Long-Term Intervention Monitoring of the Ecological Responses to Commonwealth Environmental Water Delivered to the Lower Murray River Selected Area in 2015/16

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 and Murray River (SARDI Aquatic Sciences); microinvertebrates (ALS, WRM); pump station (www.waterconnect.sa.gov.au); matter transport modelling (UoA, UoWA).

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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 two (2015/16) of the five-year Commonwealth Environmental Water Office (CEWO) Long-Term Intervention Monitoring (LTIM) project. In 2015/16, ~814 GL of Commonwealth environmental water was delivered to the South Australian section of the Murray River, termed the LMR. Flow delivery to the LMR was coordinated through a series of watering events across the southern connected Basin to achieve multi-site environmental outcomes. Environmental watering assisted in maintaining river flow at 9,600–11,700 ML day^{-1} from mid-September to late October 2015, and at ~9,800 ML day^{-1} during mid- to late February 2016 in the LMR. Commonwealth environmental water also supported weir pool raising (WPR) events in Weir Pools 2 and 5. Furthermore, environmental watering supplemented freshwater flows to the Lower Lakes and Coorong from September 2015, with Commonwealth environmental water contributing to 100% of barrage releases between September 2015 and June 2016.

Seven indicators were used to evaluate the ecological response to Commonwealth environmental water in the main river channel of the LMR Selected Area. Category 1 indicators aimed to evaluate Basin-scale objectives and outcomes, as well as local (Selected Area) objectives, while Category 3 indicators aimed to address local evaluation questions. 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)

Key ecological outcomes

Monitoring in 2015/16 identified a number of ecological responses associated with the delivery of Commonwealth environmental water in the LMR. Key findings, in relation to CEWO short-term evaluation questions, are summarised in Table 1. Results from the 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 2015/16. Key findings for Category 1 Hydrology (channel) are not presented as they did not have specific Selected Area evaluation questions. Objectives and Selected Area-specific hypotheses for each indicator are provided in Appendix A. CEW = Commonwealth environmental water, WPR = weir pool raising.

INDICATORS	CEWO SHORT-TERM EVALUATION QUESTIONS AND ANSWERS	KEY FINDINGS
Category 1: Stream Metabolism	What did CEW contribute to: • Patterns and rates of primary productivity and decomposition? There were enhanced gross primary production and respiration rates associated with WPR in Weir Pool 5 and return flows from Chowilla, both of which were supported by CEW. Integrated ecosystem net production was near zero, indicating that organic material was derived from aquatic production with little enhancement from external supplies that could have further increased food supplies. • Dissolved oxygen levels? Oxygen concentrations did not fall below acceptable levels (>50% saturation).	There was enhanced metabolic activity associated with WPR in Weir Pool 5 and return flows from Chowilla, with potential benefits for food webs. Integrated ecosystem net production was near to zero, suggesting that the source of organic material was largely aquatic photoautotrophs with little enhancement from external supplies. Cycles of metabolic activity at the site below Lock 1 were due to probe error (biofouling). Progressive increases in metabolic activity over the whole monitoring season were related to changes in water quality, particularly continuous reductions in turbidity which influences the availability of sunlight in the water column. The reason for the turbidity reduction was not obvious but may be related to the different sources of water being supplied (e.g. Murrumbidgee River or Murray River water), or a shift in relative contributions from run-off and water storages. Further analyses are required of upstream flow deliveries and turbidities to understand the role of water delivery in supporting the improved metabolic conditions. The quality of the environmental water was sufficient that its contribution to the flow conditions helped retain oxygen concentrations at acceptable levels.

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 SA's Long Term Environmental Watering Plan (Appendix I).	Small-bodied fish abundance and diversity remained high in 2015/16, while there was an increase in the abundances of exotic goldfish and common carp, and a decrease in native bony herring, relative to 2014/15. Based on length frequency data, there was no recruitment (to age 0+) of golden perch, silver perch or freshwater catfish in 2015/16. The absence of recruitment of golden perch and silver perch in association with the 2015/16 flow regime (i.e. low, stable flows) is consistent with our contemporary understanding of the life histories of these flow-cued spawners. For the second consecutive year, small Murray cod (<150 mm TL, likely age 0+) were sampled in the LMR Selected Area during 2015/16, indicating successful recruitment. Furthermore, there was persistence of the age 0+ cohort from 2014/15 as age 1+ in 2015/16. The mechanisms behind the recruitment of cohorts of Murray cod from 2014/15 and 2015/16 remain unclear.
Category 3: Hydrological Regime*	What did CEW contribute to: • Hydraulic diversity within weir pools? Increase in weir pool median water velocities of ~0.1 m s^{-1} during winter and spring compared to without CEW, with some cross sections in the weir pool ranging 0.17–0.3 m s^{-1}. • Variability in water levels within weir pools? Increases in water levels in weir pools of up to 0.3 m in the upper reaches for weir pools without WPR, and up to 0.7 m in weir pools with WPR.#	CEW contributed to an increase in median water velocities of ~0.1 m s^{-1} during winter and spring. Velocities for some cross sections in the LMR increased to 0.17–0.3 m s^{-1} due to CEW. The range in velocities within a weir pool increased with the CEW contribution, representing an increase in hydraulic diversity. Restoring flowing habitat is critical for the rehabilitation of riverine biota and ecological processes in the lower River Murray. For weir pools without WPR events, the water level was assumed to be the same at the weir (downstream end) with CEW delivery, but CEW increased water levels up to 0.3 m in the upper reaches of these weir pools. For weir pools that had WPR events (Weir Pools 2 and 5), the ~0.5 m WPR raising at the weir increased water levels up to 0.7 m in the upper reaches of the weir pool due to CEW delivery. Periodic increases in water levels could improve the condition of riparian vegetation and increase biofilm diversity.

Category 3: Matter Transport*	What did CEW contribute to: • Salinity levels and transport? Reduced salinity concentrations in the Murray River Channel, Lower Lakes and, in particular, the Murray Mouth. Increased export of salt from the Murray River Channel and Lower Lakes, and decreased net import of salt to the Coorong. • Nutrient concentrations and transport? 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. • Water quality to support aquatic biota and normal biogeochemical processes? Reduced salinity concentrations in the Lower Lakes and Murray Mouth may have improved habitat for freshwater and estuarine biota in the region. • Ecosystem function? Increased exchange of nutrients and phytoplankton between critical habitats of the Lower Murray would may have supported primary and secondary productivity in the region and in doing so supported food webs of the LMR, Lower Lakes and Coorong.	The modelling suggests that environmental water impacted positively on the concentrations of dissolved and particulate matter. This was observed through: • Minor reduction in salinity in the Murray River Channel and Lower Lakes and a significant reduction in salinity levels in the Murray Mouth, with median salinities of 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 higher silica in the Lower Lakes with CEW. 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 and Lower Lakes, and decreased net import of salt to the Coorong. CEW contributed to 41% and 87% of the total modelled export of salt from the Murray River Channel and Lower Lakes, respectively. There was a net modelled import of salt to the Coorong of 1,850,028 tonnes with all water during 2015/16, but the modelling suggests that without CEW this would have been 6,441,297 tonnes. • Increased exports of nutrients from the Murray River Channel, Lower Lakes and Murray Mouth. The most apparent differences in exports associated with environmental water were for silica, with CEW contributing 41% and 95% of the total silica exports from the Murray River Channel and Lower Lakes, respectively. Whilst 395 tonnes of silica was exported from the Lower Lakes to the Coorong with all water, without CEW there was only 20 tonnes of silica exported. Silica is a particularly important nutrient for supporting the growth of diatoms, a phytoplankton group that is generally considered to be of high nutritional quality in coastal and riverine ecosystems. As such, the

		increased export of silica associated with CEW may have supported increased secondary productivity in the Murray River Channel, Lower Lakes and Coorong. • Increased exports of phytoplankton biomass from the Murray River Channel, Lower Lakes and Murray Mouth. 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).
Category 3: Micro-invertebrates	What did CEW contribute: • To microinvertebrate diversity? Peaks in microinvertebrate diversity below Lock 6 and Lock 1 aligned with peaks in river discharge and CEW delivery. Most in-channel taxa were not true potamoplankton (plankton of flowing waters), but transported taxa from floodplain or riparian sources (e.g. Chowilla). • Via upstream connectivity to microinvertebrate communities of the LMR Selected Area? Many (25%) microinvertebrates from 2015/16 were not recorded during 2014/15. Some will have originated from littoral margins in the LMR (e.g. WPR or Chowilla return flows), but some likely originated from further upstream, including novel taxa for the continent or for the LMR. • The timing and presence of key species in relation to the diet of large-bodied native fish larvae?	Differences in microinvertebrate diversity between all sampling events at sites below Lock 6 (floodplain zone) and Lock 1 (gorge zone) reflect the short generation times of the protist/rotifer-dominated microinvertebrate assemblages, seasonal succession, and transport of mixed assemblages from different upstream Murray River sources (including CEW), thereby increasing taxonomic diversity. Increases in density and diversity below Lock 6 during early November were likely triggered by antecedent high river flow and CEW flushing littoral (epiphytic and epibenthic) taxa to the main channel microinvertebrate assemblages. Increases in density and diversity below Lock 1 were associated with high river flow due to CEW delivery, but steeply decreased following a decline in river flow, recession in water level and return flow from WPR in Weir Pool 2. Cool water taxa such as *Filinia terminalis* likely originated from Goulburn sources. The origin of hitherto Amazonian *Hexarthra braziliensis* and *Keratella americana*, and Northern Hemisphere *Daphnia galeata* remains unclear.

	Relationship between timing of ambient (present in the environment) microinvertebrates, driven by CEW, and their presence in fish diet could not be determined. • To microinvertebrate abundance? Flow, which CEW contributed to, was a driver of density through October 2015, particularly at Lock 1. With reduced flows and a recession of water levels, there was a proportional drop in abundance, followed by a steady increase of warm-water taxa through summer.	
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 2015/16 corresponded with limited spawning and negligible recruitment (to young-of-year, age 0+) of golden perch and silver perch.	CEW contributed to limited golden perch and silver perch spawning in the LMR Selected Area, but negligible recruitment to young-of-year (age 0+). The golden perch population in the LMR was dominated by 5 and 6 year old fish spawned in the lower River Murray and Darling River in 2010/11 and Darling River in 2009/10. The silver perch population in the LMR was comprised of 2, 4, 5 and 6 year old fish, which were spawned in the lower River Murray, mid-Murray and Darling rivers. Based on a contemporary understanding of the flow related population dynamics of golden and silver perch in the southern MDB, moderation and fragmentation of flows between the mid and lower River Murray, and an absence of flow in the Darling River, in spring–summer 2015/16, potentially diminished spawning and recruitment of golden perch and silver perch in the LMR.

* Evaluation of CEW for Hydrological Regime and Matter Transport indicators is based on modelled data.

An increase in water level of 0.45 m and 0.5 m at the downstream end of Weir Pools 5 and 2, respectively, was achieved through infrastructure operation and maintained through the delivery of CEW to compensate for losses.

Key learning and management implications

Despite the delivery of significant volumes of Commonwealth environmental water, 2015/16 was a dry year and so river flow remained relatively low in the LMR (i.e. <12,000 ML day^{-1}) throughout the year (bankfull flow is ~50,000 ML day^{-1}). Nevertheless, some hydraulic and ecological outcomes were achieved through the combination of Commonwealth environmental water delivery and infrastructure operation (see 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:

- Hydrodynamic restoration is fundamental to restoring ecosystem function of the lower River Murray (downstream of the Darling River junction). Environmental water delivery can increase hydraulic diversity (velocities and water levels), potentially leading to ecological benefits by improving habitat and restoring riverine ecosystem function.
- 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 spawning fishes in spring and summer).
- Environmental flows should 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 (as observed for microinvertebrates in this study) and potentially enhanced recruitment.
- Weir pool manipulation can be used as a management tool to complement flow delivery to enhance ecological outcomes (e.g. improved riverine productivity via increased lateral connectivity).
- Water source (i.e. origin) can alter inputs to the LMR (e.g. nutrients, phytoplankton community composition). These attributes can be further affected by river operations that re-route flow (e.g. floodplain regulators or storages). Combined, these changes can lead to changes in the structure and function of aquatic food webs.

- In the lower River Murray, maintaining the hydrological integrity (i.e. magnitude, variability and source) of flow from upstream (e.g. Darling River or mid-Murray) is critical to support system-scale processes and promote positive ecological outcomes (e.g. improved productivity, enhanced spawning and recruitment of flow-dependent fish species at >15,000 ML day^{-1}).
- Consideration should be given to using Commonwealth environmental water 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 (Kingsford 2000; Bunn and Arthington 2002; Tockner and Stanford 2002). Environmental flows have been used to re-establish key components of the natural flow regime for ecological restoration of river systems (Poff *et al.* 1997; Arthington *et al.* 2006). Understanding biological and ecological responses to flow regimes provides critical knowledge to underpin environmental flow management to achieve the best ecological outcomes (Walker *et al.* 1995; Arthington *et al.* 2006).

The southern Murray–Darling Basin (MDB) is a highly regulated river system, where natural flow regimes have been substantially modified, leading to decreased hydrological (e.g. discharge) and hydraulic (e.g. water level and velocity) variability, and reduced floodplain inundation (Maheshwari *et al.* 1995; Richter *et al.* 1996). 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) constructed in the 1930s–1940s, 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 (e.g. natural flow vs. actual flow in 2015/16 in Figure 3). These have had profound impacts on riverine processes and ecosystems (Walker 1985; Walker and Thoms 1993).

The South Australian section of the Murray River (herein, Lower Murray River, LMR) represents a significant ecological asset to be targeted for environmental watering (DEWNR 2013). This complex system 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. During the Millennium drought in the MDB (2001–2010) (Figure 2), the ecosystem of the LMR was under severe stress; much of the biota declined and the community resilience was compromised (e.g. Noell *et al.* 2009; Nicol 2010; Zampatti *et al.* 2010). Since the drought broke in 2010/11, increased flow (both natural and environmental flows) has led to some positive responses, contributing to ecological restoration (e.g. Ye *et al.* 2014; 2015a; 2015b).

