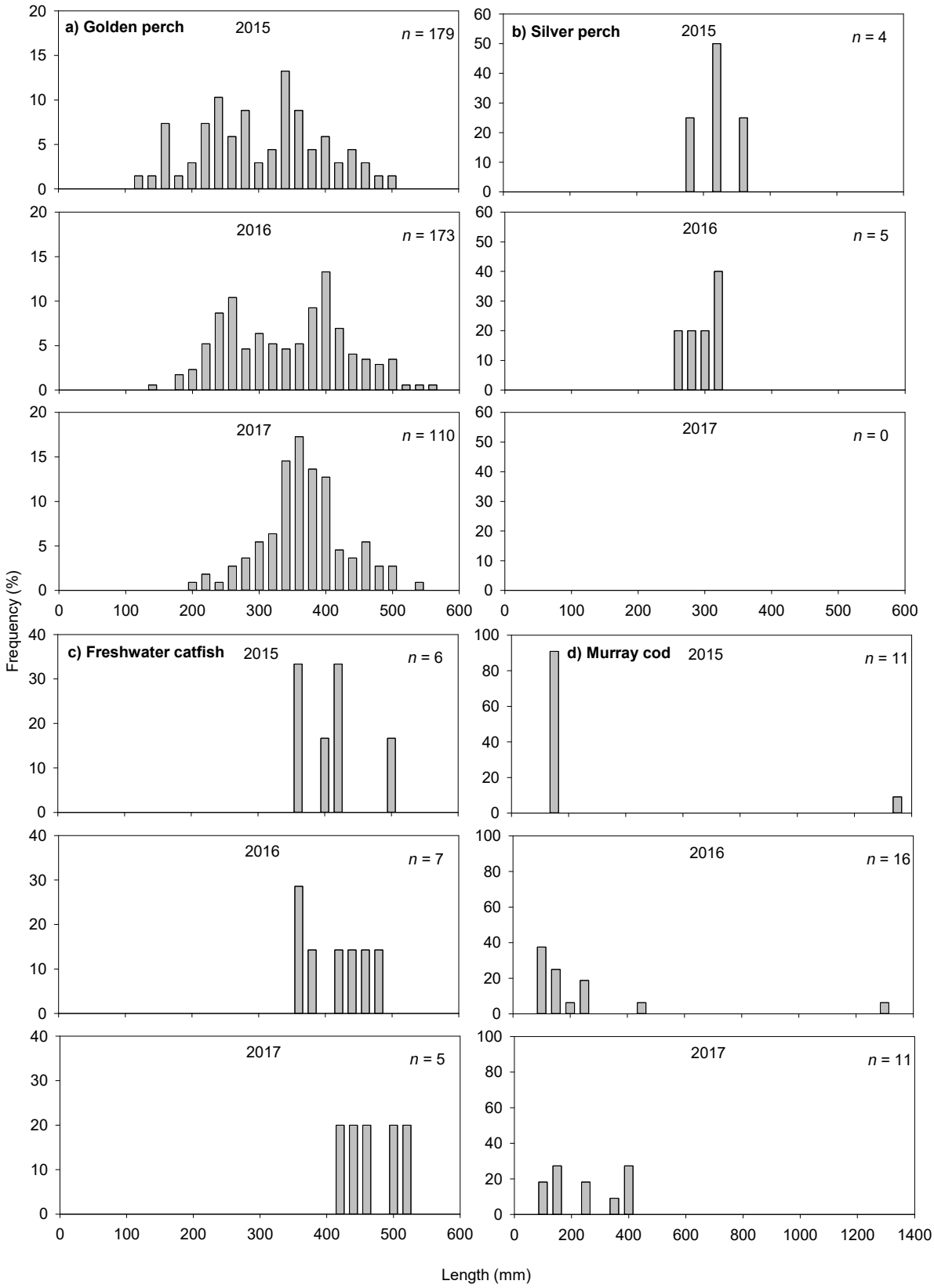


Figure D4. Length frequency distributions of periodic (a, b) and equilibrium target species collected from the gorge geomorphic zone of the LMR from 2015–2017.

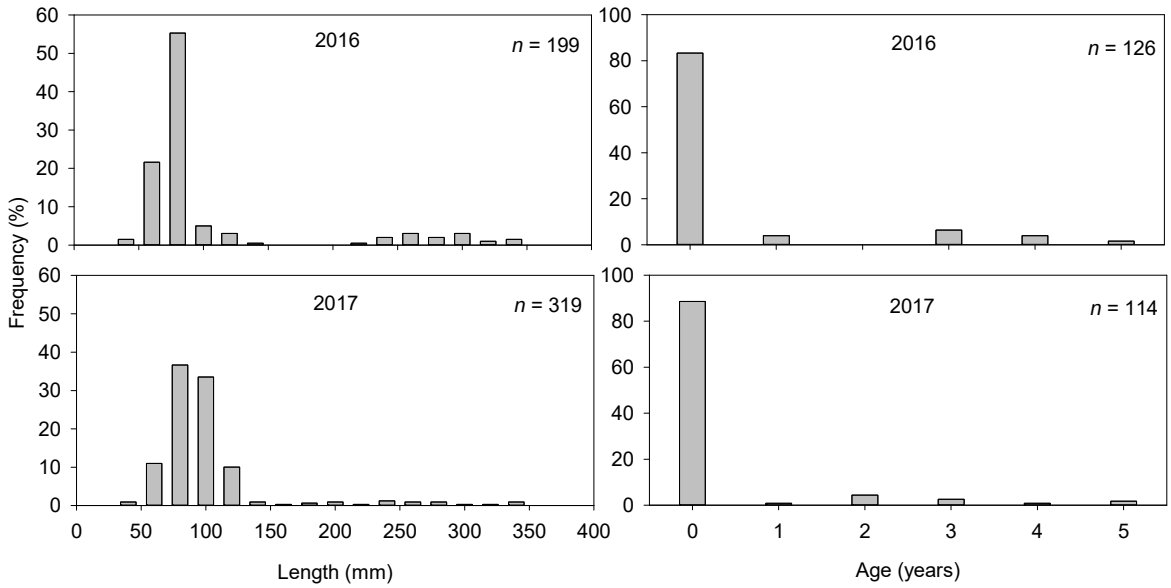


Figure D5. Fork length and age frequency distributions of bony herring collected from the gorge geomorphic zone of the LMR during 2016 and 2017.

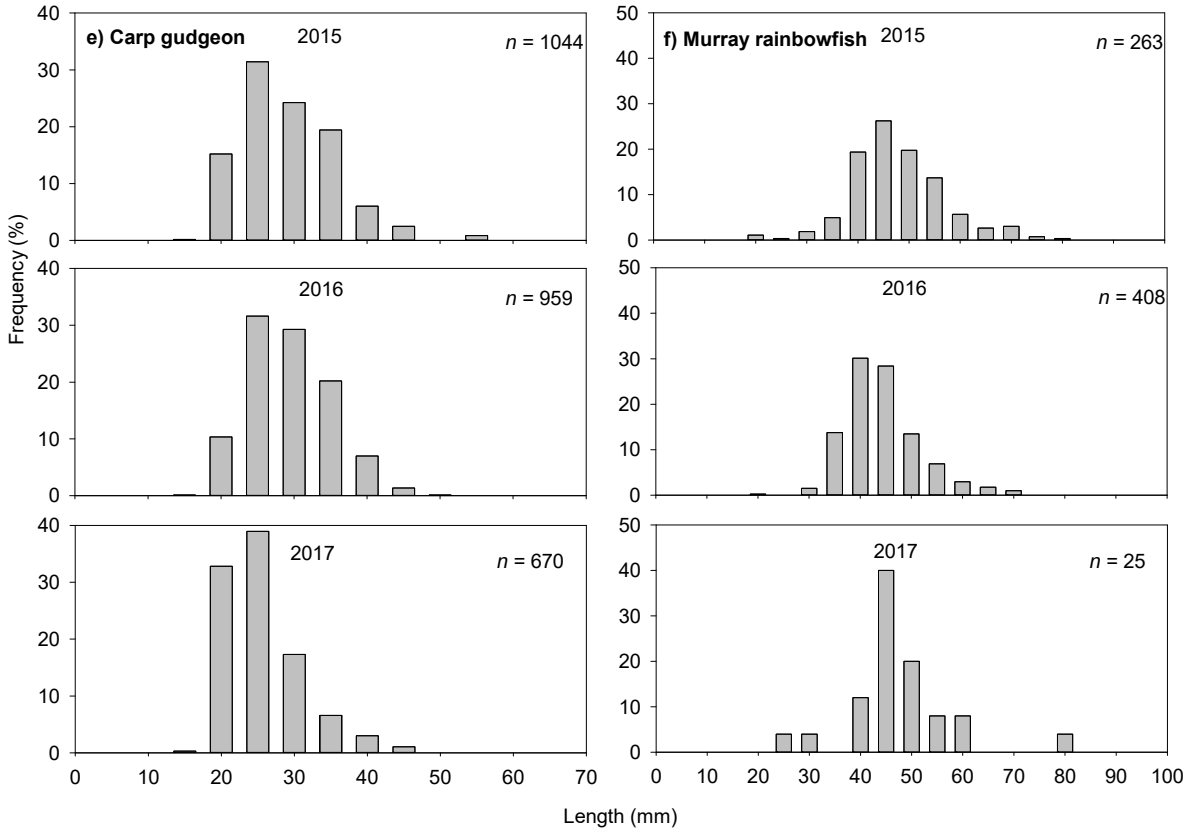


Figure D6. Length frequency distributions of opportunistic (e, f) target species collected from the gorge geomorphic zone of the LMR from 2015–2017.

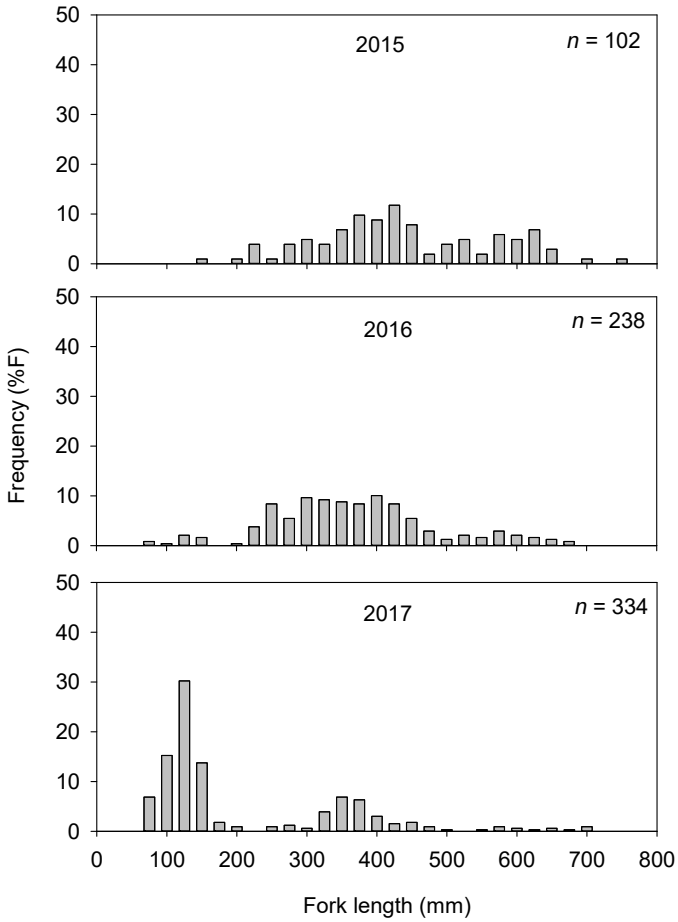


Figure D7. Length frequency distributions of common carp from the gorge geomorphic zone of the LMR from 2015–2017.

Discussion

Relatively low (<15,000 ML day^{-1}), stable flows predominated in the LMR during Year 1 (2014/15) and 2 (2015/16) of the CEWO LTIM project. Consequently, small-bodied fish abundance and diversity were high in 2015 and 2016, and there was no significant change in small-bodied fish assemblage structure from 2015 to 2016. Abundances of flow-cued species (i.e. golden perch and silver perch) remained similar in both years; however, there was a significant change in the large-bodied fish assemblage, driven primarily by a decrease in bony herring in 2016. Following overbank flows (>45,000 ML day^{-1}) in spring/summer 2016 (Year 3 of the CEWO LTIM project), there was a significant change to the small- and large-bodied fish assemblages with an overall decrease in the abundance of small-bodied species (e.g. carp gudgeon, gambusia

and Murray rainbowfish) and an increase in the abundance of common carp in 2017. Declines in the abundance of small-bodied fishes in the main channel habitats of the LMR was previously recorded in 2012, following overbank flows (Bice *et al.* 2014). 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 the decreased abundance of small-bodied fishes. Increased abundance of common carp in 2017 appeared to be driven by a large recruitment event in 2016/17 associated with overbank flows (Figure D7). Enhanced recruitment of common carp has been previously observed in the LMR (Bice *et al.* 2014) and elsewhere in the MDB (King *et al.* 2003; Stuart and Jones 2006), during floodplain inundation. Following a recession in water levels in summer 2017 (Figure 2), large numbers of age 0+ carp likely entered the main channel from off-channel floodplain and wetland habitats (their typical spawning habitat) and were captured during sampling in autumn and winter 2017.

Based on length frequency data from electrofishing, there was no recruitment (to age 0+) of golden perch and silver perch in 2014/15, 2015/16 or 2016/17 (Figure D4 in Appendix D). The lack of recruitment of golden perch and silver perch in association with the 2014/15 and 2015/16 flow regimes (i.e. low, stable flows) is consistent with our contemporary understanding of the life histories of these flow-cued spawners (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b) (also see Section 0 Category 3 Fish Spawning and Recruitment). In 2016/17, overbank flows should have been conducive to spawning of flow-cued spawners (Zampatti and Leigh 2013a); however, hypoxic (low dissolved oxygen) conditions during spring/early summer (Figure C1 in Appendix C) may have impeded the survival of eggs and larvae.

Based on length frequency data from electrofishing, there has been no recruitment (to age 0+) of freshwater catfish (*Tandanus tandanus*) from 2014/15–2016/17. Freshwater catfish spawn independent of flows (Davis 1977); however, their recruitment dynamics in the lower River Murray are poorly understood and their current spawning biomass in this region is historically low (Ye *et al.* 2015). For the third consecutive year, small Murray cod (<150 mm TL, likely age 0+) were sampled in the LMR during 2017, indicating successful recruitment. Furthermore, the cohorts from 2014/15 and 2015/16 seem to have persisted in 2016/17. In the main channel of the lower River Murray, Murray cod recruitment has been poor in association with periods

of low flow, particularly 2003–2010, and positively associated with years of elevated flow (in-channel and overbank) (Ye *et al.* 2000; Ye and Zampatti 2007; Zampatti *et al.* 2014). The mechanisms facilitating the recruitment of cohorts of Murray cod from 2014/15 and 2015/16, both low flow years, remain unclear.

Conclusion

In the main channel of the LMR, the 2014/15 and 2015/16 fish assemblages were characterised by high abundances of small-bodied species and a lack of recruitment of native, large-bodied flow-cued spawners. This fish assemblage structure was similar to that during drought in 2007–2010 (Bice *et al.* 2014) and characteristic of a low flow scenario. Following high flows in 2016/17, assemblages shifted towards one characterised by low abundances of small-bodied species and a large-bodied species, common carp. This assemblage was more typical of high flows, similar to the one in 2010–2012 (Bice *et al.* 2014). However, recruitment of native, large-bodied flow-cued spawners (e.g. golden perch) was negligible in 2016/17, despite a flow regime that was conducive to spawning of these species. Hypoxic conditions during the spring/early summer spawning season may have impeded the survival of their eggs and larvae.

APPENDIX E: HYDROLOGICAL REGIME

Model calibration

To represent the high flow conditions that occurred in 2016/17 accurately, modifications to the models used in previous LTIM reports (Ye *et al.* 2016a; 2017) were required.

For Weir Pools 1 and 2 the same 1-dimensional model used in Ye *et al.* (2017) was used, but the overbank roughness was modified to provide a better representation of the higher water levels that occurred. The Manning's roughness of n = 0.027 used previously was maintained in the channel, and for higher water levels that were overbank, a roughness of n = 0.032 and n = 0.035 was used for Weir Pool 1 and 2, respectively. The resulting water levels can be seen in Figure E1.

For Weir Pools 3–5, coupled 1 and 2-dimensional MIKE FLOOD models recently developed as part of the South Australian Riverland Floodplain Integrated Infrastructure Program (SARFIIP) were used. Details of these models and their calibration can be found in McCullough *et al.* (2017). The water levels simulated by these models for the model from Lock 3 to Lyrup can be seen in Figure E2. The performance of the models was deemed suitable for the purposes of evaluating the contributions of environmental water.

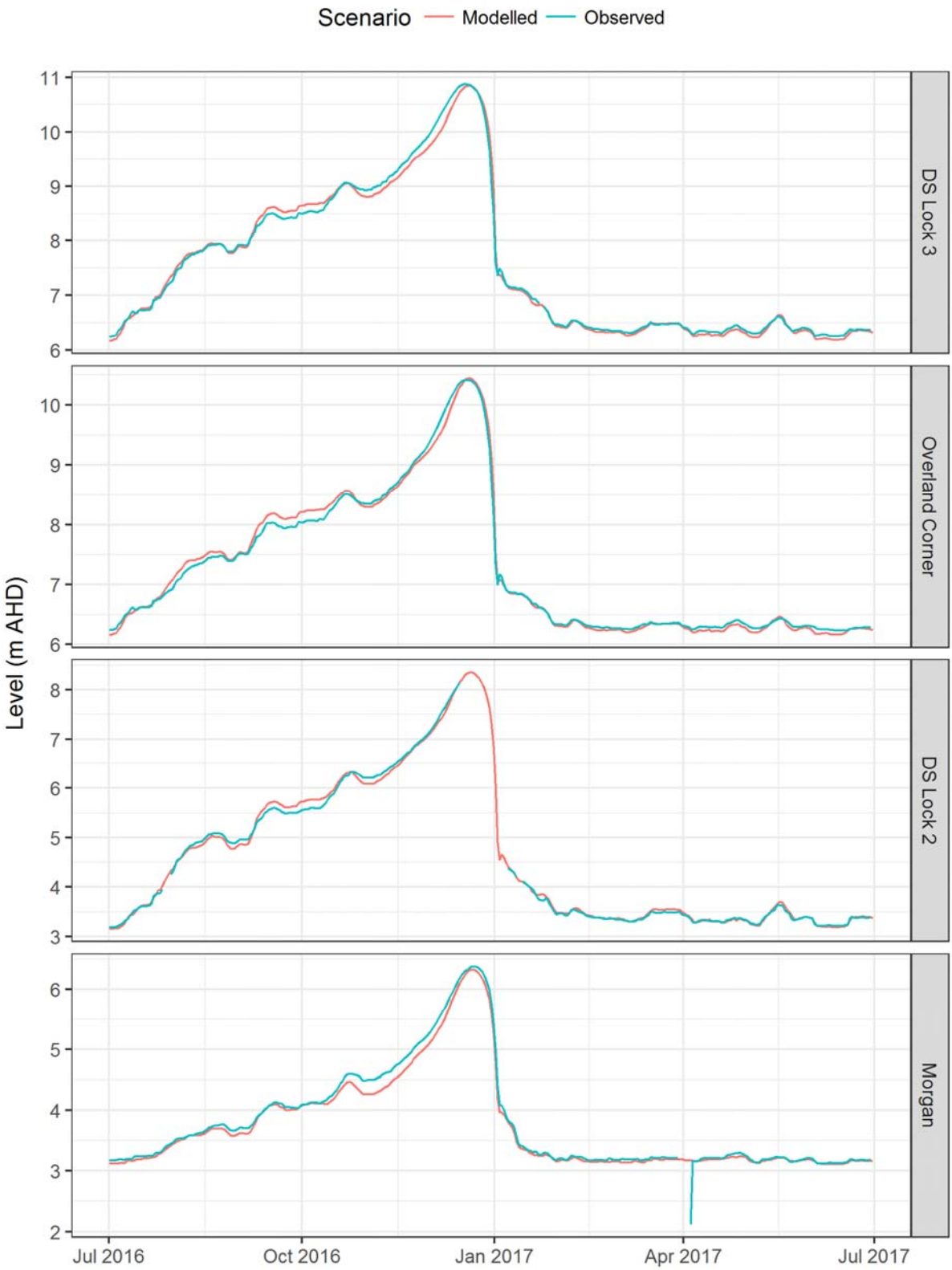


Figure E1. Water levels used for calibration of the model from Lock 1 to Lock 3.

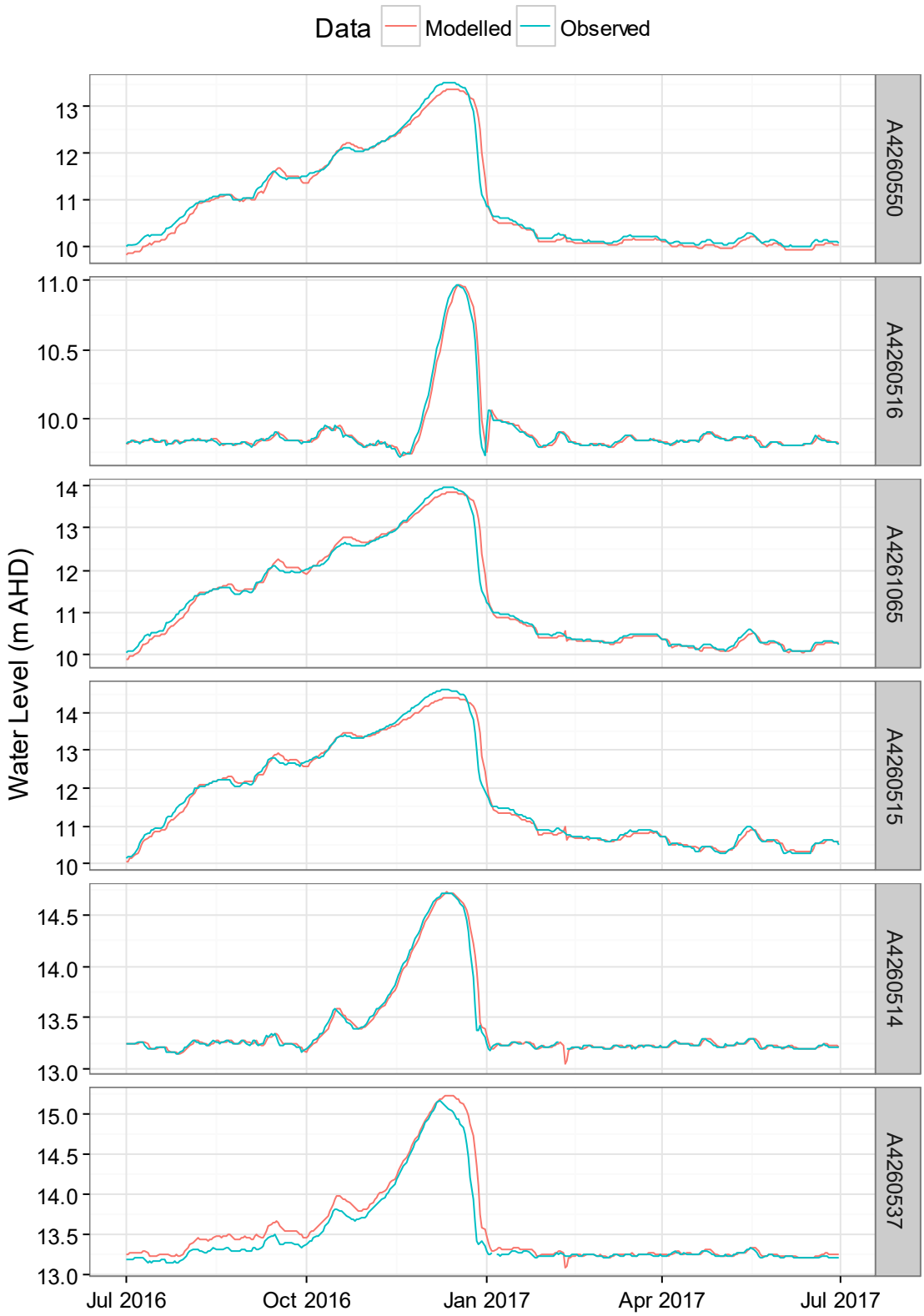


Figure E2. Water levels used for calibration of the model from Lock 3 to Lyrup.

Environmental water scenarios

After suitable model calibration was achieved, the models were used to simulate the without environmental water cases. Three scenarios have been considered:

- With all water. This is the observed conditions, as used for model calibration.
- Without Commonwealth environmental water. This allows the contribution of Commonwealth environmental water to the hydraulic variables to be quantified.
- Without any environmental water. This allows the collaborative outcomes across all environmental water holders to be quantified.

The flow time series for these scenarios were provided by the MDBA. The relevant environmental water contribution (without Commonwealth environmental water or without environmental water) was used as the upstream boundary for each model, with all other settings kept the same. The MDBA outputs were used to account for the changes in diversions within South Australia with and without environmental water.

The observed water levels were maintained as the downstream boundary in all model runs for two reasons:

- It was agreed by the LMR Selected Area Working Group that the weir pool raising events that occurred prior to high flows would have been undertaken even if environmental water was not available to underwrite the water use from these events.
- When the weirs were removed due to high flows, only small volumes of environmental water were delivered. As such water levels were expected to be similar at these times when the weirs were not controlling the water level.

Water level

Results for the three scenarios at the upper end of each weir pool can be seen in Figure E3. During flows when the weirs are controlling water levels (below 54,000 ML day^{-1} to 67,000 ML day^{-1} depending on the weir), the upper reaches of the weir pool are the most responsive to changes in flow, and therefore show the maximum change in water level due to the environmental water.

Unregulated flow conditions in the first half of the year resulted in little environmental water delivery beyond some small return flows, and as such little difference between the scenarios in Figure E3. Environmental water was delivered in late December 2016 and January 2017 to slow the recession in water levels after high flows (Figure 3). Figure E4 presents a version of Figure E3 focused on this period, highlighting the date and peak water level modelled to have occur in December, and the date and water level at the point where the rate in water level decline reduced noticeably. It can be seen that with the environmental water the water level decrease over this two to three-week period was between 2.5 m at Weir Pool 3, 4 and 5 to 3. 8 m at Weir Pool 1, and without the environmental water, the water level drop would have been an additional 0.7–0.9 m over the same period.

Following high flows the environmental water delivered in the first six months of 2017 can be seen in to increase water levels for the remainder of the year. The largest increases occurred in late March 2017 of between 0.2–0.4 m, as return flows from a pulse in the Goulburn River from the Commonwealth and Victorian Environmental Water Holders, as well as River Murray Improved Flows coincided (Figure 5).

Velocity

The results for the velocity in each weir pool can be seen in Figure E5. The median velocity in each weir pool on each day is presented as the solid lines, with the range represented by the 10th and 90th percentiles represented by the shaded area.

The velocities calculated for Weir Pool 1, 2 and 5 represent the average velocity across a river cross section at each computation point. As these points are not necessarily equally spaced along the river, a length weighted velocity was adopted to calculate the 10th, 50th (median) and 90th percentile velocities within the reach. This approach assumes a constant velocity between computation points, which may not be accurate; however, there is no better information available without adding further cross sections to these models.

For Weir Pools 3–5, the models are based on a flexible mesh of small elements (variable in size, but in the range of 10 s of metres), and as such there is much greater variability in the velocity results within the weir pool each day due to this finer resolution modelling.

It can be seen from Figure E5 that the provision of environmental water on the recession of the flood slowed the reduction in velocity during January 2017, as well as water levels. After this event, without environmental water, median velocities tended to be less than 0.1 m s^{-1} (representing lentic habitat) across the weir pools, with the exception of an unregulated flow event in May 2017. For the case with environmental water in the second half of the year, weir pool median velocity increased to a small degree (typically ~0.05-0.07 m s^{-1}), with some sections of the river greater than 0.17 m s^{-1}, particularly during the event in March 2017, when environmental water increased the flow to South Australia to 10,000 ML day^{-1}.

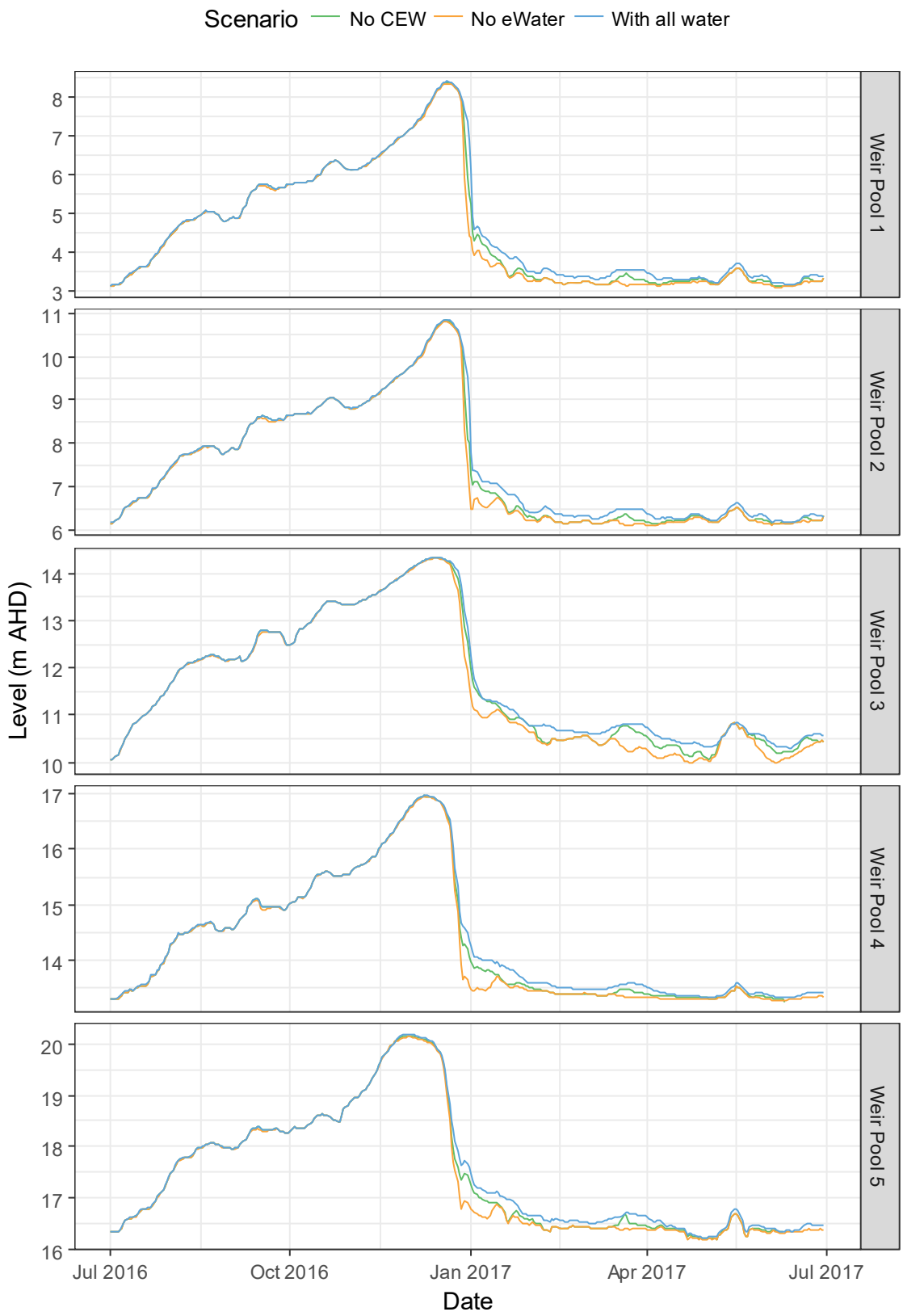


Figure E3. Modelled water level at the upstream end of each weir pool without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

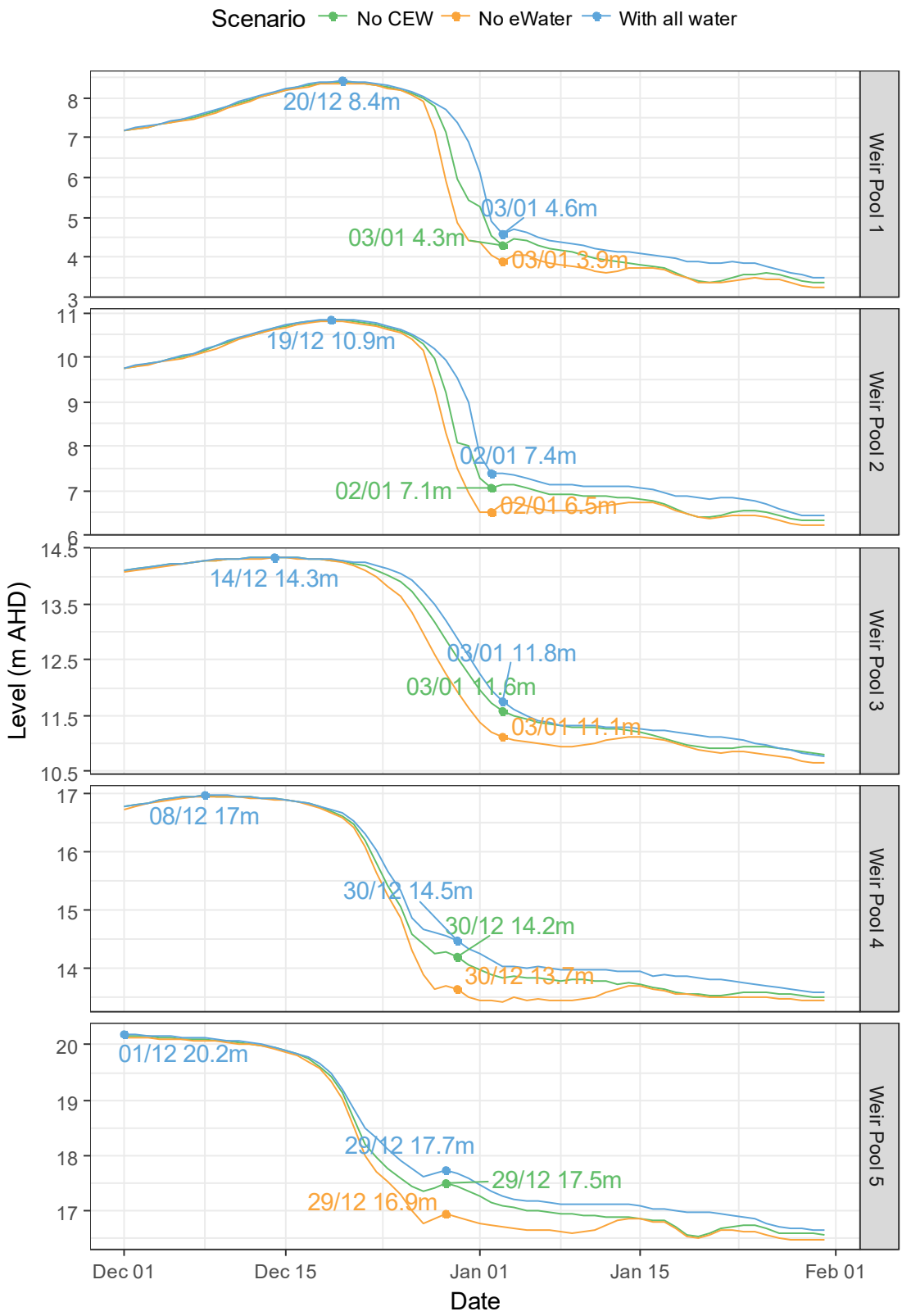


Figure E4. Modelled water level at the upstream end of each weir pool without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue) for December 2016 and January 2017 only, to highlight the differences during the flood recession.

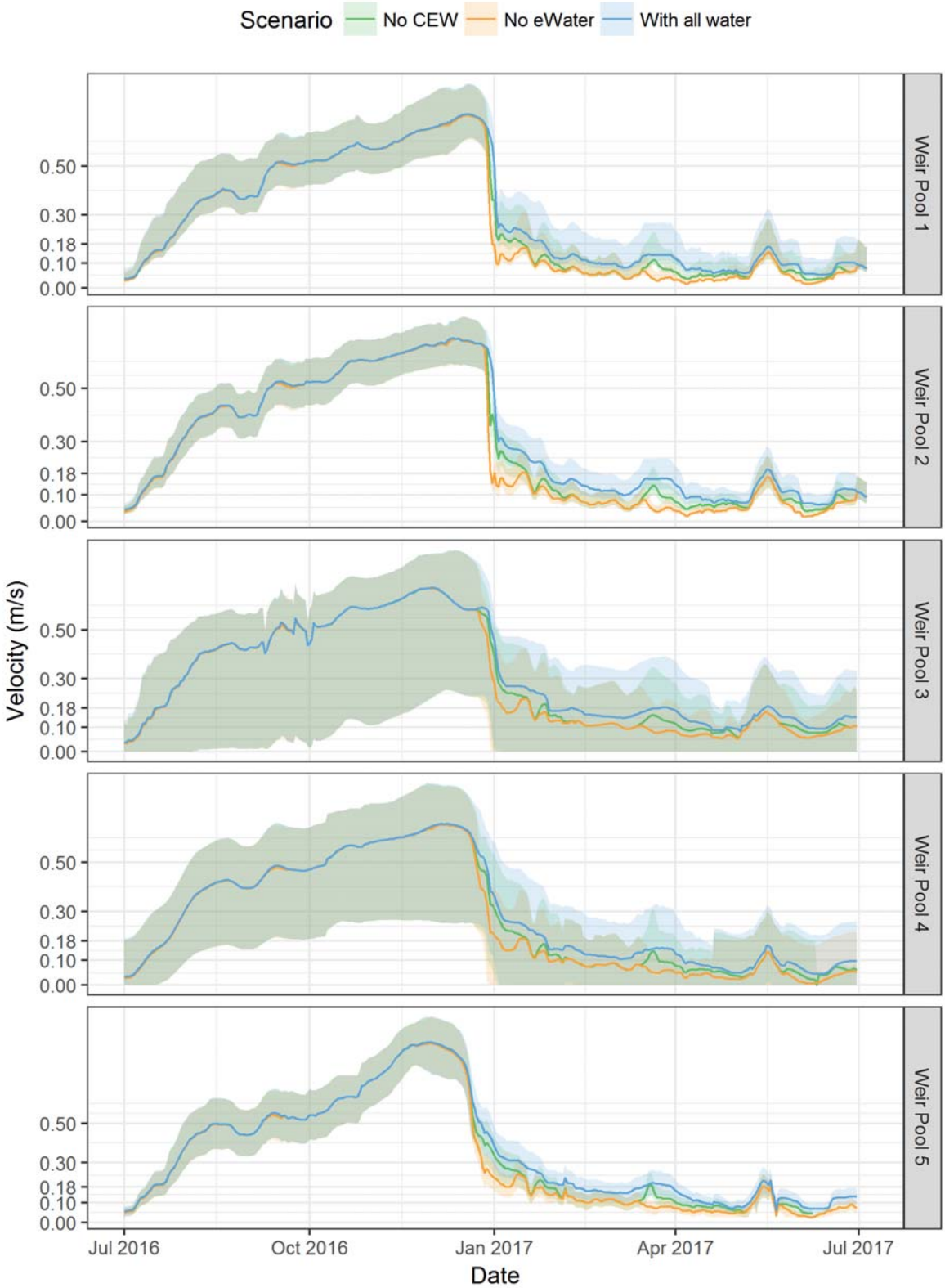


Figure E5. Median modelled velocity in each weir pool (line), with the 10th and 90th percentile the shaded band. Scenarios presented are without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue). Note that the larger range for Weir Pool 3 compared to Weir Pools 1 and 2 is due to a more detailed model used in this reach.

APPENDIX F: MATTER TRANSPORT

Background

Flow provides habitat and resources for aquatic organisms by altering the concentrations and transport of dissolved and particulate matter. Here we consider dissolved and particulate matter to include:

- Salinity, which is a measure of total dissolved salts and is a key parameter governing the distribution and abundance of aquatic biota. Salinity is strongly influenced by flow through the alteration of groundwater inputs, evapoconcentration and intrusions of seawater (Brookes *et al.* 2009; Aldridge *et al.* 2011; 2012; Mosley *et al.* 2012).
- Dissolved inorganic nutrients, which are essential resources for the growth and survival of biota and are readily assimilated (Poff *et al.* 1997). Nitrogen, phosphorus and silica are particularly important because they often control the productivity of aquatic ecosystems. Flow results in the mobilisation and transport of dissolved nutrients through the leaching of nutrients from dried sediments and dead organic matter.
- Particulate organic nutrients (phosphorus and nitrogen), which are those nutrients incorporated into the tissue of living and dead organisms. Flow can influence particulate organic nutrient concentrations and transport through a number of mechanisms, including through increased productivity associated with elevated dissolved nutrient concentrations.
- Chlorophyll *a*, which is a measure of phytoplankton biomass, with phytoplankton being an important primary producer of riverine ecosystems. Flow can influence chlorophyll *a* concentrations and transport through increased phytoplankton productivity.

Altering the flow regime of riverine systems can alter the concentrations and transport of dissolved and particulate matter (Aldridge *et al.* 2012). For example, reduced flow can result in salinisation through evapoconcentration and the intrusion of saline water; reduced nutrient concentrations due to decreased mobilisation of nutrients from the floodplain; reduced primary productivity because of nutrient limitation; and thus

reduced secondary productivity. Such observations have been made in the Murray River, including the LMR, Lower Lakes and Coorong (Brookes *et al.* 2009; Aldridge *et al.* 2011; 2012; Mosley *et al.* 2012).

Environmental flow deliveries may be used to reinstate some of the natural processes that control the concentrations and transport of dissolved and particulate matter (Aldridge *et al.* 2012; 2013; Ye *et al*. 2015a; 2015b; 2016a). In doing so, these flows may provide ecological benefits through the provision of habitat and resources for biota. To assess the contribution of environmental water use to matter transport in 2016/17, a hydrodynamic-biogeochemical model was applied for the region below Lock 1 to the Murray Mouth. The model was validated with water quality data.

Water quality sampling and analyses

Water quality was monitored for the Murray River Channel (at Wellington), Lower Lakes and Coorong between July 2015 and June 2016, and for the Murray River Channel (at Morgan) between July 2016 and June 2017 (Table F1). At each sampling site, measurements of water temperature, electrical conductivity, dissolved oxygen, pH and turbidity were taken. In addition, integrated-depth water samples were collected and sent to the Australian Water Quality Centre, an accredited laboratory of the National Association of Testing Authorities. Samples were analysed for filterable reactive phosphorus (hereafter referred to as phosphate), total phosphorus, nitrate, ammonium, total Kjeldahl nitrogen, dissolved silica and chlorophyll *a* using standard techniques. Organic nitrogen was calculated as the difference between total Kjeldahl nitrogen and ammonium.

Table F1. Sampling sites within each water-body

Water-body	Sampling site	Sampling frequency	Data source
Murray River Channel	Morgan	Approximately weekly between 01/07/2016 and 30/06/17	SA Water
	Wellington	Approximately four times between 01/07/2015 and 30/06/16	Murray Futures (DEWNR)
Lower Lakes	Lake Alexandrina Opening		
	Poltalloch		
	Milang		
	Lake Alexandrina Middle		
	Point McLeay		
	Finniss River		
	Currency Creek		
	Goolwa Barrage		
	Lake Albert Opening		
	Lake Albert Middle		
	Meningie		
Coorong	Monument Road		
	Murray Mouth		
	Ewe Island		
	Tauwitchere		
	Mark Point		
	Long Point		
	Parnka Point		
	Villa de Yumpa		
	Jack Point (north)		
	Salt Creek (south)		

Hydrodynamic–biogeochemical modelling

To assess the effects of the environmental water delivery on salt and nutrient transport between Lock 1 and the Southern Ocean, a hydrodynamic-biogeochemical model was set-up and applied. The model platform used was the coupled hydrodynamic-biogeochemical model TUFLOW-FV-AED, developed by BMTWBM and the University of Western Australia. TUFLOW-FV is now used extensively in the region for hydrological purposes, and was used to assess the contribution of environmental water to dissolved and particulate matter during 2013/14, 2014/15 and 2015/16 (Ye *et al*. 2016a; 2016b;

2017). A single model domain was applied spanning Lock 1 to the Southern Ocean, including the Coorong (Table F1). The TUFLOW-FV model (BMTWBM) adopts an unstructured-grid model that simulates velocity, temperature and salinity dynamics in response to meteorological and inflow dynamics. In this application, AED was configured to simulate the dynamics of light, oxygen, nutrients, organic matter, turbidity and phytoplankton.

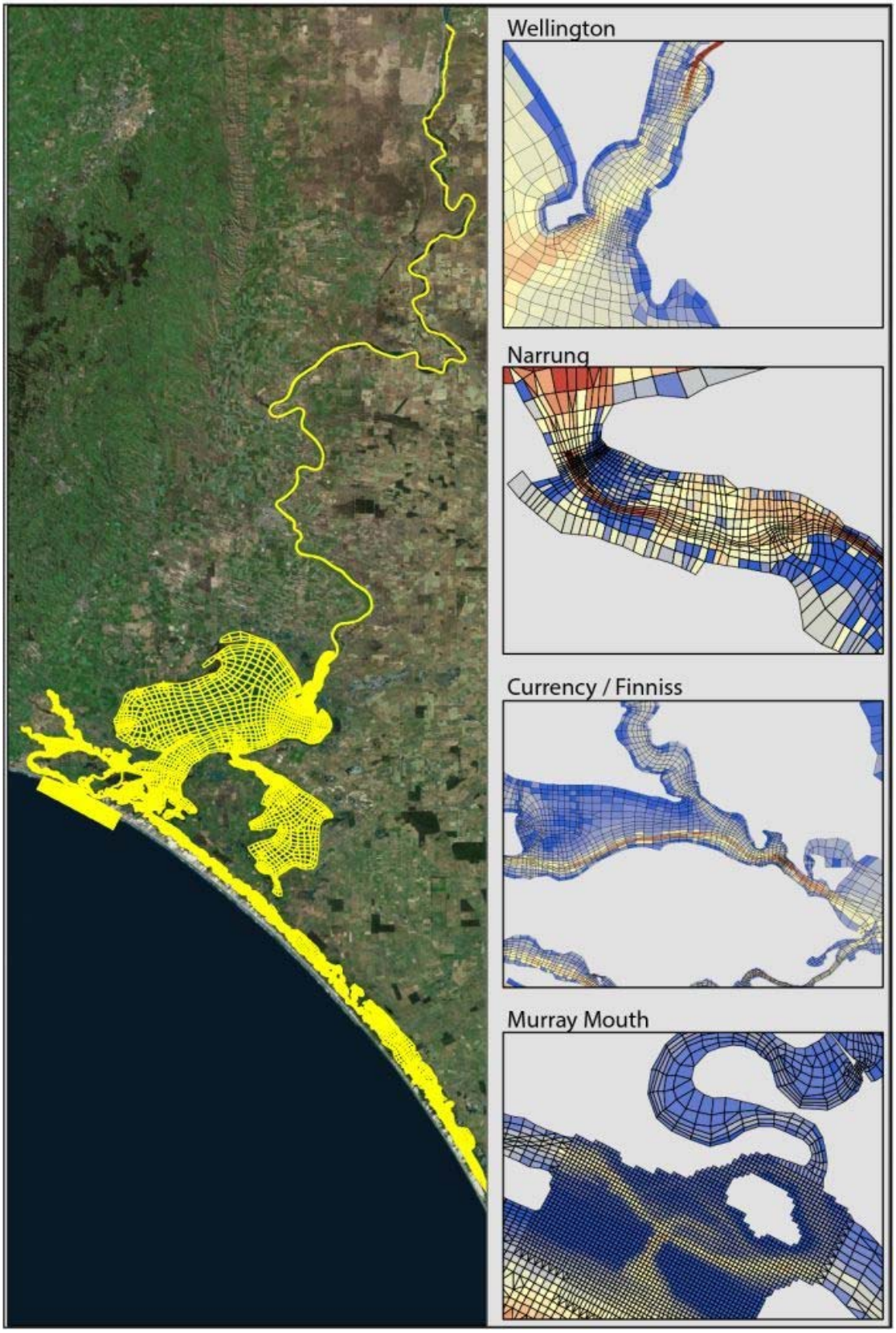


Figure F1. Overview of model domain applied in this study using TUFLOW-FV. Grid provided courtesy of Department of Environment, Water and Natural Resources. Coloured grids in maps on the right-hand side represent depths, i.e. increasing depth from cool (blue) to warm (red).

The model runs were initialised with data from a range of data sources. Inflow data (Lock 1), used to drive the main river domain, were provided by the Murray–Darling Basin Authority for three scenarios (Figure F2):

- 'with all water' (i.e. observed, including all environmental and consumptive water);
- without Commonwealth environmental water ('no CEW'); and
- without any environmental water ('no eWater').

These simulations were run for the period between July 2016 and June 2017.

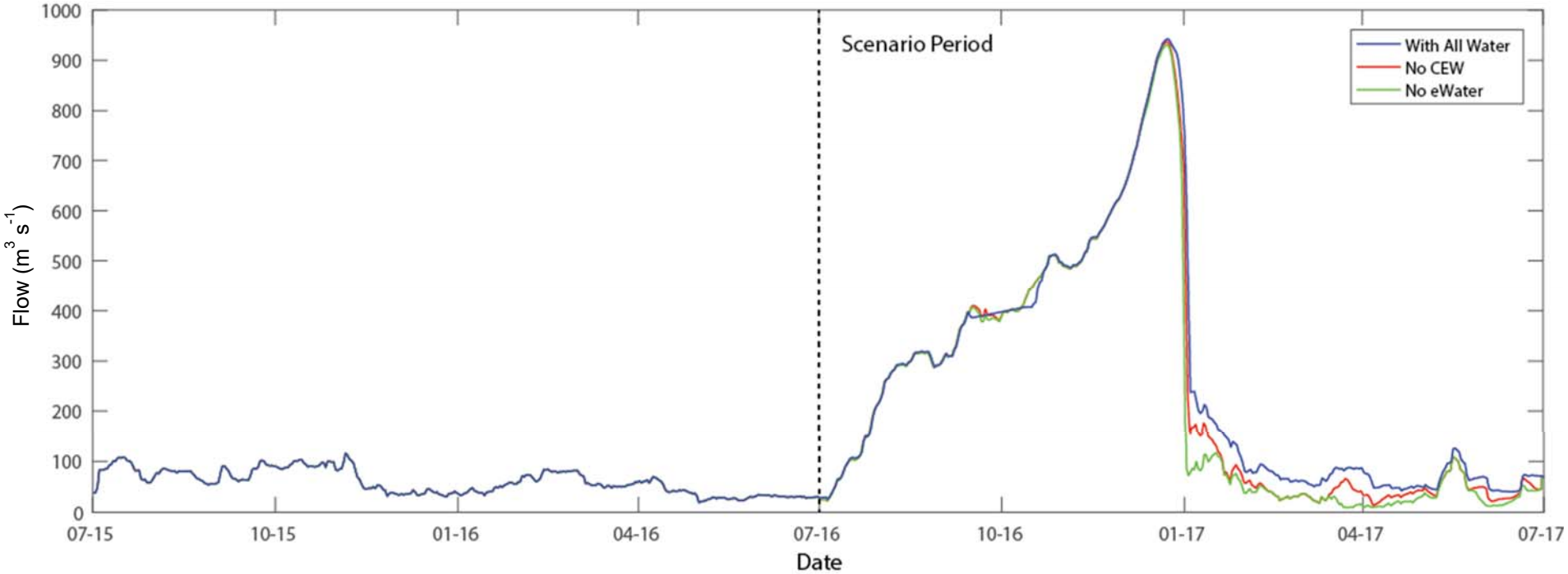


Figure F2. Overview of the three flow scenarios assessed by the model simulations. Scenarios include flow with all water, flow without Commonwealth environmental water (no CEW) and flow without any environmental water (no eWater). Flows were applied to the model at the upstream boundary which is at Lock 1.

Additional flow specifications for SA Water off-takes were also included. Irrigation return flows were assumed to be negligible over this period and were not included in the model. Similarly, flows from Eastern Mount Lofty Ranges were not included since their contribution to the Lower Lakes is considered to be relatively minor (Cook *et al.* 2010). Meteorological conditions were based on data from Narrung. Between Lake Alexandrina and the Coorong four barrages were included (Goolwa, Mundoo, Ewe Island and Tauwitchere) and set with a spill-over height of 0.72 m AHD. The barrage operation was set to include gate operation based on operational information provided through discussions with representatives of Department of Environment, Water and Natural Resources. At the bottom of the domain, two open boundaries were specified, one at the Murray Mouth and one at Salt Creek. Murray Mouth water level was based on Victor Harbor tidal data, which is available at 10 min resolution. Salt Creek flow data was set based on available flow data from the WaterConnect website (DEWNR).

Water quality conditions for both boundary points were set based on a linear interpolation of the measured nutrient and salinity data collected as part of this study. Water quality conditions for the river inflow at Lock 1 were determined based on interpolation of available data from Lock 1 or Morgan. For water quality properties for the without environmental water scenarios, rating curves were developed for flow and concentration. Based on the daily flow difference, a scaled concentration was estimated for water quality parameters including salinity, phosphate, ammonium, nitrate, total nitrogen and silica. The physico-chemical information at other sites was used to validate the model.

The influence of environmental water on the concentrations of matter was assessed through a comparison of modelled concentrations for the various scenarios for the Murray River Channel (Wellington), Lower Lakes (Lake Alexandrina Middle) and Coorong (Murray Mouth). Modelled concentrations are presented as medians of modelled cells within areas surrounding sampling sites (Figure F3). A range in concentrations within those cells is also presented for the 'with all water' scenario.

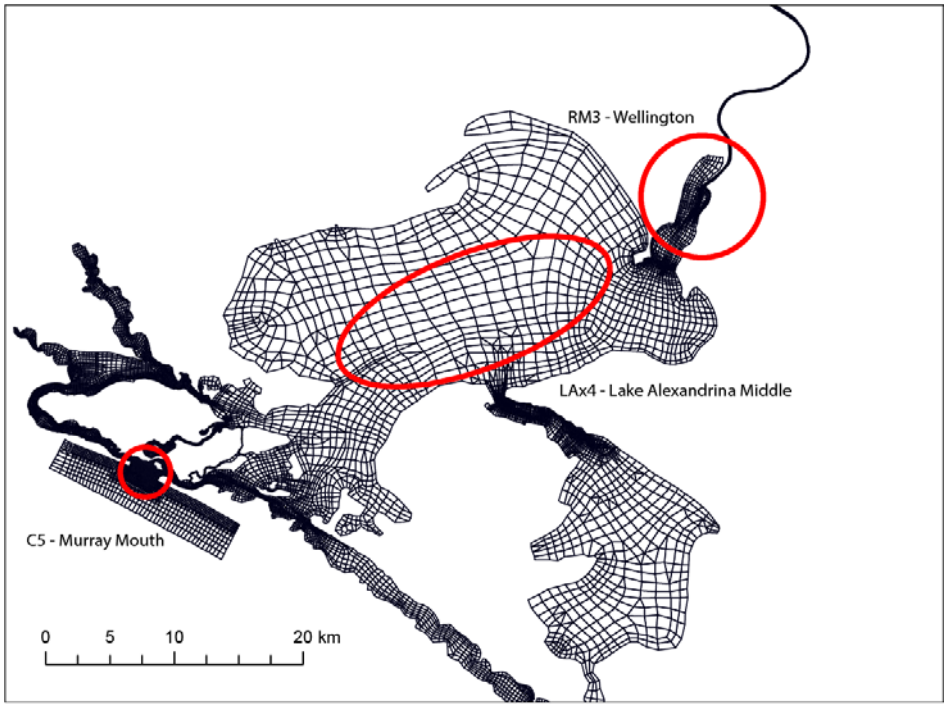


Figure F3. Modelled cells (circled) used for calculating the modelled concentration of nutrients or salt at the Wellington, Lake Alexandrina Middle and Murray Mouth sites.

The transport of matter was assessed through modelled exports from the Murray River Channel (Wellington), Lower Lakes (Barrages) and Coorong (Murray Mouth). Findings are presented for salinity, ammonium, phosphate, dissolved silica, organic nitrogen, organic phosphorus and chlorophyll *a*. Salinity is presented as practical salinity units (PSU), a measurement of the measured conductivity to standard potassium chloride (KCl) conductivity. PSU was used for validating model outputs as it overcomes observed differences in electrical conductivity caused by changes in water temperature. One PSU is approximately equal to part per thousand.

The inflow data that were used to drive the main river domain are treated as indicative only as they do not account for all complexities associated with water accounting, water attenuation through the system and different management decisions that may have been made if the volume of environmental water provided had not been available (Neville Garland, MDBA, pers. comm.). Assumptions made to address these complexities result in uncertainty in the model outputs and so outputs are not to be treated as absolute values (refer to Aldridge *et al.* 2013 for more detail). When assessing the relative differences between scenarios, the uncertainties are considered to influence the accuracy of each scenario equally and so the model outputs are used to assess the general response to environmental water delivery.

Results

The findings are discussed in Section 0 Matter Transport. This appendix includes a detailed presentation of data on Matter Transport including field collected data used for model validation.

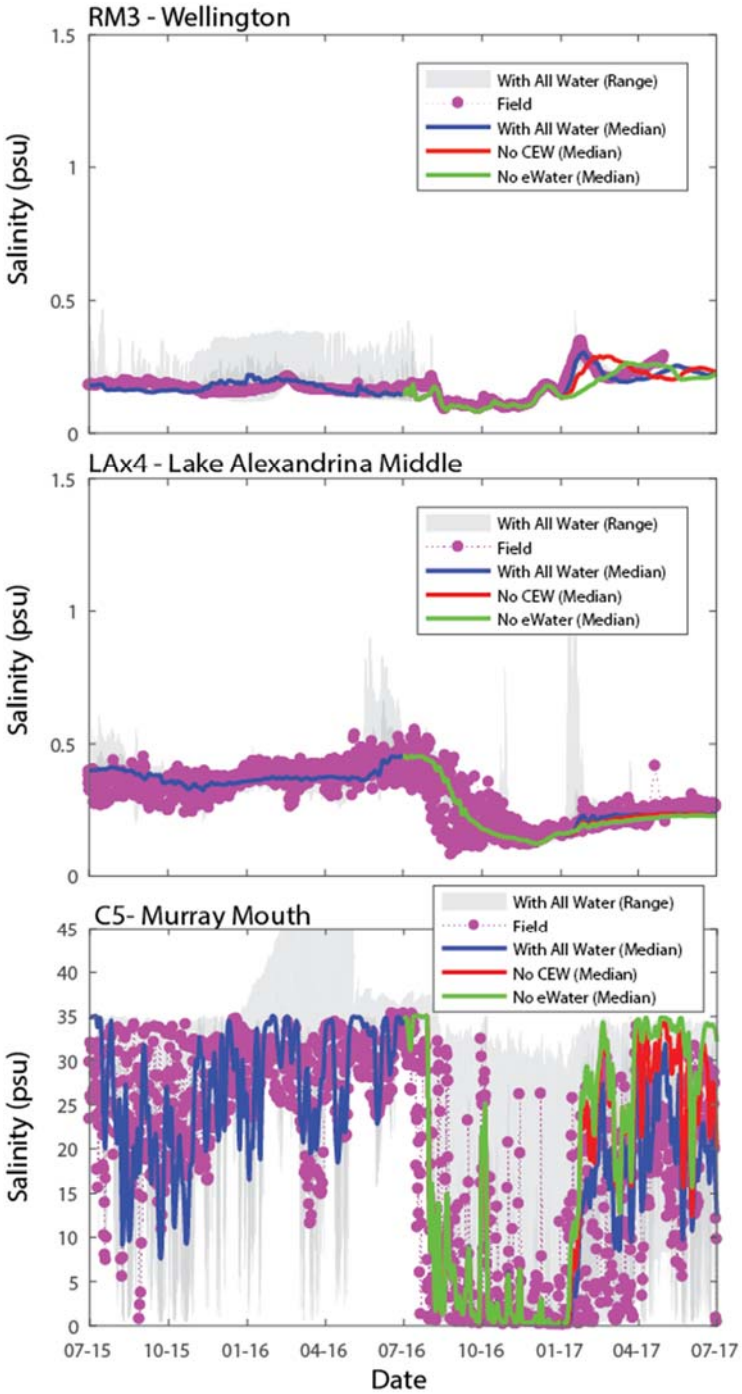


Figure F4. Observed and modelled practical salinity units (PSU) at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). Median values represent that of selected modelled cells surrounding sampling sites. Field measurements are depicted as solid circles.

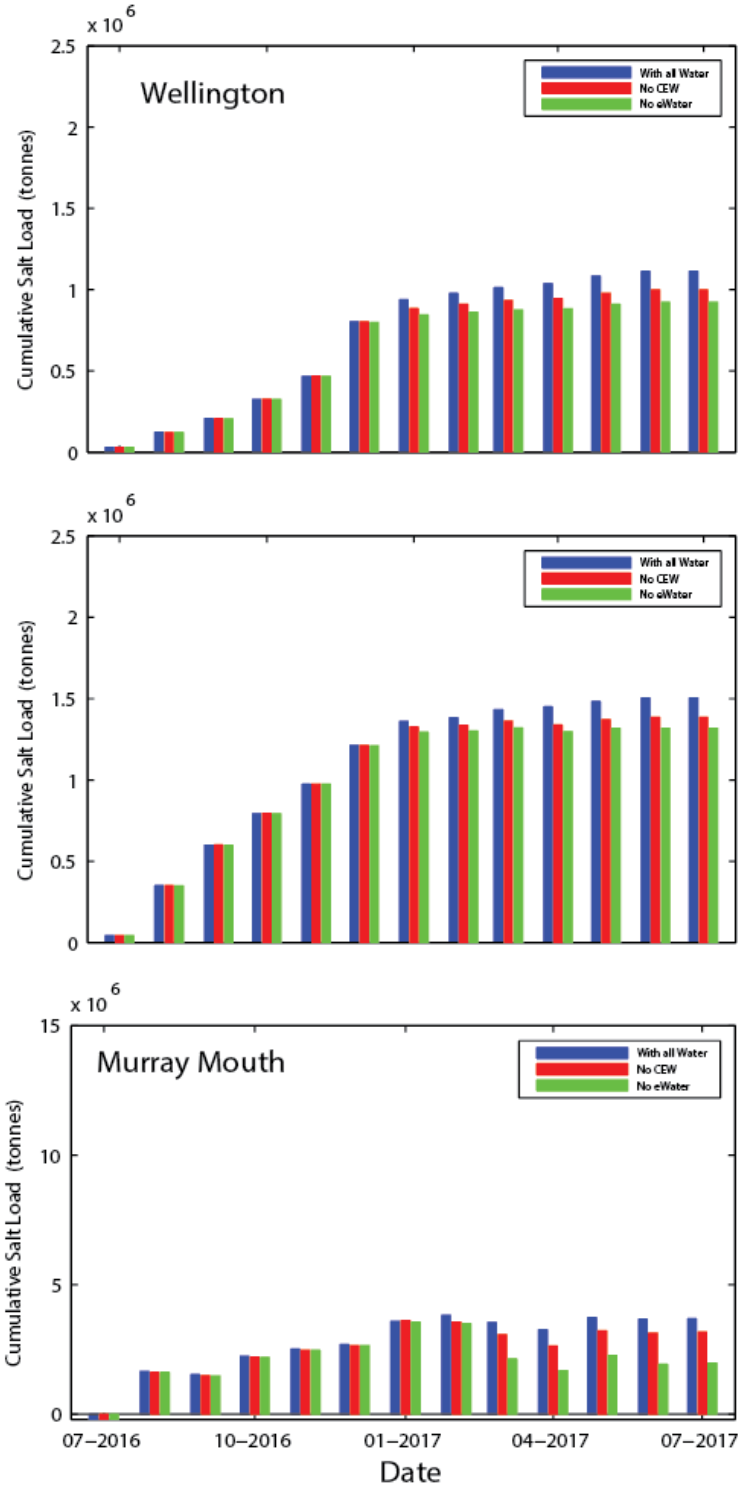


Figure F5. Modelled cumulative salt exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

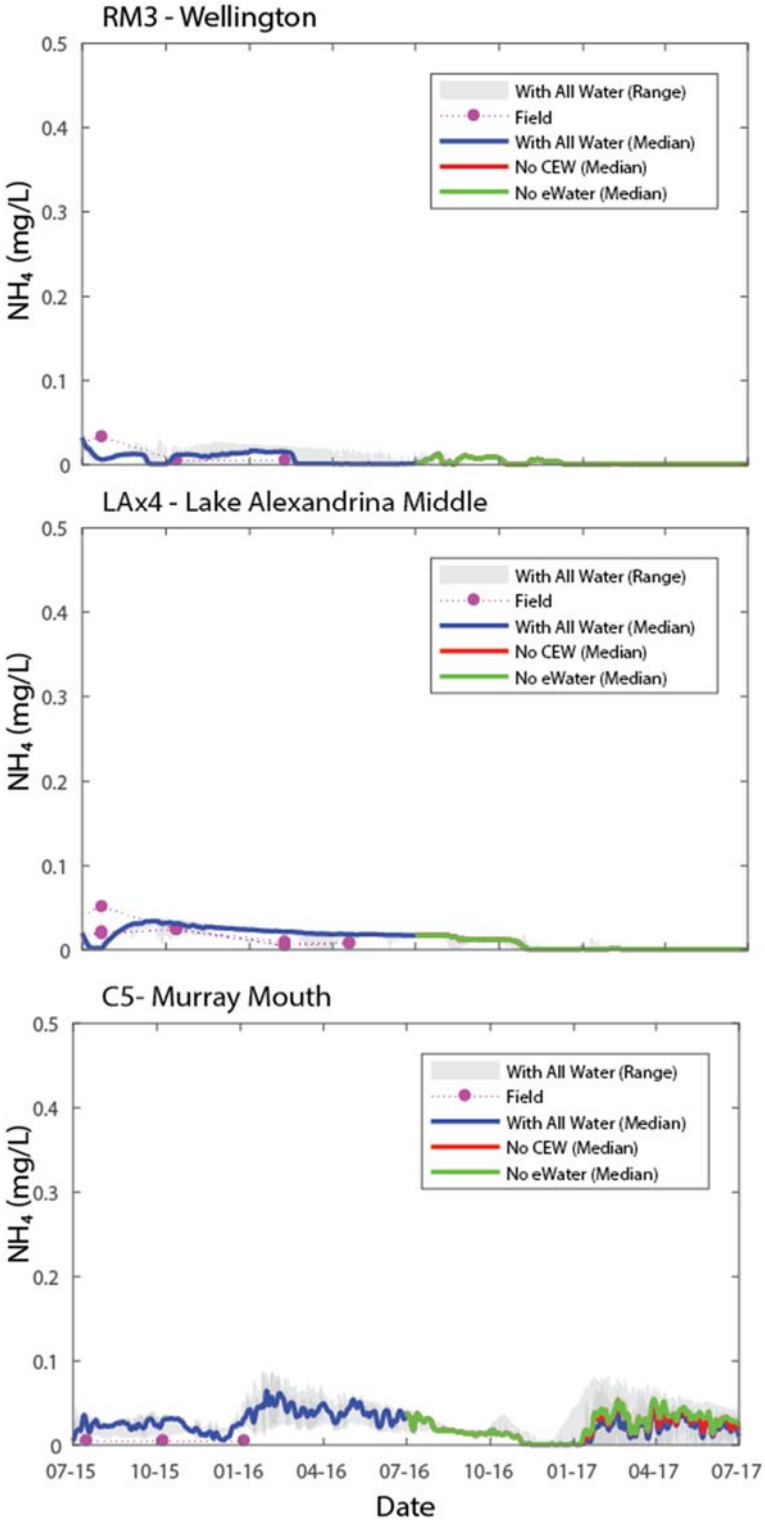


Figure F6. Observed and modelled ammonium (NH4) concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). The data presented are the median of selected modelled cells surrounding the sampling sites.

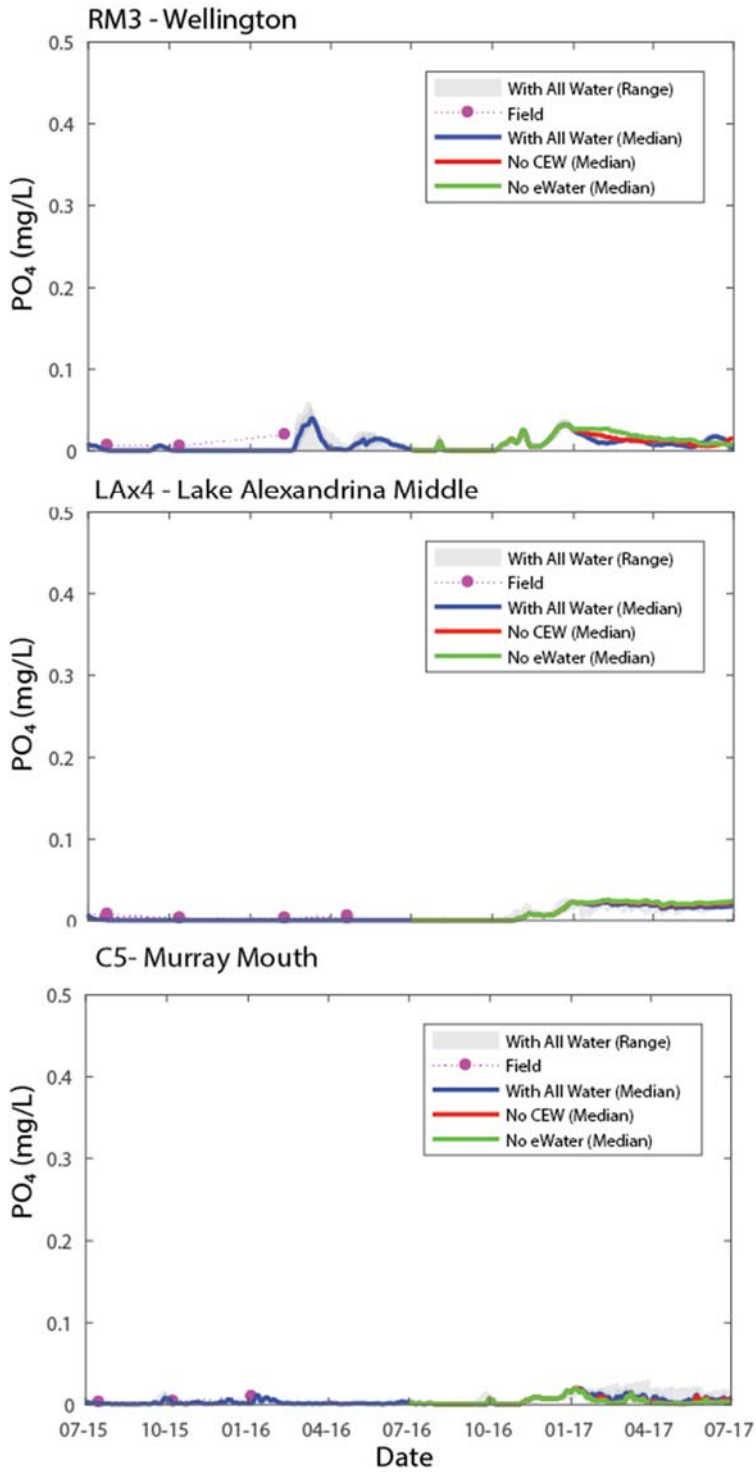


Figure F7. Observed and modelled phosphate (PO4) concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). The data presented are the median of selected modelled cells surrounding the sampling sites.