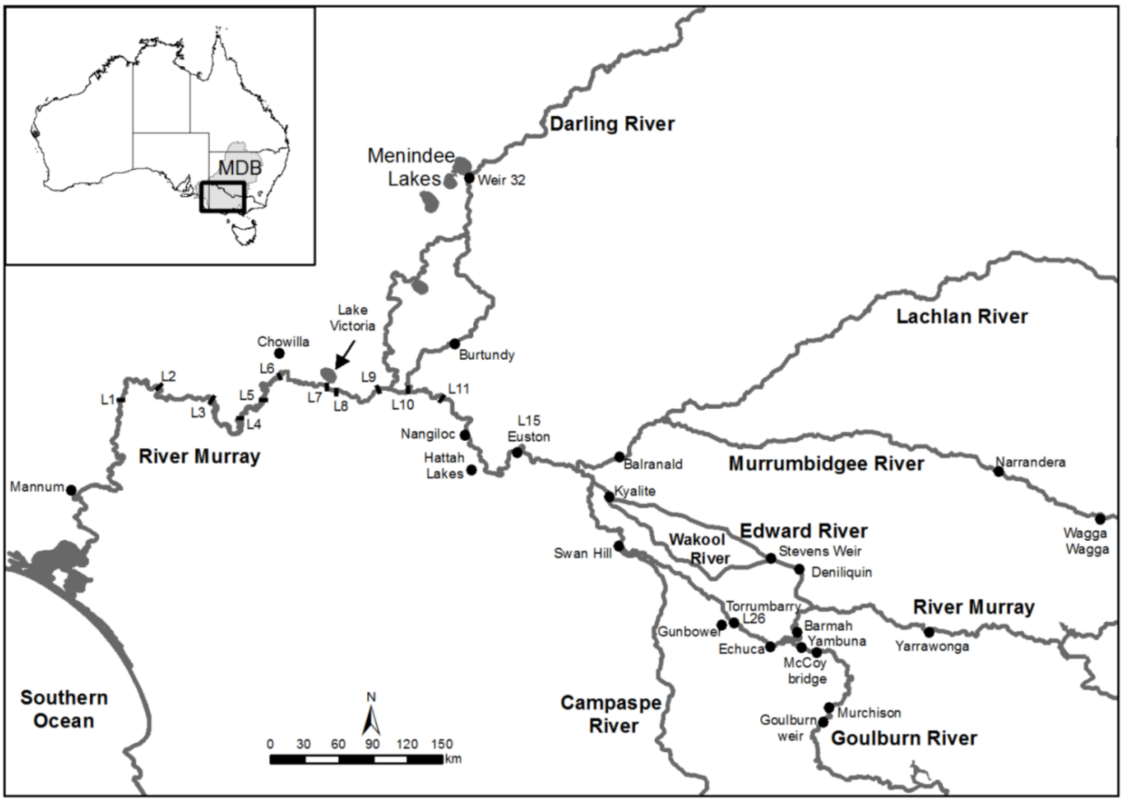

Figure 1. Map showing the location of the Murray–Darling Basin and the major rivers that comprise the southern Murray-Darling Basin, the numbered Locks and Weirs (up to Lock 26, Torrumbarry), the Darling, Lachlan, Murrumbidgee, Edward–Wakool, Campaspe and Goulburn rivers and Lake Victoria, an off-stream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

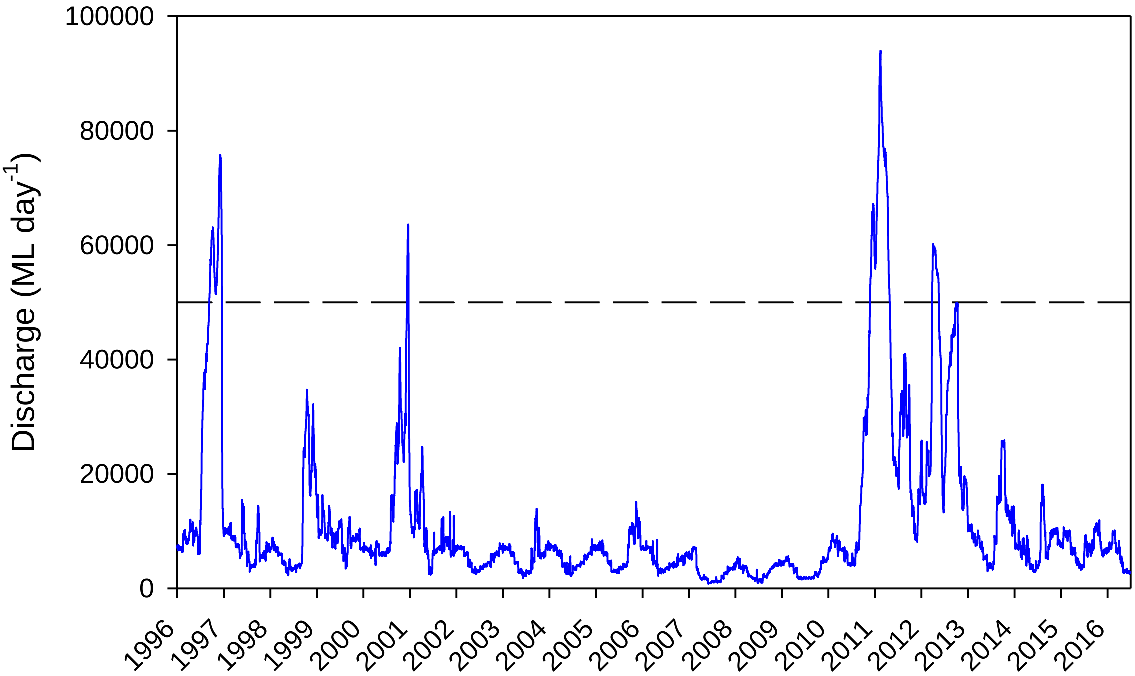

Figure 2. Daily flow (ML day^{-1}) in the LMR at the South Australian border from January 1996 to July 2016. Dotted line represents approximate bankfull flow in the main channel of the LMR.

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 (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 2015 have demonstrated the ecological benefits in the LMR (Ye *et al.* 2015a; 2015b; 2016a; 2016b).

2015/16 was a dry year (<12,000 ML day^{-1}, Figure 2) and quite unusual, where full entitlement flow was provided, but there was no unregulated flow (Figure 3). During this year, ~814 GL of Commonwealth environmental water was delivered to the LMR from 1 July to 30 November 2015, and from 2 January to 30 June 2016. This included 15.8 GL of Commonwealth environmental water used for wetlands and weir pool raising (WPR) within South Australia, with the remaining ~798 GL flowing through the main channel.

Commonwealth environmental water delivered to South Australia in July and August 2015, largely consisting of return flows from water at Barmah–Millewa Forest and flow pulse events in the Goulburn River, increased flow in the LMR (discharge at the South Australian border, QSA) from entitlement flows (~3,200–3,700 ML day^{-1}) to ~5,500–9,200 ML day^{-1} (Figure 3). From September to November 2015, Commonwealth environmental water was delivered to the LMR in combination with The Living Murray environmental water, of which the Commonwealth environmental water proportion comprised ~77% (Figure 4). Commonwealth environmental water delivery peaked at ~5,900 ML day^{-1} on 21 September and then at ~6,700 ML day^{-1} on 28 October 2015, maintaining river flow at a peak of 9,600–11,700 ML day^{-1} (Figure 3). The delivery of Commonwealth environmental water during this period also supported the raising of Weir Pools 2 (between Locks 2 and 3) and 5 (between Locks 5 and 6), resulting in an additional inundation area of 175 and 894 ha for Weir Pools 2 and 5, respectively

(Figure 5; refer to Appendix B for more detail). In addition, Commonwealth environmental water assisted The Living Murray environmental water in supporting the operation of the Chowilla regulator to promote an in-channel rise in water levels in the Chowilla Anabranch system (Figure 5; Appendix B).

Following late October 2015, with reduced environmental water, flow in the LMR steadily decreased to 5,600 ML day^{-1} by end November 2015, where it remained at entitlement flow through to early January 2016 (Figure 3). Commonwealth environmental water was directly traded into the LMR to enable barrage flow to occur over January to March, which also increased river flow from ~3,000 ML day^{-1} to ~9,800 ML day^{-1} during February 2016. Further return flows of Commonwealth environmental water occurred in the last three months of the water year. Outputs from modelling indicate that Commonwealth environmental water contributed to all barrage releases from September 2015 to June 2016.

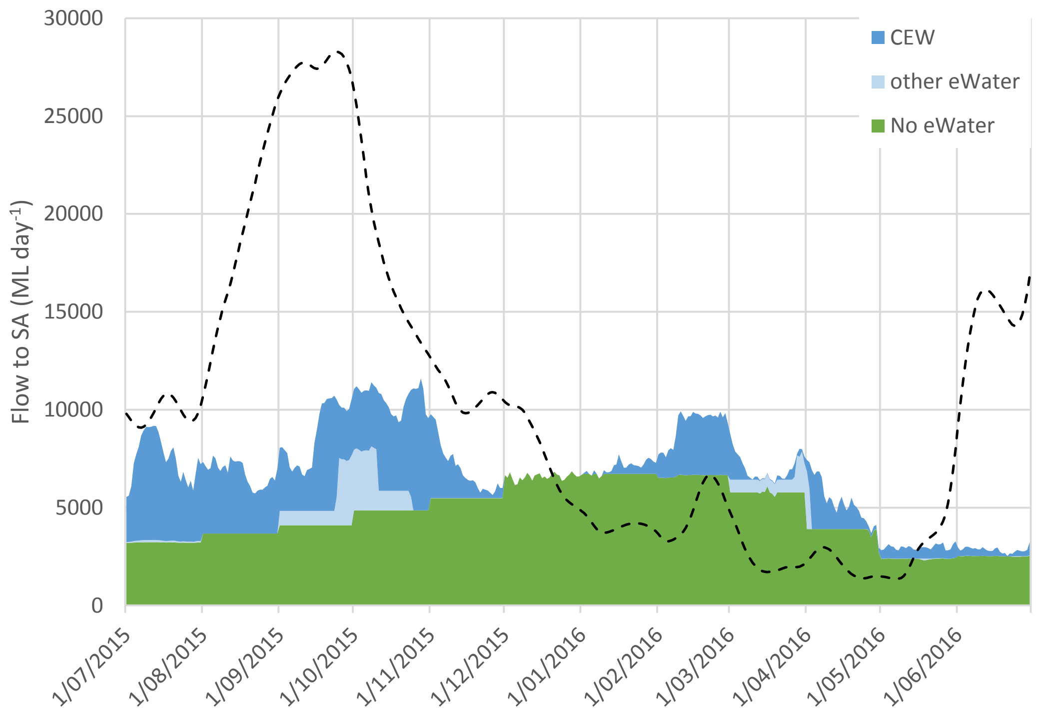

Figure 3. Flow to South Australia from July 2015 to June 2016 (stacked area chart) compared to modelled flow under natural conditions (black dotted line). CEW = Commonwealth environmental water; other eWater = other eWater such as The Living Murray and Victorian Environmental Water Holder.

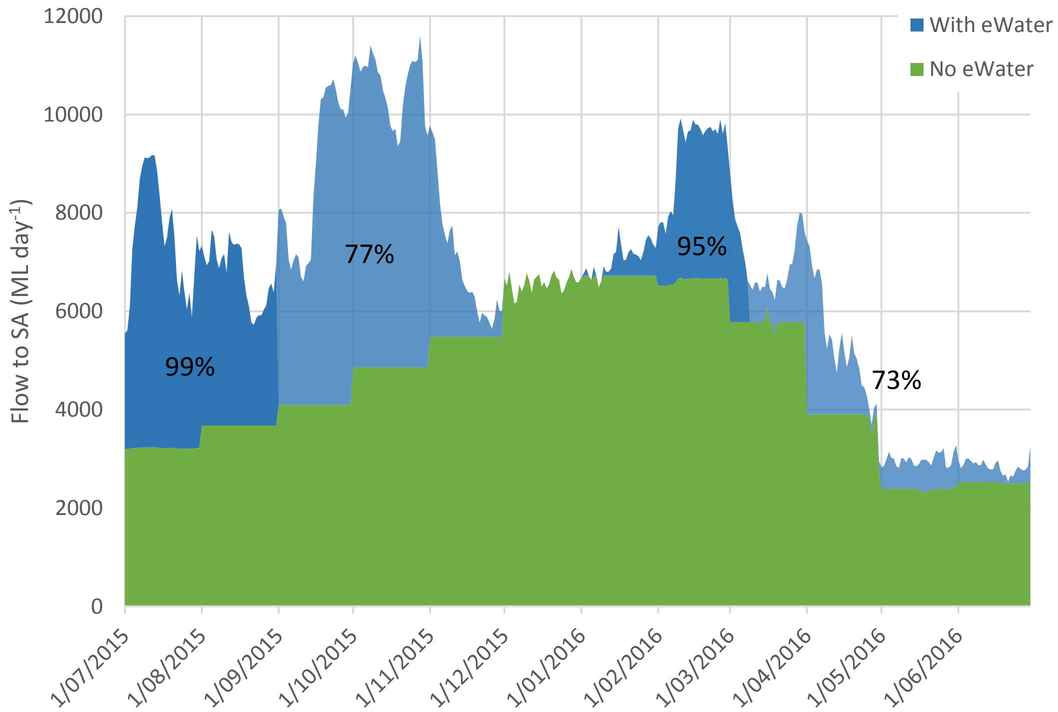

Figure 4. Commonwealth environmental water contribution to main watering events in 2015/16. Shading of the blue environmental water area represents the proportion of Commonwealth environmental water. There was not a case in 2015/16 where environmental water was provided without some of that water being Commonwealth environmental water.

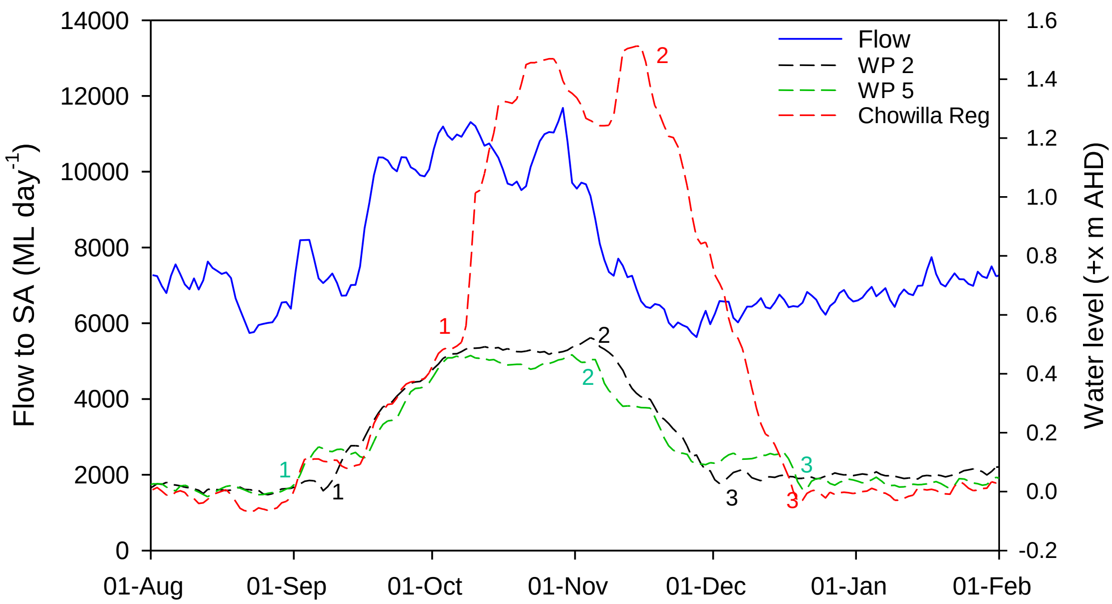

Figure 5. Water levels (dotted lines) in the Lock 2 (US Lock 2, +6.1 m AHD) and Lock 5 (US Lock 5, +16.3 m AHD) weir pools and Chowilla Anabranch (US Chowilla Regulator, +16.4 m AHD) between August 2015 and February 2016 (DEWNR), demonstrating weir pool raising and Chowilla in-channel rise events in the LMR. Flow (solid line) at the South Australian border (QSA) is overlaid. Numbers corresponding to water levels indicate the: (1) commencement of water level raising, (2) maximum level before the start of the recession and (3) return to normal pool levels at the end of the recession.

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 2015/16 can be seen in Figure 6[a].

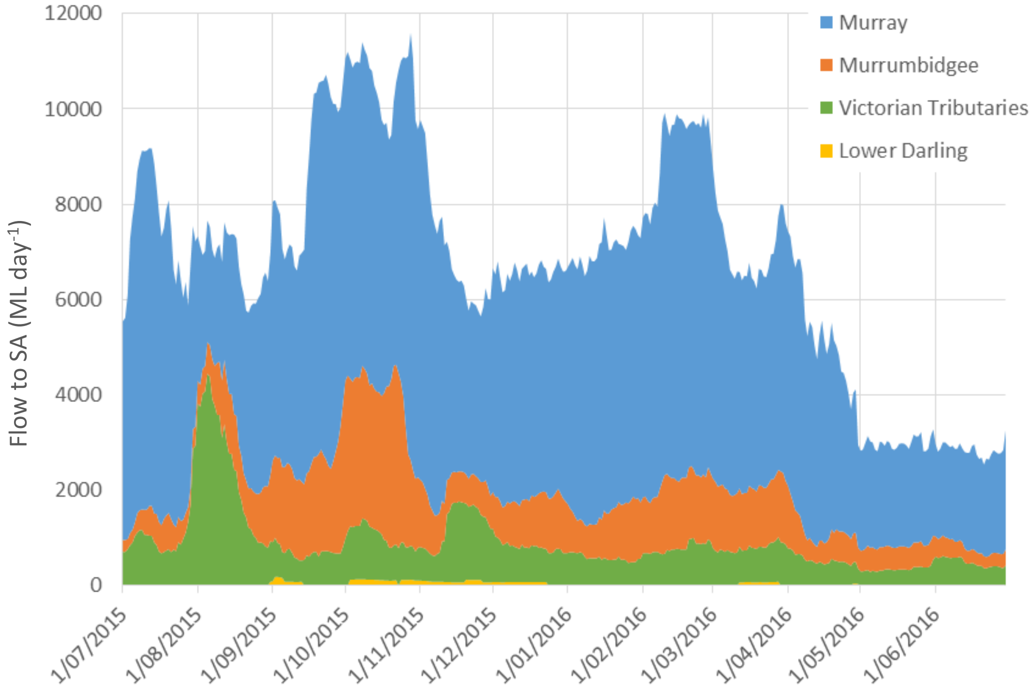

Figure 6. Source of all (environmental and consumptive) water delivered to the South Australian border (MDBA). Caveats for estimated water delivery time are mentioned above. Refer to Figure 1 for location of tributaries and rivers, relative to the LMR.

Concurrently with the environmental water deliveries described above, there were other management interventions that occurred upstream of the Selected Area (e.g. manipulations of Weir Pools 7, 8, 9 and 15, and Barmah–Millewa Forest inundation), which may have affected ecological responses in the LMR Selected Area. Refer to Appendix B for more information.

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[a] 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.

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 7). 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.

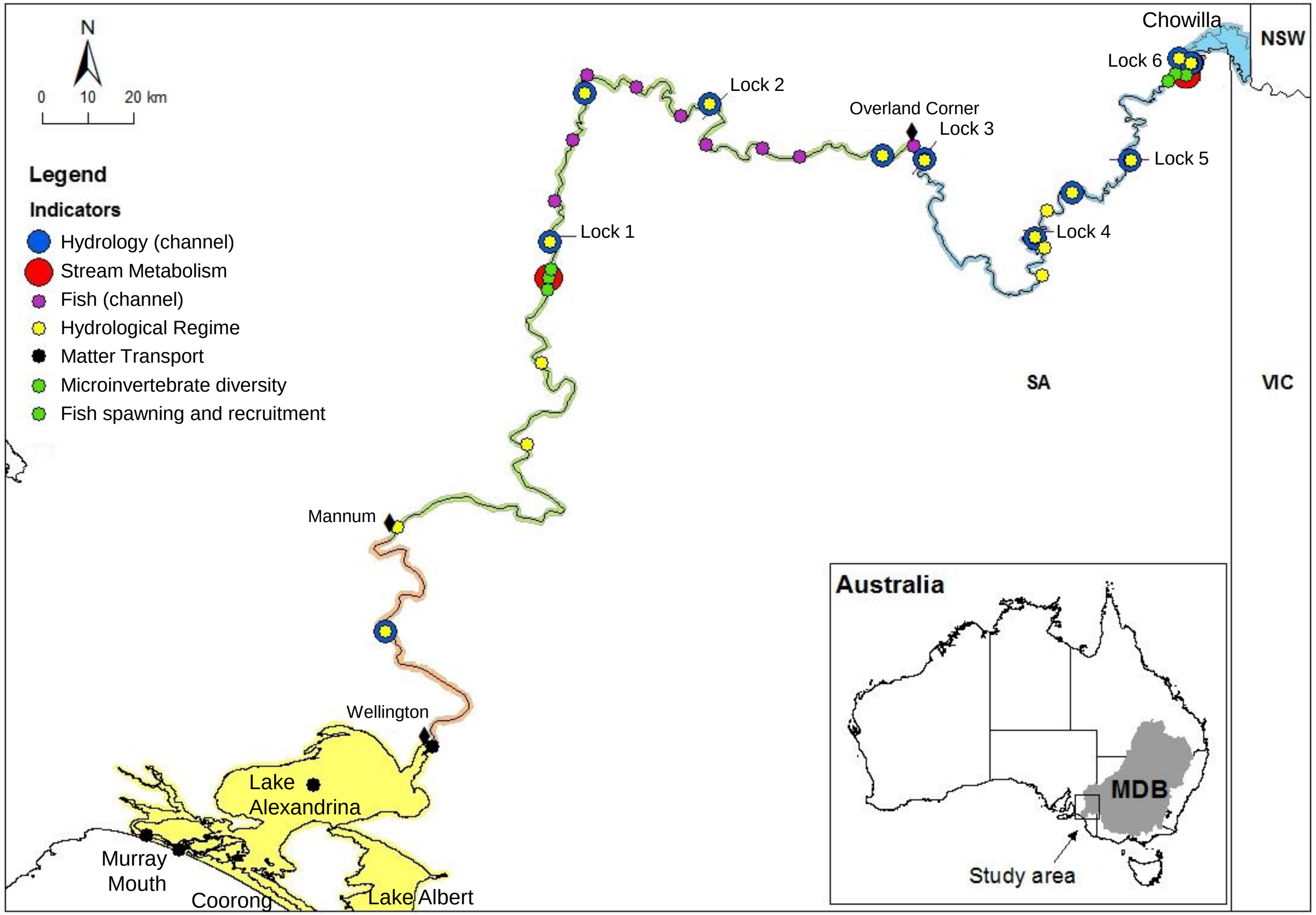

Figure 7. Map of the LMR Selected Area showing the floodplain (blue), gorge (green) and swamplands (orange) geomorphic zones, and the Lower Lakes, Coorong and Murray Mouth (yellow). Sampling sites are indicated by coloured circles. Fish Spawning and Recruitment sites represent larval sampling only.

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 Selected Area (LMR LTIM M&E Plan), 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 (Appendix A). The hypotheses were developed based on our conceptual understanding of the life histories of relevant biota and ecological processes and the effect of flow on them. The following conceptual diagram illustrates our current understanding of how river ecosystems are affected by the key ecosystem driver (flow regime), subject to flow management and climate effects, and how these indicators contribute toward a holistic understanding of ecosystem responses to flow management and ecological benefits (Figure 8).

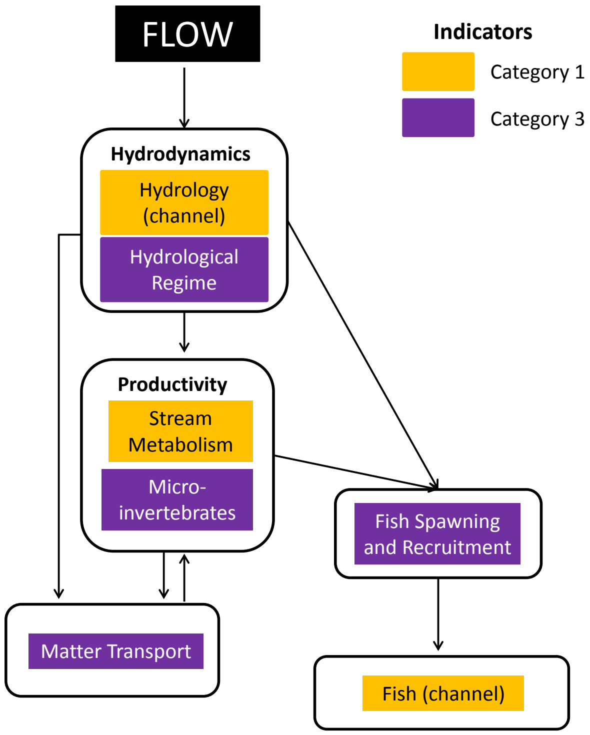

Figure 8. Conceptual diagram of how the main channel of river systems are affected by the key ecosystem driver (flow regime), subject to flow management and climate effects, and how complementary monitoring components (indicators) contribute toward a holistic understanding of ecosystem responses to flow management and ecological benefits in the LMR Selected Area. Magnitude, timing and duration are factors of flow (in black).

1.4 Key findings from the CEWO LTIM project for Year 1 (2014/15)

During Year 1 (2014/15) of the CEWO LTIM project, ~581 GL of Commonwealth environmental water was delivered to the LMR Selected Area, in conjunction with other sources of environmental water (e.g. MDBA The Living Murray). Environmental water delivery helped to maintain river flow at 9,000–10,000 ML day^{-1} during October and November 2014 and from mid-January to mid-March 2015 in the LMR. The watering events also supplemented flows to the Lower Lakes and barrage releases to the Coorong from September 2014 to June 2015.

Commonwealth environmental water delivery contributed to a number of short-term ecological outcomes in the LMR Selected Area during 2014/15:

- Increased hydraulic diversity, reflected by increased median velocity (generally from 0.1 to 0.15 m s^{-1}), with some cross sections in the weir pool transforming from 0.11–0.17 m s^{-1} to 0.17–0.3 m s^{-1}.
- Increased water levels of up to 0.2 m in the upper reaches of weir pools, 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 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 the return flows from Chowilla Floodplain.
- Reduced salinity concentrations in the Murray River Channel, Lower Lakes and, in particular, the Murray Mouth; increased salt export from the Murray River Channel and Lower Lakes; and reduced salt import into to the Coorong.

However, there was limited golden perch spawning and recruitment in 2014/15 due to the absence of favourable hydrological characteristics, such as spring–summer in-channel flow variability or overbank flows. More detail on the outcomes of the 2014/15 monitoring is available in last year's annual evaluation report for the LMR Selected Area (Ye *et al.* 2016a).

1.5 Purpose of the CEWO LTIM report for Year 2 (2015/16)

This synthesis report presents a summary of the second year's (2015/16) key findings of indicators for the LMR Selected Area (Section 2), and answers CEWO short-term (one-year) evaluation questions (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 also discussed in this report (Appendix I). 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 I). The Basin-scale evaluation for fish community responses to Commonwealth environmental water are being undertaken by the Monitoring and Evaluation (M&E) Advisors (LMR LTIM M&E Plan). General recommendations for environmental flow management in the LMR are provided in Section 4, based on monitoring and evaluation outcomes, and expert scientific opinion. As stated in the LMR LTIM M&E Plan, monitoring and evaluation of Commonwealth environmental water delivery in the LMR Selected Area focused on spring/summer given this was the primary period for biological response monitoring in the LMR; therefore, our findings and recommendations on environmental water management are most relevant to this period. Nevertheless, the annual cycle of flow is important for maintaining and restoring ecological integrity of riverine ecosystems thus environmental water allocation may be required beyond spring/summer. More detailed information (e.g. methodology, statistics, etc.) for each indicator in the LMR are provided in the Appendices and LMR LTIM M&E Plan.

2 KEY FINDINGS

2.1 Category 1

Stream Metabolism

River metabolism measurements estimate in-stream rates of gross primary production (GPP) and ecosystem respiration (ER), and provide information on the energy processed through river food webs (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). In the main channel of the Murray River, the production of organic material is largely due to photosynthesis by phytoplankton (Oliver and Merrick 2006), but this is augmented by the transport of organic material from the floodplain during floods. These food resources are consumed and respired in aquatic food webs. Ecosystem net production (ENP), the difference between the formation and breakdown of organic material by photosynthesis and respiration, respectively, helps identify whether food resources have come from within the river (autochthonous) or the surrounding landscape (allochthonous). Analyses of metabolism measurements enables an assessment of the fundamental trophic energy connections that characterise different food web types (e.g. detrital, autotrophic, planktonic), and the size of the food web and 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 irradiance were undertaken at single river sites in the gorge (downstream of (below) Lock 1) and floodplain (below Lock 6) geomorphic zones of the LMR Selected Area in 2015/16 (refer to LMR LTIM M&E Plan). Discrete water quality samples were collected approximately every four weeks and analysed for chlorophyll-*a*, total nitrogen (TN, the sum of all forms of nitrogen), nitrate and nitrite combined (NO_X, the oxides of nitrogen), ammonium (NH_4), total phosphorus (TP, the sum of all forms of phosphorus), dissolved forms of phosphorus (PO_4), and dissolved organic carbon (DOC). 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).

During the 2015/16 monitoring period, oxygen concentrations were maintained at acceptable levels (>50% saturation, where 100% saturation in the Murray River is typically 7–8 mg L^{-1} during summer). At the site below Lock 1, three periods of reduced oxygen concentration were recorded reaching minimum values of 5 mg L^{-1} (Figure C1 in Appendix C). Nevertheless, it is suspected that these resulted from biofouling of the oxygen sonde housing (probe error) as extensive analyses excluded other likely environmental explanations (Appendix C).

At both sampling sites, metabolic activity (GPP and ER) gradually increased in magnitude over the sampling period, although there were differences in specific patterns between the two sites (Figures C2 and C6 in Appendix C). Metabolic activity was enhanced at the site below Lock 6 (i.e. in Weir Pool 5) from early October to early November 2015, when this weir pool was being raised, supported by Commonwealth environmental water (Figure 9; Figure B1 in Appendix B). It is suggested that the changes in metabolism reflected the increased connection between the river and the floodplain resulting from WPR. As water levels in Weir Pool 5 began to recede following WPR, there was a decrease in ER to mid-November. However, as the rate of decline slowed, ER began to increase again with a peak occurring ~26 November, aligning with the cessation of water level decline (Figure 9). These increased respiration rates following the return of the weir pool to operating height aligned with the period of the drawdown of water levels in the Chowilla Anabranch, following a regulated in-channel rise event (Figure 5; Figure C3b in Appendix C; Appendix B). This return of water from the anabranch was also associated with an increase in GPP and so may have contributed to the general increase in metabolism, although the junction is downstream of the sampling site and so its direct influence is difficult to assess.