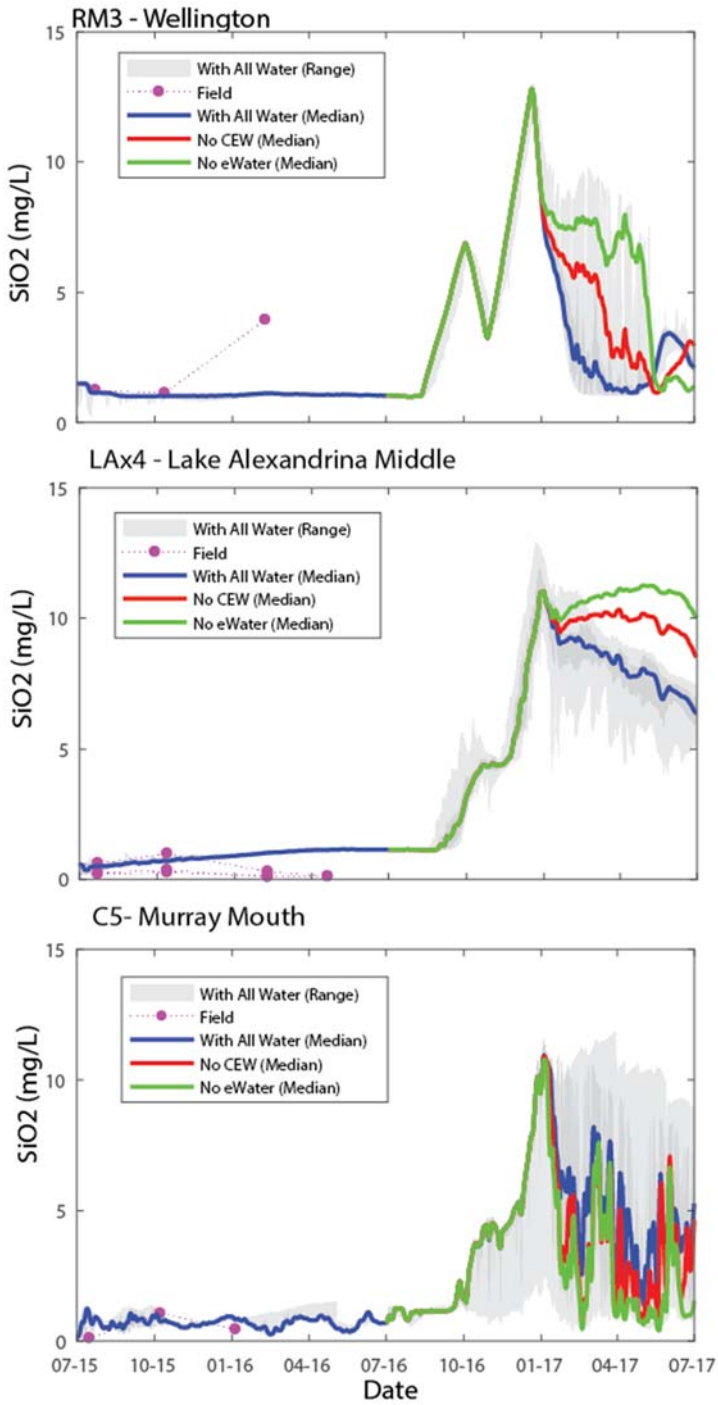


Figure F8. Observed and modelled silica concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). The data presented are the median of selected modelled cells surrounding the sampling sites.

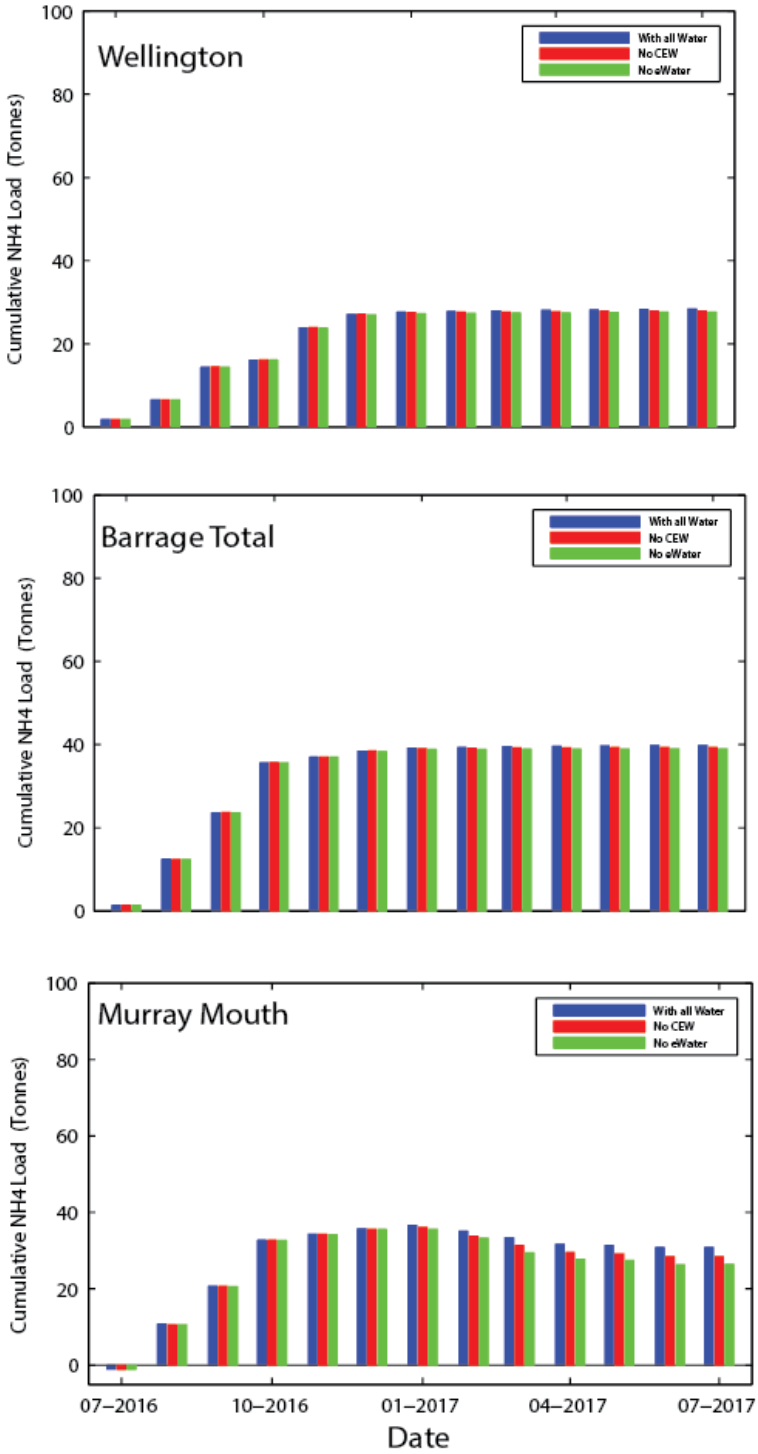


Figure F9. Modelled ammonium (NH4) loads at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

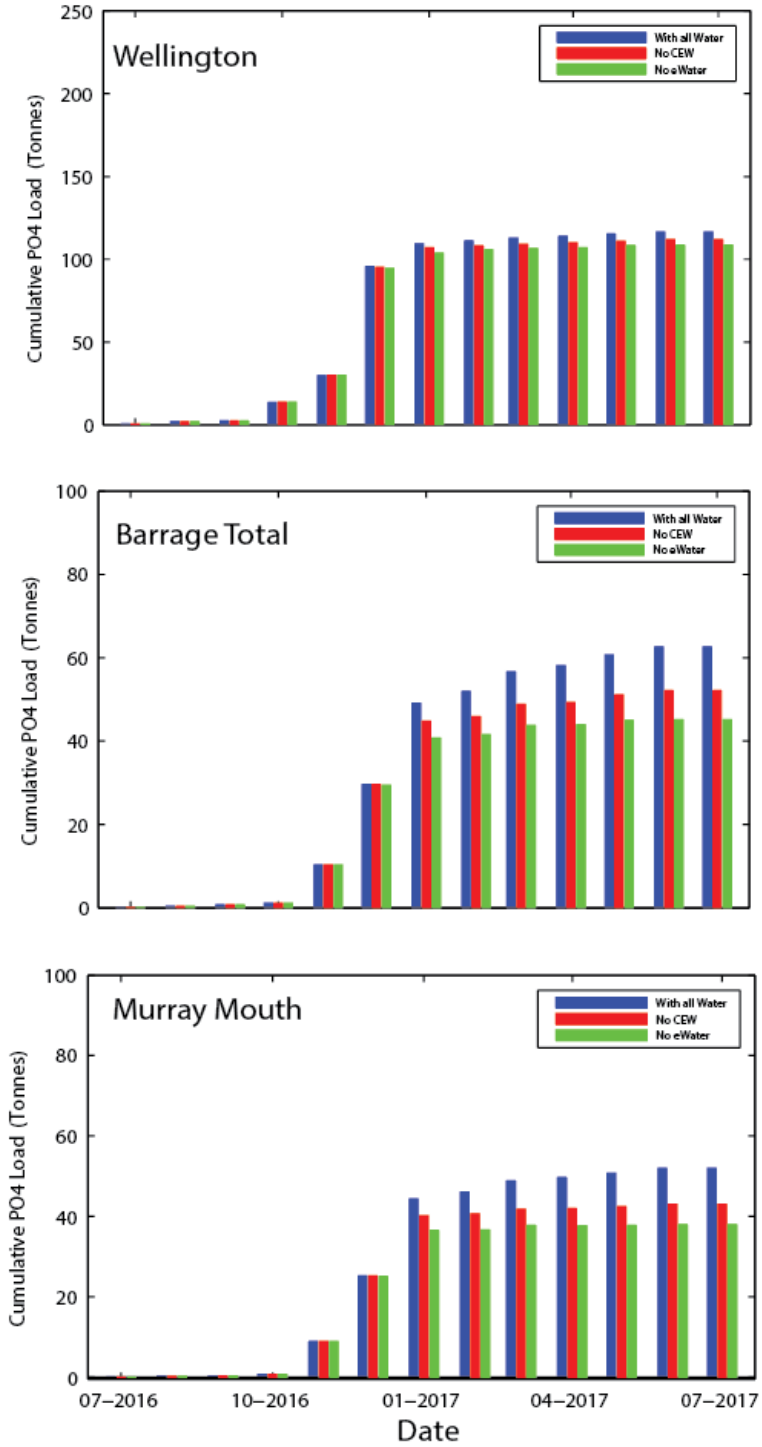


Figure F10. Modelled phosphate (PO4) loads at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

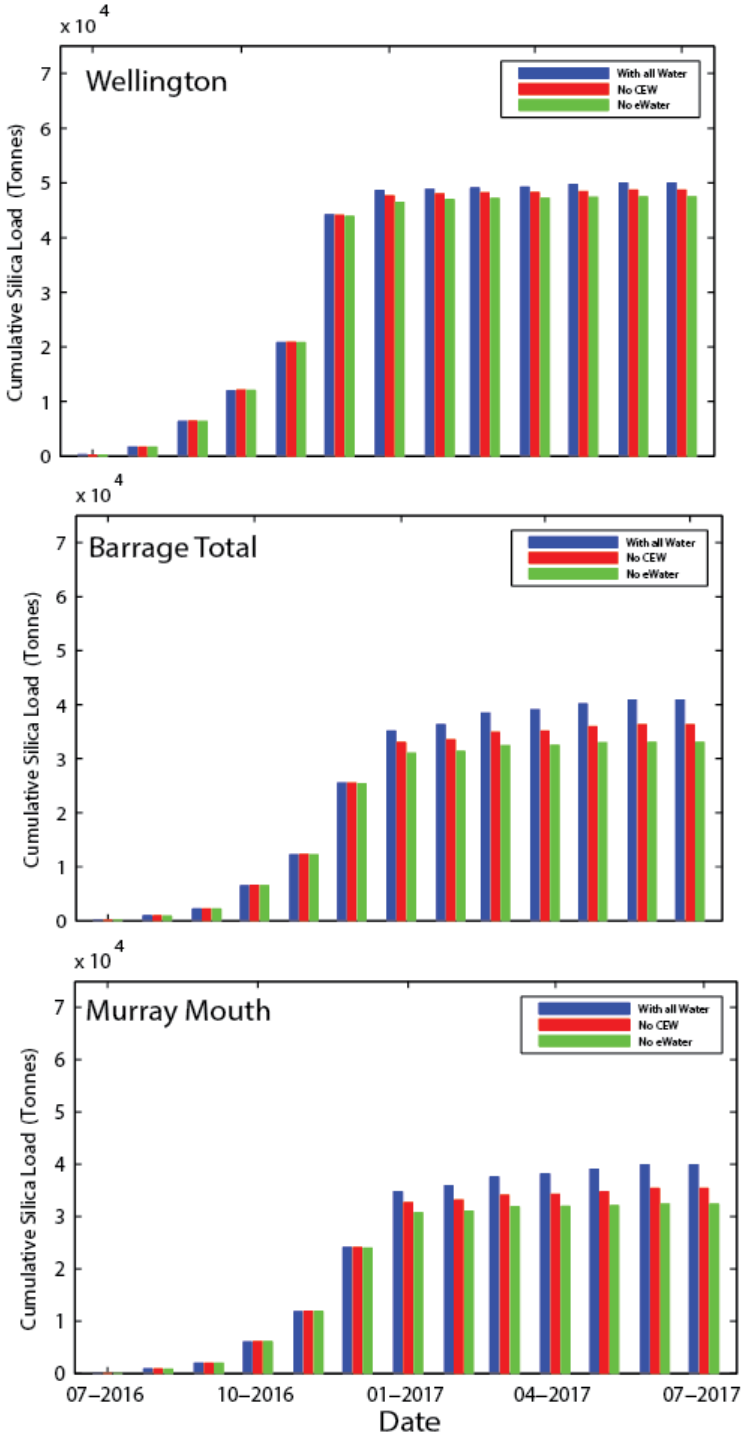


Figure F11. Modelled silica loads at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

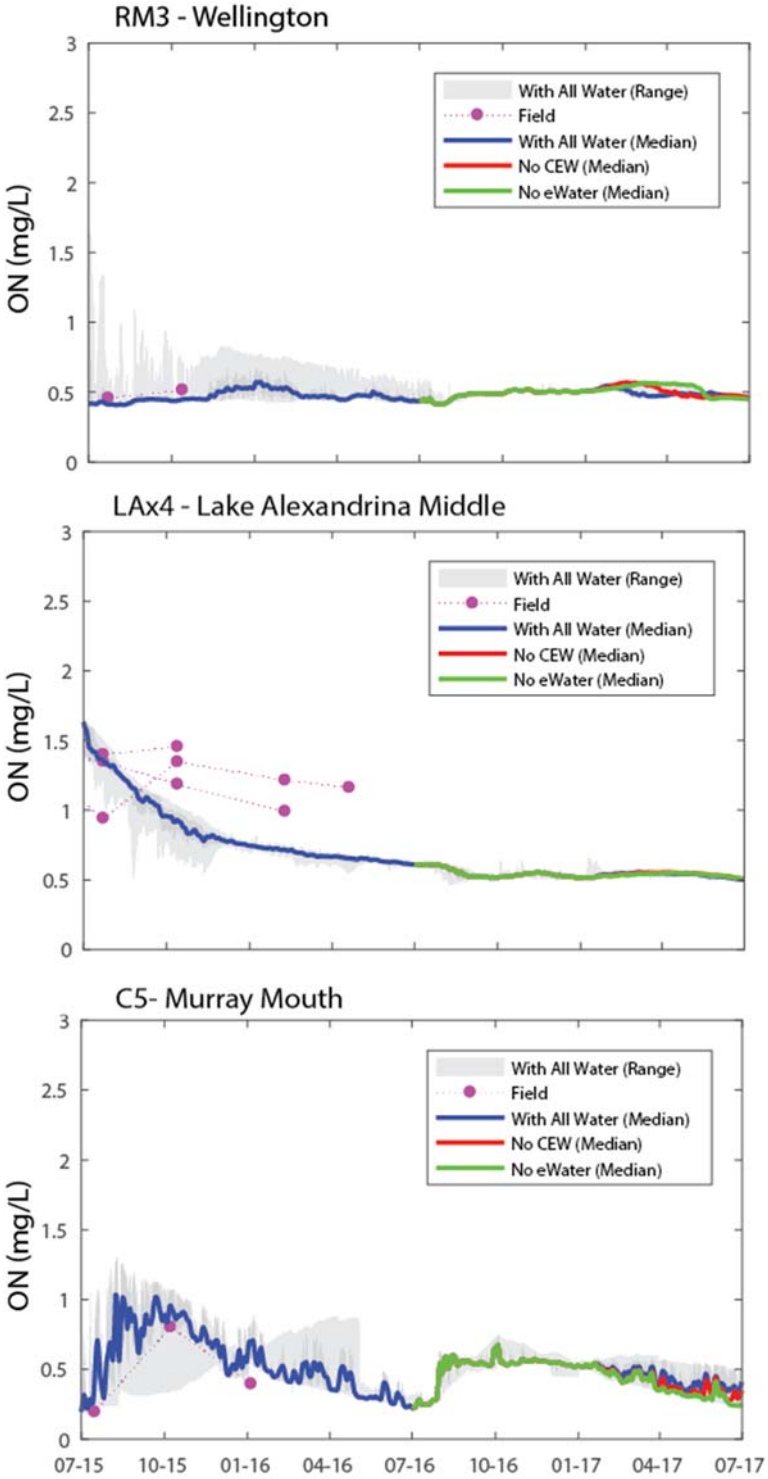


Figure F12. Observed and modelled particulate organic nitrogen (ON) concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). The data presented are the median of selected modelled cells surrounding the sampling sites. Measured concentrations are solid circles.

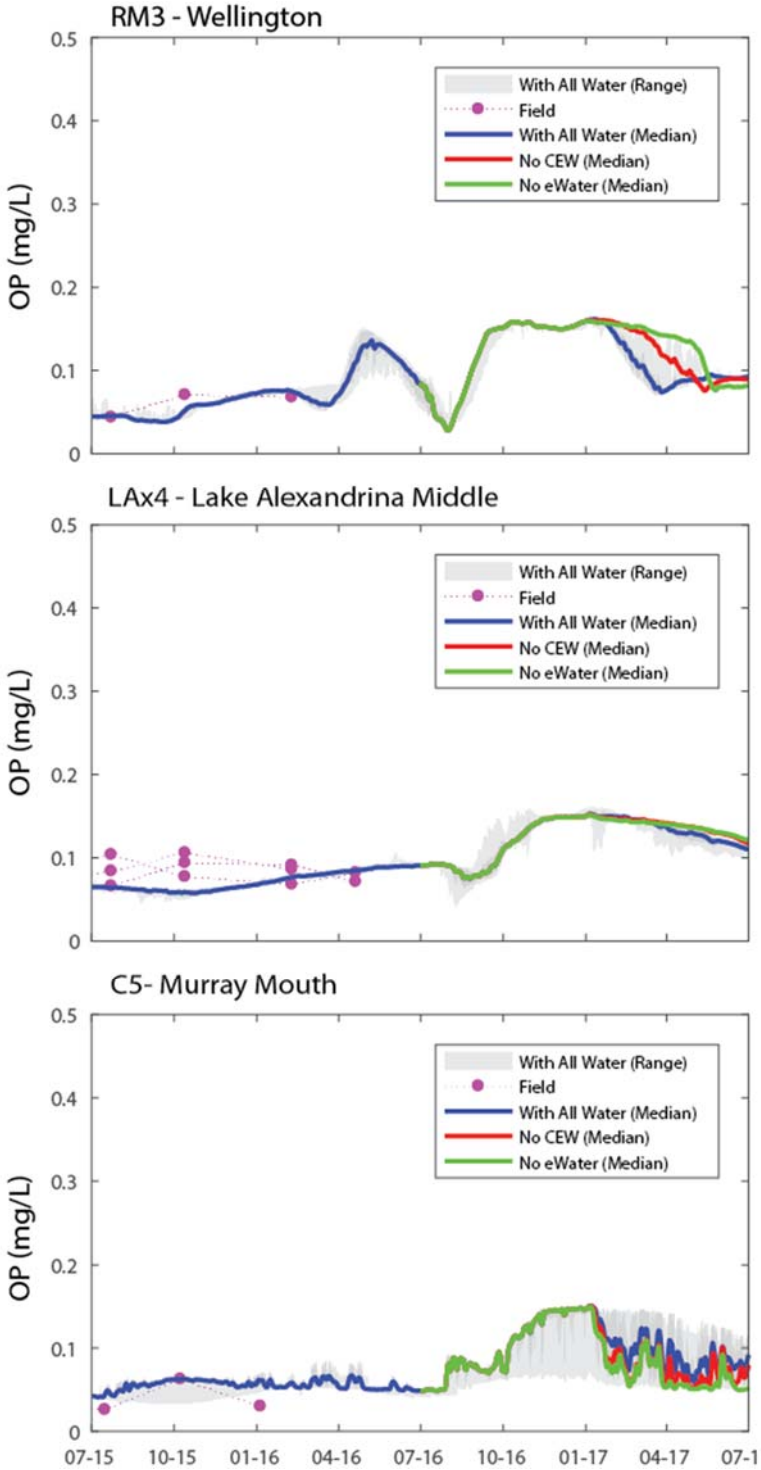


Figure F13. Observed and modelled particulate organic phosphorus (OP) concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). The data presented are the median of selected modelled cells surrounding the sampling sites. Measured concentrations are solid circles.

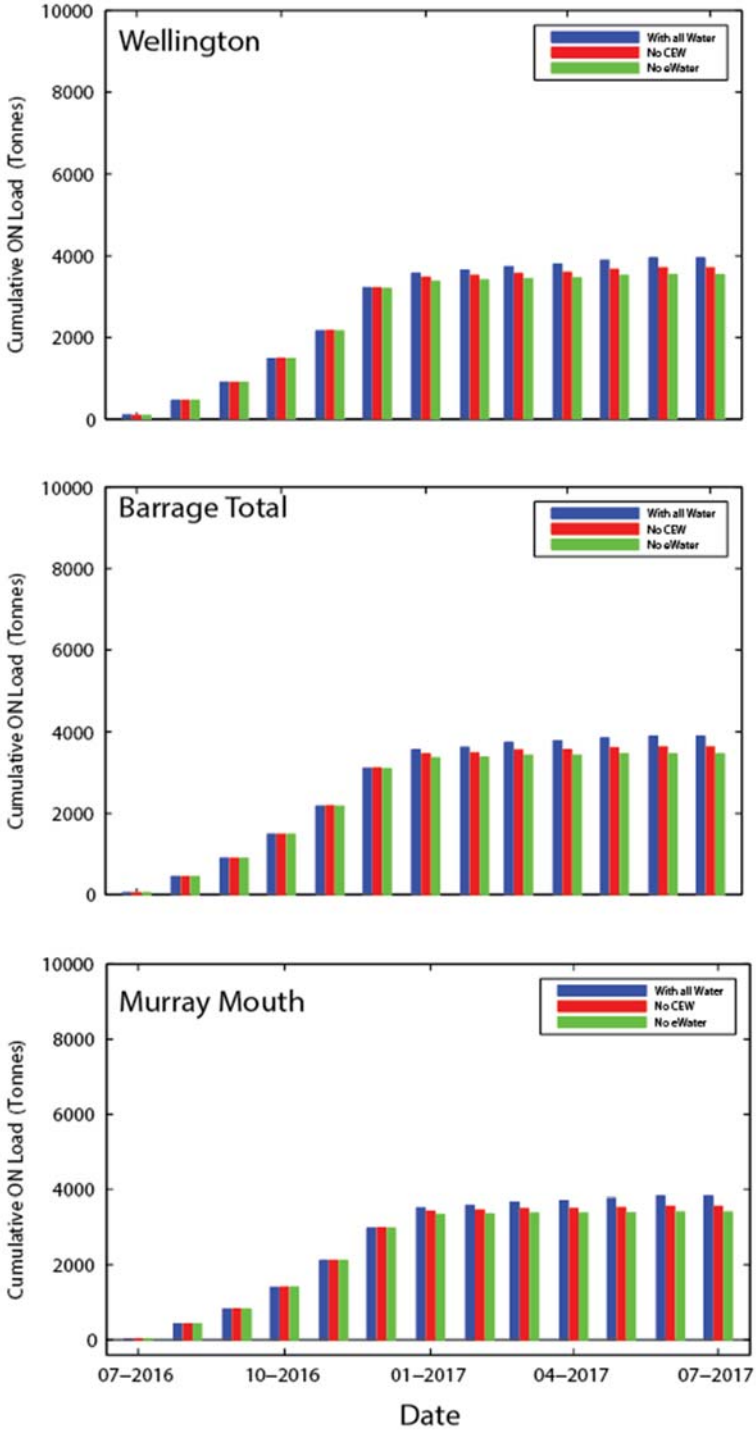


Figure F14. Modelled cumulative particulate organic nitrogen (ON) export (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

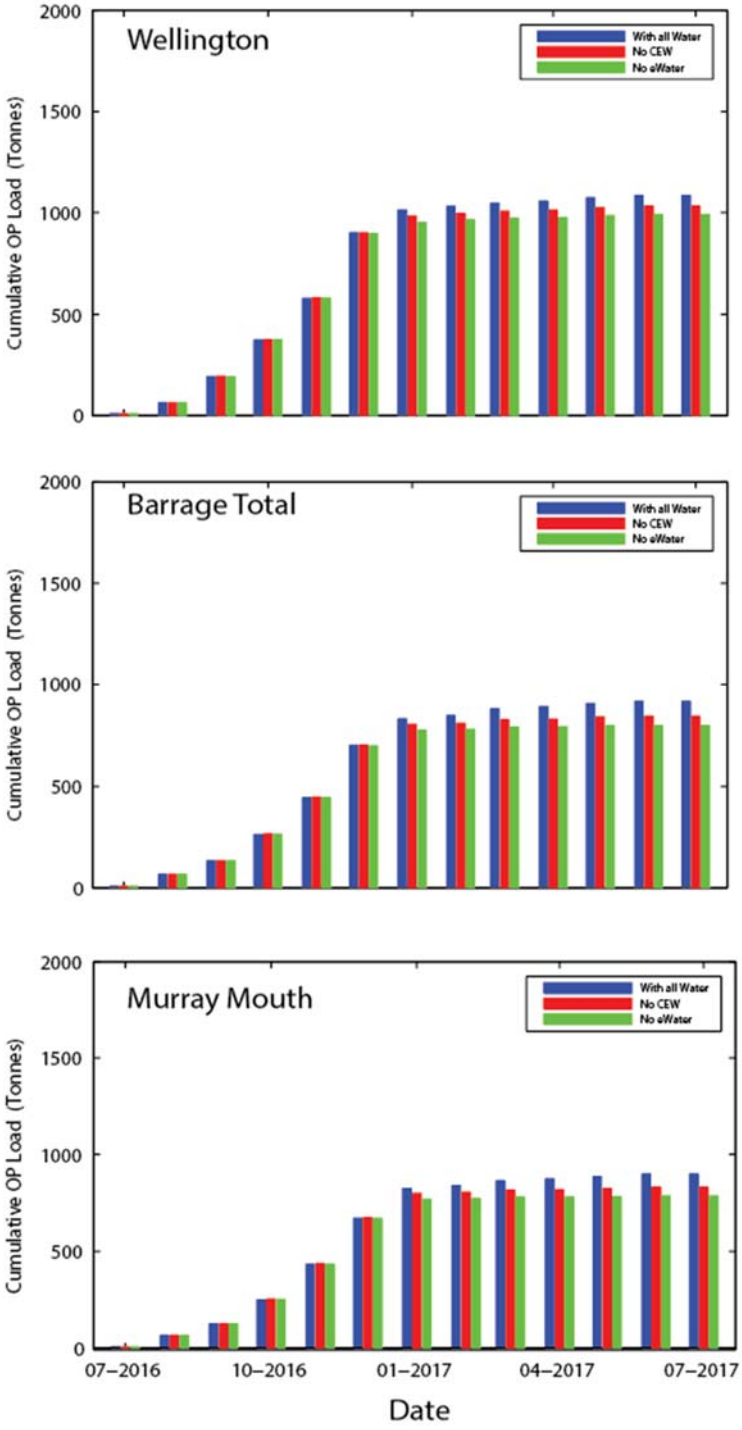


Figure F15. Modelled cumulative particulate organic phosphorus (OP) export (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

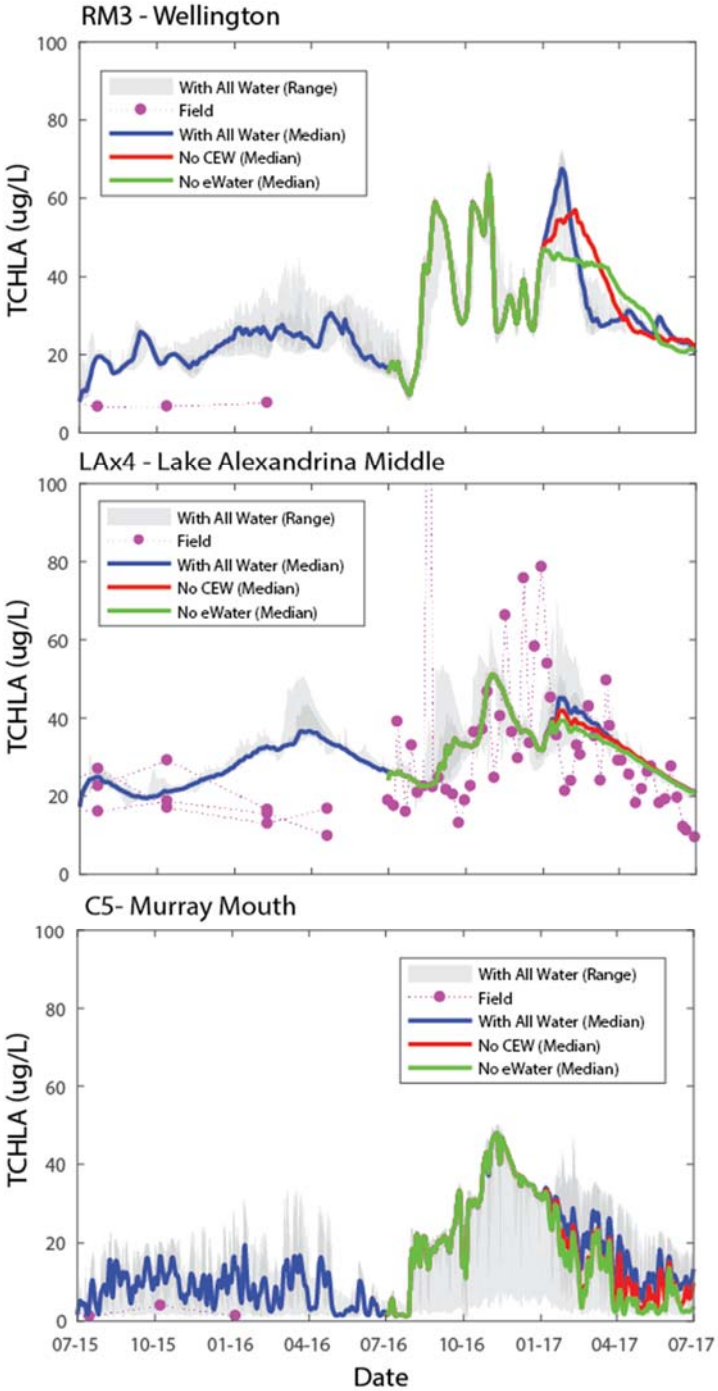


Figure F16. Observed and modelled chlorophyll *a* concentrations. The modelled scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater). The data presented are the median of selected modelled cells surrounding the sampling sites. Measured concentrations are solid circles.

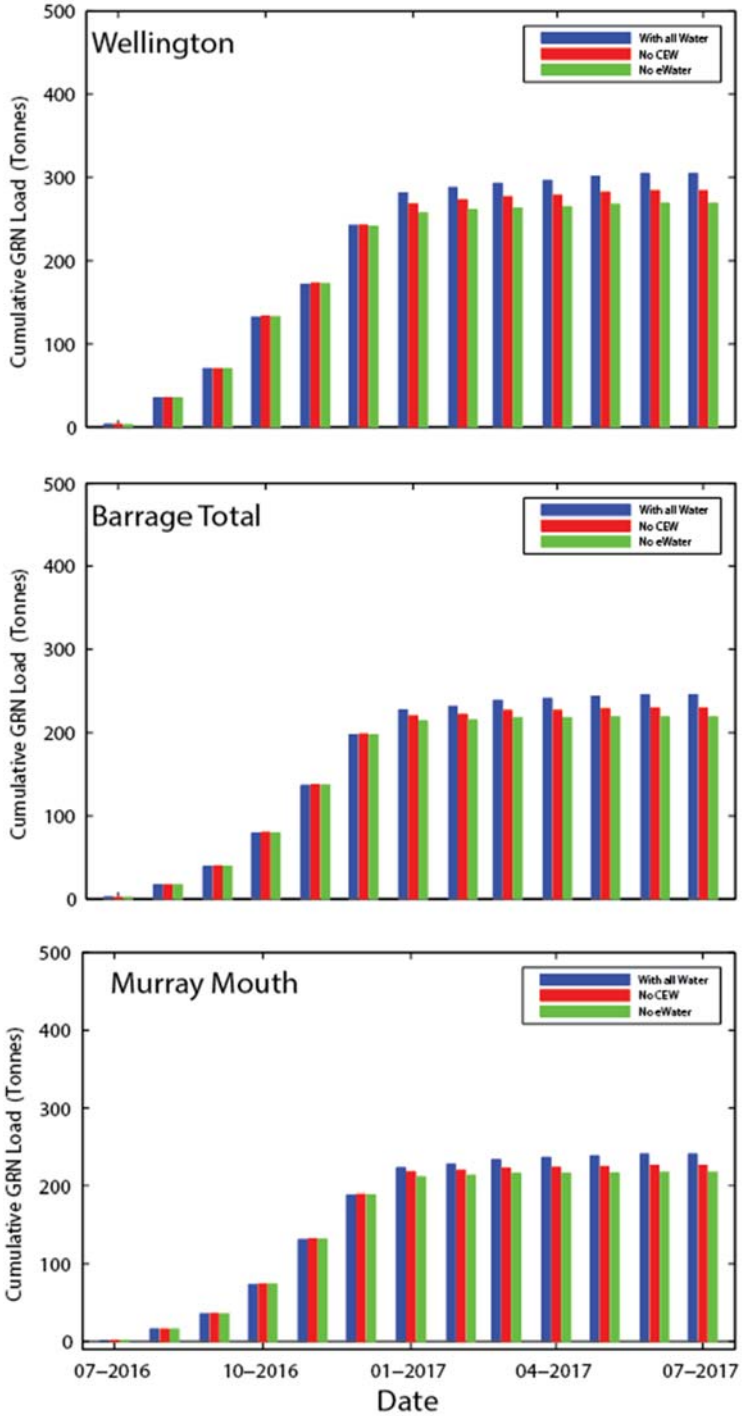


Figure F17. Modelled cumulative phytoplankton (GRN, as measured by carbon) net exports with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (no CEW) and without any environmental water (no eWater).