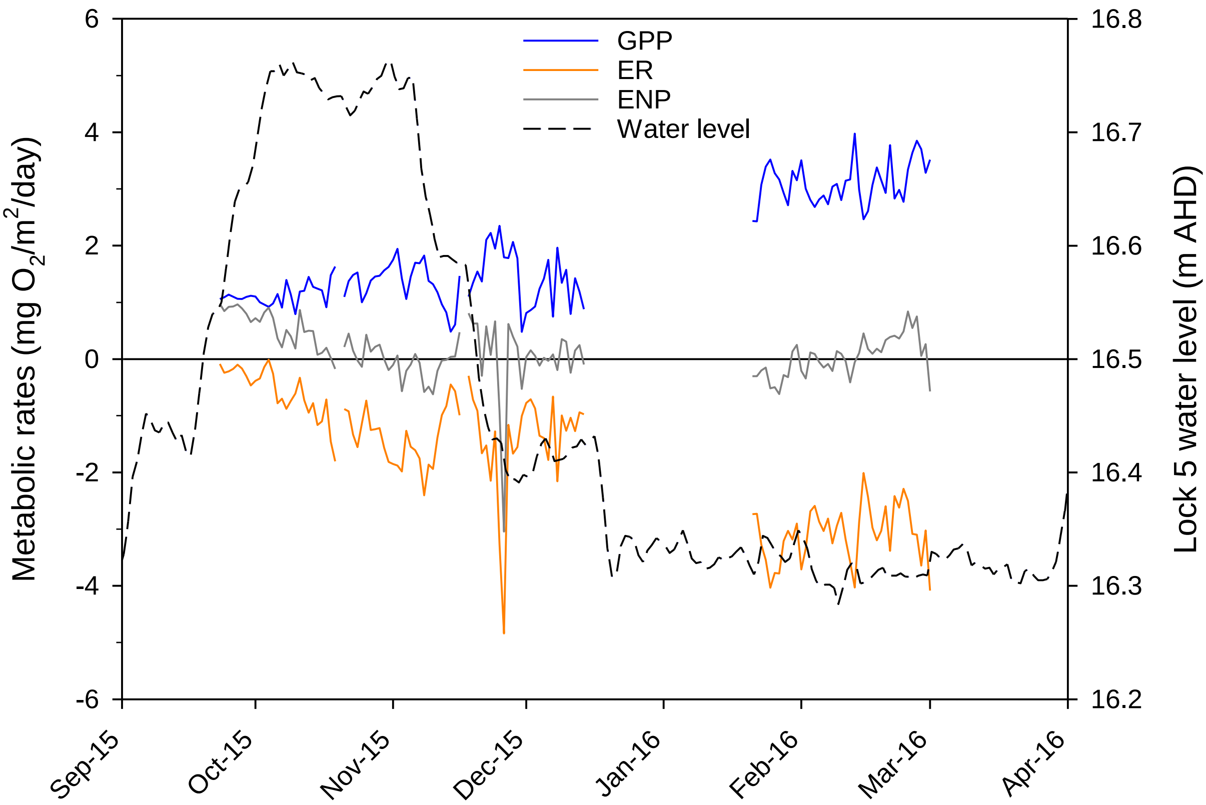

Figure 9. Comparison of time series of rates of gross primary production (GPP), ecosystem respiration (ER) and ecosystem net production (ENP) with water levels in the weir pool upstream of Lock 5 (m AHD).

Below Lock 6, integration of the metabolic responses over the total duration of the monitoring period produced an ENP close to zero (Figure C5 in Appendix C), suggesting that the flooded area was enhancing aquatic phototrophic production, and that this was largely returned to the river. This response is important as intermittent periods of increased supplies of organic material are important to the food webs of the Murray River. Their decline in frequency, duration and extent has been proposed as a major cause of reductions of aquatic biota, through decreases in food supplies (Oliver and Merrick 2006; Oliver and Lorenz 2010).

Below Lock 1, there were several cycles of increasing and then decreasing metabolic activity that appeared to be responsible, at times, for driving down oxygen concentrations (Figures C1 and C6 in Appendix C). These results were attributed to the unusual extent of biofouling that was not observed at the site below Lock 6. Further discussion relating to probe error is provided in Appendix C.

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 can influence riverine hydraulics and structural habitat, which may influence fish assemblage structure (Bice *et al.* 2014).

During March–April 2016, small- and large-bodied fish assemblages were sampled from the gorge geomorphic zone of the LMR Selected Area (Figure 7) using fyke nets and electrofishing, respectively. 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 M&E Advisors. While there is no CEWO local (Selected Area) evaluation questions for this indicator, we analysed monitoring data from the LMR Selected Area to investigate temporal variation in fish assemblage and population structure between Year 1 (autumn 2015, herein 2014/15) and 2 (autumn 2016, herein 2015/16) (Appendix D).

Relatively low (<15,000 ML day^{-1}), stable flows predominated in the LMR Selected Area during 2014/15 and 2015/16. Consequently, small-bodied fish abundance and diversity remained high in 2015/16 (Figure 10b; Table D2 in Appendix D), and there was no significant change in small-bodied fish assemblage structure from 2014/15 to 2015/16. Abundances of flow-cued spawning 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 an increase in exotic goldfish and a decrease in bony herring, in 2015/16, relative to 2014/15 (Figure 10a; Table D1 in Appendix D). Increased abundances of exotic, large-bodied species may reflect their generalist/opportunistic life histories.

Based on length frequency data, there was no recruitment (to age 0+) of native, large-bodied golden perch, silver perch or freshwater catfish in 2015/16 (Figure D4 in Appendix D). The absent 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 2.2

Category 3 Fish Spawning and Recruitment). For the second consecutive year, small Murray cod (<150 mm TL, likely age 0+) were sampled in the LMR Selected Area during 2015/16 (Figure D4 in Appendix D), indicating successful recruitment. Furthermore, there was persistence of the age 0+ cohort (100–150 mm TL) from 2014/15 to age 1+ (196–232 mm TL) in 2015/16. 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. The mechanisms facilitating the recruitment of cohorts of Murray cod from 2014/15 and 2015/16, both low flow years, remain unclear.

In the main channel of the LMR Selected Area, 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. The current fish assemblage structure is similar to that during drought (i.e. 2007–2010) (Bice *et al.* 2014) and characteristic of a low flow scenario. Persistent low flow conditions are likely to favour generalist/opportunistic species (e.g. small-bodied and exotic species) that are adapted to benign hydraulic conditions and abundant aquatic vegetation. Continued low flows in the lower River Murray are likely to have further negative effects on the recruitment of flow-cued spawning species and, in turn, lead to a decline in their population resilience and abundance.

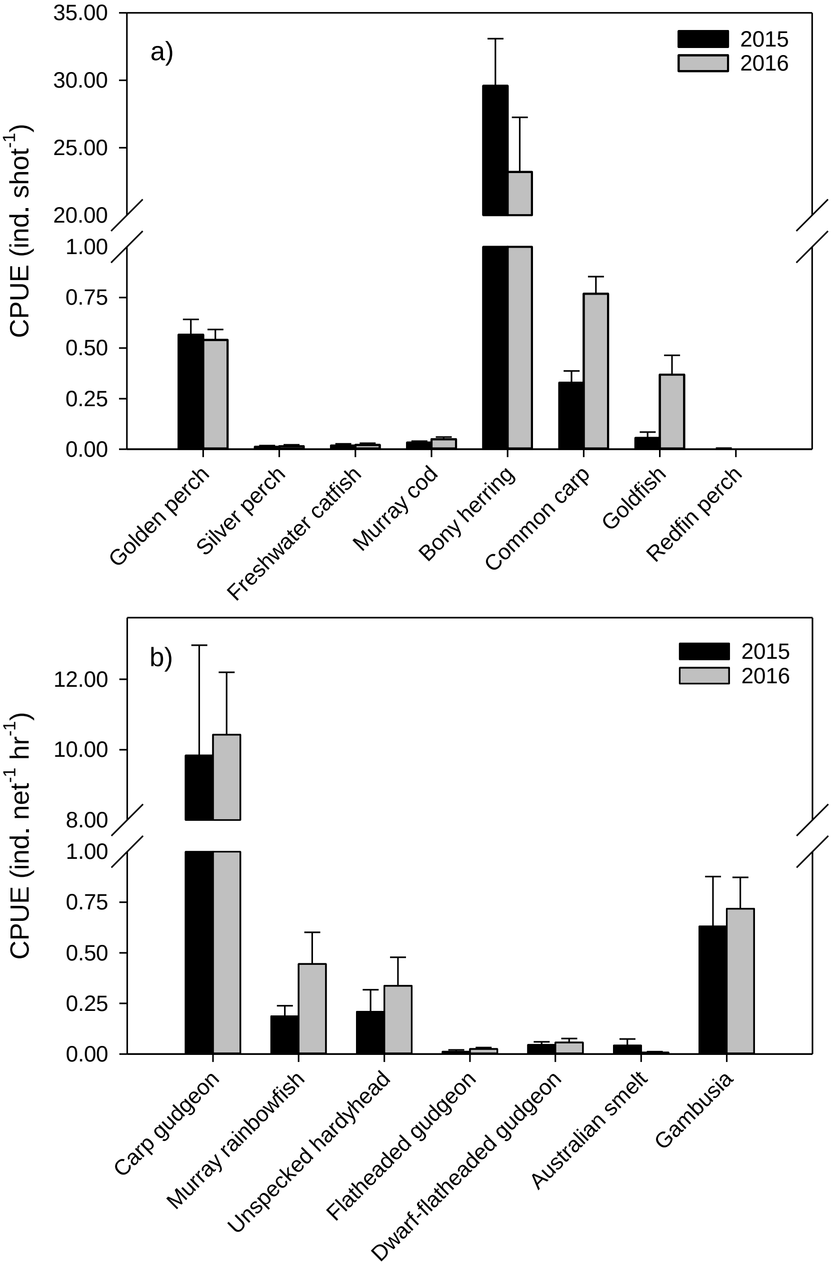

Figure 10. Mean catch-per-unit-effort (CPUE) ± standard error of (a) large-bodied fish species captured using electrofishing (individuals per 90 second shot) and (b) small-bodied fish species captured using fine-mesh fyke nets (individuals per net per hour) in the gorge geomorphic zone (10 sites) of the LMR Selected Area during 2014/15 and 2015/16.

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 riverine environment characterised by water velocities ranging ~0.4–0.8 m s^{-1}, even at discharges <10,000 ML day^{-1}. Post 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 riverine habitats are important for ecological and life history processes for many native biota. These include reproductive activity in flow-cued spawning species (e.g. golden perch), facilitating downstream drift and transportation of plankton, macroinvertebrates and larvae, and providing 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) in the lower River Murray.

The Hydrological Regime indicator used models to convert the discharge delivered to the LMR Selected Area in 2015/16 to water levels and velocities. 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.

Water level and velocity results for a weir pool in the gorge (Weir Pool 1, Lock 1–2) and floodplain (Weir Pool 5, Lock 5–6) zones (Figure 7) are presented in Figures 11 and 12. 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 2 for the Weir Pool 1 case) has been presented, as the upper end of the weir pool is the least influenced by the lock, and hence most responsive to changes in discharge. Water level results for a location in the middle of the weir pool, and at both upper and middle locations for all weir pools, are presented in Appendix E. Further investigation into the Commonwealth environmental water contribution to increase in water levels through the WPR events is provided in Section 4.

In 2015/16, Commonwealth environmental water was critical in undertaking WPR at Weir Pools 2 and 5. As such, it was assumed that the WPR would not have occurred in the 'without environmental water' cases. This can be seen in Figure 11 as the difference in water level of almost 0.7 m in October 2015 in Weir Pool 5, of which 0.45 m was due to WPR, with the remainder of the increase in water level due to the increase in discharge resulting from the environmental water.

The results indicate that environmental water resulted in increases in water level of up to 0.3 m in the upper reaches of the weir pool for Weir Pools 1 and 4 in winter and spring. This increased up to 0.7 m in spring for Weir Pools 2 and 5, during the WPR events. For velocity, it can be seen from Figure 12 and Figure E8 (in Appendix E) that, without environmental water, the median velocity was less than 0.1 m s^{-1} across the weir pools for all of 2015/16 (with some exceptions in Weir Pool 5). Velocities less than 0.1 m s^{-1} represent slow-flowing habitat, and as such the without environmental water case (i.e. entitlement flow all year), represents close to stagnant weir pools. In contrast, the 'With CEW' case represents an increase in weir pool median velocity of ~0.1 m s^{-1} in winter and spring across all weir pools. The shaded blue areas in Figure 12 and Figure E8 (in Appendix E) indicate that, with Commonwealth environmental water, there were some cross sections in each weir pool (typically the upper reaches) greater than 0.17 m s^{-1}, between July and November 2015 as well as in February 2016, coinciding with the delivery of Commonwealth environmental water increasing over the South Australian border (QSA) to over 9,000 ML day^{-1} (Figure 3). Hydraulic diversity, if defined as the range between the 10^{th} and 90^{th} percentile velocities (i.e. the shaded areas in Figure 12 and Figure E8 in Appendix E), can be seen to have been increased by the contribution of Commonwealth environmental water to the LMR.