APPENDIX G: MICROINVERTEBRATES

Microinvertebrates

Background

The aquatic microinvertebrate communities of the MDB are rapid responders to environmental flows. Floodplain plankton communities respond within hours of inundation, with egg production stimulated, resting propagules triggered, and resulting emergence changing the species composition and diversity of the resident assemblage within days (Tan and Shiel 1993). To date, LTIM in 2014/15 and 2015/16 has demonstrated changes in microinvertebrate species composition as a result of littoral (epiphytic and epibenthic) and floodplain taxa being flushed into the main channel from flooded littoral margins or inundated floodplains (Ye *et al.* 2016a; 2017). To assess the responses of microinvertebrates in the LMR to delivery of Commonwealth environmental water in the LMR during 2016/17, the following evaluation questions were addressed:

What did Commonwealth environmental water contribute:

- to microinvertebrate diversity?
- to microinvertebrate abundance (density)?
- via upstream connectivity to microinvertebrate communities of the LMR?
- to the timing of microinvertebrate productivity and presence of key species in relation to diet of golden perch larvae?

Methods

Sampling sites and procedure

Microinvertebrate sampling was conducted approximately fortnightly between 26 September 2016 and 11 January 2017 at the three core LTIM sites within each of the floodplain and gorge geomorphic zones of the LMR (Figure 6; Table G1), concurrent with larval fish sampling. Three replicate samples were taken at each site during the day, while three replicate samples were taken at night at the sites 5 km downstream of Lock 1 and 6 only. During the 2016/17 monitoring period, three extra monitoring sites were included to complement existing LTIM monitoring and evaluation, and to investigate the influence of weir pool raising at Locks 2 on microinvertebrate diversity

and density (Appendix B). The extra monitoring sites were situated upstream and downstream of Lock 2 (Lock 2u and Lock 2d, respectively) and downstream of Lock 3 (Figure B1 and Table B1 in Appendix B).

Table G1. Details of microinvertebrate sampling sites downstream (DS) of Lock 1 and 6 in the LMR.

Zone	Site	Latitude	Longitude
Floodplain	5 km DS Lock 6	S34.01902	E140.87572
Floodplain	7 km DS Lock 6	S34.01764	E140.85461
Floodplain	9 km DS Lock 6	S34.0319	E140.84062
Gorge	5 km DS Lock 1	S34.4052	E139.61723
Gorge	7 km DS Lock 1	S34.42263	E139.61293
Gorge	9 km DS Lock 1	S34.44596	E139.61102

A Perspex Haney plankton trap (4.5-litre capacity) was used mid-channel (by boat) to collect surface and bottom volumes (9-litres), 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 (Figure G1). The filtrate was then preserved in the field (100% ethanol) to a final concentration of ~75%, and a volume <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 autopipette. This 1 ml 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.

Figure G1. Perspex Haney trap used for sampling microinvertebrate assemblages in the main channel of the Lower Murray River.

Statistical analyses

All statistical analyses were conducted on day-time Haney trap data from below Lock 1 and Lock 6 only as the additional monitoring sites for 2016/17 had no site replication (Appendix B). Temporal variation (between sampling trips) in microinvertebrate densities and taxa richness were analysed qualitatively for all sites using graphical plots of mean values ± standard error. Temporal variation in daytime microinvertebrate assemblage structure was investigated using a two-factor (i.e. sampling trip x lock) permutational multivariate analysis of variance (PERMANOVA) in the software package PRIMER v. 6.1.12 (Clarke and Gorley 2006) and PERMANOVA + v.1.02 (Anderson *et al.* 2008). Analyses were performed on log transformed log(x+1) data and Bray-Curtis (Bray and Curtis 1957) similarities were used to construct the similarity matrices for all multivariate analyses with a dummy variable = 1. Significance was set at a = 0.05. When significant differences occurred, PERMANOVA pair-wise comparisons were undertaken. To allow for multiple comparisons between regions and sizes, a false discovery rate (FDR) procedure (B–Y method correction) was adopted ($a = \sum_{i=1}^{n}(1/i)$; e.g. for $n_{comparisons}$ = 6, B–Y method a = 0.05/ (1/1 + 1/2 + 1/3…….+1/28) = 0.0127) (Benjamini and Yekutieli 2001; Narum 2006). The low number of samples collected for pair-wise comparisons resulted in low numbers of unique permutations and so Monte-Carlo *p*-values are presented (Anderson *et al.* 2008). Non-metric Multi-Dimensional Scaling (MDS), generated from the same matrices, was used to visualise microinvertebrate assemblages from different sampling trips. Groupings of

similarity (40%) from SIMPROF cluster analysis was overlaid on MDS ordinations to show similarity between sampling trips. When differences in microinvertebrate assemblages occurred between sampling trips for PERMANOVA, Similarity Percentages (SIMPER) analysis was used to determine the microinvertebrate taxa contributing to these differences, with a 25% cumulative contribution cut-off applied.

To model the relationship(s) between microinvertebrate assemblage structure, as described by the Bray-Curtis resemblance matrix, and one or more physico-chemical predictor variables, Distance-Based Linear Models (DistLM) were used, based on the forward stepwise selection procedure using R^2 as the selection criterion (Anderson *et al.* 2008). Automatic normalisation of environmental data occurred as part of the matrix algebra of regression in the DistLM routine (Anderson *et al.* 2008). Ordination of fitted values for DistLM was achieved through distance-based redundancy analysis (dvRDA), with vector overlays (Pearson and Spearman correlation coefficient > 0.2) to show individual water quality parameters that were important in driving variation along dbRDA axes. Six physico-chemical parameters (i.e. mean fortnightly flow, water temperature, dissolved oxygen, turbidity, electrical conductivity and pH) were included in the DistLM analysis.

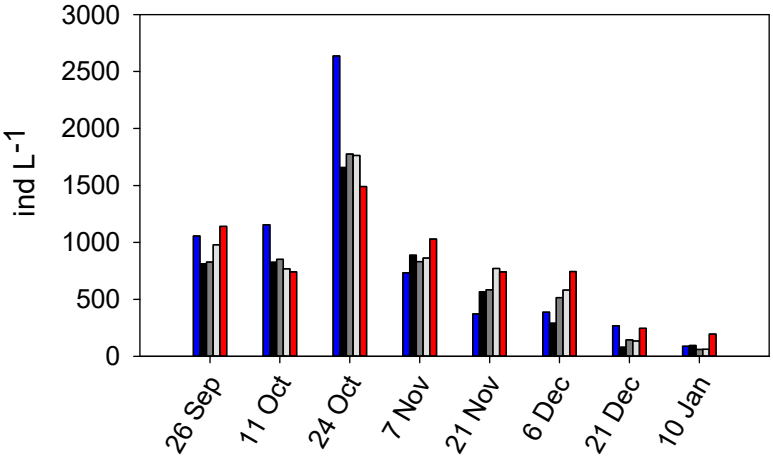
Results

Microinvertebrate catch summary, novel taxa and other observations

Over the 2016/17 sampling period, 262 microinvertebrate taxa were discriminated from 192 trap samples from the core LTIM sites in the gorge (below Lock 1) and floodplain (below Lock 6) geomorphic zones of the LMR (vs. 177 and 185 during 2015/16 and 2014/15, respectively). The 2016/17 assemblage included 108 Protista (largely testate rhizopods) (59 and 74 in 2015/16 and 2014/15), 114 Rotifera [95, 84], 16 Cladocera [11, 13], 13 Copepoda [7, 6], 1 Ostracoda [2, 2] and 9 juvenile macroinvertebrates [5, 6].

In addition to the taxa recorded from the core LTIM sites, 23 extra microinvertebrate taxa were discriminated from 72 trap samples from the three additional weir pool monitoring sites (i.e. below Lock 3, above Lock 2, below Lock 2d) in the gorge geomorphic zone of the LMR. These included 6 Protista, 15 Rotifera, 1 Cladocera and 1 Macroinvertebrate. Notably, 193 taxa (67.7%) of the assemblage were littoral, epiphytic or epibenthic in habit, incursion species in the riverine plankton.

From late September to early November 2016, the overall (all sites) in-channel assemblage was dominated by the testate tintinnid ciliate *Codonaria* (Figure G2) and a suite *of* rhizopods, with rotifers and microcrustaceans (e.g. copepods and cladocerans) notably low in abundance and diversity in comparison to previous years. From mid-November to late December 2016, a diverse rotifer assemblage, with brachionids (*Anuraeopsis, Brachionus* and *Keratella* spp.), synchaetids (*Polyarthra* and *Synchaeta* spp., and trochosphaerids (*Filinia* species) was recorded. *Codonaria* and diverse riparian rhizopods were still present, but in reduced numbers (Figure G2). Dominant taxa in the late December and January assemblage at all locations were a mix of Murray and Darling River rotifer species, such as brachionids (e.g. *Brachionus caudatus personatus, B. durgae*).



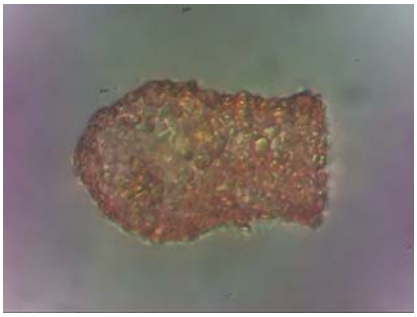


Figure G2. Mean relative abundances (ind L-1) of *Codonaria* sp. by trip and lock (daytime only) for 2016/17.

Among the diverse brachionids recorded below Lock 1, the invasive *Keratella* cf. *americana* (Figure G3) first recorded 21 October 2015, occurred in traps from all locations in low numbers through October 2016–January 2017. It appears to be established in the system. Similarly, *Hexarthra braziliensis*, another introduced species first noted in November 2015, occurred in small numbers below Lock 6 in November and December 2016. The introduced cladoceran *Daphnia galeata,* first recorded from a single individual collected in a night net tow from Lock 6A, October 2015, was collected twice, again single individuals, below Lock 2, 09 November 2016, and Lock 6, 10 January 2017. The historical replacement of native *Daphnia* when *D. galeata* is introduced is discussed by Karabanov *et al.* (2017).

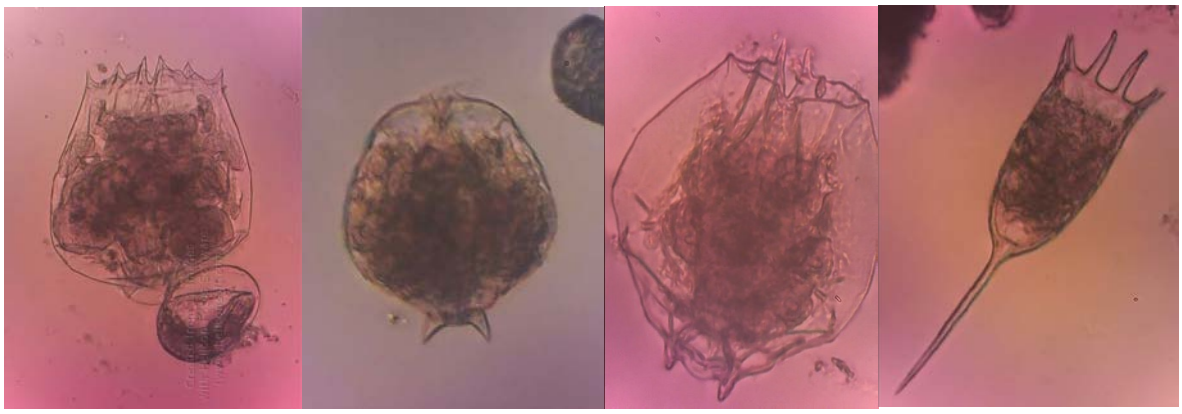


Figure G3. Novel microinvertebrate taxa sampled in the LMR. *Brachionus durgae* (left, 292 x 240 µm) from a night trap below Lock 1 during 21 December 2016; *Brachionus* n. sp. a (112 µm) (centre left) from a day trap below Lock 6 on 21 November 2016; *Brachionus* n. sp. b (160 µm) (centre right) from a day trap below Lock 6 on 7 November 2016; invasive *Keratella americana* (256 µm)(right) first recorded Oct 2015, in traps, Nov 2016-Jan 2017, at all locations.

Another first record from the continent is the brachionid rotifer *Brachionus durgae* Dhanapathi, 1974, described from India. Several individuals were recorded from traps below Lock 1 on 21 December 2016 and Lock 2 on 12 January 2017. *B durgae* is a warm-stenotherm, known to date only from India and the southwestern islands of Japan (Sudzuki 1992). It may have been long-resident in Australia, not discriminated from the *B. urceolaris* complex, which it resembles, or may have been bird-vectored more recently. It is a shallow-vegetated-pond species, i.e. heleoplanktonic rather than riverine in habit, and likely derived from a floodplain source, either Chowilla, Barmah-Millewa, or most likely, the Darling catchment.

Two new indigenous brachionids also were collected from the 2016/17 trap series. The smaller 'sp. a' (Figure G3) was first collected on 21 November 2016 below Lock 6, then in small numbers on subsequent trips until the end of sampling in January. It occurred simultaneously on all trips at Locks 2/3 sites, but was collected below Lock 1 only during the 21 November and 22 December field trips. The larger *Brachionus* n. sp. b (Figure G3) occurred in the same samples, likely deriving from the same (floodplain) source as 'sp. a'. Both are members of the *Brachionus angularis-lyratus* complex, which has several undescribed taxa known from the continent.

A single individual of a small notommatid rotifer (not figured) collected on 09 November 2016 below Lock 2, when eroded for trophi (dentition) identification, was found to have trophi conforming closely to those described for *Notommata prodota* Myers, 1933, described from Mt Desert Island in Maine, and not seen since. Given the

geographical disjunction, caution suggests that this record be left as *Notommata* cf. *prodota* until further specimens can be examined. It may be undescribed. Regardless, it is a littoral incursion.

Empty loricae of recently dead *Keratella* species (Figure G4) were abundant in trap samples from all locks through the first four sampling trips from late September to early November, also occasional dead-on-collection microcrustaceans and macroinvertebrates. Evidence of stressors affecting the microinvertebrate assemblage in late December 2016 was provided by collection of parasitised rotifers in trap samples. Rotifers are reportedly prone to infestation by sporozoan and fungal parasites when physiologically stressed, generally at maximum population densities, and are known from rivers elsewhere (e.g. Gorbunov and Kosova 2001). Most common infested species in the LMR were *Brachionus calyciflorus amphiceros*, *B. caudatus*, *Filinia* spp., and *Trichocerca* spp. (Figure G5).

Figure G4. Empty loricae of *Keratella slacki* Lock 6 (top left), *K. procurva* Lock 6 (top right), *K. australis*, Lock 1 (bottom left) and *K. tropica* Lock 1 (bottom right). All from September 2016-early November 2016.

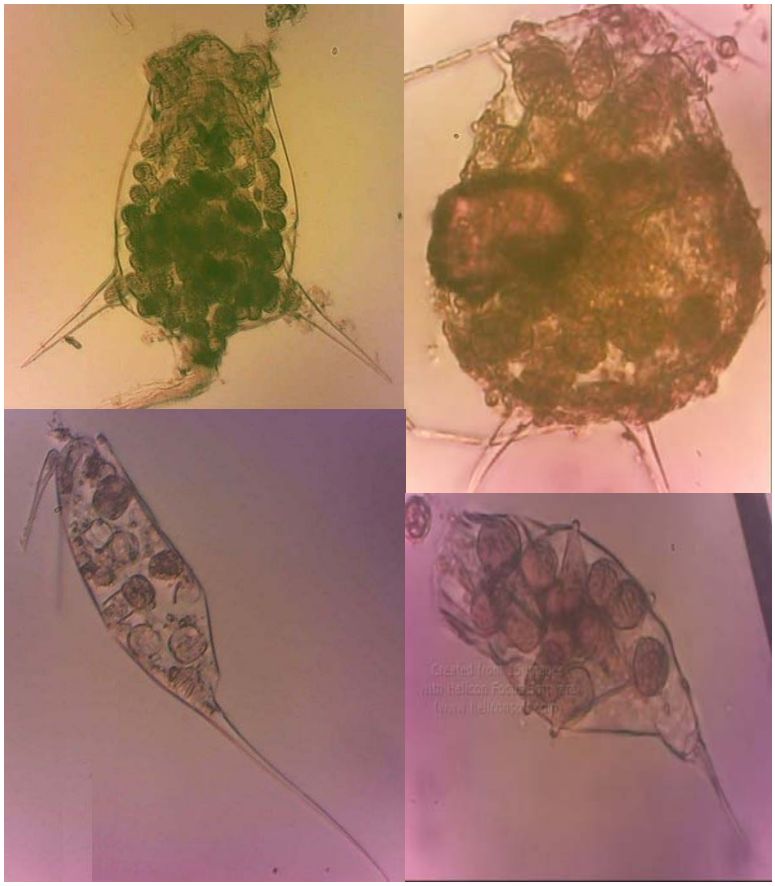


Figure G5. Parasitised rotifers: *Brachionus calyciforus amphiceros* Lock 1 (top left), *B. caudatus personatus* Lock 6 (top right), *Filinia opoliensis*, Lock 1A (bottom left) and *Trichocerca* sp. Lock 6A (bottom right). All from December 2016 and early January 2017 trips.

Densities and taxa richness

Microinvertebrate density fluctuated throughout the sampling period, with a similar pattern observed at all sites (Figure G6). Lowest densities generally occurred in late September 2016 and mid-January 2017, while highest densities occurred in late October, late November and late December 2016. Within Weir Pool 2, microinvertebrate density peaked in late December at 2,918 ind.L^{-1} (above Lock 2), coinciding with maximum water levels. Below Lock 1 and Lock 6, densities peaked in late October at 2,234 ± 154 (mean ± S.E.) and 3,133 ± 110 ind.L^{-1}, respectively, following a recession in water levels.

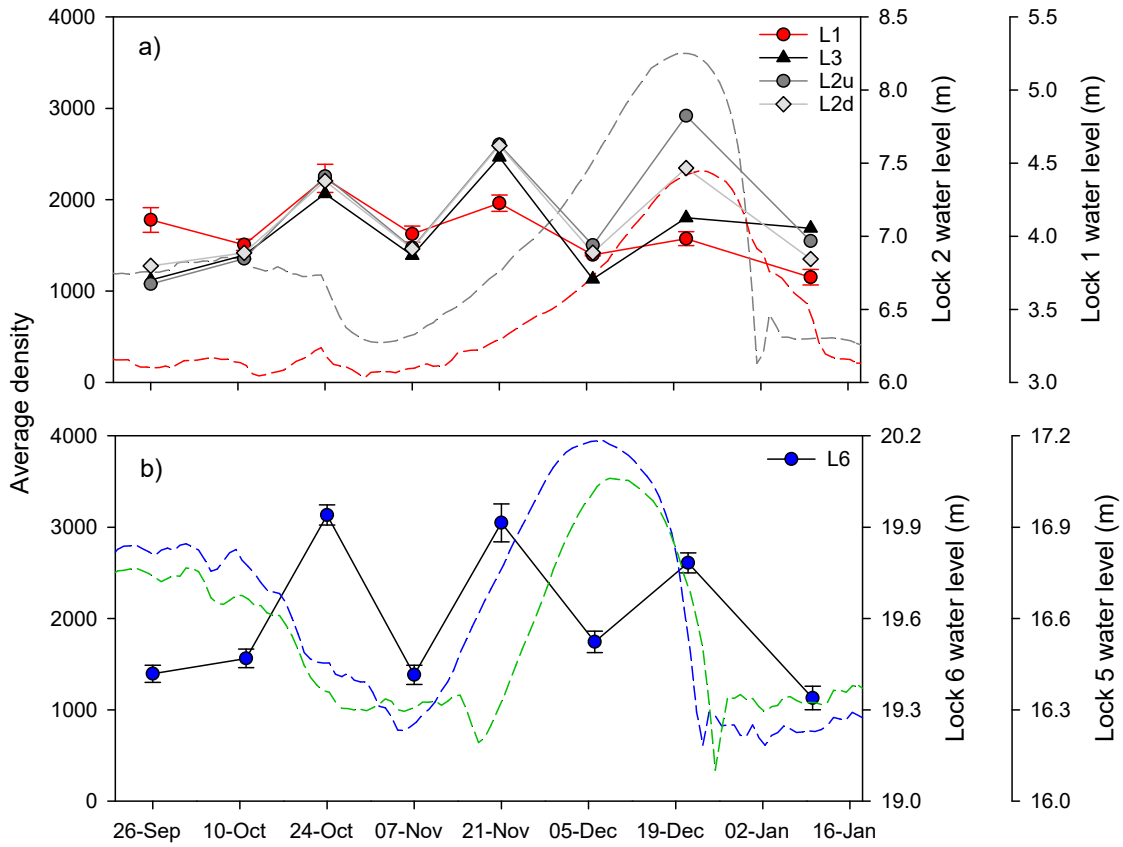


Figure G6. Average microinvertebrate density (ind.L^{-1} ±S.E.) at sites: a) below Lock 1 (L1), Lock 2 (L2d) and Lock 3 (L3), and above Lock 2 (L2u); and b) below Lock 6 (L6) in each sampling trip from late September 2016 to early January 2017. Dotted lines show water level (m AHD) variations from weir pool raising within Weir Pools 2 (upstream (US) Lock 2) and 5 (US Lock 5). Water levels are also presented for locks that are immediately upstream of the sampling sites: Lock 1 (red), Lock 2 (grey), Lock 5 (green), Lock 6 (blue).

At all sites, microinvertebrate taxa richness (indicating diversity) gradually increased from late September to late November (Figure G7). Diversity was variable after late November, where it declined in early December, increased in late December and declined again in early January. Diversity peaked in late November below Lock 1 (mean ± S.E. = 37.9 ± 1.1 spp.) and in Weir Pool 2 (above Lock 2 = 44.3 spp.), and in late December (51.8 ± 2.4 spp.) below Lock 6.

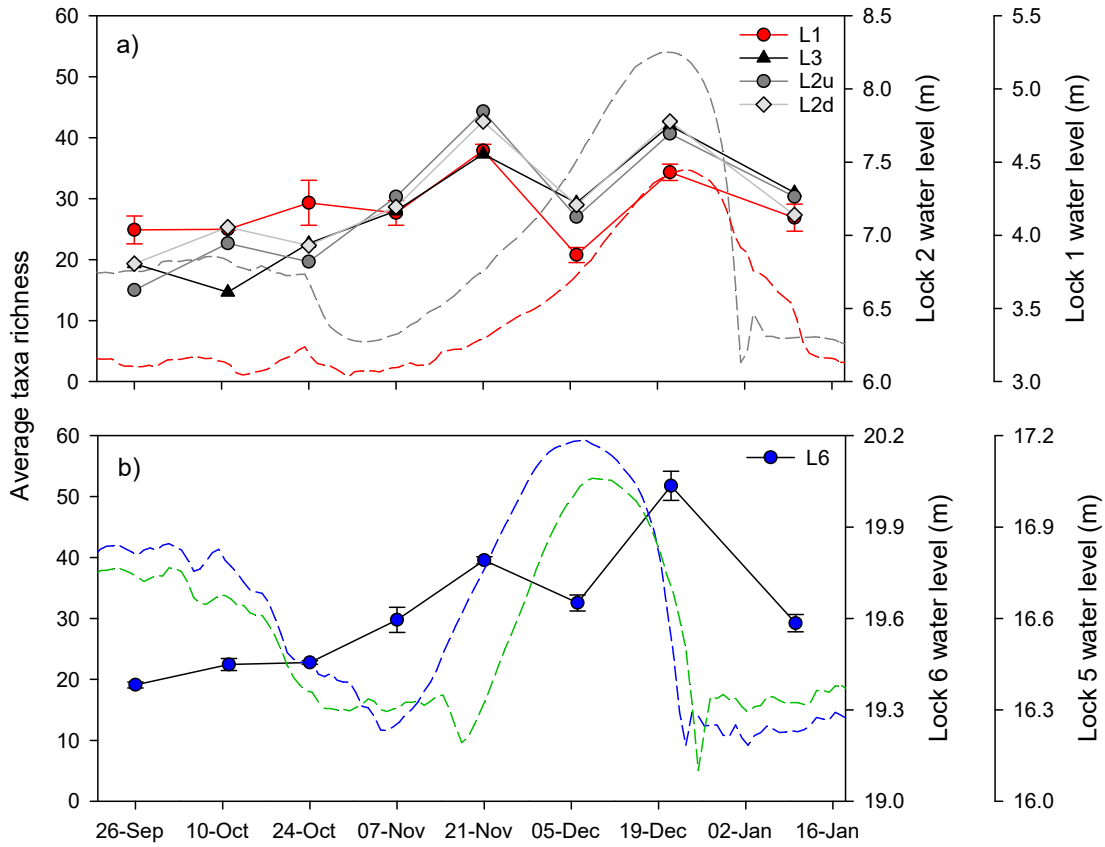


Figure G7. Average microinvertebrate taxa richness (±S.E.) at sites: a) below Lock 1 (L1), Lock 2 (L2d) and Lock 3 (L3), and above Lock 2 (L2u); and b) below Lock 6 (L6) in each sampling trip from late September 2016 to early January 2017. Dotted lines show water level (m AHD) variations from weir pool raising within Weir Pools 2 (upstream (US) Lock 2) and 5 (US Lock 5). Water levels are also presented for locks that are immediately upstream of the sampling sites: Lock 1 (red), Lock 2 (grey), Lock 5 (green), Lock 6 (blue).

Microinvertebrate assemblage structure

Microinvertebrate assemblages appeared to separate well based on sampling trip, with individual trips forming relatively tight groups and a temporal sequence noticeable across the MDS ordination (Figure G8). Assemblages sampled prior to mid-November showed a clear separation from those sampled afterwards. Within sampling trips, samples showed poor grouping by lock prior to mid-November; however, after mid-November, samples were separated by lock groups (Figure G8).

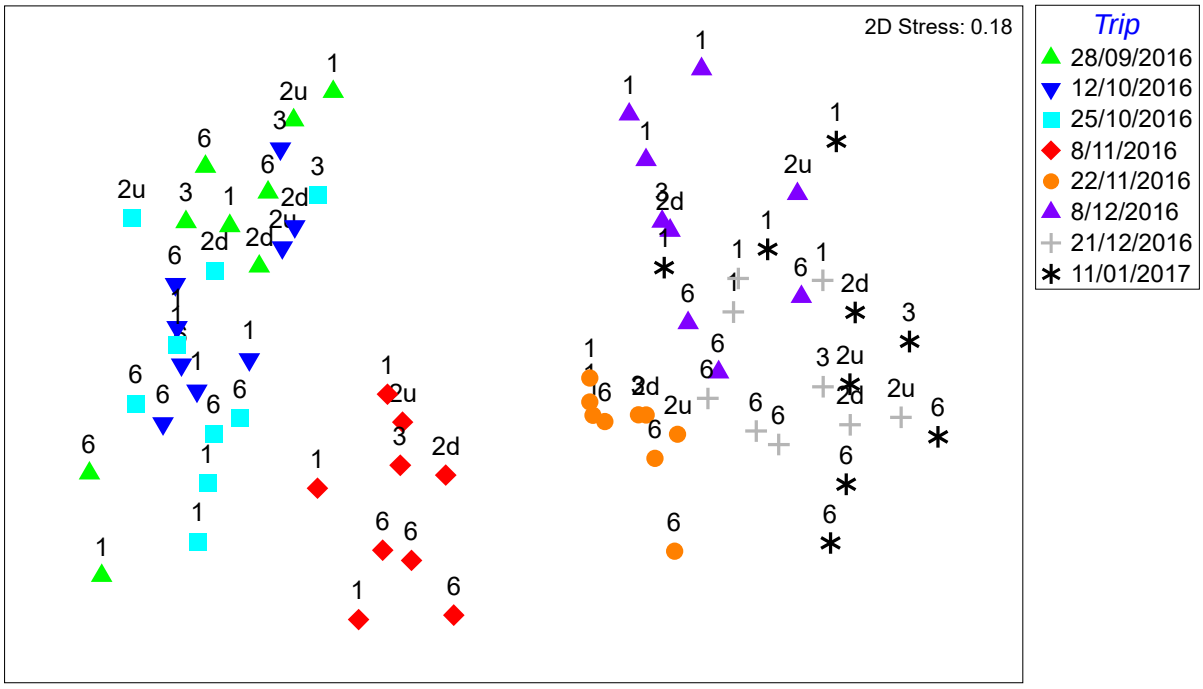


Figure G8. MDS ordination of microinvertebrate assemblage data (log transformed) for core LTIM sites below Lock 6 and Lock 1, and for additional sites below Lock 3 and 2 (2d), and above Lock 2 (2u). Additional sites were excluded from all data analyses due to low site replication.

A significant interaction was detected between locks and sampling trips (two-factor PERMANOVA; Pseudo-$F_{7,47}$ = 2.7417, p = 0.0001), suggesting inconsistent spatio-temporal variation among sampling trips between locks. Pairwise tests were conducted separately for below Lock 1 and Lock 6 to examine differences over time (i.e. between sampling trips) (Tables F2 and F5).

Lock 6

For sites below Lock 6, there were no significant differences in microinvertebrate assemblages among the first four sampling trips from late September to early November 2016 (B–Y method corrected a = 0.0127, Table G2; Figure G9). However, with the exception of one comparison, assemblages from these four sampling trips were significantly different to those from the last three sampling trips from early December 2016 to early January 2017 (Table G2 and Figure G9). Indeed, the January 2017 assemblage was significantly different to those from all other trips. Assemblages during early and late December were not significantly different from those from their preceding trip (Table G2). MDS ordination of the Lock 6 assemblages supports results from pairwise comparisons; there was strong grouping of samples by the first four

sampling trips and trips from late November and late December, and separation of all samples from early January 2017 (Figure G9).

Table G2. Within sites below Lock 6 pair-wise results of microinvertebrate log(x+1) abundance data amongst sampling trips, showing Monte-Carlo p-values. After B–Y method FDR correction, a = 0.0127 for comparisons between months (28 comparisons). * = groups significantly different.

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
12-Oct	0.2334						
25-Oct	0.0842	0.1437					
8-Nov	0.0274	0.0284	0.0445				
22-Nov	0.0166	0.0087*	0.008*	0.0121*			
8-Dec	0.0150	0.0110*	0.0072*	0.0108*	0.0332		
21-Dec	0.0094*	0.0073*	0.0055*	0.0066*	0.0114*	0.0368	
11-Jan	0.0082*	0.0053*	0.0044*	0.0049*	0.004*	0.0092*	0.0089*

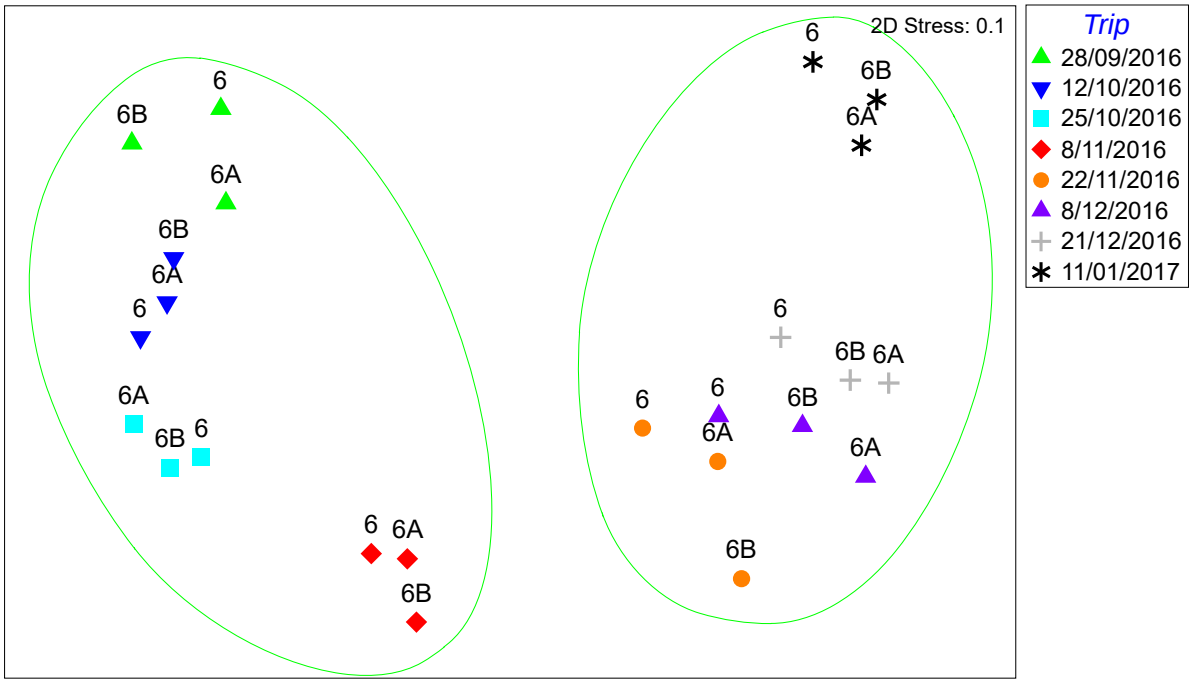


Figure G9. MDS ordination of microinvertebrate assemblage data (log transformed) from sites below Lock 6, with samples identified by sampling trip. Samples are grouped at a Bray-Curtis similarity of 40% (green circles) (SIMPROF).

SIMPER analysis was used to determine which taxa were driving the apparent differences between sampling trips. Results are provided below in Table G3. Dissimilarly between groups was primarily driven by: lower abundances of rotifers *Brachionus angularis bidens, Filinia terminalis* and *Polyarthra dolichoptera* from late

September to late October; higher abundances of rotifers *Pompholyx complanata*, *Keratella cochlearis* and *K. procurva* in early November; higher abundance of the rotifer *Keratella slacki* in late November; higher abundance of the rotifer *Anuraeopsis fissa* in late November and early December; higher abundance of the rotifer *Brachionus quadridentatus cluniorbicularis* in early December; higher abundances of rotifers *Proalides tentaculatus*, *Trichocerca pusilla* and *Asplanchna priodonta* in early and late December; higher abundances of rotifers *Trichocerca* cf. *agnatha* (not previously recorded for South Australia) and *Brachionus budapestinensis* in late December and January; and higher abundances of rotifers *Brachionus caudatus personatus*, *B. bennini* and *Trichocerca similis grandis*, and the protist *Stentor* sp., during January (Table G3).

Table G3. Microinvertebrate taxa responsible for the dissimilarity between sampling trips for sites below Lock 6 (SIMPER). Bold taxa were more abundant during the sampling trip in the respective column, while unbolded taxa were those more abundant during the sampling trip in the respective row. Average dissimilarity (%) between sampling trips is provided for each comparison. N.s. = non-significant. Some species have not been previously recorded (NR) for South Australia (SA) or Australia (Aust).

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
12-Oct	n.s.						
25-Oct	n.s.	n.s.					
8-Nov	n.s.	n.s.	n.s.				
22-Nov	n.s.	62.35% *Anuraeopsis fissa*, *Brachionus* [*angularis*] *bidens*, *Filinia terminalis*, *Polyarthra* dolichoptera, *Keratella tropica*, *Proalides tentaculatus*, *Keratella procurva*, *Keratella slacki*, *Filinia longisteta* and *Brachionus* n. sp.	60.61% *Anuraeopsis fissa* *Brachionus* [*angularis*] *bidens*, *Filinia terminalis*, *Proalides tentaculatus*, *Filinia longisteta*, *Keratella tropica*, *Trichocerca pusilla*, ***Synchaeta* sp. c** and *Keratella slacki*.	53.22% *Filinia terminalis*, *Anuraeopsis fissa* *Bosmina meridionalis*, *Brachionus* [*angularis*] *bidens*, *Brachionus* n. sp., *Arcella bathystoma*, indet. glob. ciliate c, *Proalides tentaculatus*, *Brachionus* [*quadridentatus*] *cluniorbicularis*, ***Difflugia* sp. o** and *Trichocerca pusilla*.			

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
8-Dec	n.s.	65.37% *Trichocerca pusilla*, *Brachionus* [*angularis*] *bidens*, *Anuraeopsis fissa*, indet. glob. ciliate c, *Brachionus* [*quadridentatus*] *cluniorbicularis*, *Proalides tentaculatus*, *Asplanchna priodonta*, *Brachionus lyratus*, *Polyarthra dolichoptera*, *Brachionus* n. sp. and *Arcella bathystoma*.	66.14% *Trichocerca pusilla*, *Brachionus* [*angularis*] *bidens*, *Anuraeopsis fissa*, *Proalides tentaculatus*, *Brachionus* [*quadridentatus*] *cluniorbicularis*, *Asplanchna priodonta*, indet. glob. ciliate c, *Brachionus lyratus*, ***Synchaeta* sp. c** and ***Bosmina meridionalis***.	58.87% *Trichocerca pusilla*, ***Pompholyx complanata***, ***Keratella procurva***, indet. glob. ciliate c, ***Keratella cochlearis***, *Brachionus* [*quadridentatus*] *cluniorbicularis*, *Asplanchna priodonta*, *Proalides tentaculatus*, ***Keratella australis***, *Arcella bathystoma* and *Brachionus* n. sp..	n.s.		
21-Dec	68.38% *Brachionus* [*angularis*] *bidens*, *Proalides tentaculatus*, *Polyarthra dolichoptera*, *Asplanchna priodonta*, *Brachionus budapestinensis*, *Filinia terminalis*, *Brachionus lyratus*, *Keratella tropica*, *Trichocerca* cf. *agnatha* NR for SA, *Trichocerca pusilla*, *Coleps* sp. and *Philodina alata* NR for Aust.	66.30% *Brachionus* [*angularis*] *bidens*, *Proalides tentaculatus*, *Polyarthra dolichoptera*, *Trichocerca* sp. f, *Asplanchna priodonta*, *Brachionus budapestinensis*, *Filinia terminalis*, *Brachionus lyratus*, *Trichocerca pusilla*, *Trichocerca* cf. *agnatha* NR for SA, *Keratella tropica* and *Coleps* sp.	67.38% *Proalides tentaculatus*, *Brachionus* [*angularis*] *bidens*, *Trichocerca* sp. f, *Trichocerca pusilla*, *Asplanchna priodonta*, *Brachionus budapestinensis*, *Filinia terminalis*, *Brachionus lyratus*, *Trichocerca* cf. *agnatha* NR for SA, *Conochilus* sp. b, *Polyarthra dolichoptera* and *Synchaeta pectinata* NR for SA.	58.85% *Trichocerca* sp. f, *Proalides tentaculatus*, *Asplanchna priodonta*, *Filinia terminalis*, ***Pompholyx complanata***, *Trichocerca pusilla*, *Brachionus budapestinensis*, ***Keratella cochlearis***, *Synchaeta pectinata* NR for SA, *Trichocerca* cf. *agnatha* NR for SA, ***Keratella procurva***, ***Difflugia* sp. o** and *Coleps* sp.	46.73% ***Anuraeopsis fissa***, *Brachionus budapestinensis*, *Trichocerca* sp. f, *Trichocerca* cf. *agnatha* NR for SA, *Asplanchna priodonta*, ***Keratella slacki***, ***Pompholyx complanata***, ***Bosmina meridionalis***, *Coleps* sp., *Philodina alata* NR for Aust, *Brachionus lyratus*, indet. bdelloid and *Difflugia* sp. a.	n.s.	

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
11-Jan	70.91% *Polyarthra dolichoptera*, *Brachionus budapestinensis*, *Filinia terminalis*, *Brachionus* [*angularis*] *bidens*, *Trichocerca* cf. *agnatha* NR for SA, *Stentor* sp., *Brachionus bennini*, *Brachionus caudatus personatus* and *Hexarthra* sp.	69.67% *Brachionus budapestinensis*, *Polyarthra dolichoptera*, *Filinia terminalis*, *Brachionus* [*angularis*] *bidens*, *Trichocerca* cf. *agnatha* NR for SA, *Stentor* sp., *Brachionus bennini*, *Trichocerca* [*similis*] *grandis*, *Brachionus caudatus personatus* and ***Epistylis* sp.**	71.14% *Brachionus budapestinensis*, *Filinia terminalis*, *Trichocerca* [*similis*] *grandis*, *Brachionus* [*angularis*] *bidens*, *Trichocerca* cf. *agnatha* NR for SA, *Stentor* sp., *Trichocerca pusilla*, *Brachionus bennini*, **Codonaria sp.** and *Brachionus caudatus personatus*.	63.97% *Brachionus budapestinensis*, ***Pompholyx complanata***, *Filinia terminalis*, *Trichocerca* [*similis*] *grandis*, *Stentor* sp., ***Keratella procurva***, *Brachionus bennini*, ***Keratella cochlearis***, *Brachionus caudatus personatus* and ***Epistylis* sp.**	56.56% ***Anuraeopsis fissa***, *Brachionus budapestinensis*, *Trichocerca* [*similis*] *grandis*, *Trichocerca* cf. *agnatha* NR for SA, *Stentor* sp., ***Pompholyx complanata***, ***Collotheca pelagica* NR for SA**, ***Keratella slacki***, *Brachionus caudatus personatus* and ***Polyarthra* sp. b**.	55.34% ***Anuraeopsis fissa***, *Brachionus budapestinensis*, ***Brachionus* [*quadridentatus*] *cluniorbicularis***, *Brachionus bennini*, *Brachionus caudatus personatus*, *Trichocerca* cf. *agnatha* NR for SA, ***Arcella bathystoma***, *Hexarthra* sp., *Trichocerca* [*similis*] *grandis* and *Filinia longiseta*.	50.76% ***Trichocerca* sp. f**, *Stentor* sp., *Brachionus bennini*, *Brachionus caudatus personatus*, **Collotheca *pelagica* NR for SA**, ***Proalides tentaculatus***, ***Brachionus lyratus***, ***Coleps* sp.**, ***Philodina alata* NR for Aust**, ***Polyarthra* sp. b**, ***Asplanchna priodonta***, *Trichocerca* [*similis*] *grandis* and ***Difflugia* sp. a**.

All environmental predictor variables for the microinvertebrate assemblage structure below Lock 6 were significant, except pH (Table G4). However, water temperature (30.8%) explained most of the variation (Table G4; Figure G10). Water temperature and electrical conductivity were the best environmental variables to explain the horizontal separation of the data cloud, while river flow best explained the vertical separation (Figure G11).

Table G4. DistLM marginal test results indicating which physico-chemical variable significantly contributed most the relationship with the microinvertebrate data cloud for below Lock 6. * = groups significantly different.

Variable	Pseudo-F	P	Prop.
Water temperature	9.8032	0.0001*	0.30825
Electrical conductivity	7.4875	0.001*	0.25392
Turbidity	4.9772	0.004*	0.18450
Dissolved oxygen	4.2274	0.0022*	0.16118
Mean QSA flow	4.1066	0.0014*	0.15730
pH	1.7066	0.0928	0.07199

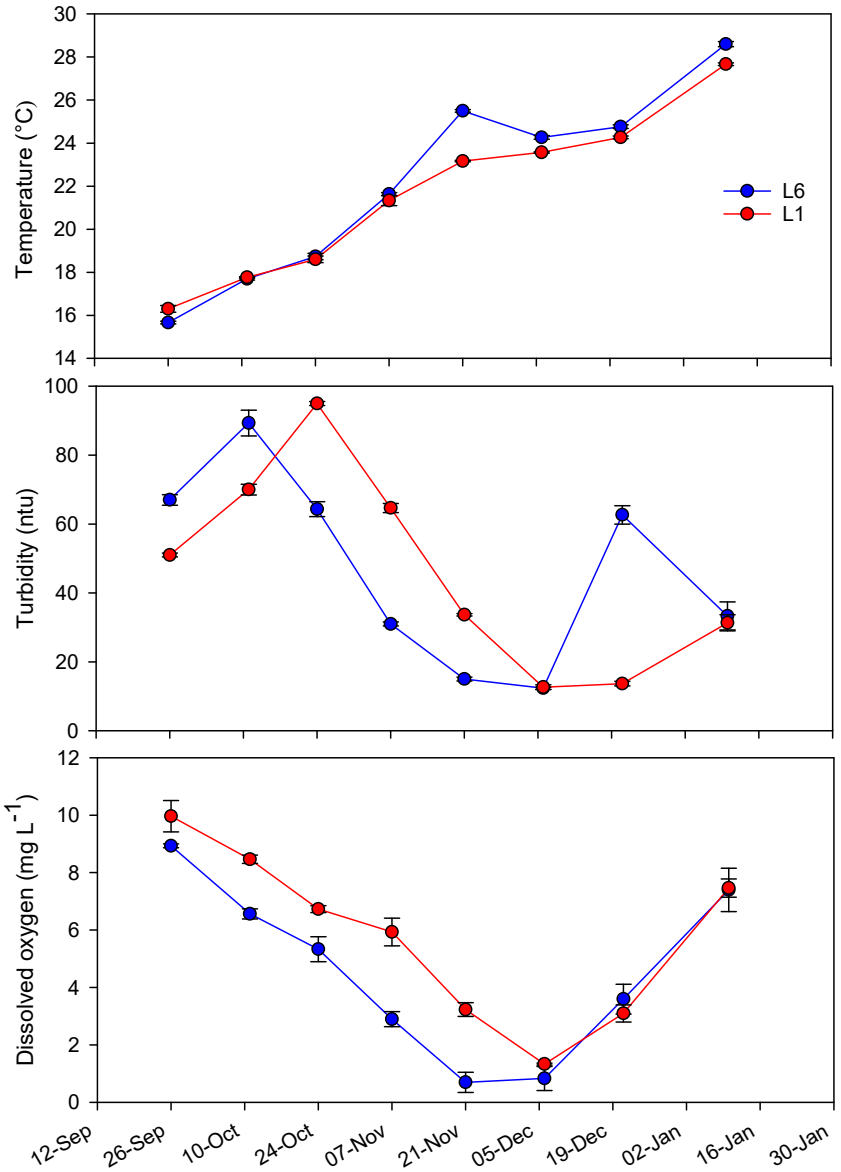


Figure G10. Mean (± standard error) water temperature (°C), dissolved oxygen (mg L^{-1}) and turbidity (ntu) measured at sites below Lock 6 (L6) and Lock 1 (L1) during sampling in 2016/17.

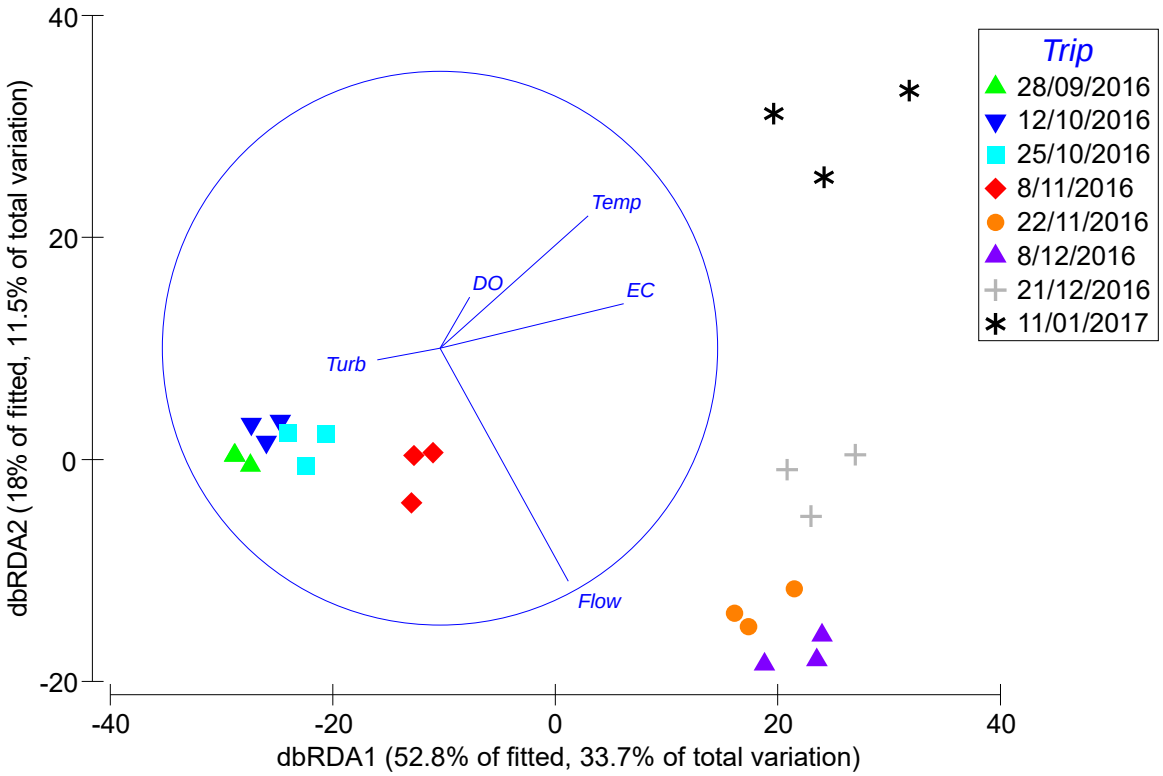


Figure G11. dbRDA ordination of the fitted model of microinvertebrate assemblage data from below Lock 6 (based on Bray-Curtis measure of log transformed data) versus the predictor variables. The vector overlay indicates multiple partial correlations (correlation coefficient > 0.2) between the predictor variables and dbRDA axes 1 and 2.

Lock 1

For sites below Lock 1, pairwise comparisons revealed significant differences in the microinvertebrate assemblage between January and trips preceding early December (B–Y method corrected α = 0.0127, Table G5; Figure G12). Similarly, the assemblage during late December was significantly different to assemblages from early October to late November. The early December assemblage was significantly different to that during early October and early November, while the late November assemblage was significantly different to that in early October (Table G5). All other comparisons were non-significant. Generally, separation between groups was high with the exception of the first three sampling trips from late September to late October, due to large variability within the late September and early October trips (Figure G12). Similar to the Lock 6 assemblages (Figure G9), the assemblages below Lock 1 were divided into two temporal groups, separated by mid-November.

Table G5. Within sites below Lock 1 pair-wise results of microinvertebrate log(x+1) abundance data amongst sampling trips, showing Monte-Carlo p-values. After B–Y method FDR correction, a = 0.0127 for comparisons between months (28 comparisons). * = groups significantly different.

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
12-Oct	0.1734						
25-Oct	0.1385	0.138					
8-Nov	0.0523	0.031	0.0876				
22-Nov	0.0210	0.0094*	0.0214	0.0313			
8-Dec	0.0246	0.0084*	0.0185	0.0108*	0.0193		
21-Dec	0.0140	0.0054*	0.0094*	0.0083*	0.0125*	0.0258	
11-Jan	0.0125*	0.0062*	0.0105*	0.0108*	0.0104*	0.0194	0.0236

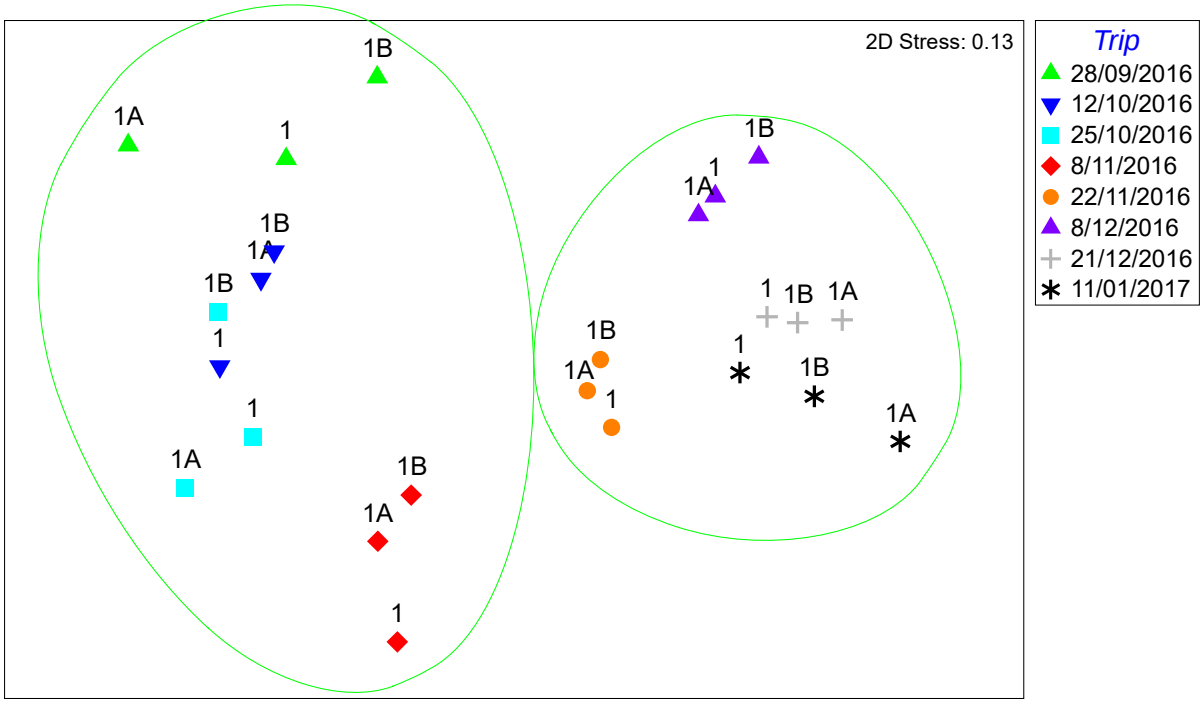


Figure G12. MDS ordination of microinvertebrate assemblage data (log transformed) from Lock 1, with samples identified by sampling trip. nMDS was based on Bray-Curtis Similarities. Samples are grouped at a Bray-Curtis similarity of 40% (SIMPROF).

Results from the SIMPER analysis comparing below Lock 1 microinvertebrate assemblages between sampling trips is provided in Table G6. Dissimilarly between groups was primarily driven by: lower abundance of rotifers *Brachionus angularis bidens* and *Polyarthra dolichoptera* in October; higher abundances of rotifers *Synchaeta* sp. b (not previously recorded for South Australia) and c, and lower abundance of the rotifer *Proalides tentaculatus*, from early October to early

November; higher abundance of the protist *Epistylis* sp. during late September and early October; higher abundances of rotifers *Pompholyx complanata* and *Keratella javana* in early November; higher abundance of rotifers *Anuraeopsis fissa*, *Keratella tropica* and *K. cochlearis* in late November; higher abundance of the protist *Difflugia* cf. *penardi* in early December; higher abundances of rotifers *Asplanchna priodonta*, *Brachionus budapestinensis* and *Conochilus dossuarius* in late December; higher abundance of rotifer *Trichocerca* cf. *agnatha* (not previously recorded for South Australia) in late December and January; and higher abundances of rotifers *Polyarthra dolichoptera* and protist *Coleps* sp. in January (Table G6).

Table G6. Microinvertebrate taxa responsible for the dissimilarity between sampling trips for sites below Lock 1 (SIMPER). Bold taxa were more abundant during the sampling trip in the respective column, while unbolded taxa were those more abundant during the sampling trip in the respective row. Average dissimilarity (%) between sampling trips is provided for each comparison. N.s. = non-significant. Some species have not been previously recorded (NR) for South Australia (SA) or Australia (Aust).

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
12-Oct	n.s.						
25-Oct	n.s.	n.s.					
8-Nov	n.s.	n.s.	n.s.				
22-Nov	n.s.	58.02% *Brachionus* [*angularis*] *bidens*, *Anuraeopsis fissa*, *Proalides tentaculatus*, *Asplanchna priodonta*, ***Bosmina meridionalis***, *Polyarthra dolichoptera*, ***Epistylis* sp.**, *Keratella tropica*, *Keratella cochlearis*, *Difflugia* cf. *penardi* and *Brachionus* n. sp.	n.s.	n.s.			
8-Dec	n.s.	64.16% ***Epistylis* sp.**, **indet. glob. ciliate b**, *Proalides tentaculatus*, ***Filinia pejleri***, *Arcella bathystoma*, ***Synchaeta* sp. b NR for SA**, ***Trichocerca* sp. f**, ***Centropyxis ecornis***, *Difflugia* cf. *penardi*, *Coleps* sp. and ***Cyphoderia ampulla***.	n.s.	63.64% ***Synchaeta* sp. c and b**, ***Pompholyx complanata***, *Conochilus* sp. b, ***Trichocerca* sp. f**, ***Keratella cochlearis***, ***Keratella javana***, *Difflugia* cf. *penardi*, ***Keratella tropica***, ***Filinia pejleri*** and *Proalides tentaculatus*.	n.s.		

Sampling trip	28-Sep	12-Oct	25-Oct	8-Nov	22-Nov	8-Dec	21-Dec
21-Dec	n.s.	67.21% *Proalides tentaculatus*, *Polyarthra dolichoptera*, *Asplanchna priodonta*, *Brachionus* [*angularis*] *bidens*, ***Epistylis* sp.**, *Trichocerca* cf. *agnatha* NR for SA, *Brachionus budapestinensis*, ***Trichocerca* sp. f**, *Brachionus lyratus*, *Conochilus dossuarius* and ***Synchaeta* sp. b NR for SA**.	67.40% *Conochilus dossuarius*, *Proalides tentaculatus*, ***Synchaeta* sp. b NR for SA**, *Conochilus* sp. b, *Brachionus* [*angularis*] *bidens*, *Trichocerca* cf. *agnatha* NR for SA, ***Synchaeta* sp. c**, *Polyarthra dolichoptera*, *Asplanchna priodonta*, *Brachionus budapestinensis* and *Brachionus lyratus*.	63.65% *Conochilus* sp. b, *Asplanchna priodonta*, ***Synchaeta* sp. c**, *Trichocerca* cf. *agnatha* NR for SA, ***Synchaeta* sp. b NR for SA**, ***Pompholyx complanata***, *Conochilus dossuarius*, *Proalides tentaculatus*, ***Trichocerca* sp. f**, ***Keratella javana*** and *Coleps* sp.	45.06% ***Anuraeopsis fissa***, ***Keratella tropica***, *Trichocerca* cf. *agnatha* NR for SA, ***Pompholyx complanata***, ***Keratella cochlearis***, ***Trichocerca* sp. f**, *Coleps* sp., *Conochilus dossuarius*, Ptygura sp., ***Difflugia* cf. *penardi***, cyclopoid nauplii and *Brachionus budapestinensis*.	n.s.	
11-Jan	67.36% *Polyarthra dolichoptera*, ***Epistylis* sp.**, ***Synchaeta* sp. b NR for SA**, ***Trichocerca* sp. f**, *Trichocerca* cf. *agnatha* NR for SA, *Proalides tentaculatus*, *Coleps* sp., cyclopoid nauplii, *Filinia opoliensis*, ***Difflugia* cf. *decloitrei*** and ***Stenosemella lacustris.***	65.23% *Polyarthra dolichoptera*, ***Epistylis* sp.**, *Proalides tentaculatus*, *Trichocerca* cf. *agnatha* NR for SA, *Brachionus* [*angularis*] *bidens*, ***Synchaeta* sp. b NR for SA**, *Coleps* sp., ***Stenosemella lacustris*** and **indet. glob. ciliate b**.	68.85% *Polyarthra dolichoptera*, ***Synchaeta* sp. b NR for SA**, *Proalides tentaculatus*, *Trichocerca* cf. *agnatha* NR for SA, *Brachionus* [*angularis*] *bidens*, *Coleps* sp., ***Synchaeta* sp. c**, ***Stenosemella lacustris***, **indet. glob. ciliate b**, cyclopoid nauplii and *Filinia opoliensis*.	62.06% ***Synchaeta* sp. b NR for SA**, *Polyarthra dolichoptera*, ***Synchaeta* sp. c**, *Trichocerca* cf. *agnatha* NR for SA, *Coleps* sp., ***Pompholyx complanata***, ***Trichocerca* sp. f**, *Filinia opoliensis*, ***Keratella javana*** and *Proalides tentaculatus*.	52.79% ***Anuraeopsis fissa***, *Coleps* sp., ***Keratella tropica***, *Trichocerca* cf. *agnatha* NR for SA, ***Brachionus* n. sp.**, *Filinia opoliensis*, ***Keratella cochlearis***, ***Trichocerca* sp. f**, ***Pompholyx complanata***, ***Stenosemella lacustris*** and ***Trichocerca pusilla***.	n.s.	n.s.

All environmental predictor variables for the microinvertebrate assemblage structure below Lock 1 were significant (Table G7). However, water temperature (28.6%) and turbidity (27.2%) explained most of the variation (Table G7; Figure G10). River flow and electrical conductivity were the best environmental variables to explain the horizontal separation of the data cloud, while water temperature best explained the vertical separation (Figure G13).

Table G7. DistLM marginal test results indicating which physico-chemical variable significantly contributed most the relationship with the microinvertebrate data cloud for below Lock 1. * = groups significantly different.

Variable	Pseudo-F	P	Prop.
Water temperature	8.8262	0.0001*	0.28632
Turbidity	8.2338	0.0001*	0.27234
Electrical conductivity	6.5835	0.0001*	0.23033
Mean QSA flow	6.3489	0.0001*	0.22395
Dissolved oxygen	5.3724	0.0002*	0.19627
pH	2.6542	0.009*	0.10766

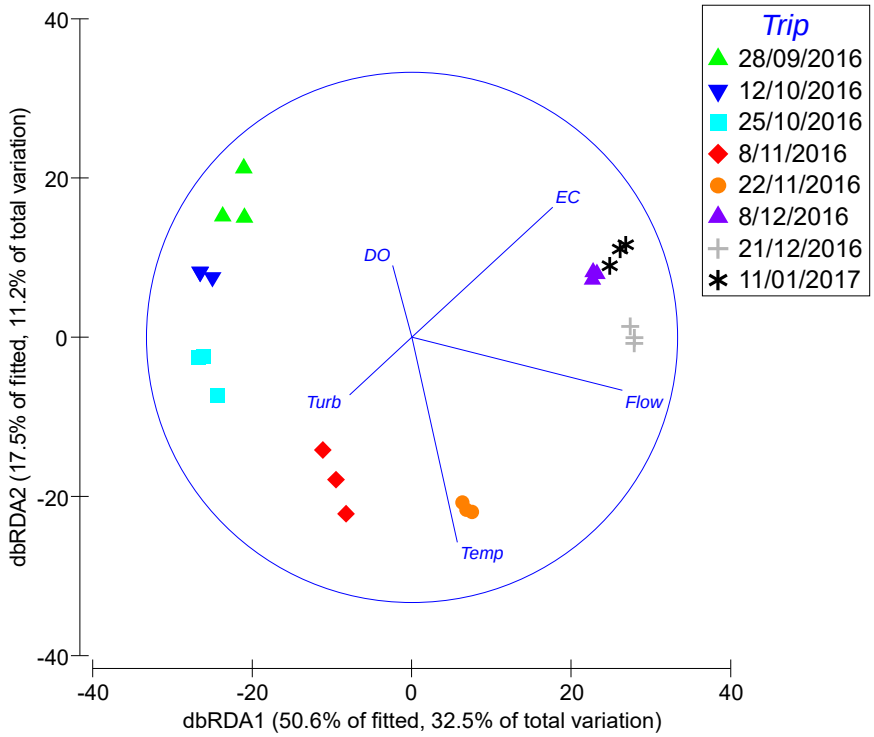


Figure G13. dbRDA ordination of the fitted model of microinvertebrate assemblage data from below Lock 1 (based on Bray-Curtis measure of log transformed data) versus the predictor variables. The vector overlay indicates multiple partial correlations (correlation coefficient > 0.2) between the predictor variables and dbRDA axes 1 and 2.

Additional weir pool monitoring sites (above and below Lock 2, and below Lock 3)

Consistent with sites below Lock 1 and Lock 6, there was high separation between sampling trips for sites in, and below, Weir Pool 2. Although the first three sampling trips from late September to late October, due to variability within the late September and late October trips (Figure G14). The microinvertebrate assemblages in, and below, Weir Pool 2 were divided into two temporal groups, separated by mid-November. No statistical analyses were performed on weir pool monitoring sites as these sites were not replicated. However, some temporal shifts in the microinvertebrate assemblages at these sites were evident, generally in accord with the temporal changes in density and diversity below Lock 6. These included protist-dominated assemblages September–October (primarily *Codonaria*), with a diverse brachionid-dominated rotifer assemblage moving through early November, and heleoplankters appearing at the Lock 2 and 3 sites, including novel brachionids, in late November. *Codonaria* and rhizopods declined through December, and were present only in small numbers by January, when warm water rotifer species appeared.

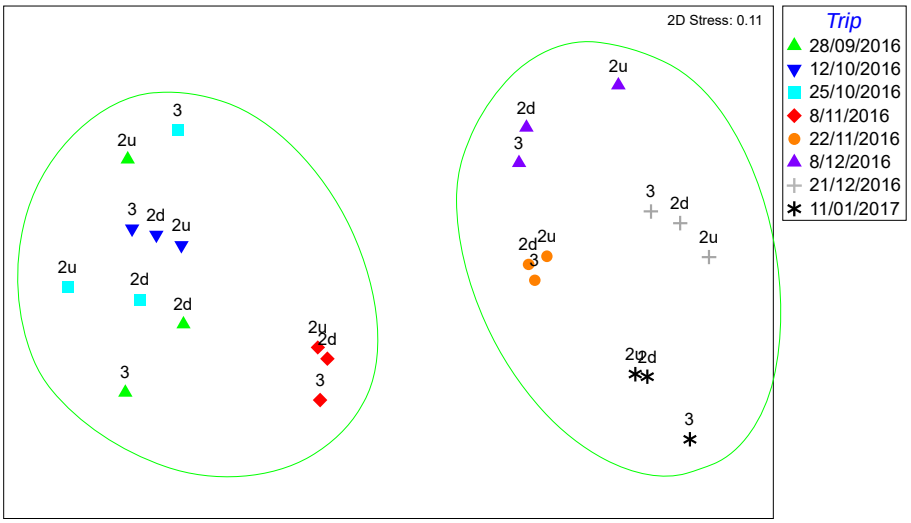


Figure G14. MDS ordination of microinvertebrate assemblage data (log transformed) from additional weir pool monitoring sites (below Lock 2 = 2d, above Lock 2 = 2u, below Lock 3 = 3), with samples identified by sampling trip. nMDS was based on Bray-Curtis Similarities. Samples are grouped at a Bray-Curtis similarity of 40% (SIMPROF).

The increasing and overbank flows impeded unequivocal interpretation of the effects of weir pool raising results in Weir Pool 2. Most of the taxa recorded for the first time from the Lock 2 and 3 samples were littoral/riparian in habit, but whether they came from weir pool margins or were transported by overbank returns from upstream is not clear.

Larval gut-content

Ambient prey assemblage

In the main channel of the lower River Murray, the microinvertebrate community is numerically dominated by protists and rotifers (Ye *et al.* 2015b; 2016a; 2017). However, the larval diets of many large-bodied fish species are comprised primarily of microcrustaceans, such as cladocerans and copepods, and insect larvae (King 2005; Kaminskas and Humphries 2009; Ye *et al.* 2015b; 2016a; 2017).

In 2016/17, the cladoceran *Bosmina meridionalis* was the most abundant microcrustacean species (Table G8). The calanoid copepod *Boeckella triarticulata* and cladocerans *Ceriodaphnia cornuta*, *Chydorus* cf. *eurynotus* and *M. micrura* were also abundant. *B. meridionalis* was highly abundant at all locks from late September to late October, and in late November at Lock 6 (Figure G15). The calanoid *B. triarticulata* and cladoceran *M. micrura* were most abundant in early December; *C. cornuta* was abundant in early January at Lock 3 and 6; and *C. eurynotus* was more abundant after mid-November. Unidentifiable copepodites and nauplii from orders Calanoida and Cyclopoida were also abundant (Table G8), with their abundances generally increasing throughout the sampling period (Figure G15).

Table G8. Mean relative abundances (ind L^{-1}) of cladocerans and copepods by lock and total (daytime only). Two species have not been recorded (NR) previously for South Australia (SA), and one for Australia (Aust).