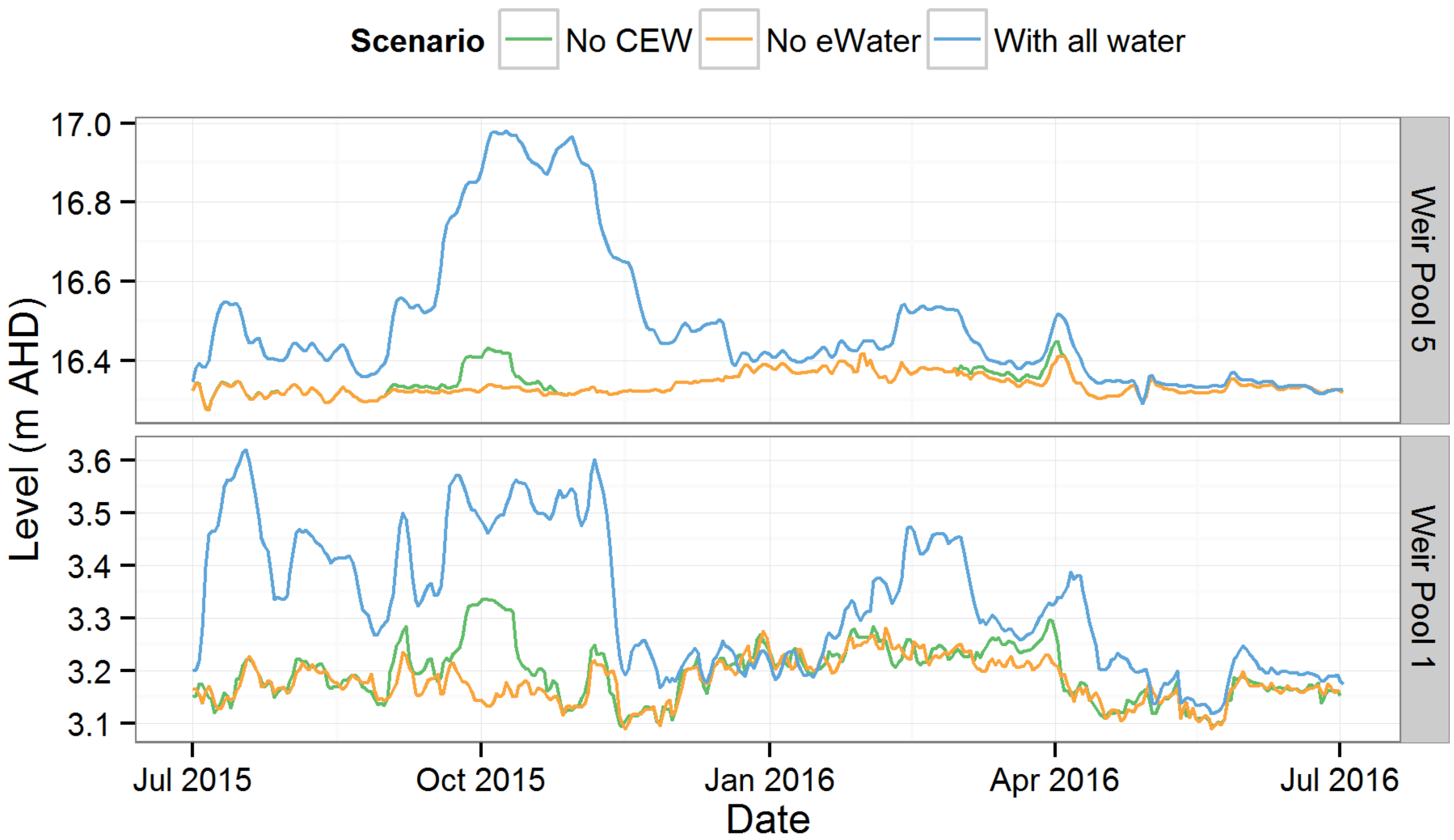

Figure 11. Water levels at the upper end of the weir pool for a weir pool in the floodplain zone (Weir Pool 5) and gorge zone (Weir Pool 1) representing observed conditions (With all water), without Commonwealth environmental water (No CEW) and without any environmental water (No eWater) for 2015/16.

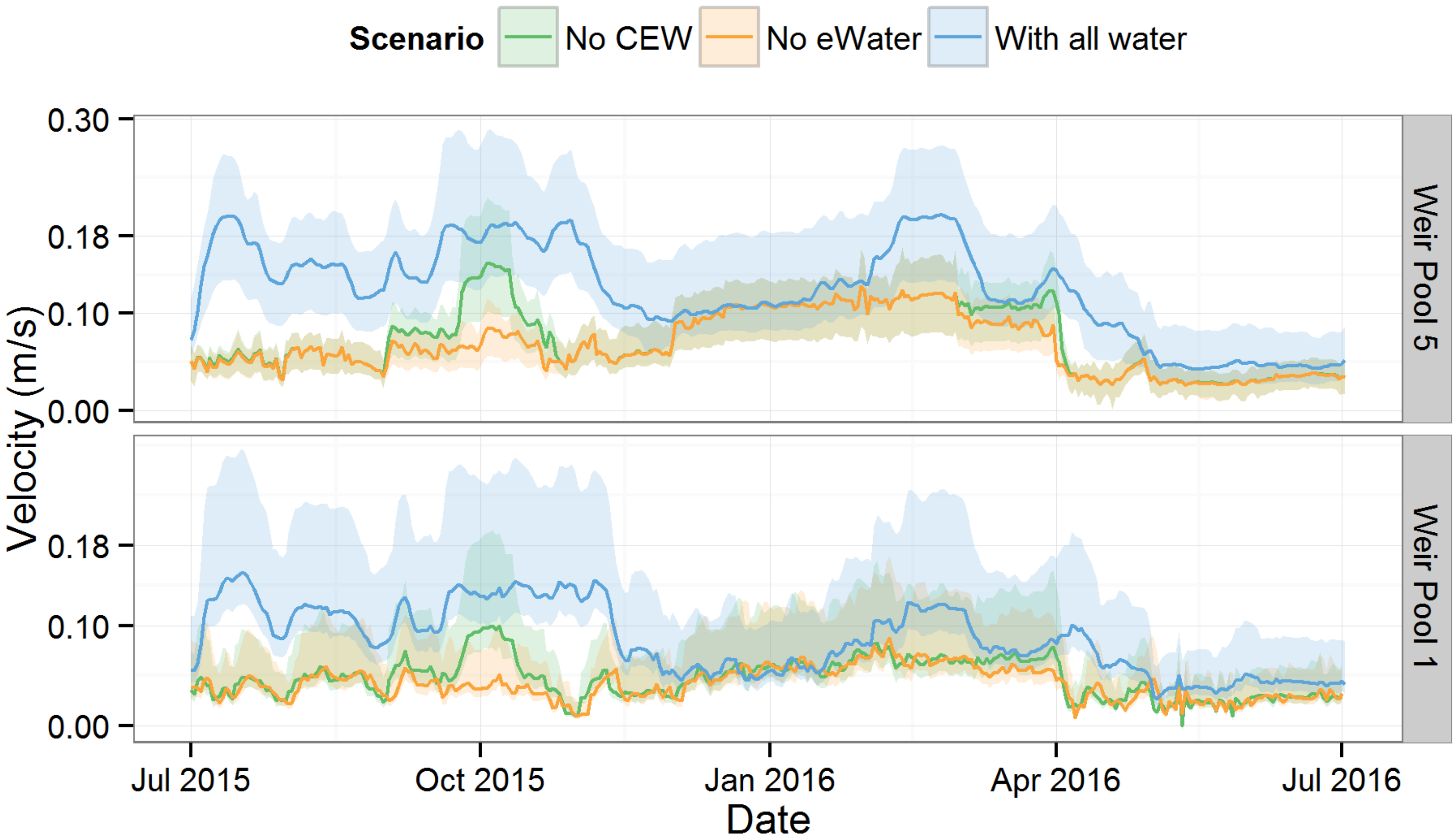

Figure 12. Cross section averaged velocities in a weir pool in the floodplain zone (Weir Pool 5) and gorge zone (Weir Pool 1) representing observed conditions (With all water), without Commonwealth environmental water (No CEW) and without any environmental water (No eWater) for 2015/16. The median velocity in the weir pool is represented by the solid line, and the range (as the 10th and 90th percentiles) represented by the shaded area.

Matter Transport

Altering the flow regime of riverine systems has altered the concentrations and transport of dissolved and particulate matter (Appendix F). For example, reduced flow can result in: salinisation through the intrusion of saline water; reduced nutrient availability due to decreased mobilisation of nutrients from the floodplain; and reduced primary productivity because of nutrient limitation. Environmental flows may be used to reinstate some of the natural processes that control the availability and transport of dissolved and particulate matter. 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, a hydrodynamic-biogeochemical model was applied for the region below Lock 1 to the Murray Mouth (see Appendix F). Assumptions made within the model result in uncertainty in the model outputs and so outputs should not be considered to be absolute values (for more detail refer to Aldridge *et al.* 2013 and Appendix F). Instead, the model outputs are used to assess the general response to environmental water delivery. For this, three simulations were run and compared for 1 July 2015 to 30 June 2016: (1) 'With all water' (i.e. observed, including all environmental and consumptive water); (2) Without Commonwealth environmental water ('No CEW'); and (3) Without any environmental water ('No eWater').

<u>*Salinity*</u>

The modelling suggests that environmental water had a minor effect on salinity levels in the Murray River Channel (i.e. Wellington) in 2015/16 (Table 2). This was also the case for Lake Alexandrina, with all environmental water reducing median salinities over 2015/16 from 0.50 practical salinity units (PSU)[b] to 0.39 PSU. Salinity within the Murray Mouth was significantly lower with environmental water, with median salinities of 27.73 PSU with all water, 35.23 PSU without Commonwealth environmental water and 35.33 PSU without any environmental water.

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[b] 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}.

Based on the modelling outputs, environmental water contributed to 117,861 tonnes (47%) and 257,485 tonnes (89%) of the total modelled export of salt from the Murray River Channel and Lower Lakes, respectively (Table 3; Figure 13). There was a modelled net import of 1,850,028 tonnes of salt to the Coorong during 2015/16 with all water. Without any environmental water there was a modelled net import of 6,649,380 tonnes of salt to the Coorong and without Commonwealth environmental water there was a modelled net import of 6,441,297 tonnes.

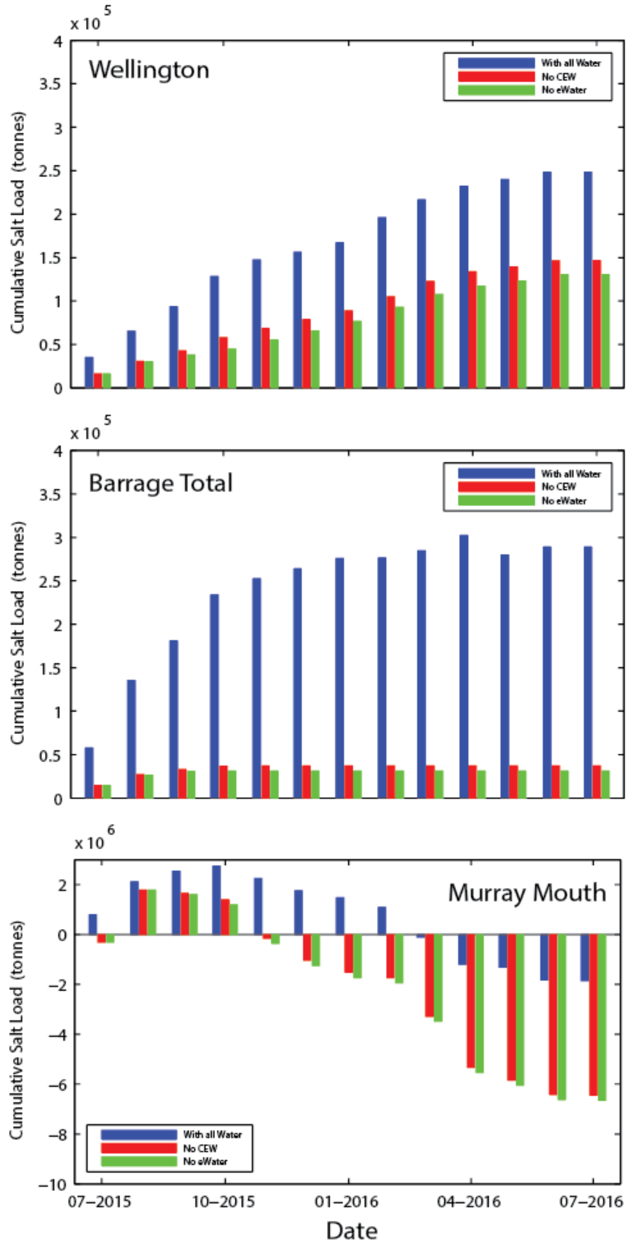

Figure 13. Modelled cumulative salt exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

Dissolved nutrients

There were only minor differences in the modelled dissolved nutrient concentrations between scenarios (Table 2; Appendix F). The most apparent differences were higher silica concentrations with environmental water within the Lower Lakes.

Since there were only small differences in concentrations of dissolved nutrients, differences in modelled exports between the scenarios were largely a result of differences in discharge (Table 3). This may have been associated with a combination of three factors. Firstly, the wetting of ephemeral habitats associated with higher discharges can mobilise additional nutrients from soils and organic material. Secondly, increased flow velocities associated with higher discharges can resuspend nutrients (e.g. silica) deposited in river sediments. Thirdly, larger volumes of water passing downstream associated with higher discharges (total export is a function of the concentration and volume). Environmental water increased exports of all dissolved nutrients from the Murray River Channel and Lower Lakes, and this was also the case in the Murray Mouth for ammonium. For phosphate and silica, there was net export from the Murray Mouth with environmental water but a net import to the Coorong without environmental water. The most apparent differences in exports associated with environmental water were for silica, with Commonwealth environmental water contributing to 41% and 95% of total silica exports from the Murray River Channel and Lower Lakes, respectively. Silica is a particularly important nutrient for supporting the growth of diatoms, a phytoplankton group that is generally considered to be of high nutritional quality in coastal and riverine systems. As such, the increased export of silica associated with environmental water may have supported increased secondary productivity along the Murray River Channel, Lower Lakes and Coorong and near-shore environment.

Particulate organic nutrients

There were no apparent differences in the modelled particulate nutrient concentrations with and without environmental water (Table 2). As such, differences in modelled exports between the scenarios were largely a result of differences in discharge (Table 3), with environmental water increasing exports. This modelling suggests that during 2015/16, Commonwealth environmental water contributed to 46% and 90% of the total exports of particulate organic nitrogen from the Murray River

Channel and Lower Lakes, respectively. With all environmental water, there was a net export of 538 tonnes of particulate organic nitrogen from the Murray Mouth, but without Commonwealth environmental water there was a net import of 224 tonnes. Similar observations were found for particulate organic phosphorus, although total exports were lower (Table 3) owing to lower concentrations. The increased export of organic nutrients associated with environmental water may have provided benefits for the Lower Lakes, Coorong and near-shore environment by providing energy to support secondary productivity.

<u>Chlorophyll a</u>

There were no apparent differences in the modelled chlorophyll *a* concentrations between scenarios (Table 2). As a result, differences in modelled exports reflected that of discharge, with environmental water resulting in additional exports (Table 3). Overall, Commonwealth environmental water contributed to 44%, 92% and 93% of the total exports of phytoplankton biomass from the Murray River Channel, Lower Lakes and Murray Mouth, respectively. The increased export of phytoplankton biomass associated with environmental water 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).

Table 2. Median concentration of salinity, nutrients and chlorophyll *a* during 2015/16 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). BDL represents model outputs that are below the analytical detection level.

Site	Scenario	Salinity (PSU)	Ammonium (mg/L)	Phosphate (mg/L)	Silica (mg/L)	Particulate organic nitrogen (mg/L)	Particulate organic phosphorus (mg/L)	Chlorophyll *a* (mg/L)
Wellington	With all water	0.16	0.017	BDL	1.02	1.17	0.11	26.5
	No CEW	0.20	0.015	BDL	1.02	1.19	0.11	27.5
	No eWater	0.23	0.017	BDL	1.02	1.18	0.11	27.9
Lake Alexandrina Middle	With all water	0.39	0.015	BDL	0.79	1.37	0.13	32.9
	No CEW	0.48	0.019	BDL	0.60	1.46	0.14	35.5
	No eWater	0.50	0.019	BDL	0.57	1.49	0.14	36.3
Murray Mouth	With all water	27.73	0.021	BDL	0.96	1.57	0.11	38.0
	No CEW	35.23	0.022	BDL	0.96	1.60	0.10	38.6
	No eWater	35.33	0.022	BDL	0.96	1.60	0.10	38.6

Table 3. Net cumulative load (tonnes) of salt, nutrients and chlorophyll a during 2015/16 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). Positive value indicates export and negative value indicates import.