Taxon	Lock 6	Lock 3	Lock 2 US	Lock 2 DS	Lock 1	Total
Cladocera	**18.1**	**15.0**	**14.5**	**18.8**	**15.9**	**16.7**
Bosminidae	13.1	10.9	9.9	15.6	13.3	12.8
Bosmina meridionalis	13.1	10.9	9.9	15.6	13.3	12.8
Chydoridae	0.7	0.5	1.1	1.3	1.4	1.0
Armatalona macrocopa	0.3				0.2	0.2
Chydorus cf. *eurynotus*	0.5		0.7	0.6	1.0	0.6
Pseudochydorus globosus				0.4		0.0
Pseudomonospilus diporus		0.5	0.4	0.4	0.2	0.2
Daphniidae	2.4	2.1	1.7	0.5	1.0	1.6
Ceriodaphnia cornuta	1.5	1.7	0.6	0.5	0.3	0.9
Ceriodaphnia sp.	0.1		0.6		0.5	0.3
Daphnia carinata s.l.	0.1	0.5			0.2	0.2
Daphnia galeata NR for Aust			0.5			0.1
Daphnia sp.	0.2					0.1
Simocephalus sp.	0.5					0.2
Ilyocryptidae	0.5	0.2	0.3			0.2
Ilyocryptus sp.	0.5	0.2	0.3			0.2
Macrotrichidae	0.2					0.1
Macrothrix sp.	0.2					0.1
Moinidae	0.7	0.5	0.7	1.4	0.1	0.6
Moina cf. australiensis					0.1	0.0
Moina micrura	0.5	0.5	0.7	1.4		0.5
Moina cf. tenuicornis	0.2					0.1
Neotrichidae	0.4	0.8	0.8		0.2	0.4
Neothrix sp.	0.4	0.8	0.8		0.2	0.4
Copepoda	**11.2**	**12.0**	**15.3**	**12.3**	**12.5**	**12.3**
Calanoida	5.7	10.2	11.1	10.8	4.9	7.1
Boeckella triarticulata	0.9		1.0	1.0	1.4	1.0
Calamoecia ampulla	0.1					0.0
calanoid copepodite	0.2	1.2		0.4	0.1	0.3
calanoid nauplii	4.5	9.0	10.1	9.4	3.3	5.8
Cyclopoida	5.3	1.9	4.1	1.5	7.6	5.2
Acanthocyclops cf. vernalis NR for SA	0.1					0.0
Mesocyclops notius NR for SA		0.2			0.2	0.1
Microcyclops varicans	0.2					0.1
Thermocyclops sp.	0.2					0.1
indet subadult	0.2				0.4	0.2
cyclopoid copepodite	1.1	0.4		0.5	1.2	0.9
cyclopoid nauplii	3.5	1.2	4.1	1.1	5.8	3.8
Harpacticoida	0.2					0.1
indet. harpac.	0.2					0.1

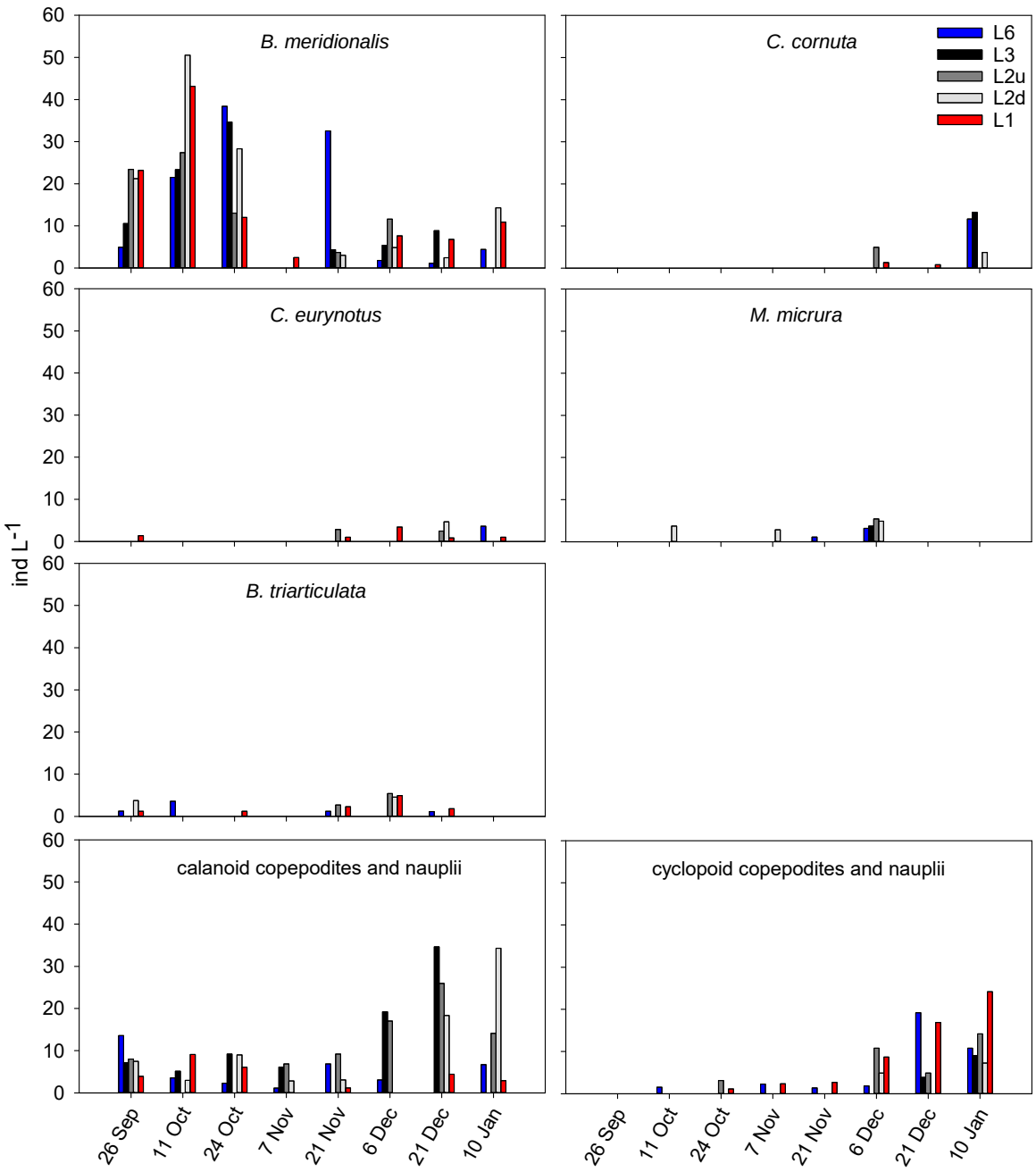


Figure G15. Mean relative abundances (ind L^{-1}) of most abundant cladocerans and copepods by trip and lock (daytime only) for 2016/17.

Larval gut analysis

This component of Category 3 Microinvertebrates aimed to determine if Commonwealth environmental water contributed to the timing of microinvertebrate productivity and presence of key species in relation to diet of large-bodied fish larvae. Gut contents of twenty Murray cod post-larvae, collected opportunistically through

larval fish sampling as part of Category 3 Fish Spawning and Recruitment (Table G9), were analysed using traditional taxonomic methods. Most Murray cod (*n* = 18) had empty guts, except the only two individuals collected on 10 January 2017 below Lock 6 (Table G9). The cladoceran *C. cornuta* was the only prey that was consumed by both individuals, which numerically contributed to 60% of the overall diet (Table G10). This was the most abundant (11.7 ind L^{-1}) prey item sampled at Lock 6 in early January (Figure G15). High abundance of this 'tropical' species (a warm stenotherm) in January, along with its absence in sampling prior to December, suggests it may have been transported from the Darling River (Shiel 1985) during increasing Darling flows to the LMR in late-December and January (involving Commonwealth environmental water and The Living Murray water) (Figure A7 in Appendix A). Alternatively, this species could have come from impounded Darling water from Lake Victoria, which was released from November 2016 (Figure A3 in Appendix A). This potentially highlights the importance of flows from other sources (e.g. Darling) contributing towards a diverse prey assemblage in the LMR for larval fishes.

Low sample sizes of larvae and patchiness of samples at temporal and spatial scales in 2016/17 (Table G8) did not allow for a quantitative comparison of fish diet to ambient microinvertebrate prey composition to determine feeding selectivity or temporal variation in feeding. In turn, the contribution of Commonwealth environmental water on the dietary composition of large-bodied fish larvae could not be evaluated.

Table G9. Catch details for Murray cod larvae that were analysed for gut-content. The Lock 1 site is situated 5 km below Lock 1. The Lock 6 and 6A sites are situated 5 and 7 km below Lock 6, respectively. The presence of food in guts is indicated by x. Total lengths (TL) were rounded to the nearest mm.

TL (mm)	Site	Date	Gut contents
11	6	7/11/2016	
11	6	7/11/2016	
12	6	7/11/2016	
12	6	7/11/2016	
12	6	7/11/2016	
12	6A	7/11/2016	
12	6	7/11/2016	
11	1	8/11/2016	
11	1	8/11/2016	
12	1	8/11/2016	
12	1	8/11/2016	
12	1	8/11/2016	
12	1	8/11/2016	
13	1	8/11/2016	
13	1	8/11/2016	
13	1	8/11/2016	
10	1	22/11/2016	
11	1	22/11/2016	
8	6	10/01/2017	X
9	6	10/01/2017	X

Table G10. Summary of gut content analysis of post-flexion Murray cod (*n* = 2; TL = 7.8 and 9.3 mm). %N represents the numerical proportion of a prey item towards the total within each species.

Prey	Presence	%N
Copepoda		
Calanoida		
Boeckella triarticulata	1/2	10
copepodites	1/2	20
Cladocera		
Daphniidae		
Ceriodaphnia cornuta	2/2	60
Chydoridae		
Chydorus cf. *eurynotus*	1/2	10

APPENDIX H: FISH SPAWNING AND RECRUITMENT

Background

Restoring flow regimes with environmental water allocations has become a central tenet of ecosystem restoration in the Murray–Darling Basin (MDB) (MDBA 2012a; Koehn *et al.* 2014). To be effective, however, flow restoration to benefit aquatic ecosystems, including fish, requires an empirical understanding of relationships between hydrology, life history and population dynamics (Arthington *et al*. 2006). Spawning and recruitment of golden perch (*Macquaria ambigua*) in the southern MDB has been associated with overbank flows and increased discharge that remains in-channel (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b). Similarly, abundant year classes of silver perch in the southern MDB correspond with increased in-channel discharge (Mallen-Cooper and Stuart 2003). As such, throughout the MDB, both golden perch and silver perch are considered candidate species to inform, and measure ecological response to, environmental water delivery.

Understanding the influence of hydrology on the population dynamics of golden perch and silver perch is reliant on accurately determining the hydrological conditions at the time and place of crucial life history processes. For example, to be able to accurately determine the hydrological conditions associated with spawning, the time and place of spawning must be known. This can be achieved by the *in situ* collection of eggs immediately post-spawning or by retrospectively determining the spatio-temporal provenance of larval, juvenile and adult fish (i.e. *when* and *where* a fish was spawned).

The Commonwealth Environmental Water Holder (CEWH) is using large volumes (>500 GL) of environmental water to augment flow regimes in the MDB to improve the health of aquatic ecosystems (Table 2). In the LMR, Commonwealth environmental water will primarily be used to contribute to increased base flows and freshes (i.e. increases in flow contained within the river channel), either complementing *natural* freshes or creating freshes (SARDI *et al.* 2016). Through the delivery of these flows, the CEWH aims to contribute to increased spawning and/or recruitment of flow-dependent fish species in the LMR.

Over the term of this project (5 years) we aim to identify potential associations between reproduction (spawning and recruitment) of native, flow-cued spawning fishes and environmental water delivery (e.g. magnitude, timing and source). The specific objectives are to compare and contrast the spawning and recruitment of golden perch in the LMR to various environmental water delivery scenarios, including identifying the timing of spawning and source (i.e. natal origin) of successful recruits to enable accurate association of ecological response with hydrology; and to explore population connectivity between regions of the southern connected MDB. We expect that: 1) increases in flow (in-channel or overbank) above regulated *entitlement* flow (QSA nominally >15,000 ML day^{-1}) in spring–summer will promote the spawning and recruitment (to young-of-year, YOY) of golden perch, and 2) multiple years of enhanced spring–summer flow will increase the resilience of golden perch populations in the LMR. The same objectives and hypotheses apply to silver perch, which are also investigated in this report; however, low sample sizes limit some analyses.

Sites

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

To determine spatio-temporal variation in water strontium (Sr) isotope ratios ($^{87}Sr/^{86}Sr$) over the spring/summer of 2016/17, water samples were collected weekly–monthly from eleven sites across the southern MDB (Table H1; Figure H1).

Table H1. Location of water sample collection for $^{87}Sr/^{86}Sr$ analysis.

River	Location	Sampling period	Total number of samples
Murray	Lock 1	12/09/16–30/01/17	11
Murray	Lock 6	13/09/16–14/02/17	11
Murray	Lock 9	27/09/16–14/02/17	11
Murray	Lock 11	12/09/16–18/02/17	12
Murray	Torrumbarry	26/09/16–13/02/17	11
Murray	Barmah	08/11/16–04/12/16	3
Darling	Weir 32	24/09/16–01/04/17	17
Edward–Wakool	Deniliquin	12/09/16–02/02/17	12
Murrumbidgee	Narrandera	05/09/16–15/02/17	10
Goulburn	Yambuna	18/10/16–06/12/16	5
Goulburn	Pyke Road	11/11/16–06/12/16	3

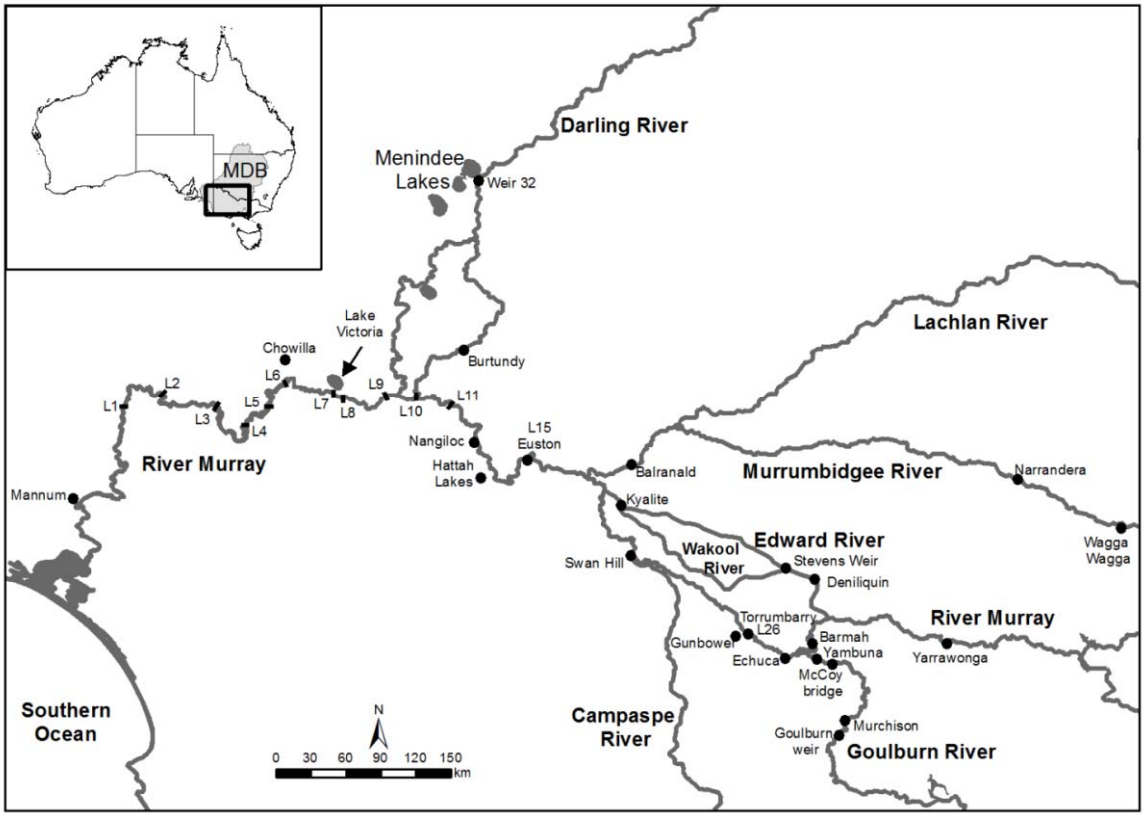


Figure H1. Map showing the location of the Murray–Darling Basin and the major rivers that comprise the southern Murray-Darling Basin, the numbered Locks and Weirs (up to Lock 26, Torrumbarry), the Darling, Lachlan, Murrumbidgee, Edward–Wakool, Campaspe and Goulburn rivers and Lake Victoria, an off-stream storage used to regulate flows in the lower Murray River.

Sampling eggs and larvae

Larval fish sampling was conducted at three sites within the floodplain and gorge geomorphic zones of the LMR (Figure 6; Table H2).

Table H2. Details of larval fish sampling sites downstream (DS) of Lock 1 and 6 in the LMR.

Zone	Site	Latitude	Longitude
Floodplain	5 km DS Lock 6	S34.01902	E140.87572
Floodplain	7 km DS Lock 6	S34.01764	E140.85461
Floodplain	9 km DS Lock 6	S34.0319	E140.84062
Gorge	5 km DS Lock 1	S34.4052	E139.61723
Gorge	7 km DS Lock 1	S34.42263	E139.61293
Gorge	9 km DS Lock 1	S34.44596	E139.61102

Sampling YOY and population age-structure

Adult and juvenile golden perch and silver perch were sampled by boat electrofishing at four and twelve sites in the floodplain and gorge geomorphic zones of the LMR, respectively, (Table H3).

Table H3. Details of boat electrofishing sites in the LMR.

Zone	Site	Latitude	Longitude
Floodplain	Murtho Forest	S34.07974	E140.75085
Floodplain	Plushes Bend	S34.22775	E140.74009
Floodplain	Rilli Island	S34.39145	E140.59164
Floodplain	Cobdogla	S34.21724	E140.36522
Gorge	Overland Corner A	S34.15942	E140.33556
Gorge	Overland Corner B	S34.1801	E140.27827
Gorge	Lowbank A	S34.18245	E140.11108
Gorge	Lowbank B	S34.1645	E140.03712
Gorge	Waikerie	S34.15823	E139.9241
Gorge	Qualco	S34.1019	E139.87569
Gorge	Cadell	S34.04371	E139.78645
Gorge	Morgan	S34.02087	E139.69016
Gorge	Scott Creek	S34.14839	E139.66095
Gorge	Blanchetown	S34.27104	E139.62602
Gorge	Swan Reach	S34.55317	E139.60809
Gorge	Caurnamont	S34.83723	E139.57341

Methods

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

Immediately after sampling, river water samples for Sr isotope analysis (unfiltered, not acidified) were refrigerated and transferred to the University of Melbourne. An aliquot (20 ml) of each sample was filtered through a pre-contaminated 0.25 µm Acrodisc syringe-mounted filter into a clean beaker, weighed, mixed with pure ^{84}Sr spike and dried overnight in a HEPA-filtered fume cupboard. Filtering in the laboratory rather than in the field simplifies sampling and avoids contamination problems. Tests with waters for which both field-filtered and laboratory-filtered splits were available showed no difference in dissolved $^{87}Sr/^{86}Sr$ even after periods of several months between collection and laboratory filtering. This is consistent with the findings of Palmer and Edmond (1989).

Strontium was extracted from filtered water samples using a single pass over a small (0.15 ml) bed of ElCHROM Sr resin (50–100 µm). Following Pin *et al*. (1994), samples were loaded in 2M nitric acid, followed by removal of matrix elements from the resin with 2M and 7M nitric acid, and collection of a Sr fraction in 0.05M nitric acid. The total blank, including syringe-filtering, is ≤0.1 ng, implying sample to blank ratios of ≥4000; blank corrections were therefore insignificant. Strontium isotope ratios were measured on a "Nu Plasma" multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS, Nu Instruments, Wrexham, UK), with sample uptake via an ARIDUS desolvating nebulizer. Instrumental mass bias was corrected by normalising to $^{88}Sr/^{86}Sr$=8.37521 using the exponential law as part of an on-line iterative spike-stripping/internal normalisation procedure, and $^{87}Sr/^{86}Sr$ results reported relative to a ratio of 0.710230 for the SRM987 Sr isotope standard. A typical analysis (at least 30 ten-second integrations) has an internal within-run precision of 0.000020 (±2se) while the external precision of the data is ±0.000040 (2sd). The rock standards BCR-2 and BHVO-2 average 0.704996±51 (2sd) and 0.703454±43 (2sd), respectively, while modern seawater Sr (coral EN-1 from Enewetak Atoll) averages 0.709155±37 (2sd); all results are consistent with published TIMS and MC-ICPMS reference data.

Sampling eggs and larvae

Larval fish sampling was conducted approximately fortnightly between 11 October 2016 and 11 January 2017. Three day-time and three night-time plankton tows were undertaken on the same day at sites 5 km below each lock, while one day-time plankton tow was undertaken at all other sites (Table H2). For each sampling trip, sites were sampled within a two-day period. 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 (Figure H2). 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. Fish in all 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).

Sampling YOY and population age-structure

Adult and juvenile golden perch (and silver perch) were sampled by boat electrofishing using a 7.5 kW Smith Root (Model GPP 7.5) electrofishing unit (Figure H3). Sampling was undertaken in April and July/August 2017 to maximise the likelihood of collecting YOY spawned in the spring–summer 2016/17 spawning season. Electrofishing was conducted during daylight hours and all available littoral habitats were fished. At each site the total time during which electrical current was applied ranged from approximately 676 to 2880 seconds. All individuals were measured to the nearest mm (total length, TL) and a subsample of golden perch (n = 47–66) proportionally representing the length-frequency of golden perch collected from the gorge and floodplain geomorphic zones of the LMR was retained for ageing. All silver perch (n = 6) collected from floodplain geomorphic zone were retained for ageing, whilst no silver perch were caught in the gorge geomorphic zone.

Ageing

Larvae and YOY

To estimate the spawn date of larval and YOY golden perch and silver perch, daily increment counts in otolith microstructure were examined. Larvae/juveniles were measured to the nearest millimetre and sagittal otoliths were removed. Otoliths were mounted individually in Crystalbond™, proximal surface downwards, and polished down towards the primordium (within 20 μm of the core) using a graded series of wetted lapping films (15, 9 and 3 μm). Crystalbond was heated and otoliths were flipped with proximal surface facing upwards. Otoliths were then polished down towards the primordium (within 20 μm of the core) using a graded series of wetted lapping films (15, 9 and 3 μm).

Sections were examined using a compound microscope (x 400) fitted with a digital camera and Olympus Stream image analysis software (version 1.9.1, Olympus Corporation, Munster, Germany). Increments were counted blind with respect to fish length and capture date. Estimates of age were determined by counting the number of increments from the primordium to the otolith edge (Figure H3). Three successive counts were made by two readers for one otolith from each fish. If these differed by more than 10%, or differed by more than 3 days in the case of very young fish (<30 days), the otolith was rejected, but if not, the mean was used as an estimate of the number of increments. Increment counts were considered to represent true age of larval and juvenile golden perch (Brown and Wooden 2007) and spawn dates were determined by subtracting the estimated age from the capture date (Zampatti and Leigh 2013a; 2013b).

Juveniles and adults

Golden perch exhibit considerable variation in length-at-age in the MDB (Anderson *et al.* 1992). Therefore, to accurately assess the age structure and year-class strength of golden perch (and silver perch), we investigated both length and age-frequency distributions. Golden perch (n = 113) and silver perch (n = 6) 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 (x 25) 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

Larvae, YOY and adult otolith preparation

Sagittal otoliths were dissected and mounted individually in Crystalbond™, proximal surface upwards, on an acid-washed glass slide and polished down towards the primordium (within 20 µm of the core) using a graded series of wetted lapping films (9, 5 and, 3 µm). The slide was then reheated and the polished otolith transferred to a 'master' slide, on which otoliths from all collection sites were combined and arranged randomly to remove any systematic bias during analysis. The samples were rinsed in Milli-Q water (Millipore) and air dried overnight in a class 100 laminar flow cabinet at room temperature.

LA-ICPMS

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 experimental system consisted of a 'Nu Plasma' multi-collector ICPMS (Nu Instruments, Wrexham, UK), coupled to a 'RESOlution' 193 nm excimer laser ablation system (formerly Resonetics, USA, now distributed by Australian Scientific Instruments, Canberra). Otolith mounts were placed in the sample cell and the primordium of each otolith was located visually via a 400× objective and video imaging system. The intended ablation path on each sample was digitally plotted using GeoStar v6.14 software (Resonetics, USA). After pre-ablation to clean the surface of the intended analysis path, and a 20–30 sec background measurement, each otolith was ablated along a transect from the primordium to the dorsal margin at the widest radius using a 6 × 100 um rectangular laser slit. The laser was operated with a fluence of around 2-3 Jcm^{-2}, pulsed at 10 Hz and scanned at 5 and 10 µm sec^{-1} (depending on the size of the otolith) across the sample. Ablation was performed under a pure helium (He) atmosphere followed by rapid transport of the ablated products to the MC-ICPMS in the argon carrier gas. After online correction for isobaric interferences (Kr, Rb, Ca argides, Ca dimers) and mass bias (internal normalisation to $^{88}Sr/^{86}Sr$ = 8.37521, Woodhead *et al*. 2005), further data reduction was done offline using the Iolite software (v.2.13, Paton *et al*. 2011).

A modern marine mollusc shell was analysed during set-up and after every 10 otolith ablations, to check data accuracy and reproducibility. Solution-mode Sr isotope data for this shell indicate a $^{87}Sr/^{86}Sr$ of 0.70916, identical to the composition of modern seawater Sr (0.709160, MacArthur and Howarth, 2004, relative to SRM987 = 0.710230). Typical within-run precision of individual ablations of this mollusc shell was ± 0.00005 (±2se), and $^{87}Sr/^{86}Sr$ averaged 0.70918±0.00017 (±1sd, *n* = 24).

Results

Water $^{87}Sr/^{86}Sr$ and hydrology

Water sample collection commenced in early September 2016 and extended, at the majority of sites, through until early February 2017. Throughout the period of collection, water $^{87}Sr/^{86}Sr$ remained reasonably stable in the Darling River and the Murray River, and its tributaries, upstream of the Darling River junction, with the highest ratios (>0.7190) measured in the Murray River at Barmah and the Edward River, and the lowest (<0.7080) in the Darling River (Figure H4). Water $^{87}Sr/^{86}Sr$ in the lower River Murray was temporally variable, with water $^{87}Sr/^{86}Sr$ generally decreasing with increased Darling River discharge (Figure H5). Water $^{87}Sr/^{86}Sr$ also generally decreased longitudinally along the Murray River as tributaries with distinct and relatively temporally stable $^{87}Sr/^{86}Sr$ (e.g. Goulburn River) contribute to discharge. There was, however, overlap in water $^{87}Sr/^{86}Sr$ between some tributary and main-stem Murray River sites; for example, $^{87}Sr/^{86}Sr$ in the Murrumbidgee River was similar to $^{87}Sr/^{86}Sr$ at Lock 9 in the lower River Murray from late October to mid-December, and Lock 6 in the LMR in late September and early November. Water $^{87}Sr/^{86}Sr$ was most variable at Lock 6 in the LMR (0.7097–0.7162), particularly after mid-January 2017 (Figure H4).

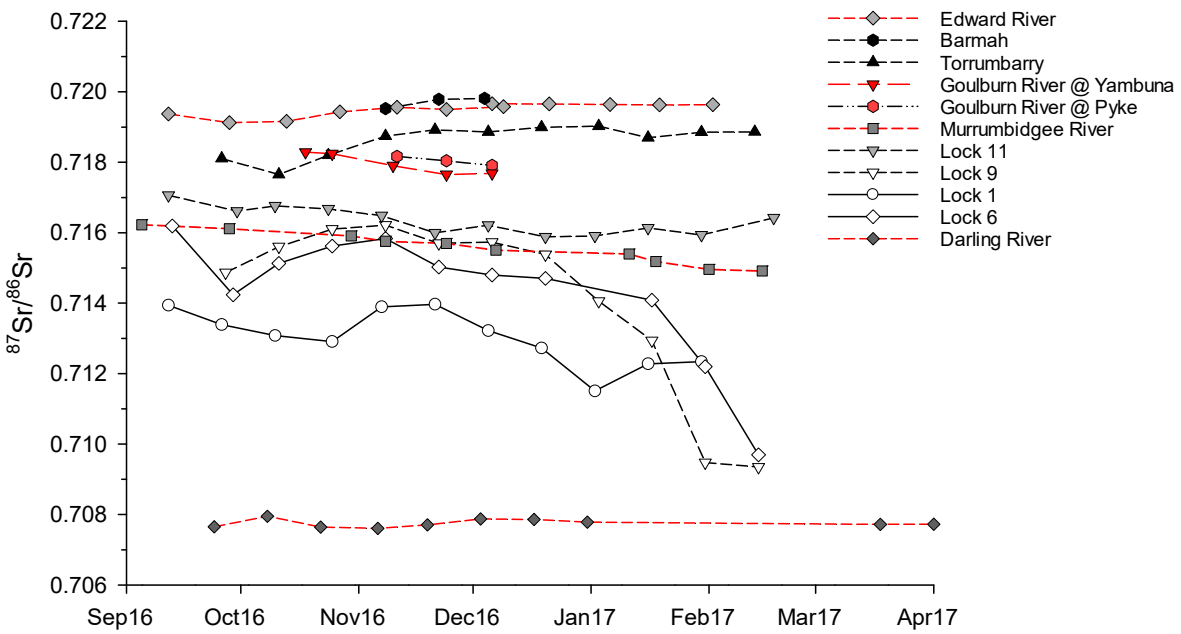


Figure H4. $^{87}Sr/^{86}Sr$ ratios in water samples collected from mid-September 2016 to April 2017 in the Murray (Lock 1, 6, 9, 11 Torrumbarry and Barmah), Darling, Goulburn, Edward and Murrumbidgee rivers.

From mid-September 2016 to early March 2017, flow in the LMR (discharge at the South Australian border, QSA) ranged approximately 7,500–94,600 ML day^{-1} (Figure H5). From mid-September to late October 2016, flow ranged 33,100–48,500 ML day^{-1} before increasing to a peak of ~94,600 ML day^{-1} in late November 2016. Shortly after this peak, flow sharply decreased to 17,300 ML day^{-1} in early January 2017, before declining further to 7,500 ML day^{-1} by early March 2017. QSA was mainly comprised of flow from the upper Murray River, Murrumbidgee River and Victorian tributaries of the Murray River from July to mid-December 2016 (Figure 5). From mid-December 2016 to mid-May 2017, however, the relative proportion of flow from the Darling River increased.

Flow in the mid-reaches of the Murray River at Euston was similar to flow in the LMR, peaking at 113,300 ML day^{-1} in mid-November 2016 then rapidly decreasing to 6,200 ML day^{-1} in late January 2017, where it remained <7,000 ML day^{-1} through the remainder of summer (Figure H5). Flow in the Darling River at Burtundy ranged approximately 800–1,800 ML day^{-1} from mid-September 2016 to mid-January 2017, before increasing to a peak of 5,100 ML day^{-1} in late January 2017 (Figure H5). Flow then steadily decreased to 1,300 ML day^{-1} by early April 2017.

From July to mid-December 2016, during high unregulated flows, the contribution of Commonwealth environmental water (excluding South Australian held entitlement

flow) to flow at the South Australian border remained <300 ML day^{-1}. No Commonwealth environmental water (on top of South Australian held entitlement flow) was delivered from 20 August to 12 November 2016. From mid-December 2016 to early March 2016, the contribution of Commonwealth environmental water to flow at the South Australian border ranged 1,100–8,100 ML day^{-1}, peaking at ~8,100 ML day^{-1} on 22 December 2016 and then at ~7,700 ML day^{-1} on 19 January 2017 (Figure 3). Environmental water from the MDBA's The Living Murray program was delivered during several events from 8 September 2016 to 21 June 2017. The largest volume was delivered from 11 December 2016 and 31 January 2017 (~156 GL), peaking at ~8,200 ML day^{-1} on 27 December 2016 (Figure 3).

From September to mid-December 2016, $^{87}Sr/^{86}Sr$ in water samples collected from Lock 9, 6 and 1 in the lower River Murray reflected water delivery from the mid-Murray River. From January to mid-February 2017, water $^{87}Sr/^{86}Sr$ at Lock 9 and 6 reflected increased discharge from the Darling River (Figures H4 and H5).

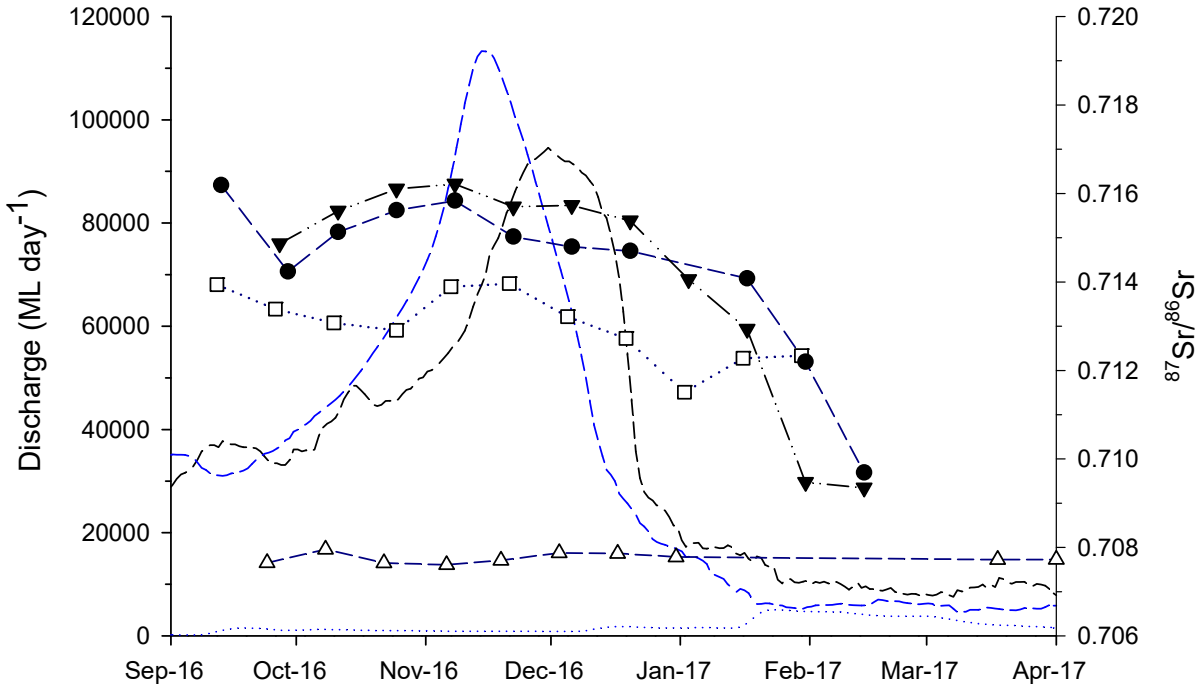


Figure H5. Mean daily discharge (ML day^{-1}) in the Murray River at the South Australian border (dashed black line) and Euston (dashed blue line). $^{87}Sr/^{86}Sr$ in water samples collected from mid-September 2016 to April 2017 in the lower River Murray at Lock 9 (solid triangles), Lock 6 (solid circles) and Lock 1 (open squares), and the Darling River at Menindee (Weir 32) (open triangles).

Larval fish assemblage

A total of 655 larvae from five small-bodied species and 198 larvae from five large-bodied species were sampled by plankton tows from three sites in each of the gorge and floodplain geomorphic zones (combined) of the LMR (Tables H2 and H4). This was considerably less than the total abundances of small- and large-bodied species sampled in 2015/16 (18,499 and 3,736 larvae, respectively) due to reduced abundance of all small-bodied species and bony herring in 2016/17. Flathead gudgeons (*Philypnodon* spp.) and carp gudgeon were the most abundant small-bodied species, while common carp was the most abundant large-bodied species. No silver perch or freshwater catfish (*Tandanus tandanus*) were sampled in 2016/17.

Table H4. Total catches from larval fish sampling conducted between 11 October 2016 and 11 January 2017. Three day-time and three night-time plankton tows were undertaken on the same day at sites 5 km downstream (DS) each lock, while one day-time plankton tow was undertaken at sites that were 7 km and 9 km downstream each lock.

	Lock 1				Lock 6				
Site	5km DS	7 km DS	9 km DS	**Total**	5km DS	7 km DS	9 km DS	**Total**	**Grand total**
Small-bodied									
Flatheaded gudgeons#	207	21	14	**242**	173	32	26	231	**473**
Carp gudgeon	55	4	8	**67**	60	12	4	76	**143**
Australian smelt	6	0	0	**6**	20	3	5	28	**34**
Unspecked hardyhead	0	0	0	**0**	1	0	0	1	**1**
Gambusia	0	0	0	**0**	3	1	0	4	**4**
Large-bodied									
Common carp	44	13	13	**70**	38	5	12	55	**125**
Bony herring	10	0	0	**10**	20	0	4	24	**34**
Murray cod	11	0	0	**11**	8	0	0	8	**19**
Golden perch	3	0	0	**3**	14	2	0	16	**19**
Golden perch eggs*	*	*	*	*	*	*	*	*	*
Perch hatchlings*	*	*	*	*	*	*	*	*	*

'Flatheaded gudgeons' include flatheaded gudgeon (*Philypnodon grandiceps*) and dwarf flatheaded gudgeon (*Philypnodon macrostomus*).

* Fish eggs suspected to be golden or silver perch were collected below Lock 1 from 12 October to 22 November 2016 and below 6 from early 11 October to 7 November 2016. These eggs were hatched out and confirmed to be golden perch. Perch hatchlings were golden perch or silver perch that were too small to be identified to species. Their presence or absence is indicated in the table.