Site	Scenario	Salt	Ammonium	Phosphate	Silica	Particulate organic nitrogen	Particulate organic phosphorus	Phytoplankton (as carbon)
Wellington	With all water	248,267	29.5	13.8	1760	1480	105	39.9
	No CEW	146,443	17.9	11.6	1035	795	61	22.5
	No eWater	130,406	16.0	11.2	919	688	54	19.6
Barrage	With all water	288,516	8.2	0.9	395	832	62	23.5
	No CEW	36,884	0.5	0.2	20	84	5	1.9
	No eWater	31,031	0.3	0.2	16	70	4	1.5
Murray Mouth	With all water	-1,850,028	9.4	0.2	155	538	44	19.6
	No CEW	-6,441,297	4.4	-0.4	-245	-224	-7	1.4
	No eWater	-6,649,380	4.3	-0.4	-253	-237	-7	1.2

Microinvertebrates

Aquatic microinvertebrates (protists, rotifers and microcrustaceans) 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 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 2015/16 using a Haney trap at sites below Lock 1 and Lock 6, in the gorge and floodplain geomorphic zones, respectively (Appendix G; LMR LTIM M&E Plan).

Over the 2015/16 sampling period (October 2015 to January 2016), 177 microinvertebrate taxa (rotifer/protist dominated) were discriminated from 192 trap samples from sites below Lock 1 and Lock 6 (Appendix G). The microinvertebrate assemblages, below each lock, were significantly different between all sampling events (Appendix G). However, it should be kept in mind that the majority of microinvertebrates predominating in the LMR have life cycles that are shorter than the inter-event timeframe (2 weeks), and that species compositional changes are standard with plankters responding to spatial and temporal changes in physico-chemistry. Longitudinal successional/compositional changes in entrained microinvertebrate assemblages in the moving LMR system below Lock 6 have been documented (Shiel *et al.* 1982, Ye *et al.* 2016a). Microinvertebrate taxa driving the dissimilarity between sampling events below both locks are tabulated in Appendix G and described below in context with seasonal changes and watering events.

Below Lock 1, mean microinvertebrate density increased to a peak in mid-October (2,230 ind. L^{-1}) and then steeply declined to 116 ind. L^{-1} in mid-November, where density remained low (<400 ind. L^{-1}) until mid-December 2016 (Figure 14). Microinvertebrate diversity (taxa richness) followed a similar trend, where mean diversity increased to ~53 taxa in early November, and then fell to ~19 taxa in mid-November (Figure 14). Peaks in density and diversity aligned with increased Commonwealth environmental water delivery and increased river discharge (Figure 3; Figure 14), which was a significant driver of assemblage structure (Figure G9 in

Appendix G). Conversely, the steep decreases in density and diversity were associated with: declining river flows (Figure 3; Figure 14); a recession of water levels at Lock 1 (Figures G3 and G4 in Appendix G); and, potentially, the downstream passage of water from Weir Pool 2 following WPR (Figures G3 and G4 in Appendix G), which was supported by Commonwealth environmental water (Appendix B). It is unclear if the declines in diversity and density were related to the effects of the drawdown from Weir Pool 2 (e.g. toxic leachates from a newly wetted area, Portinho *et al.* 2016), or a 'new' in-channel pulse low in microinvertebrates. Reductions in dissolved oxygen measurements below Lock 1 following the drawdown of water from Weir Pool 2 during the sampling period are not evident (Figure C1 in Appendix C). While densities and diversities remained low and stable below Lock 1 during the water level recession in Weir Pool 2 (lag effect of travel time taken into account), there was an increase in the number of littoral taxa (e.g. testate amoebae and littoral rotifers, e.g. *Trichocera*) during this period in mid-December. These littoral taxa could have originated from flushed littoral margins of Weir Pool 2 from WPR or from a suite of management interventions further upstream, such as raisings or lowering of Weir Pools 5, 7, 8, 9 and 15, Chowilla in-channel rise or Barmah–Millewa Forest floodplain inundation (Appendix B).

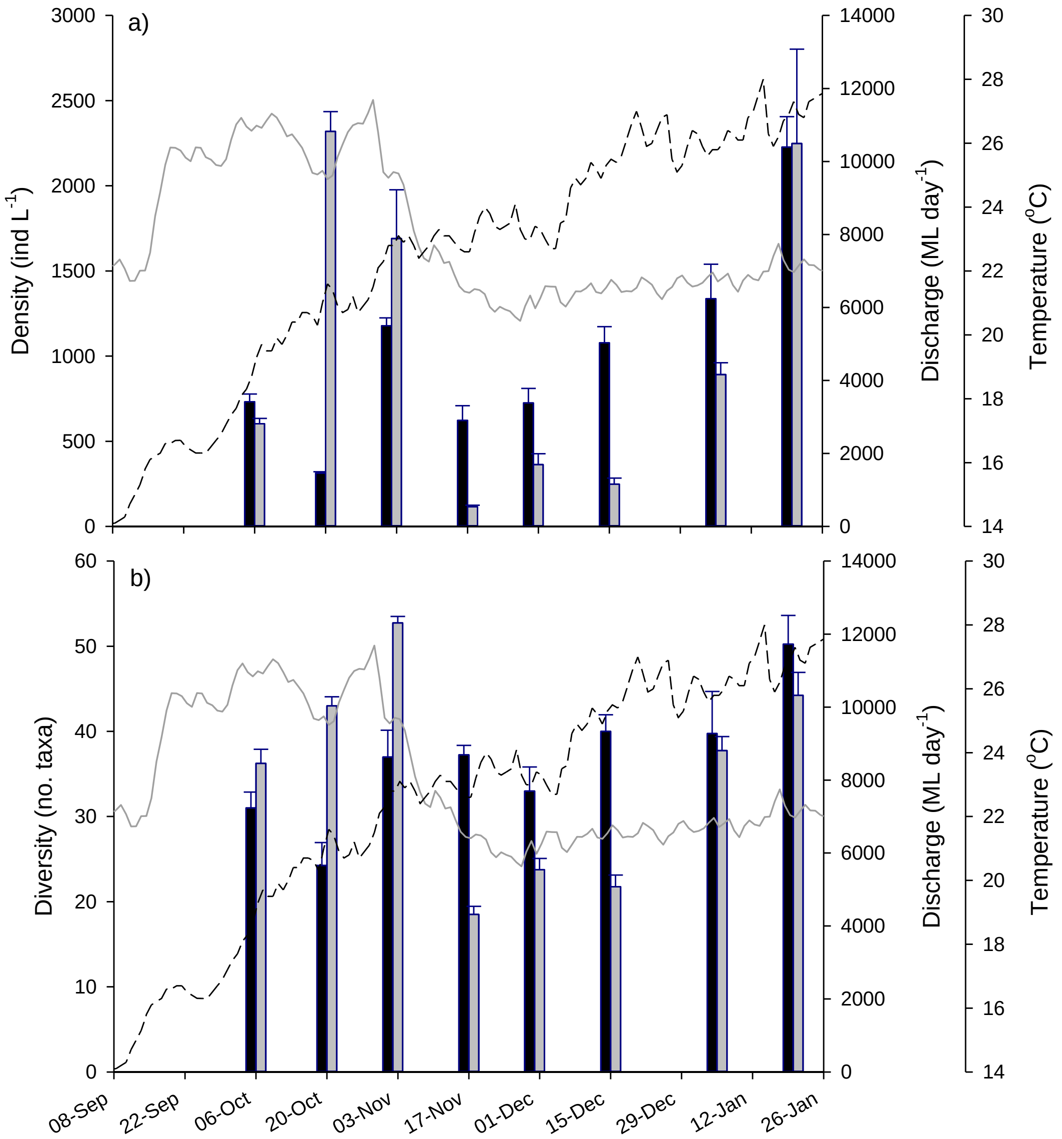

Figure 14. Mean (±S.E.) (a) density and (b) taxa richness of microinvertebrates collected in the LMR Selected Area at sites below Lock 1 (light bars) and Lock 6 (dark bars) in 2015/16, plotted against discharge (ML day^{-1}) in the LMR at the South Australian border (solid grey line) and water temperature (°C) (dashed black line). Sampling was undertaken approximately fortnightly from 6 October 2015 to 21 January 2016.

While not as abrupt as below Lock 1, microinvertebrate density below Lock 6 showed a similar pattern; there was an increase from October to early November (1,178 ind. L^{-1}) and a decline in mid-November (623 ind. L^{-1}) (Figure 14). Mean diversity below Lock 6 increased from mid-October (~24 taxa) to early November, where it remained constant (~33–40 taxa) through to early January. Increases in densities and diversity during early November below Lock 6 were likely influenced by multiple factors including antecedent high river flow, raised water levels of Weir Pool 5 (Figures

G3 and G4 in Appendix G), flows from the Chowilla in-channel rise event or upstream management interventions (e.g. upstream weir pool manipulation or return flows from Barmah–Millewa Forest, Appendix B). Littoral testates and rotifers (e.g. *Lecane*, *Cephalodella*, *Trichocerca*) in trap samples at this time were evidence of incursions from littoral margins. In early October, obligate littoral taxa (chydorids *Picripleuroxus, Pseudochydorus* and *Pseudomonospilus*) also were recorded. Raised water levels (either in weir pools or Chowilla Creek) likely led to longer residence time of microinvertebrates, potentially permitting densities to increase in early November, which then led to increased diversity after these littoral taxa were flushed from littoral margins. The presence of littoral taxa (e.g. chydorids and testates) at sites in the main river channel of the LMR downstream, and not the immediately upstream, of the Chowilla junction (Figure 7) suggests that Chowilla was likely a source of these littoral taxa (e.g. return flows from the in-channel rise event, Appendix B). Similar to below Lock 1, a decline in microinvertebrate density below Lock 6 in mid-November/early December aligned with declining river flow (Figure 14) and receding water levels following WPR (Figures G3 in Appendix G). However, diversity remained high (~33–40 taxa) during this period due to the influx of littoral taxa from a number of possible sources.

With warmer temperatures in January, density and diversity below Lock 1 and Lock 6 increased (Figure 14), within ranges comparable to those from 2014/15 (Ye *et al.* 2016a). Until the end of sampling (late January), the microinvertebrate assemblage reverted to a protist/rotifer dominant potamoplankton from a mixed littoral/limnetic assemblage. Warmer temperatures favoured warm-water species such as *Codonaria* and other ciliates, brachionid and synchaetid rotifers below Lock 1, and *Keratella lenzi* below Lock 6 (Appendix G). As for 2014/15 (Ye *et al.* 2016a), population density increases of microinvertebrates below Lock 1 and Lock 6 during January were likely attributable to downstream passage of upstream assemblage, additional taxa collected moving downstream, and instream reproduction.

Notably, 25% (44 taxa) of the 2015/16 microinvertebrates (6 Rhizopoda, 27 Rotifera (mostly littoral in habit), 3 Cladocera, 3 Copepoda and 2 macroinvertebrates) recorded in the trap samples were not recorded in the previous year. Of these, rotifers *Hexarthra braziliensis* and *Keratella americana,* and cladoceran *Daphnia galeata* ('notable taxa' in Appendix G) are new to the continent, *Brachionus caudatus, B.*

lyratus, Keratella javana and Filinia brachiata among other rare floodplain incursion species, and the chydorids *Pseudochydorus* and *Picripleuroxus* recorded from the LMR for the first time.

Fish Spawning and Recruitment

Spawning and recruitment of golden perch (*Macquaria ambigua ambigua*) 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, the time and place of spawning must be known.

In 2015/16, 814 GL of Commonwealth environmental water was delivered to the LMR Selected Area with environmental water peaking at ~6,700 ML day^{-1} on 28 October 2015, during a peak in total discharge of ~11,600 ML day^{-1} (Figure 3). To evaluate the contribution of Commonwealth environmental water to the spawning and recruitment of golden perch and silver perch in the LMR Selected Area, we sampled larval and young-of-year (YOY) (Figure 15) at sites in the gorge and floodplain geomorphic zones (Figure 7); used otolith microstructure and chemistry, specifically strontium (Sr) isotope ratios ($^{87}Sr/^{86}Sr$), to retrospectively determine the time and place of spawning of larvae and YOY; and used electrofishing to collect a representative subsample of the golden perch and silver perch populations in the LMR Selected Area to enable determination of population demographics.

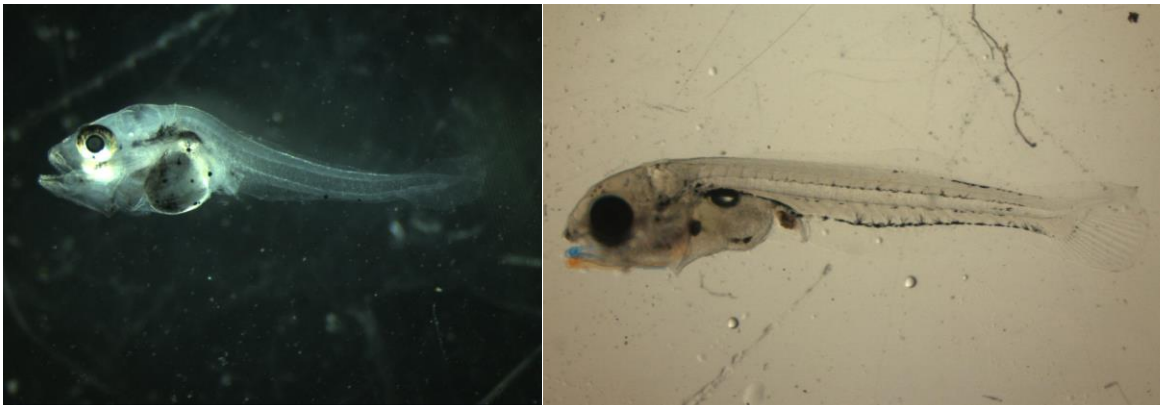

Figure 15. Larval golden perch (left) and silver perch (right) were sampled as an indicator for spawning, while young-of-year were sampled as an indicator for recruitment.

In 2015/16, golden perch and silver perch spawning, as indicated by the collection of larvae and retrospective determination of age and spawning location of larvae and YOY fish (i.e. age 0+), occurred from mid-October to mid-November 2015 in the LMR Selected Area. Larval abundances were low with a total of two golden perch and one silver perch larvae collected over eight fortnightly sampling events between October 2015 and January 2016. Spawning in the LMR Selected Area coincided with water temperatures ≥20 ˚C and periods of decreasing discharge in October 2015 (~10,500–9,500 ML day^{-1}) and November 2015 (~11,500–7,000 ML day^{-1}).

Assessment of the resilience of golden perch and silver perch populations requires an understanding of survival and population demographics. Sampling of golden perch and silver perch populations in the LMR Selected Area in 2016 revealed an absence of age 0+ and 1+ fish, indicating that recruitment to YOY, following spawning from October to November 2015, was poor for both species. In combination, spawning and recruitment data indicate that the flow regime in the lower River Murray in spring–summer 2015/16 (including Commonwealth environmental water) led to minimal spawning and negligible recruitment of golden perch and silver perch in the LMR Selected Area.

Despite the absence of age 0+ and 1+ fish, a broad range of age-classes of golden perch were collected, with fish ranging from age 2+ to 19+ years. Throughout the LMR, however, the sampled golden perch population was dominated by age 6+ and 5+ fish, spawned in 2009/10 and 2010/11, respectively, in both the Darling River and lower River Murray (as determined by otolith $^{87}Sr/^{86}Sr$, Appendix H). Sequential year classes from 2010–2013 conferred resilience on the golden perch population in the LMR following episodic recruitment throughout the Millennium drought (2001–2010) (Zampatti and Leigh 2013b). Nevertheless, negligible recruitment in 2015 and 2016, reveal the vulnerability of this species to flow regulation.

Silver perch were collected in low abundance (n = 18) in the LMR Selected Area in 2016, and the sampled population (n = 9) ranged in age from 2+ to 6+ years. These ages corresponded to spawning from years 2009/10 to 2013/14 in the lower River Murray, mid-Murray and Darling rivers (as determined by otolith $^{87}Sr/^{86}Sr$, Appendix H). As for golden perch, negligible recruitment of silver perch in 2015 and 2016 also reveals the vulnerability of this species to flow regulation.

3 SYNTHESIS AND EVALUATION

The delivery of Commonwealth environmental water aims to increase the magnitude and/or duration of natural freshes and overbank flows. Over the long-term, this is expected to make a significant contribution to achieving ecological outcomes in the LMR Selected Area, through restoring ecological processes and improving habitat for biota in the main channel and floodplain/wetlands (see Figure 8). 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 (LMR LTIM M&E Plan) to be relevant for this Selected Area. In this second year's report of the five-year monitoring and evaluation project, the ecological outcomes of the 2015/16 Commonwealth environmental water delivery are focused on addressing CEWO short-term (one-year) evaluation questions (Table 4). 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.

Overall, 2015/16 was a relatively dry year. A total of ~814 GL of Commonwealth environmental water was delivered to the LMR, in conjunction with other sources of environmental water (e.g. MDBA The Living Murray). Flow was delivered through a series of targeted watering events to this region (along with return flows from the Murrumbidgee River and Victorian tributaries) to achieve multi-site environmental outcomes. Environmental watering helped to maintain river flows in the LMR of 9,600–11,700 ML day^{-1} from mid-September to late October 2015, and at ~9,800 ML day^{-1} from mid-to late February 2016. Environmental water also facilitated WPR events at Weir Pools 2 and 5, and supported the use of The Living Murray water to instigate an in-channel rise event in Chowilla Anabranch. Furthermore, Commonwealth environmental water supplemented freshwater flows to the Lower Lakes and Coorong, maintaining barrage releases from September 2015 to June 2016. The environmental water delivery, in combination with infrastructure operation, contributed to a number of short-term ecological outcomes in the LMR Selected Area (Table 4). Key outcomes are summarised below.

Commonwealth environmental water contributed to increased water velocities and hydraulic diversity in the LMR Selected Area in 2015/16. This was reflected by generally increased median velocity in weir pools of ~0.1 m s^{-1} during winter and spring. These increased water velocities may have provided suitable habitat for fishes with life histories adapted to lotic (flowing water) habitats (e.g. golden perch, Murray cod). Nevertheless, during this dry year, median velocities in the lower River Murray remained substantially less than pre-regulation main channel velocities, which ranged from ~0.4–0.8 m s^{-1}, even at discharges <10,000 ML day^{-1} (Mallen-Cooper and Zampatti 2015). Consequently, environmental responses to increased water velocities associated with Commonwealth environmental water may be limited. Commonwealth environmental water in 2015/16 also contributed to increases in water levels of up to 0.3 m in the upper reaches for weir pools that were not subjected to WPR, and up to 0.7 m for weir pools that were. The higher water levels resulted in increased inundation of the littoral zone of the river channel, and increased connection with the floodplain in some areas.

Additional environmental/ecological outcomes in the LMR, associated with Commonwealth environmental water delivery in 2015/16, included:

- Increased transport of nutrients and phytoplankton, which would likely stimulate primary and secondary productivity in downstream ecosystems.
- Intermittent increases in supplies of organic material through improved connection with the floodplain, which are deemed important to the food webs of rivers. The increases were linked to WPR in Weir Pool 5, and possibly return water from the Chowilla Floodplain, both facilitated by Commonwealth environmental water.
- Increased microinvertebrate diversity and abundance. However, high abundances and diversity were short-lived and sharply decreased following a reduction in river flow and recession in water levels.
- Reduced salinity concentrations in the Murray Mouth and increased export from the Murray River Channel and Lower Lakes and reduced the import of salt to the Coorong.

However, there was limited spawning and negligible recruitment (to YOY, age 0+) of golden perch and silver perch during 2015/16. These findings support contemporary conceptual models of the flow-related ecology of golden perch and silver perch in the lower River Murray, with spawning and recruitment being associated with spring–

summer in-channel flow variability (nominally greater than 15,000 ML day^{-1}) and overbank flows in the lower River Murray or substantial flow pulses (e.g. 2,000–3,000 ML day^{-1} down the lower Darling River) (Zampatti and Leigh 2013a; Zampatti *et al*. 2015). Such hydrological characteristics were absent in 2015/16. Moderation and fragmentation of flow between the mid-Murray River and lower River Murray in 2015 (through the operation of Lake Victoria) (Figure 16), and an absence of flow in the Darling River, potentially diminished spawning and/or recruitment of golden perch and silver perch in the LMR Selected Area.

Table 4. 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. CEW = Commonwealth environmental water, WPR = weir pool raising.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 1. Stream Metabolism	What did CEW contribute to patterns and rates of primary productivity and decomposition?	There were enhanced gross primary production and respiration rates associated with WPR in Weir Pool 5 and return flows from Chowilla, both of which were supported by CEW. Integrated ecosystem net production was near zero, indicating that organic material was derived from aquatic production with little enhancement from external supplies that could have further increased food supplies.
	What did CEW contribute to dissolved oxygen levels?	Oxygen concentrations did not fall below acceptable levels (>50% saturation).
Category 3. Hydrological Regime*	What did CEW contribute to hydraulic diversity within weir pools?	Increase in weir pool median water velocities of ~0.1 m s^{-1} during winter and spring compared to without CEW, with some cross sections in the weir pool ranging 0.17–0.3 m s^{-1}.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 3. Hydrological Regime*	What did CEW contribute to variability in water levels within weir pools?	Increases in water levels in weir pools of up to 0.3 m in the upper reaches for weir pools without WPR, and up to 0.7 m in weir pools with WPR. [#]
Category 3. Matter Transport*	What did CEW contribute to salinity levels and transport?	Reduced salinity concentrations in the Murray River Channel, Lower Lakes and, in particular, the Murray Mouth. Increased export of salt from the Murray River Channel and Lower Lakes, and decreased net import of salt to the Coorong.
	What did CEW contribute to nutrient concentrations and transport?	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.
	What did CEW contribute to ecosystem function?	Increased exchange of nutrients and phytoplankton between critical habitats of the Lower Murray would may have supported primary and secondary productivity in the region and in doing so supported food webs of the LMR, Lower Lakes and Coorong.
	What did CEW contribute to water quality to support aquatic biota and normal biogeochemical processes?	Reduced salinity concentrations in the Lower Lakes and Murray Mouth may have improved habitat for freshwater and estuarine biota in the region.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 3. Micro-invertebrates	What did CEW contribute to microinvertebrate diversity?	Peaks in microinvertebrate diversity below Lock 6 and Lock 1 aligned with peaks in river discharge and CEW delivery. Most in-channel taxa were not true potamoplankton (plankton of flowing waters), but transported taxa from floodplain or riparian sources (e.g. Chowilla).
	What did CEW contribute via upstream connectivity to microinvertebrate communities of the LMR Selected Area?	Many (25%) microinvertebrates from 2015/16 were not recorded during 2014/15. Some will have originated from littoral margins in the LMR (e.g. WPR or Chowilla return flows), but some likely originated from further upstream, including novel taxa for the continent or for the LMR.
	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 the environment) microinvertebrates, driven by CEW, and their presence in fish diet could not be determined.
	What did CEW contribute to microinvertebrate abundance (density)?	Flow, which CEW contributed to, was a driver of density through October 2015, particularly at Lock 1. With reduced flows and a recession of water levels, there was a proportional drop in abundance, followed by a steady increase of warm-water taxa through summer.
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 2015/16 corresponded with limited spawning and negligible recruitment (to YOY, age 0+) of golden perch and silver perch.

* Evaluation of CEW for Hydrological Regime and Matter Transport indicators is based on modelled data.

An increase in water level of 0.45 m and 0.5 m at the downstream end of Weir Pools 5 and 2, respectively, was achieved through infrastructure operation and maintained through the delivery of CEW to compensate for losses.

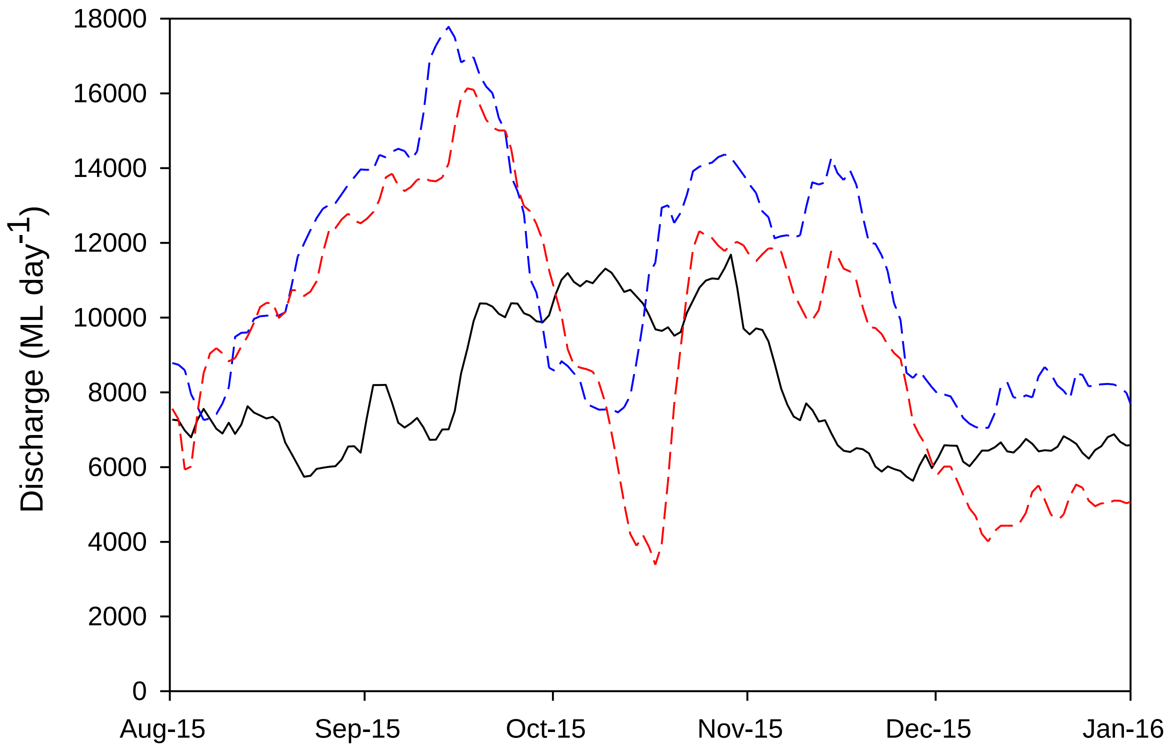

Figure 16. The changes in flow peaks measured at Euston (blue dotted line), above the Darling River junction, and Lock 10 (red dotted line), above Lake Victoria, to that measured at the South Australian border (black solid line). Differences are attributed primarily to the regulation of the flow into Lake Victoria.

4 MANAGEMENT IMPLICATIONS AND RECOMMENDATIONS

Monitoring outcomes from the LTIM Project, in conjunction with our contemporary understanding of ecological response to flow management in the LMR, underpin the adaptive management of Commonwealth environmental water and river operation, aiming to maximise ecological benefits from available water. This study reveals that the delivery of environmental water, e.g. to provide freshes or enhance in-channel flows, can increase hydraulic diversity (velocities and water levels), which has potential benefits for riverine ecosystems in the LMR. When the timing of flow delivery aligns with biological requirements (e.g. reproductive season of flow-cued fish species), significant ecological outcomes can be achieved. In addition, other measures such as weir pool manipulation could be used to complement flow management to enhance environmental outcomes.

Environmental flows should be delivered to promote both longitudinal and lateral connectivity, which will increase the productivity in the LMR through increased carbon and nutrient inputs. 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 (as observed for microinvertebrates in this study) and potentially enhanced recruitment. Also important is the source of water (i.e. origin), which can influence biological responses and ecological processes. For instance, changing the source of the water can alter the turbidity and the amount and form of nutrients, changing the amount and composition of primary producers (e.g. phytoplankton) and affecting secondary production. Water quality and biological attributes can be further affected by river operations that re-route (e.g. through floodplains or wetlands) or fragment the flow (e.g. by diversions or storages), leading to potential changes in the structure and function of aquatic food webs.

Managing environmental flow to increase carbon and nutrient transport and primary productivity in the LMR may be achieved through coordinating water source and by river operations that increase floodplain connectivity. However, broader water quality characteristics need to be considered due to the potential risks to aquatic biota of poor water quality (e.g. low dissolved oxygen). Furthermore, maintaining hydrological integrity (i.e. magnitude, variability and source) of flow from upstream (e.g. Darling River or mid-Murray) to the lower River Murray is critical to support system-scale processes and promote positive ecological outcomes (e.g. improved productivity, enhanced spawning and recruitment of flow-dependent fishes). Specific management considerations from indicators are provided below, which relate to the key findings in Section 2.

Hydrology and Hydrological Regime

In a dry year without unregulated flow, and within current system constraints, it may be challenging to substantially improve on the hydrodynamic outcomes observed in 2015/16 with environmental water. The benefits of WPR undertaken in 2015/16, one river operation option achievable within current constraints, has been further investigated here.

Modelled results of water levels and velocities in the weir pool with and without Commonwealth environmental water, and with and without WPR, on the day corresponding to the largest raising and largest Commonwealth environmental water contribution are presented for Weir Pool 2 in Figure 17 (see Appendix E for Weir Pool 5). The 0.5 m WPR can be seen to increase the water level by this amount in Weir Pool 2, at the downstream end (right hand side of the upper plot of Figure 17). Without Commonwealth environmental water, the flow over Lock 3 on this day was modelled to be 785 ML day^{-1}. This was a short-term drop in flow, with the average flow for the two weeks around this date was 2070 ML day^{-1}. As such the water are predominately lentic or slow-flowing (black lines in the bottom plot of Figure 17), with a stable water maintained at the pool level throughout the weir pool (black lines in the top plot of Figure 17). With Commonwealth environmental water, the flow on this day increased to 8,760 ML day^{-1}, resulting in faster flowing water and a water level 0.2 m higher at the upper end of the weir pool with WPR, and 0.7 m without WPR, without Commonwealth environmental water. One concern with WPR is the potential for reduced velocities, due to increasing the cross sectional area for the same discharge. This can be seen to occur in Figure 17, with lower velocities for the with WPR case compared to without. Two main observations can be made based on the modelling of the 2015/16 WPR events:

1) The combination of Commonwealth environmental water delivery and WPR (blue solid line in Figure 17) resulted in both higher water levels and higher velocities compared to the without Commonwealth environmental water and without WPR (black solid line in Figure 17); and

2) The reduction in velocity due to WPR was relatively small compared to the increase in velocity due to the delivery of Commonwealth environmental water.