Golden perch and silver perch larval collection and spawn dates

In 2016/17, 19 golden perch larvae were collected in the gorge and floodplain geomorphic zones of the LMR (Table H5). The majority of larvae (*n* = 14) were collected at Lock 6 on 10 January 2017, while two larvae were collected on 24 October 2016 at Lock 6 and one individual was collected from Lock 1 on 25 October 2016, 22 November 2016 and 10 January 2017. Ages of these larvae ranged 2 (pre-flexion) to 27 days (post-flexion), corresponding to spawn dates of 22–23 October 2016, 20 November 2016, 14 December 2016, 24 December 2016–1 January 2017 and 7 January 2017 (Table H5; Figure H6). Two YOY golden perch were also collected by fyke nets between Lock 2 and 3 (gorge geomorphic zone) on 4–5 April 2017 (Table H5). Age of the smallest YOY golden perch (47 mm) was 124 days, corresponding a spawn date of 2 December 2016 (Table H5; Figure H6). The larger fish (61 mm) was unable to be accurately aged (at a daily resolution).

Table H5. Capture location and date, length (mm), age (days), spawn date and otolith core $^{87}Sr/^{86}Sr$ values for larval golden perch collected from the floodplain and gorge geomorphic zones of the LMR by larval tows and fyke nets (*). It was not possible to obtain a daily age and spawn date for the largest (61 mm) golden perch. Red text indicates that age was estimated based on ages of golden perch with similar total lengths.

Species	Zone	Capture location	Capture date	Length (mm)	Age (days)	Spawn date	$^{87}Sr/^{86}Sr$
Golden perch	Floodplain	Lock 6	24/10/2016	5	2	22/10/2016	
Golden perch	Floodplain	Lock 6	24/10/2016	5	2	22/10/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	8	12	29/12/2016	0.7146
Golden perch	Floodplain	Lock 6	10/01/2017	6.5	11	30/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	7	15	26/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	10	17	24/12/2016	0.7145
Golden perch	Floodplain	Lock 6	10/01/2017	8	13	28/12/2016	0.7142
Golden perch	Floodplain	Lock 6	10/01/2017	7.5	11	30/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	7	13	28/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	6	15	26/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	8	11	30/12/2016	0.7145
Golden perch	Floodplain	Lock 6	10/01/2017	7	14	27/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	8	14	27/12/2016	0.7143

Species	Zone	Capture location	Capture date	Length (mm)	Age (days)	Spawn date	$^{87}Sr/^{86}Sr$
Golden perch	Floodplain	Lock 6	10/01/2017	6.5	11	30/12/2016	
Golden perch	Floodplain	Lock 6	10/01/2017	5.5	9	1/01/2017	
Golden perch	Floodplain	Lock 6	10/01/2017	13	27	14/12/2016	0.7078
Golden perch	Gorge	Lock 1	25/10/2016	5	2	23/10/2016	
Golden perch	Gorge	Lock 1	22/11/2016	4.5	2	20/11/2016	
Golden perch	Gorge	Lock 1	11/01/2017	4	4	7/01/2017	
Golden perch	Gorge	Lowbank d/s*	4/04/2017	61			0.7140
Golden perch	Gorge	Overland Corner d/s*	5/04/2017	47	124	2/12/2016	0.7096

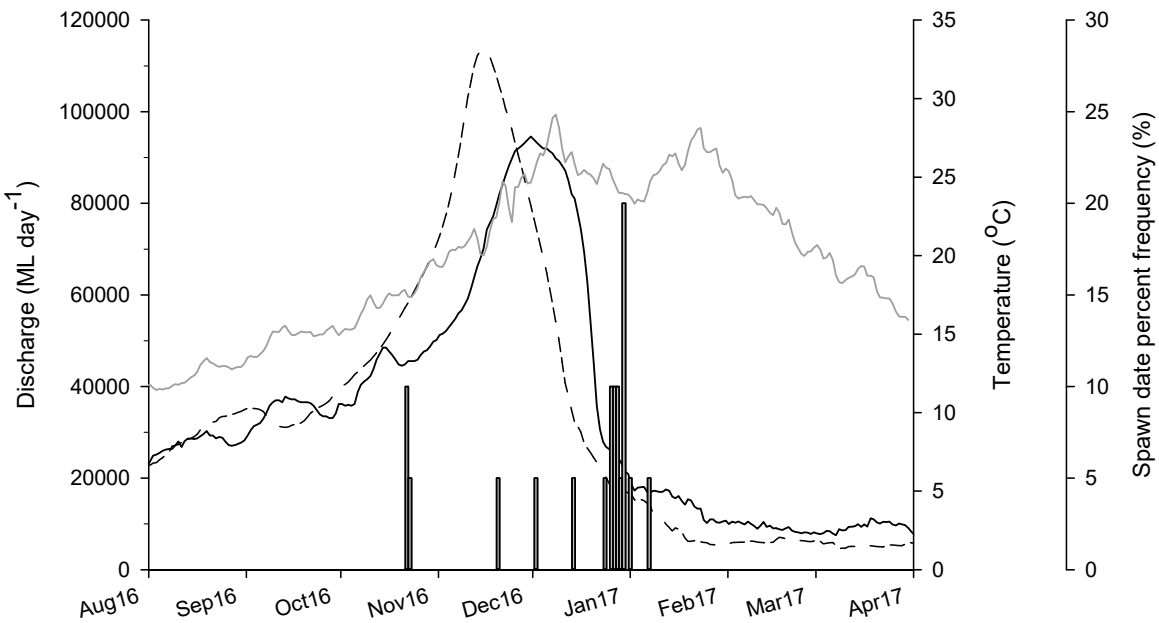


Figure H6. Back-calculated spawn dates for larval and young-of-year golden perch (grey bars; *n* = 20) captured from the LMR during 2016/17, plotted against discharge (ML day^{-1}) in the Lower Murray River at the South Australian border (solid black line) and Euston (dashed black line), and water temperature (°C) (grey line).

Otolith $^{87}Sr/^{86}Sr$ of larval golden perch and silver perch

A sample of eight of the 21 golden perch larvae/YOY were analysed for $^{87}Sr/^{86}Sr$ (Table H5). The otoliths of most remaining larval golden perch were too small for LA-ICPMS

analysis. Larvae collected at Lock 6 on 10 January 2017, which were spawned 24–30 December 2016, had otolith core $^{87}Sr/^{86}Sr$ indicative of the lower River Murray in the vicinity, or in the region upstream (i.e. Lock 6–Lock 9), of their capture location, below Lock 6 (i.e. 0.7142–0.7146) (Table H5; Figure H7). In contrast, one larvae collected at Lock 6 on 10 January 2017, which had a spawn date of 14 December 2016, had otolith core $^{87}Sr/^{86}Sr$ of 0.7078, indicative of the Darling River.

The two YOY golden perch collected between Lock 2 and 3 in early April 2017 had different otolith core $^{87}Sr/^{86}Sr$. The YOY golden perch (47 mm, 124 days old) that was spawned on 2 December 2016 exhibited otolith core $^{87}Sr/^{86}Sr$ slightly greater than the Darling River, but lower than Murray River water $^{87}Sr/^{86}Sr$ values (Figure H7), suggesting this fish may have been spawned in the Murray River close to the Darling confluence. The larger YOY golden perch (61 mm) with unknown spawn date had otolith core $^{87}Sr/^{86}Sr$ (0.7140) similar to those larvae spawned on 24–30 December 2016, indicative of the LMR, in the region of Lock 1–Lock 6 (Table H5; Figure H7).

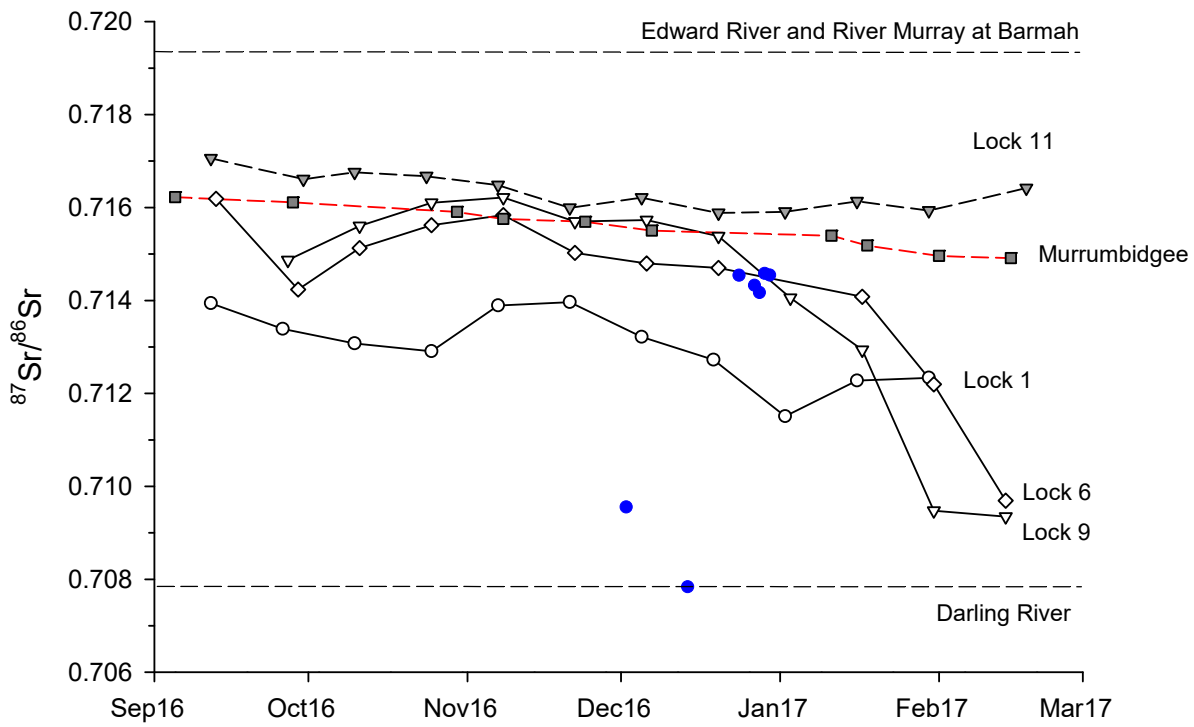


Figure H7. $^{87}Sr/^{86}Sr$ in water samples collected from late September 2016 to late February 2017 at sites in the southern MDB. $^{87}Sr/^{86}Sr$ in the Darling River and Edward River/Murray River at Barmah are presented as dashed straight lines as these were temporally stable and represent the maximum and minimum $^{87}Sr/^{86}Sr$ measured in water samples in the southern MDB in 2016/17. Closed blue circles represent spawn date and otolith core $^{87}Sr/^{86}Sr$ of larval golden perch collected in the LMR from December 2016 to January 2017.

Transects of $^{87}Sr/^{86}Sr$ from the otolith core to edge can elucidate the movement history of golden perch from birth to death, but may also reflect temporal variability in ambient $^{87}Sr/^{86}Sr$ in water. The transect of otolith $^{87}Sr/^{86}Sr$ for the YOY golden perch captured below Lock 3 in early April 2017 (spawned 2 December 2016) indicates a spawning origin between the Darling River and Lock 9 (Figure H7) and subsequent movement downstream to its capture location in the vicinity of Lock 3 (Figure H8b). In contrast, the transect of otolith $^{87}Sr/^{86}Sr$ for the other YOY golden perch captured between Lock 3 and 2 (unknown spawn date) indicates that this individual was spawned in the lower River Murray, likely between Lock 1 and Lock 6 (Figure H7), and moderation of otolith $^{87}Sr/^{86}Sr$ represents downstream movement and/or variation in lower Murray River water $^{87}Sr/^{86}Sr$ over the fish's life (Figure H8a).

Transects of otolith $^{87}Sr/^{86}Sr$ for the five golden perch larvae captured below Lock 6 that were spawned 24–30 December 2016 indicated that these individuals were spawned in the lower River Murray, likely above Lock 6, and remained in this region throughout their early life (e.g. Figure H8c). In contrast, the transect of otolith $^{87}Sr/^{86}Sr$ for the 27 day old golden perch larvae captured below Lock 6, with a spawn date of 14 December 2016, indicated that this individual was spawned in the Darling River and subsequently moved (passively/actively) to the capture location in the Murray River below Lock 6 (Figure H8d).

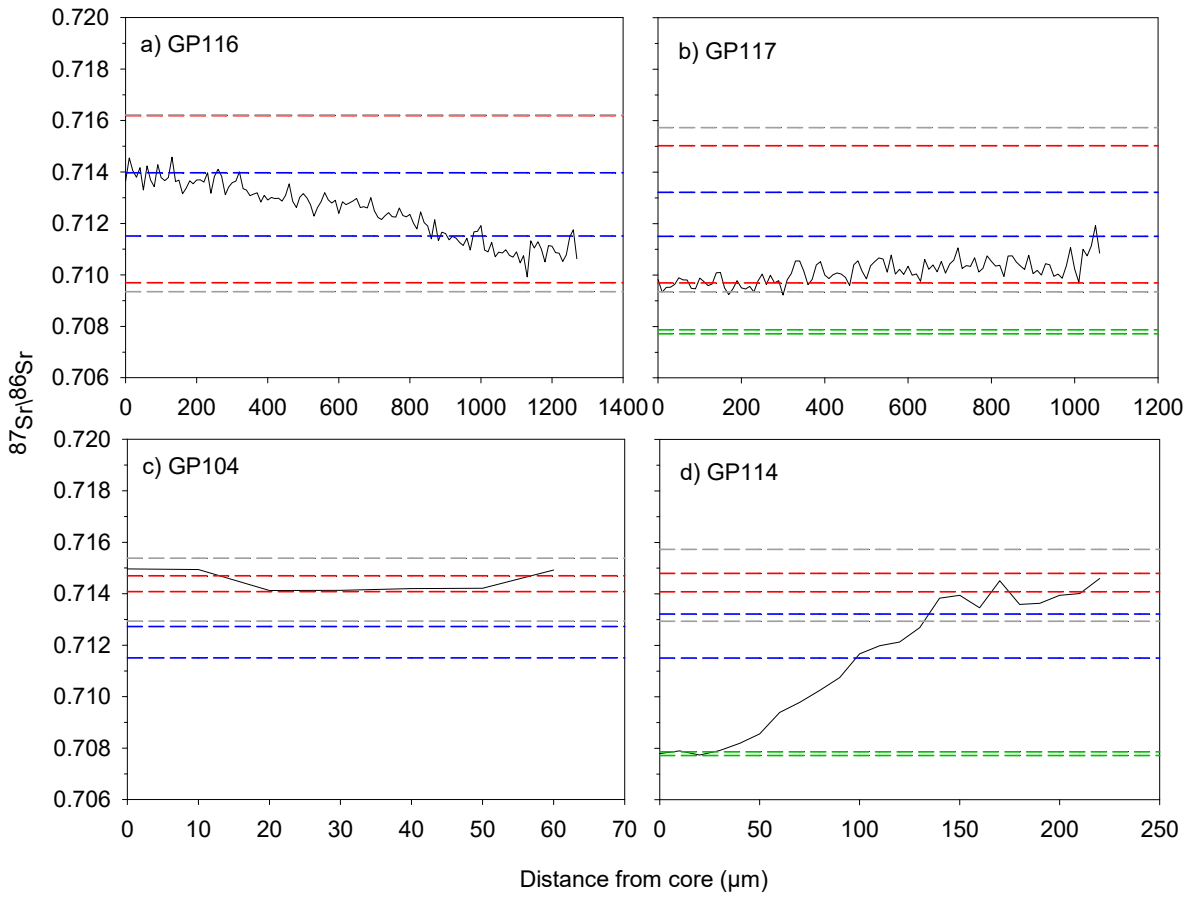


Figure H8. Individual life history profiles based on otolith Sr isotope transects (core to edge) for young-of-year golden perch aged (a) unknown and (b) 124 days, collected between Lock 2 and 3 in the gorge zone of the LMR, and golden perch larvae aged (c) 17 days and (d) 27 days, collected below Lock 6 in the floodplain zone of the LMR. Dashed lines denote minimum and maximum $^{87}Sr/^{86}Sr$ ratios in the Murray River at Lock 1 (blue), Lock 6 (red) and Lock 9 (grey), and in the Darling River (green) between spawn and capture dates of each individual. Note that the four other larvae that were analysed, which were spawned 24–30 December 2016, had similar otolith Sr isotope transects to the 17 day old larvae in (c).

Golden perch and silver perch length and age structure

In 2017, no YOY golden perch or silver perch were collected during Category 1 and 3 Fish LTIM electrofishing in the LMR. Although two YOY golden perch (47 and 61 mm TL) were collected during Category 1 Fish LTIM fyke netting. Golden perch sampled in the gorge and floodplain geomorphic zones of the LMR ranged in age from 0+ to 20+ years, with dominant cohorts of age 5+, 6+ and 7+ fish, spawned in 2011/12, 2010/11 and 2009/10 and, respectively. Age 6+ fish comprised 51 and 33% of the sampled population in the floodplain and gorge geomorphic zones, respectively. Age 7+ fish comprised 9 and 30% of the population in the floodplain and gorge zones, respectively, whilst age 5+ fish comprised 26 and 15% in the floodplain and gorge

zones, respectively (Figure H9). In the gorge geomorphic zone, age 16+ and 20+ fish spawned in 2000/01 and 1996/97 collectively comprised 11% of the sampled population (Figure H9).

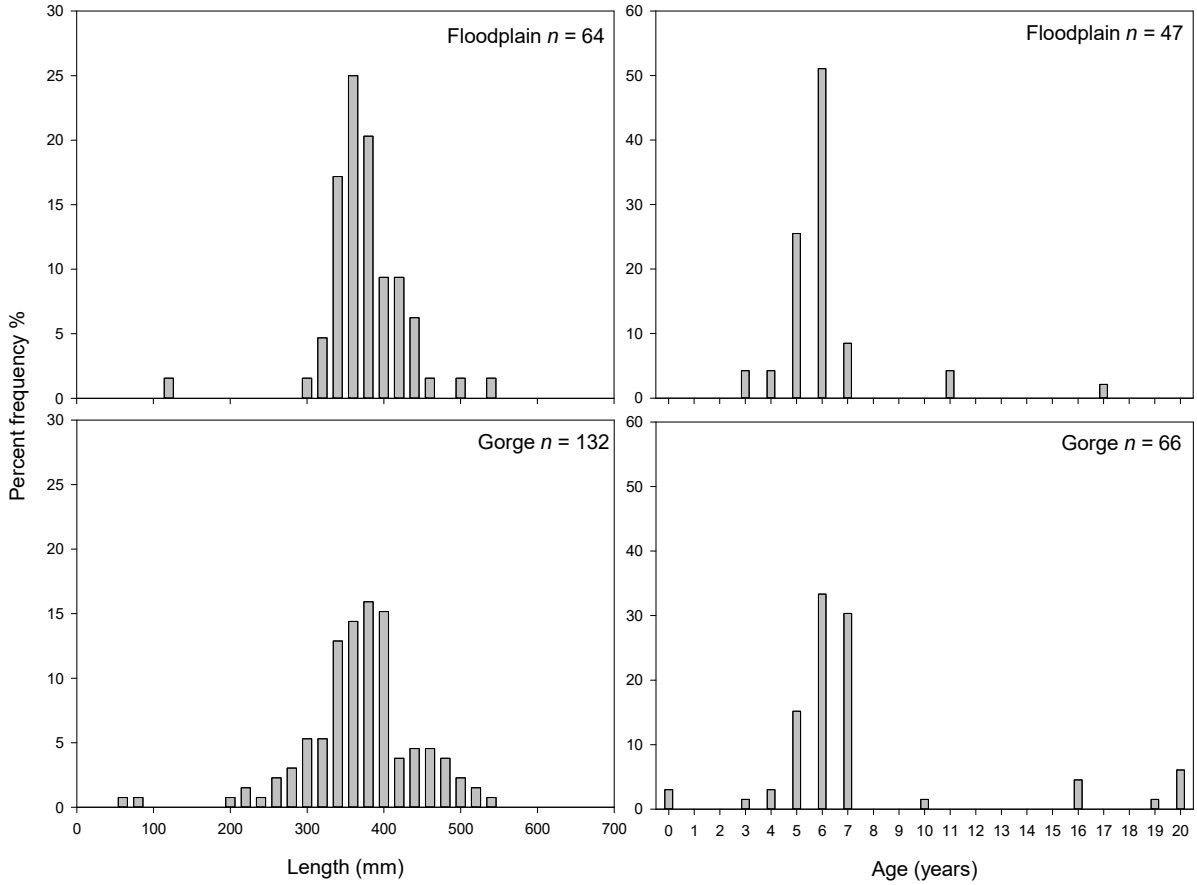


Figure H9. Total length (left column) and age (right column) frequency distribution of golden perch collected by boat electrofishing from the floodplain (top) and gorge (bottom) geomorphic zones of the LMR in autumn/winter 2017.

In 2017, no silver perch were sampled from the gorge geomorphic zone of the LMR, whilst low numbers (*n* = 6) were sampled from the floodplain geomorphic zone. Silver perch sampled in the floodplain geomorphic zone ranged from age 3+ to 7+ (Figure H10).

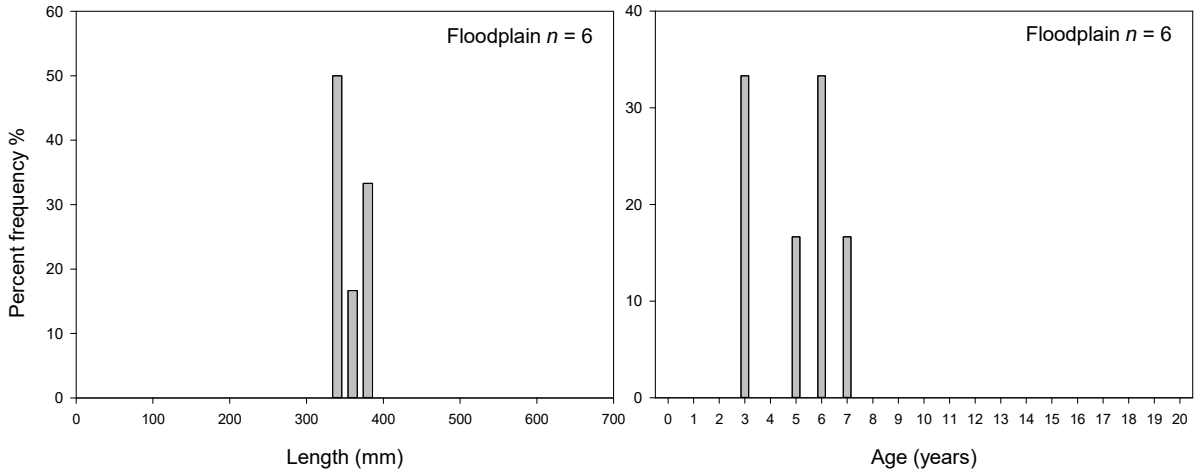


Figure H10. Fork length (left column) and age (right column) frequency distribution of silver perch collected by boat electrofishing from the floodplain geomorphic zone of the LMR in autumn/winter 2017. No silver perch were captured in the gorge geomorphic zone.

Otolith $^{87}Sr/^{86}Sr$, natal origin and migration history of golden/silver perch

Golden perch

To investigate the natal origin and migration history of dominant cohorts (Figure H9) of golden perch in the LMR (gorge and floodplain geomorphic zones) in 2016/17, we analysed $^{87}Sr/^{86}Sr$ from the otolith core to edge in a subsample of fish from age 3+ (*n* = 3), 4+ (*n* = 4), 5+ (*n* = 20), 6+ (*n* = 20) and 7+ (*n* = 20) cohorts (Table H6; Figures H13–17). We compared these transects to water $^{87}Sr/^{86}Sr$ measured at sites across the southern MDB from 2011–2017 (Zampatti *et al.* 2015; this report; SARDI unpublished data) (Figures H11 and H12).

Table H6. Capture location and geomorphic zone, length (mm), age (years) and spawn year, and otolith core $^{87}Sr/^{86}Sr$ of 67 golden perch collected from the lower River Murray in 2017. Life history profiles are shown for individuals marked with *.

Zone	Capture location	Length (mm)	Age (years)	Spawn year	Core $^{87}Sr/^{86}Sr$
Floodplain	Overland Corner u/s	193	3	2013/14	0.711679
Floodplain	Murtho Forest	352	3	2013/14	0.710644
Floodplain	Plushes Bend	336	3*	2013/14	0.710018
Floodplain	Overland Corner u/s	278	4	2012/13	0.710638
Floodplain	Plushes Bend	395	4*	2012/13	0.707638
Floodplain	Plushes Bend	322	4	2012/13	0.709814
Gorge	Qualco	293	4*	2012/13	0.710207
Floodplain	Overland Corner u/s	260	5	2011/12	0.707105
Floodplain	Overland Corner u/s	326	5	2011/12	0.707304
Floodplain	Cobdogla	362	5	2011/12	0.707095
Floodplain	Cobdogla	341	5	2011/12	0.707095
Floodplain	Murtho Forest	325	5	2011/12	0.707052
Floodplain	Murtho Forest	394	5	2011/12	0.707295
Floodplain	Plushes Bend	301	5	2011/12	0.713576
Floodplain	Plushes Bend	364	5*	2011/12	0.7131
Floodplain	Rilli Island	387	5	2011/12	0.707209
Floodplain	Rilli Island	361	5	2011/12	0.707416
Floodplain	Rilli Island	335	5	2011/12	0.707244
Floodplain	Rilli Island	321	5	2011/12	0.712
Gorge	Qualco	295	5	2011/12	0.707299
Gorge	Lowbank u/s	445	5	2011/12	0.708532
Gorge	Lowbank u/s	295	5	2011/12	0.707089
Gorge	Lowbank u/s	312	5	2011/12	0.706983
Gorge	Caurnamont	362	5	2011/12	0.709594
Gorge	Caurnamont	368	5	2011/12	0.707762
Gorge	Caurnamont	381	5	2011/12	0.707288
Gorge	Blanchetown	403	5*	2011/12	0.707202
Floodplain	Cobdogla	365	6	2010/11	0.70909
Floodplain	Cobdogla	398	6	2010/11	0.707244
Floodplain	Murtho Forest	359	6	2010/11	0.708791
Floodplain	Murtho Forest	371	6	2010/11	0.713056
Floodplain	Murtho Forest	431	6	2010/11	0.707363
Floodplain	Plushes Bend	344	6	2010/11	0.711912
Floodplain	Plushes Bend	318	6	2010/11	0.712293
Floodplain	Rilli Island	311	6	2010/11	0.71185
Floodplain	Rilli Island	366	6	2010/11	0.71149
Floodplain	Rilli Island	338	6	2010/11	0.707958
Gorge	Qualco	360	6	2010/11	0.707314
Gorge	Scott's Creek	340	6	2010/11	0.712334
Gorge	Scott's Creek	255	6	2010/11	0.710989

Zone	Capture location	Length (mm)	Age (years)	Spawn year	Core $^{87}Sr/^{86}Sr$
Gorge	Overland Corner d/s	325	6	2010/11	0.711867
Gorge	Lowbank u/s	345	6*	2010/11	0.707294
Gorge	Swan Reach	290	6	2010/11	0.711127
Gorge	Cadell	365	6*	2010/11	0.71157
Gorge	Cadell	236	6	2010/11	0.710957
Gorge	Waikerie	373	6	2010/11	0.711249
Gorge	Morgan	335	6	2010/11	0.711284
Floodplain	Plushes Bend	451	7	2009/10	0.707912
Floodplain	Rilli Island	416	7*	2009/10	0.710805
Floodplain	Rilli Island	368	7	2009/10	0.710312
Floodplain	Rilli Island	381	7	2009/10	0.707441
Gorge	Qualco	423	7	2009/10	0.710914
Gorge	Qualco	380	7	2009/10	0.709241
Gorge	Scott's Creek	380	7	2009/10	0.707201
Gorge	Scott's Creek	393	7	2009/10	0.707512
Gorge	Overland Corner d/s	300	7	2009/10	0.707602
Gorge	Lowbank u/s	372	7	2009/10	0.707143
Gorge	Caurnamont	378	7	2009/10	0.707692
Gorge	Swan Reach	381	7	2009/10	0.708381
Gorge	Swan Reach	411	7	2009/10	0.709743
Gorge	Swan Reach	464	7	2009/10	0.707686
Gorge	Blanchetown	388	7	2009/10	0.707643
Gorge	Blanchetown	342	7	2009/10	0.707636
Gorge	Cadell	373	7	2009/10	0.711128
Gorge	Cadell	336	7	2009/10	0.710564
Gorge	Waikerie	399	7*	2009/10	0.707531
Gorge	Morgan	352	7	2009/10	0.708029

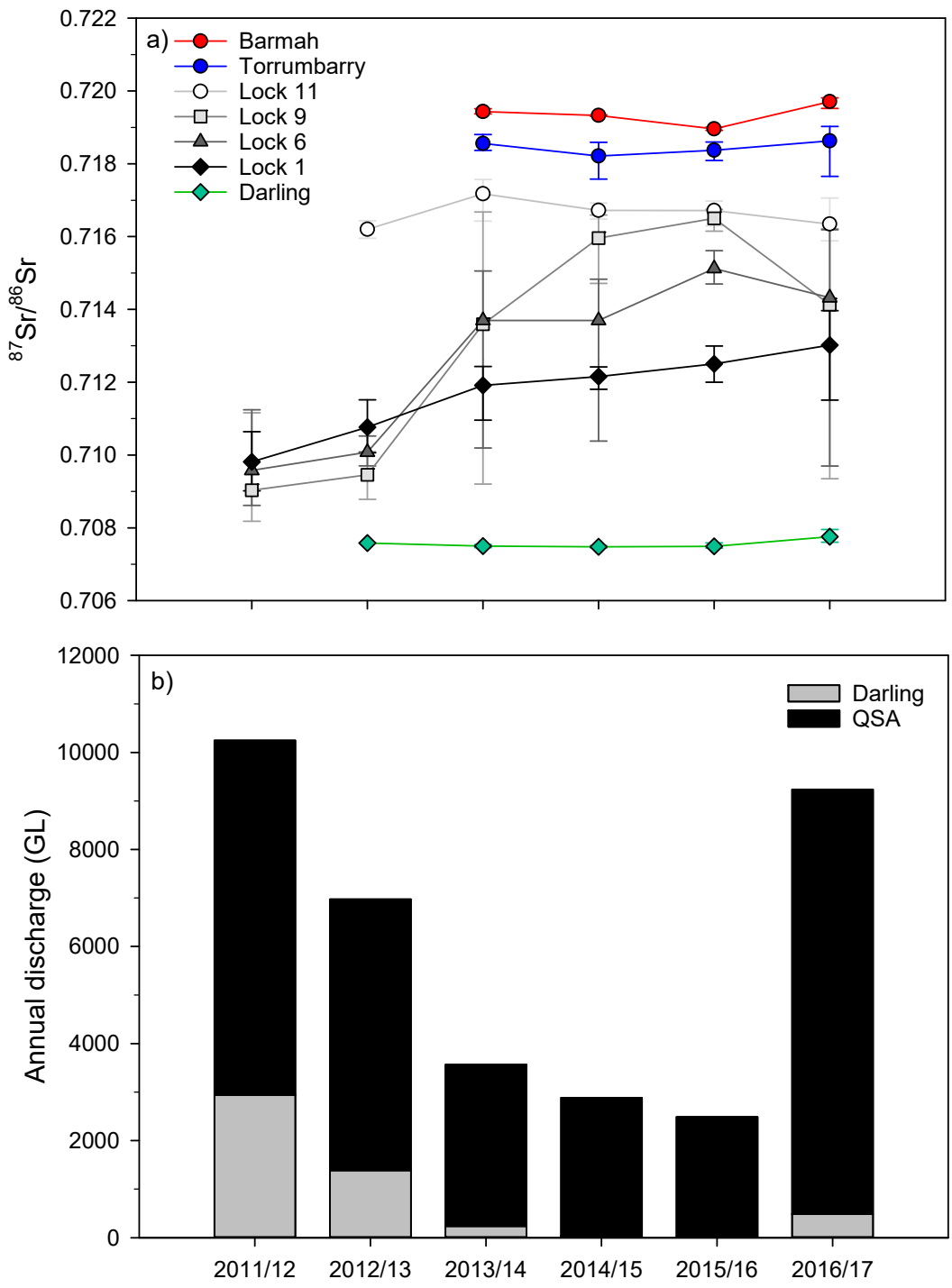


Figure H11. (a) Mean $^{87}Sr/^{86}Sr$ (with minimum and maximum values as error bars) in water samples collected from spring/summer in the mid-Murray (Barmah, Torrumbarry and Lock 11), lower Murray (Lock 9, 6 and 1) and Darling Rivers from 2011 to 2017, and (b) annual discharge (GL) in the Murray River at the South Australian border (QSA) and the proportion of discharge from the Darling River at Burtundy that contributed to QSA.

All three age 3+ golden perch (spawned 2013/14) collected from the floodplain geomorphic zone of the LMR exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to water $^{87}Sr/^{86}Sr$ in the lower River Murray (~0.7080–0.7140) (Figures H11 and H12), indicating these fish were spawned in the lower River Murray. Transects of otolith $^{87}Sr/^{86}Sr$, indicate all fish spent their entire lives in the lower River Murray (Figure H13).

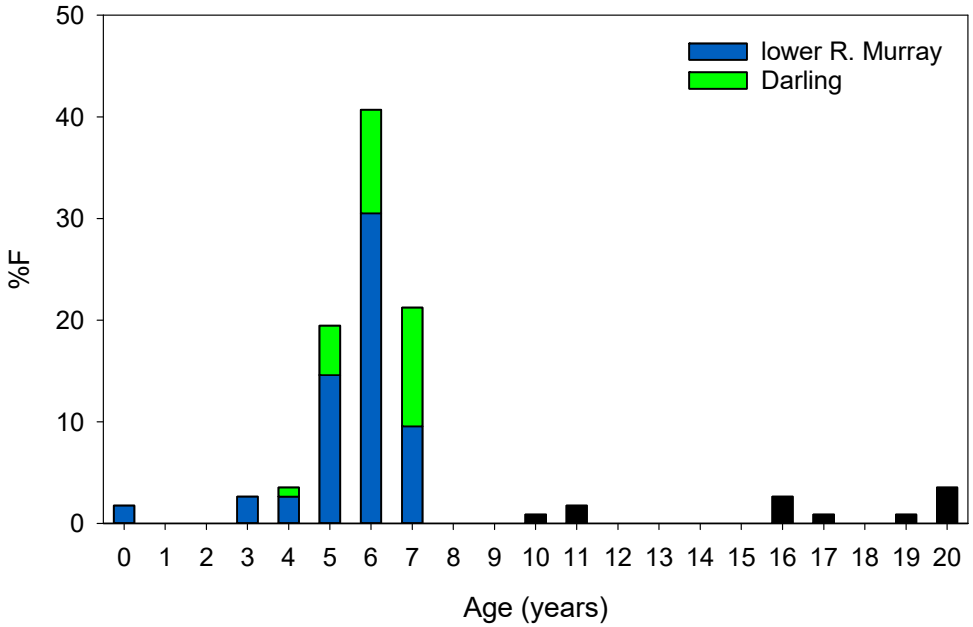


Figure H12. Age frequency distribution of golden perch from the LMR (floodplain and geomorphic zones combined) in autumn/winter 2017 (*n* = 113) showing the natal origins (i.e. lower River Murray and Darling River) of dominant cohorts inferred from otolith core signatures of the sampled fish from 2017 (Table H6) in comparison to the water sample reference collection (Figure H11). Percentage of origin for each cohort are based on the subsampled population. Age cohorts with black bars were not assessed for natal origin.

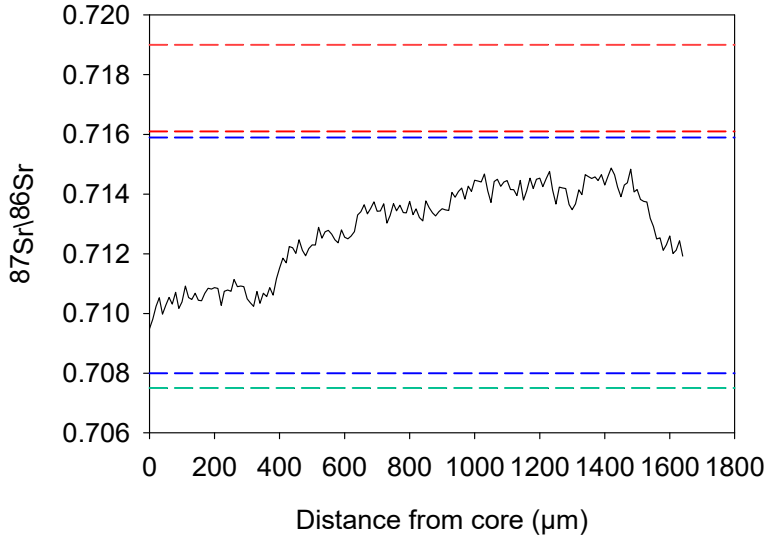


Figure H13. An individual life history profile based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of an otolith from an age 3+ golden perch collected from Plushes Bend in the floodplain geomorphic zone of the lower River Murray. Green dashed line indicates the temporally stable water $^{87}Sr/^{86}Sr$ 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 River Murray (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).

Of the four age 4+ golden perch (spawned 2012/13) analysed, three exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to water $^{87}Sr/^{86}Sr$ in the lower River Murray (~0.7080–0.7140) (Figures H11 and H12), indicating these fish were spawned in the lower River Murray. Transects of otolith $^{87}Sr/^{86}Sr$, indicate all fish spent their entire lives in the lower River Murray (Figure H14). The other fish exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to the distinct Darling River water $^{87}Sr/^{86}Sr$ of ~0.7075, indicating this fish was spawned in the Darling River. Transects of otolith $^{87}Sr/^{86}Sr$, indicate that this fish transitioned from the Darling River into the lower River Murray in its first year of life (i.e. age 0+) (Figure H14).

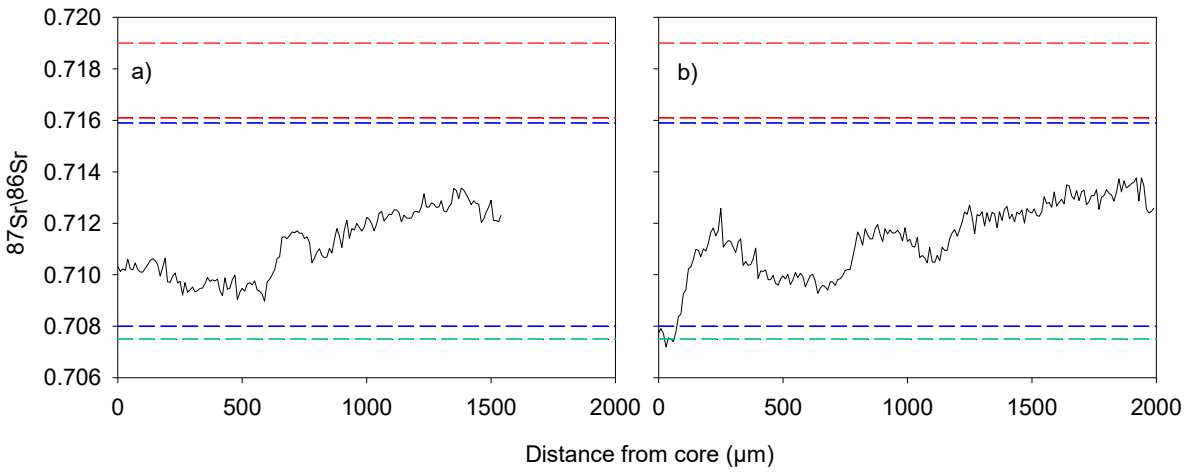


Figure H14. An individual life history profile based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of an otolith from age 4+ golden perch collected from (a) Qualco and (b) Plushes Bend in the gorge and floodplain geomorphic zones, respectively, of the lower River Murray. Green dashed 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 River Murray (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).

The majority (75%, *n* = 15) of age 5+ golden perch (spawned 2011/12) exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to the Darling River water $^{87}Sr/^{86}Sr$ of ~0.7075, indicating these fish were spawned in the Darling River (Figures H11 and H12). Transects of otolith $^{87}Sr/^{86}Sr$ indicate these fish transitioned from the Darling River into the lower River Murray in their first (i.e. age 0+, *n* = 2) or second year (i.e. age 1+, *n* = 13) of life (Figure H15). The remaining five fish had higher, but variable core $^{87}Sr/^{86}Sr$ (Table H6), comparable to water $^{87}Sr/^{86}Sr$ in the lower River Murray (~0.7080–0.7140) (Figure H11), indicating these fish were potentially spawned in various locations in the lower River Murray. Transects of otolith $^{87}Sr/^{86}Sr$, indicate all six fish had spent their entire lives in the lower River Murray (Figure H15).

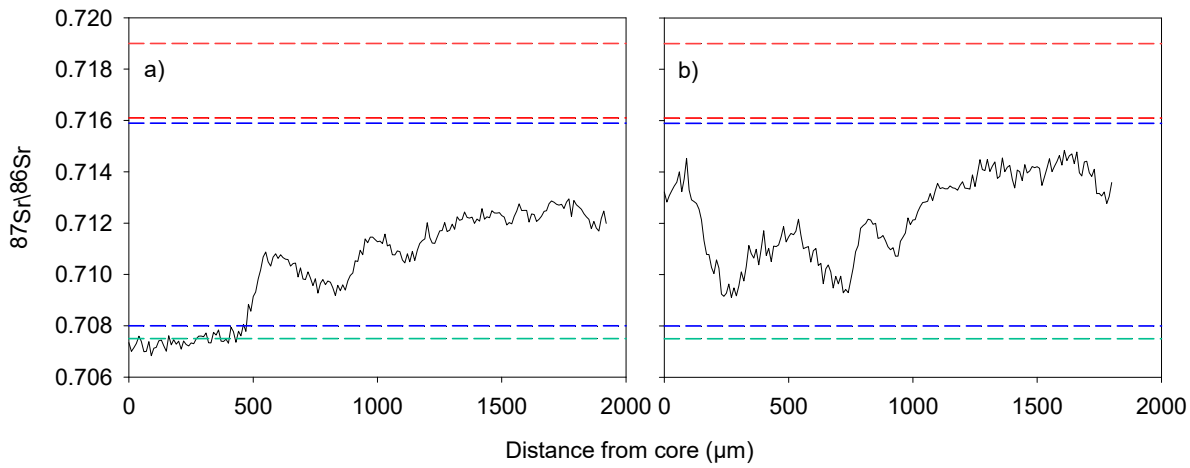


Figure H15. An individual life history profile based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of an otolith from an age 5+ golden perch collected from (a) Plushes Bend and (b) Blanchetown in the floodplain and gorge geomorphic zones, respectively, of the lower River Murray. Green 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 River Murray (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).

Of the age 6+ golden perch (spawned 2010/11), 75% (*n* = 15) exhibited variable otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to water $^{87}Sr/^{86}Sr$ in the lower River Murray (~0.7080–0.7140) (Figures H11 and H12), indicating these fish were potentially spawned in various locations in the lower River Murray. Transects of otolith $^{87}Sr/^{86}Sr$ indicated all fifteen fish spent their entire lives in the lower River Murray (Figure H16). The remaining five age 6+ fish exhibited otolith core $^{87}Sr/^{86}Sr$ comparable to the distinct Darling River water $^{87}Sr/^{86}Sr$ of ~0.7075, indicating these fish were spawned in the Darling River. Transects of otolith $^{87}Sr/^{86}Sr$ indicate that age 6+ fish spawned in the Darling River transitioned into the lower River Murray as age 0+ (Figure H16b) and 1+ and remained in this region until capture in 2017.

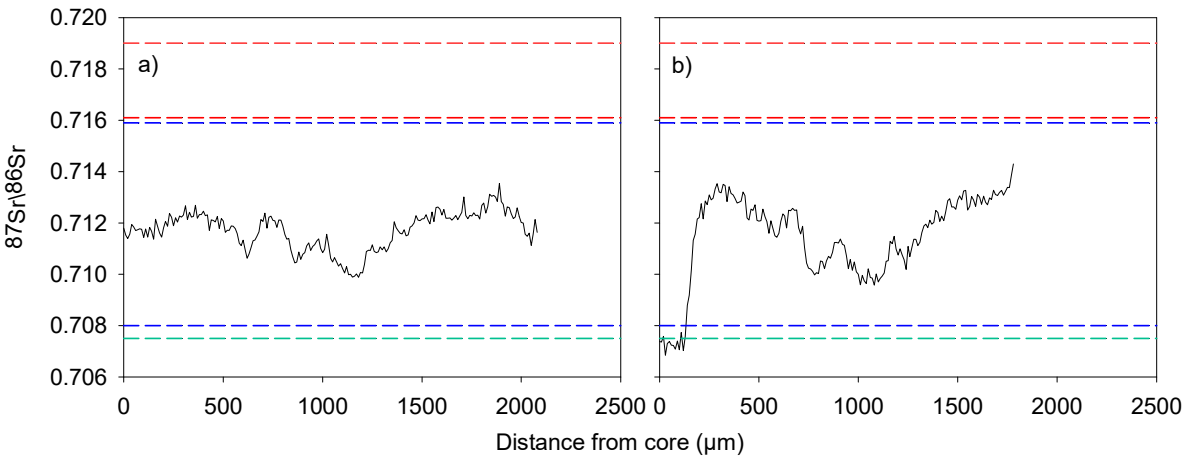


Figure H16. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from two age 6+ golden perch collected from (a) Cadell and (b) upstream of Lowbank in the gorge geomorphic zone of the lower River Murray. Green dashed line indicates the temporally 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 River Murray (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).

Eleven (55%)of the age 7+ golden perch (spawned 2009/10), exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to the Darling River water $^{87}Sr/^{86}Sr$ of ~0.7075 (Figure H11), indicating these fish were spawned in the Darling River. The remaining eight age 6+ fish exhibited otolith core $^{87}Sr/^{86}Sr$ comparable to the lower River Murray (~0.7080–0.7140) (Figure H11 and H12). Transects of otolith $^{87}Sr/^{86}Sr$, indicate that age 7+ spawned in the Darling River transitioned into the lower River Murray as age 0+ and 1+ (Figure H17a), and remained in this region until capture in 2017.

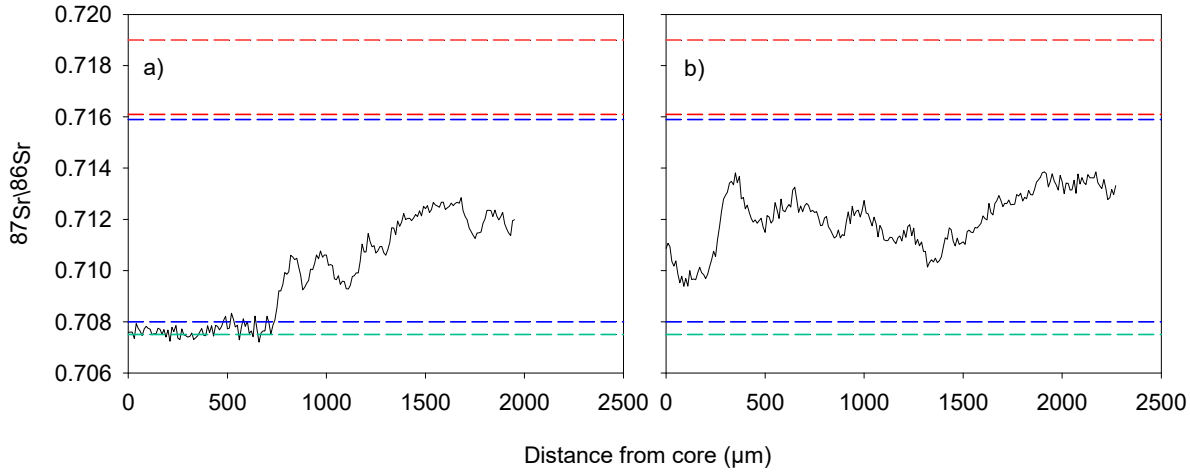


Figure H17. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from two age 7+ golden perch collected from (a) Waikerie and (b) Rilli Island in the gorge and floodplain geomorphic zones, respectively, of the lower River Murray. Green dashed 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 River Murray (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).

Silver perch

In 2016/17, we analysed $^{87}Sr/^{86}Sr$ from the otolith core to edge of silver perch from age 3+ (*n* = 2), 5+ (*n* = 1), 6+ (*n* = 2) and 7+ (*n* = 1) cohorts (Table H7; Figures H18). All age 3+, 5+ and 6+ silver perch, spawned in 2013/14, 2011/12 and 2010/11, respectively, exhibited otolith core and transect $^{87}Sr/^{86}Sr$ indicative of a lower River Murray spawning origin and occupation of this region throughout their lives (Table H7; Figure H18a–e; Figure H19). In contrast, the age 7+ silver perch (spawned 2009/10) exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H7) indicative of a mid-Murray River spawning origin (upstream of the Darling River confluence and downstream of Torrumbarry) (Figure H11). Transects of otolith $^{87}Sr/^{86}Sr$ indicate that this fish transitioned into the lower River Murray as age 0+ (Figure H18f).

Table H7. Capture location and geomorphic zone, length (mm), age (years) and spawn year, and otolith core $^{87}Sr/^{86}Sr$ of 6 silver perch collected from the floodplain geomorphic zone of lower River Murray in 2017. Life history profiles are shown for individuals marked with *.

Zone	Capture location	Length (mm)	Age (years)	Spawn year	Core $^{87}Sr/^{86}Sr$
Floodplain	Rili Island	336	3*	2013/14	0.71064
Floodplain	Rili Island	322	3*	2013/14	0.71089
Floodplain	Rili Island	370	5*	2011/12	0.710019
Floodplain	Murtho Forest	326	6*	2010/11	0.711748
Floodplain	Rili Island	341	6*	2010/11	0.712331
Floodplain	Plushes Bend	368	7*	2009/10	0.716321

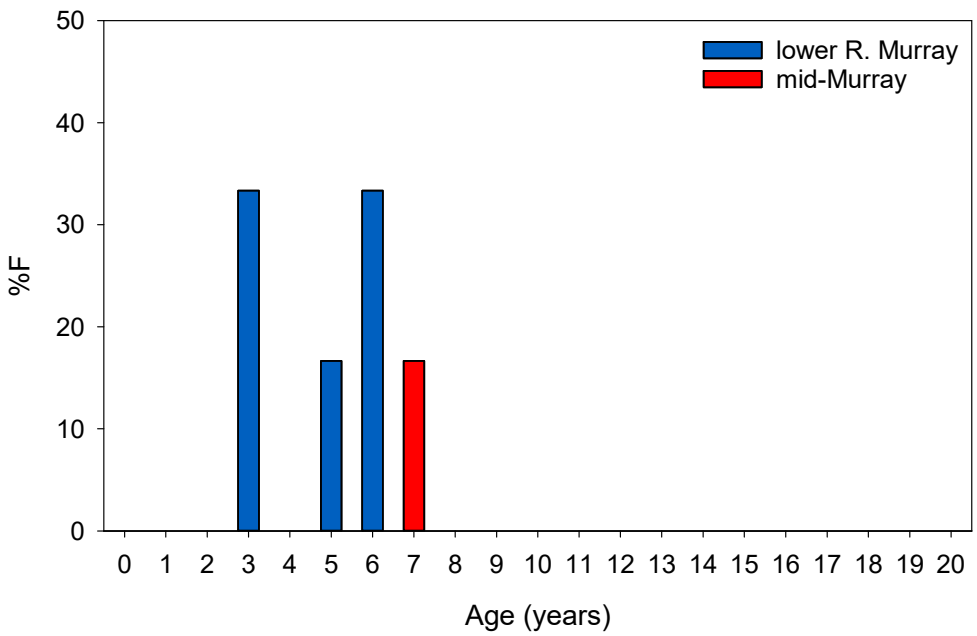


Figure H18. Age frequency distribution of silver perch from the LMR (floodplain and geomorphic zones combined) in autumn/winter 2017 (*n* = 6) showing the natal origins (i.e. lower River Murray and mid-Murray River) of dominant cohorts inferred from otolith core signatures of the sampled fish from 2017 (Table H7) in comparison to the water sample reference collection (Figure H11). Age cohorts with black bars were not assessed for natal origin.

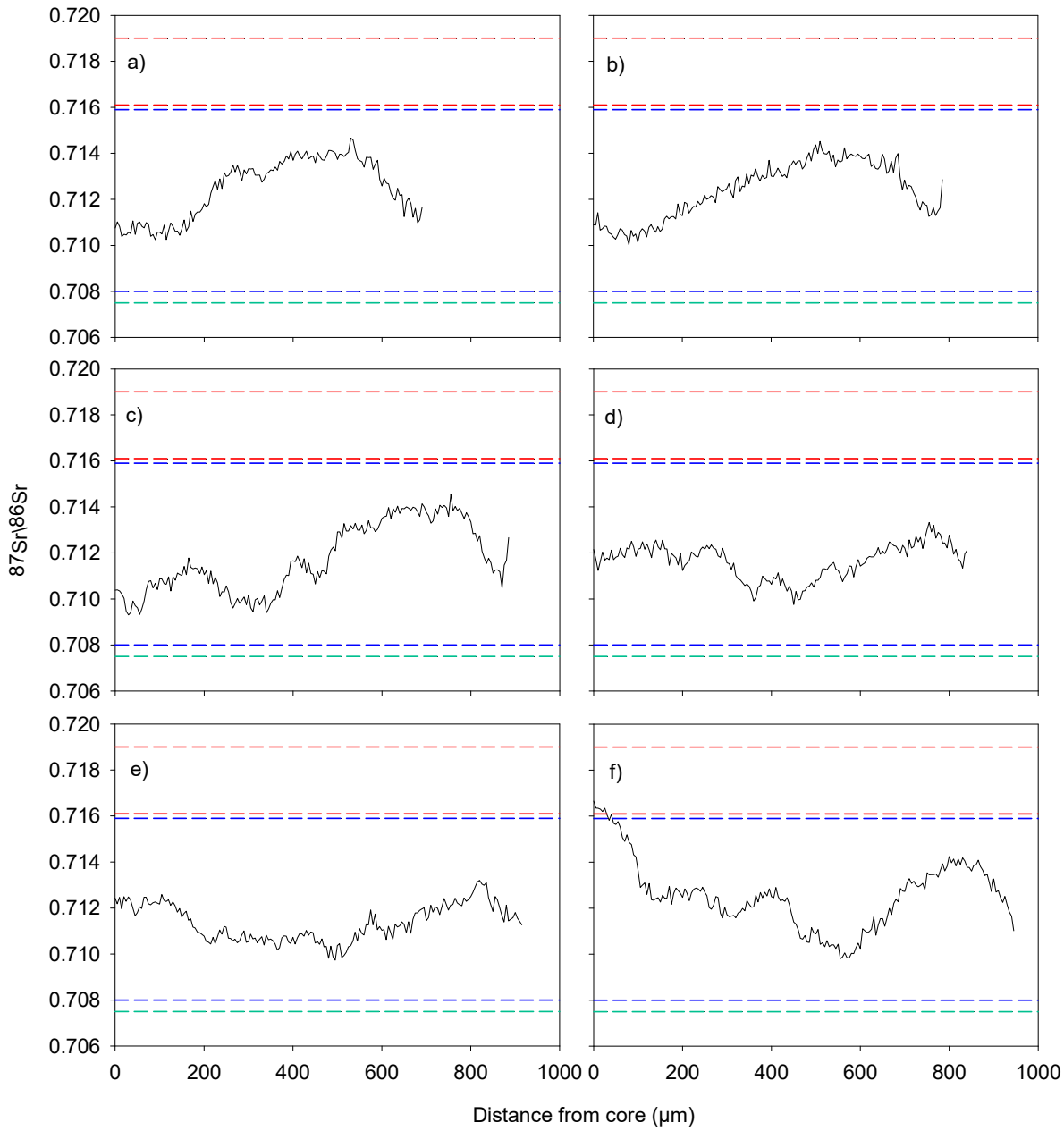


Figure H19. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from (a,b) two age 3+ silver perch collected from Rilli Island, (c) an age 5+ silver perch collected from Rilli Island, two age 6+ silver perch collected from (d) Murtho Forest and (e) Rilli Island, and (f) an age 7+ silver perch collected from Plushes Bend in the floodplain geomorphic zone of the lower River Murray. 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 River Murray (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).

Discussion and evaluation

In 2016/17, flow in the LMR increased steadily from ~30,000 ML day^{-1} in early September to a peak of ~94,600 ML day^{-1} in late November 2016. Flow then decreased rapidly to <20,000 ML day^{-1} in January 2017 and <10,000 ML day^{-1} by March 2017.

In 2016/17, golden perch eggs and larvae were collected from the gorge and floodplain geomorphic zones of the LMR between October 2016 and January 2017, with the majority of larvae (*n* = 14) collected downstream of Lock 6 on 10 January 2017. The age of these larvae (predominantly 9–17 days) and otolith $^{87}Sr/^{86}Sr$ indicate these fish were spawned from 24 December–1 January, in the lower River Murray between the Darling River junction and Lock 6. One 27-day old golden perch larvae was also collected at Lock 6 on 10 January 2017, and otolith $^{87}Sr/^{86}Sr$ indicated this fish was spawned in the Darling River.

Overall, the presence of eggs and larvae with a lower River Murray provenance indicates that in 2016/17, golden perch spawning in the lower River Murray extended from October to early January and occurred in association with the ascending and descending limbs of a peak flow of ~94,600 ML day^{-1}.

In 2017, the golden perch population in the floodplain and gorge geomorphic zones of the LMR was dominated by age 7+, 6+ and 5+ fish. No age 0+ golden perch were collected by electrofishing, although two YOY golden perch were collected incidentally in fyke nets. There was, however, a general absence of age 0+ golden perch in the LMR in 2017 indicating negligible recruitment from spawning in spring–summer 2016/17.

In 2017, the sampled silver perch population in the LMR was comprised of age 3+–7+ fish spawned from 2010–2014 in association with in-channel and overbank increases in flow in the lower River Murray, mid-Murray River and the Darling River. No age 0+–2+ silver perch were collected indicating negligible recruitment in 2014/15–2016/17.

Conclusions

These findings augment contemporary conceptual models of the flow-related ecology of golden perch and silver perch in the Murray River. Previous investigations indicate that golden perch and silver perch recruitment in the LMR is promoted by spawning associated with spring–summer increases in flow (in-channel and overbank) in the lower and mid-Murray River, and lower Darling River (Zampatti and Leigh 2013a; Zampatti *et al*. 2015; Ye *et al*. 2017). As well as local spawning, immigration of age 0+ or 1+ fish can substantially enhance populations, particularly during years of high flow (Zampatti and Leigh 2013b; Zampatti *et al*. 2015).

In spring–summer 2016/17, golden perch (but not silver perch) spawning occurred in the lower River Murray in association with overbank flooding (QSA peak flow ~94,600 ML day^{-1}). Recruitment to YOY in 2017, however, was negligible, indicating localised recruitment failure and low levels of immigration from spatially distinct spawning sources such as the lower Darling and mid Murray rivers. The mechanisms contributing to localised recruitment failure were not explored as a component of this project, but the coincidence of a hypoxic blackwater event with spawning may have contributed to larval mortality. This could include the direct impacts of low dissolved oxygen concentrations on larval survival and/or indirectly through the impacts of hypoxia on food resources (Gehrke 1991; Section 4 Microinvertebrates).

APPENDIX I: DEWNR SHORT-TERM EVALUATION QUESTIONS

Table I1. DEWNR short-term (one-year) evaluation questions for CEWO LTIM Category 1 and 3 indicators. Evaluation questions are based on ecological targets from the Long-Term Environmental Watering Plan (LTWP) for the South Australian Murray River. DEWNR evaluation questions serve as 'additional' questions as there may be some CEWO questions that are also relevant to DEWNR's targets from the LTWP. CEW = Commonwealth environmental water.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 1. Stream Metabolism	What did CEW contribute to temporarily shifting open water productivity towards heterotrophy?	A marked increase in ecosystem respiration at the site below Lock 6 aligned with an increased delivery of naturally turbid water from the Darling River, which CEW contributed to. It is thought that the reduced light penetration altered the metabolic balance of the phytoplankton, a natural response to the conditions. The quality of environmental water has a significant influence on stream metabolism and the accumulative long-term influences of water quality attributes need to be assessed especially in respect to timing, frequency and size of flow delivery.
	What did CEW contribute to increased nutrients and DOC levels?	The data suggested that CEW contributed little to increased nutrients or DOC concentrations, especially relative to the large changes associated with overbank flows.
	What did CEW contribute to maintaining dissolved oxygen levels above 50% saturation throughout the water column at all times?	Overbank flows reduced dissolved oxygen levels to below 50% saturation (~4.5 mg L^{-1}). Environmental water that supplemented releases from Lake Victoria maintained oxygen levels above 4 mg L^{-1} in the Rufus River.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 1. Fish (channel)#	Did the length-frequency distribution for Murray cod in the Gorge zone reflect recent recruits, sub-adults and adults?	During autumn/winter 2017, recent recruits (i.e. <300 mm TL, 64%) and sub-adults (i.e. 300–600 mm TL, 36%) were sampled in the Gorge geomorphic zone of the LMR; however, adults (>600 mm TL) were not sampled.
	Did a YOY cohort represent >50% of the Murray cod population from the Gorge zone?	No. During autumn/winter 2017, a YOY cohort (i.e. <150 mm TL) of Murray cod represented less than 50% (45%) of the population in the Gorge geomorphic zone of the LMR.
	Did the length-frequency distribution for bony herring, Murray rainbowfish and carp gudgeon, include size classes representing YOY in the Gorge zone?	Yes. During autumn/winter 2017, length-frequency distributions indicated YOY were present for bony herring, Murray rainbowfish and carp gudgeon.
	Did the relative abundance of common carp in the Gorge zone increase during the current year, relative to the previous year, whilst the relative abundances of flow-dependent native species decreased?*	There was an increase in the ratio (total abundance) of common carp to flow-dependant, native species (golden perch and silver perch) at all five sites sampled in autumn 2017, relative to the previous year. During 2016 the mean site ratio was 1.43 carp (± 0.38 S.E.) to every 1 flow-dependant, native species. In 2017, this ratio increased to 19.33 carp (± 4.45) to every 1 flow-dependant, native species.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 1. Fish (channel) #	Did the estimated biomass of common carp in the Gorge zone increase during the current year, relative to the previous year, whilst the estimated biomass of flow-dependent native species decreased?*	In contrast to relative abundance, there was a decrease in the ratio (total biomass) of common carp to flow-dependant, native species (golden perch and silver perch) at four of the five sites sampled in autumn 2017, relative to the previous year. In 2017, the site where the estimated biomass of carp increased at a greater rate than flow-dependant, native species was Overland Corner A. During 2016, the mean site ratio was 2.10 kg of carp (± 0.65 S.E.) to every 1 kg of flow-dependant, native species. In 2017, this ratio decreased to 1.48 kg of carp (± 0.33) to every 1 kg of flow-dependant, native species.
Category 1. Hydrology (channel)	What did CEW contribute to providing a seasonal hydrograph that encompassed variation in discharge, velocity and water levels?	In 2016/17 a substantial high flow event provided a seasonal hydrograph that encompassed variation in discharge, velocity and water levels. Environmental water supplemented this event by reducing the recession in water levels in the order of 0.7–0.9 m and resulted in an increase variability in lower flows over the first six months of 2017.
Category 3. Hydrological Regime	What did CEW contribute to providing diverse hydraulic conditions and complex habitat for flow dependant biota and processes?	Environmental water provided on the flood recession slowed the reduction in velocity during January. After this event, environmental water increased weir pool median velocities to a small degree (0.05 – 0.07 m s^{-1}), with some sections of the river being greater than 0.17 m s^{-1}, particularly during an event in March.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 3. Hydrological Regime	What did CEW contribute to providing diverse hydraulic conditions over the range of velocity classes in the lower third of weir pools so that habitat and processes for dispersal of organic and inorganic material between reaches are maintained?	Discharge exceeding 10,000 ML day^{-1} is expected to result in a well-mixed column where negatively buoyant propagules would be maintained in suspension (Wallace *et al.* 2014). In 2016/17, CEW contributed to create these conditions for short periods in late January, February, March and May. Further research is required to determine relationships between velocity classes and a well-mixed water column, for dispersal of organic and inorganic material between reaches.
Category 3. Matter Transport	What did CEW contribute to maintaining water quality to support aquatic biota and normal biogeochemical processes?	The modelling suggests that environmental water impacted positively on the concentrations of dissolved and particulate matter. This was observed through a considerable reduction in salinity in the Coorong, where there was a modelled median salinity of 12.97 practical salinity units (PSU) with all water during 2016/17, compared to 17.46 PSU without CEW. Salinity is known to have a significant impact upon biogeochemical processes and so maintaining salinities in the Coorong within that of normal estuarine conditions may have maintained normal biogeochemical processes for this region. Furthermore, reduced salinity concentrations in the Coorong, likely improved habitat for estuarine biota. The higher flows in 2016/17 (peak ~94,600 ML day^{-1}) compared with 2015/16 (peak 28,000 ML day^{-1}) maintained salinity at much lower concentrations: median salinity in 2015/16 was 27.73 PSU.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 3. Matter Transport	What did CEW contribute to providing for the dispersal of organic and inorganic material and organisms between river and wetlands?	The modelling suggests that CEW increased the export of dissolved and particulate matter. This was observed through: • Increased salt export from the Murray River Channel and Lower Lakes. Total salt export through the Murray Mouth in 2016/17 was 3,679,277 tonnes. CEW contributed 519,292 tonnes of salt export through the Murray Mouth, which equates to 14% of total salt export. • Increased exports of nutrients from the Murray River Channel, Lower Lakes and Coorong/Murray Mouth. The most apparent differences in exports associated with environmental water were for silica. CEW contributed 18% of total silica export through the Murray Mouth in 2016/17. This was less than the proportional contribution of CEW in 2015/16, which comprised 95% of the total silica exports. This highlights the importance of CEW delivery, particularly in years with lower flows. • Increased exports of phytoplankton biomass from the Murray River Channel, Lower Lakes and Coorong/Murray Mouth. It is important to remember than nutrients are a resource that drive productivity and fuel food webs. The increased transport of dissolved and particulate matter may have provided benefits for the Lower Lakes, Coorong and near-shore marine environment by providing energy to ecosystem productivity, as nutrients and phytoplankton are consumed by higher trophic organisms.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 3. Micro-invertebrates	What did CEW contribute to increased microinvertebrate input from floodplain to the river and thus reducing the reliance of in-stream food webs on autochthonous productivity? What did CEW contribute to increased dispersal of organisms between river and wetlands?	Of ~285 taxa recorded from the LMR main channel in 2016/17, 193 (67.7%) were not true potamoplankton, but littoral/epiphytic/epibenthic incursions, flushed into the main channel from floodplain or riparian sources. However, the majority of these taxa were sampled during increasing and overbank flows prior to mid-December when most of the sampling occurred, and when CEW delivery was mostly absent. No wetland samples were collected in 2016/17 to ascertain CEW dispersal of microinvertebrates from the main channel flows.
Category 3. Fish Spawning and Recruitment	What did CEW contribute to the population age structure of golden perch in the LMR? What did CEW contribute to the population age structure of silver perch in the LMR?	CEW delivery in 2016/17 did not contribute to the presence of any new cohorts (age 0+) of golden perch in the LMR, despite spawning during spring/summer 2016. The mechanisms leading to recruitment failure of golden perch in 2017 remain unexplored, but may in part be associated with the hypoxic blackwater, which may have impacted directly on egg and larval development, or indirectly via the effect of food resources in the LMR. CEW delivery in 2016/17 did not contribute to the presence of any new cohorts (age 0+) of silver perch in the LMR. No silver perch spawning was detected in 2016/17.

Indicator	One-year evaluation question(s)	Answers to one-year evaluation question(s)
Category 3. Fish Spawning and Recruitment	Did CEW contribute to a YOY or age 1+ cohort that represented >30% of the golden perch population in the LMR?	No. Age 0+ (2016/17) and 1+ (2015/16) cohorts represented <30% of the golden perch population in the LMR during autumn/winter 2017. In 2016/17, there was spawning of golden perch, but negligible recruitment. The mechanisms leading to recruitment failure of golden perch in 2017 remain unexplored, but may in part be associated with the hypoxic blackwater, which may have impacted directly on egg and larval development, or indirectly via the effect of food resources in the LMR.
	Did CEW contribute to a YOY or age 1+ cohort that represented >30% of the silver perch population in the LMR?	No. No age 0+ (2016/17 cohort) or 1+ (2015/16 cohort) silver perch were detected during electrofishing in the LMR during autumn/winter 2017. No silver perch spawning was detected in 2016/17.

Category 1 Fish (Channel) data have been consolidated to evaluate a number of fish targets of DEWNR's LTWP. These questions and answers do not relate to evaluation of flow or CEW. Furthermore, the LTIM Category 1 Fish monitoring program is not designed to determine what is facilitating changes in population dynamics of fish species for DEWNR's LTWP evaluation questions, e.g. spawning and recruitment of Murray cod or common carp.

* To remove sampling season bias, only sites sampled during autumn 2017 were used in carp ratio calculations. Site ratios of common carp to flow-dependant, native species were calculated by dividing the total biomass or number of individuals (abundance) of carp for that site by the total biomass or number of individuals (abundance) of golden perch and silver perch for the same site, respectively. The mean site ratio for a particular year was calculated by averaging the site ratios. 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).

8 ACRONYMS

ADF	Additional Dilution Flows
AHD	Australian Height Datum
CEW	Commonwealth environmental water
CEWH	Commonwealth Environmental Water Holder
CEWO	Commonwealth Environmental Water Office
DEWNR	Department of Environment, Water and Natural Resources
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
NPL	Normal pool level
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

9 GLOSSARY

ADF	Additional Dilution Flows: aims to reduce river salinities in South Australia without significantly impacting on water availability. The intent is that additional water is delivered to South Australia rather than be lost as evaporation from Menindee Lakes. The ADF procedures are triggered when the storage in Menindee Lakes exceeds the specified volume within the given month and the combined storage in Hume and Dartmouth Reservoirs exceeds 2000 GL.
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.
Blackwater	Water that is black in colour due to low dissolved oxygen concentrations (hypoxic). Often associated with the decomposition of large amounts of organic matter on floodplains.
Flood or flooding	Refers to flows that are overbank. In South Australia, this is deemed to be above 45,000 ML day^{-1}.
Epibenthic	Organisms living on the surface of sediment.
Epiphytic	Organisms that are attached to plants.
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^{-1}.
Littoral	The margin along the bank of the river.
Lotic	Refers to flowing water, typically median velocities of approximately ≥0.3 m s^{-1}.
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).
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.