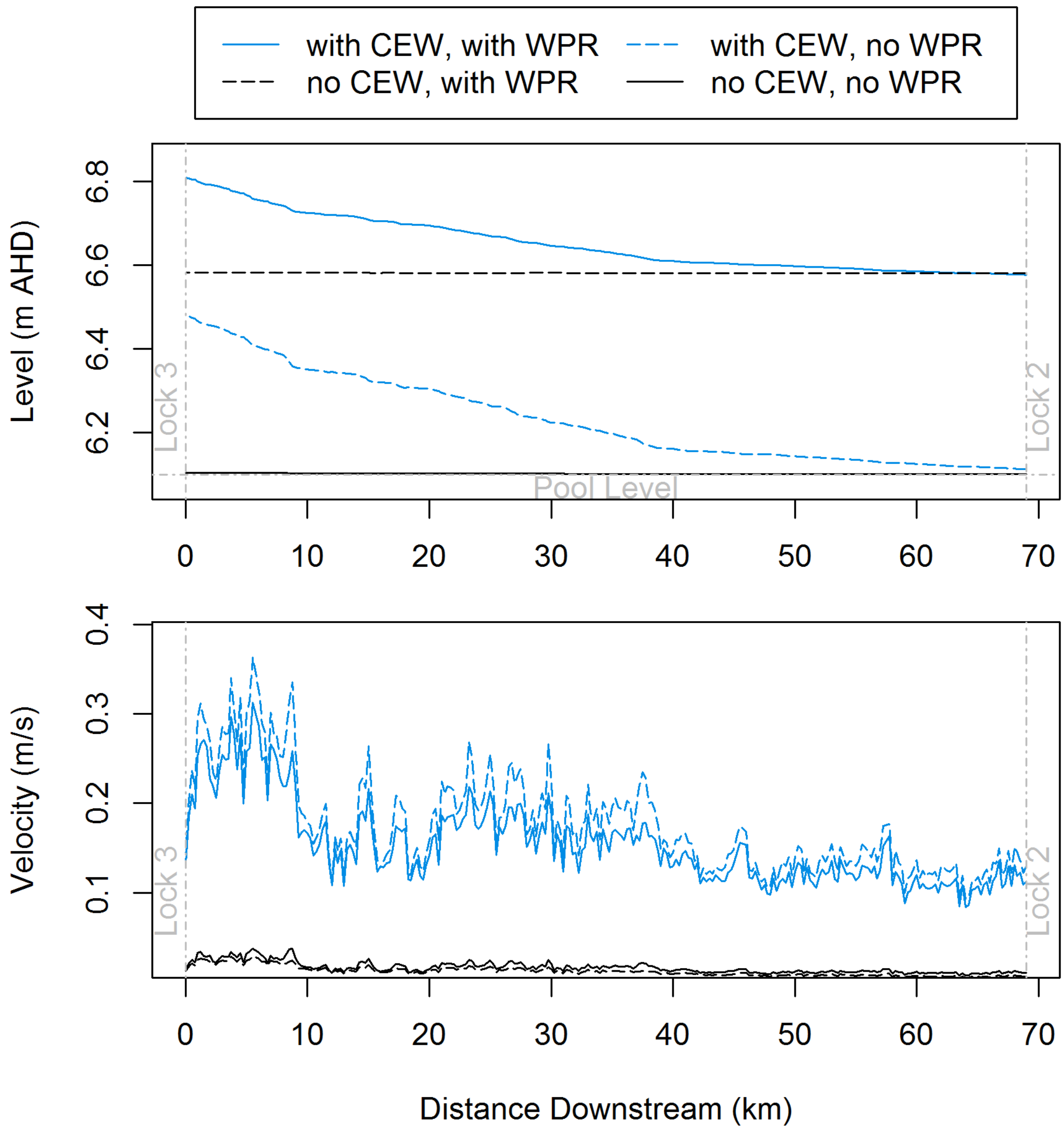

Figure 17. Change in water level and velocity along the weir pool with and without CEW and with and without the weir pool raising (WPR) for Weir Pool 2 on 31/10/15. This day represents the largest difference between the scenarios, with the weir pool at the maximum raising, and the largest volume of CEW delivered at the time of the raising.

Stream Metabolism

The changes in metabolism below Lock 6 during WPR were reflective of an increased connection between the river and the floodplain, resulting from WPR. Enhanced aquatic phototrophic production in the flooded area was largely returned to the river and expected to benefit food webs in the main channel of the LMR. Application of WPR as a management technique to a wider range of sites and more frequently might broadly enhance metabolic activity with expected benefits to food webs, although further investigations are required to identify the beneficiaries and to optimise the delivery of organic materials and nutrients within ranges that do not generate detrimental effects such as deoxygenation. This will rely on characteristics such as the periods of floodplain return flows, area flooded and duration of inundation. The continuing collection of data through the LTIM project should help to better understand these processes.

Below Lock 1, the observed cycles of enhanced metabolism were the result of biofouling of the oxygen sondes, despite precautions to avoid this problem. Further adjustments are being made to the probes to overcome this issue.

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 5 years, including Aldridge *et al*. (2013), Ye *et al*. (2015b) and Ye *et al*. (2016a) 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;
- 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-large floods (e.g. >40,000 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 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

Environmental water delivery through floodplains translocates entrained microinvertebrates from upstream main channel assemblages. Environmental water stimulates microinvertebrate productivity from propagules deposited into floodplain sediments, which has been demonstrated for Chowilla (Furst *et al.* 2014) and Barmah–Millewa (Gigney *et al.* 2006). High population densities can develop in relatively short timeframes as a result of the rapid life cycles of the component organisms; hours to days for the protists and rotifers, days to weeks for the microcrustaceans. Drawdown post-watering returns the reconstituted assemblage to downstream food webs.

In years of low flows, where extensive floodplain watering is precluded, WPR can achieve similar cueing of propagule emergence along flooded weir pool margins and shallow backwaters. In areas with reduced flow, a planktonic microinvertebrate assemblage can develop, with newly wetted margins providing fresh resources. To what extent will be influenced by the retention time. WPR and the Chowilla Anabranch in-channel rise during 2015/16, both supported by Commonwealth environmental water, likely contributed to the return of a similar number of taxa, at comparable density and diversity, to those recorded during the Chowilla Floodplain inundation event in 2014/15, which used The Living Murray environmental water and was supported by Commonwealth environmental water. Differences in constituent organisms can be attributed to different sources of environmental water during 2015/16.

Fish Spawning and Recruitment

The flow regime of the lower River Murray is highly modified, with in-channel freshes conspicuously absent (Maheshwari *et al.* 1995; Zampatti and Leigh 2013a). In spring–early summer 2015/16, freshes present in the mid-Murray River were regulated out of the flow regime of the lower River Murray (Figure 16). Considering a contemporary understanding of the flow-related ecology of golden and silver perch in the southern MDB, this tempering of the flow regime, and an absence of flow in the Darling River, potentially diminished spawning and recruitment of golden perch and silver perch in the lower River Murray, including the LMR Selected Area. At the same time, however, these actions promoted hydrological conditions conducive to the spawning and recruitment of goldfish and common carp. Disadvantaging native fishes whilst encouraging invasive species is a pervasive symptom of river regulation. Consequently, in the LMR Selected Area, supporting positive outcomes for native fishes reliant on lotic habitats and/or flow-mediated spawning, through the delivery of Commonwealth environmental water, will require consideration of maintaining the longitudinal integrity of flow regimes in the Murray River. To restore these hydrological features (i.e. spring–early summer flow pulses), 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).

5 CONCLUSION

2015/16 was a dry year (<12,000 ML day^{-1}) with no unregulated flow whilst full South Australian entitlement flow was provided to the LMR. During this year, the delivery of Commonwealth environmental water (~814 GL) to this Selected Area, in conjunction with other environmental water, helped to maintain river flow at 9,600–11,700 ML day^{-1} during spring and ~9,800 ML day^{-1} in late summer, and supported WPR in Weir Pools 2 and 5. Environmental watering led to a range of ecological outcomes including: increased hydraulic diversity (velocity and water levels) in the river channel (weir pools); intermittent increases in the supplies of organic materials that are critical food resources to riverine food webs; increased transport of nutrients and phytoplankton, likely stimulating primary productivity in downstream ecosystems; increased microinvertebrate diversity and abundance; reduced salinity concentrations in the Murray Mouth and increased export and reduced import of salt. Nevertheless, the flow regime in this dry year (2015/16), despite environmental water delivery, did not result in successful spawning and recruitment (to age 0+) of golden perch and silver perch in the LMR. This outcome concurs with contemporary flow-related ecological models for these flow-dependent species.

The LMR is heavily regulated with substantially altered hydrology and hydrodynamics compared to the historical natural flow regime. Environmental water delivery to improve hydraulic and habitat diversity can provide benefits for ecological restoration in the LMR. WPM could be applied as a complementary measure to flows to enhance ecological outcomes (e.g. enhancing riverine productivity by improving lateral connectivity). For environmental watering, the timing of flow delivery is important, which should continue to align with ecological objectives and consider biological processes and life history requirements throughout the year, particularly in spring and summer (e.g. reproductive season of flow-cued spawning species). Environmental flows that promote both longitudinal and lateral connectivity (despite operational limitations) will increase the productivity in the riverine ecosystem and facilitate the transport and dispersion of aquatic biota (e.g. microinvertebrates, fish larvae) to and throughout the LMR. These can potentially increase species diversity and enhance recruitment. The source of water (i.e. origin) should also be considered, which can influence the ecological processes and biological response. Additionally, managing environmental flow to enhance carbon and nutrient transport and primary productivity in the LMR (e.g. through coordinating water source and by river operations that increase floodplain connectivity) should consider broader water quality effects due to the potential risks to aquatic biota of poor water quality (e.g. low dissolved oxygen). Furthermore, environmental flow delivery should, where possible, maintain hydrological integrity of flow from upstream (e.g. Darling or mid-Murray) to the lower River Murray, which will support system-scale processes and promote positive ecological outcomes (e.g. improved productivity, enhanced spawning and recruitment of flow-dependent species).

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7 APPENDICES

APPENDIX A: SELECTED AREA OBJECTIVES AND HYPOTHESES FOR EACH INDICATOR

Indicator-specific objectives and hypotheses for the LMR Selected Area were created by the LMR project team during the planning phase of the project. These objectives and hypotheses, which appear in the LMR LTIM M&E Plan, are provided below for Category 1 and 3 indicators.

Category 1

Hydrology (channel)

Objective: The recorded daily discharge and water level at locations within the selected area will inform the assessment of other indicators and evaluation.

Stream Metabolism

Objective: Assess how environmental water influences primary production and ecosystem respiration in the river channel.

Hypotheses: Increased flow into the LMR (peak and duration) in spring/summer will:

- Not enhance the transport of organic material from the floodplain if delivered as in-channel flows so that autochthonous carbon captured in-stream through photosynthesis will be the major source of energy to the aquatic food webs.
- Alter metabolic rates if water quality changes influence the growth of aquatic plants (microalgae and macrophytes) by modifying light and nutrient availability and this will alter the supply of autochthonous organic carbon to food webs.
- Enhance the supply of allochthonous organic carbon to the river channel if increasing flow better connects the channel with riparian, wetland or floodplain areas, leading to increased energy supplies and enhanced ecosystem respiration rates due to decomposition.
- Reduce dissolved oxygen concentrations to levels below those required by aquatic organisms, with potentially lethal effects if flows carry excessive loads of organic carbon that increase respiration and decomposition rates unless water quality is appropriately managed along with flows.

- Lead to increased food web size and complexity that can support larger populations of organisms dependent on aquatic systems for food supplies if flows lead to increased energy supply due to enhanced aquatic photosynthetic production or enhanced supply of externally sourced organic carbon.

Fish (channel)

Objective: Determine presence or absence, relative abundance and age or size class structure for nominated species (Hale *et al.* 2014).

Category 3

Hydrological Regime

Objective: Assess how Commonwealth environmental water has contributed to an increase in discharge, velocity and depth of flow at a high spatial and temporal resolution.

Hypothesis: Commonwealth environmental water will increase metrics representing desirable conditions, for example increased velocities and increased variability in water levels.

Matter Transport

Objective: Assess whether Commonwealth environmental water has increased the transport and export of salt, nutrients and phytoplankton biomass through the LMR Selected Area.

Hypotheses: Commonwealth environmental water will increase:

- The mobilisation of salts from the Basin and increase the transport of salt passing from Lock 1 through the LMR Selected Area (and through the Lower Lakes and Murray Mouth)
- The mobilisation of nutrients from the Basin and increase nutrient loads passing from Lock 1 through the LMR Selected Area (and through the Lower Lakes and Murray Mouth)
- Phytoplankton biomass loads passing from Lock 1 through the LMR Selected Area (and through the Lower Lakes and Murray Mouth).

Microinvertebrates

Objectives:

- Compare and contrast potamoplankton assemblages pre- and post-Commonwealth environmental water deliveries
- Compare and contrast littoral microcrustacean assemblages pre- and post-Commonwealth environmental water deliveries
- Compare and contrast propagule deposition (egg-bank) in riparian sediments post-environmental deliveries
- Identify pre- and post-Commonwealth environmental water delivery dietary items of juvenile fish collected concurrently with microinvertebrate samples
- Compare pre- and post-Commonwealth environmental water delivery dietary item proportions to ambient microinvertebrate composition to determine selectivity of feeding.

Hypotheses:

- Microinvertebrate taxonomic diversity will increase in inundated habitats due to increases in available habitat by triggering propagules deposited in sediments
- Microinvertebrate abundance will increase in inundated habitats in response to increased egg production by resident or transported populations
- Microinvertebrate propagule density and diversity in riparian sediments will increase post-environmental water delivery
- Microinvertebrate assemblage responses will be reflected in the dietary components of fish larvae (golden perch).

Fish Spawning and Recruitment

Objectives:

- Compare and contrast spawning response to various environmental water deliveries.
- Compare and contrast recruitment success in response to various environmental water deliveries.
- Compare and contrast the timing of spawning and source (i.e. natal origin) of successful recruits in response to various environmental water deliveries.
- Identify potential associations between reproduction (spawning and recruitment) and environmental water delivery (e.g. magnitude, timing and source).

- Determine population connectivity between regions (e.g. larvae spawned in the Goulburn recruiting to LMR Selected Area populations).

Hypotheses:

- Increases in flow above regulated entitlement flow (in-channel or overbank) in spring–summer will promote the spawning and recruitment (to young-of-year) of golden perch and silver perch.
- Multiple years of enhanced spring–summer flow will increase the resilience of golden perch and silver perch populations in the LMR.

APPENDIX B: OVERVIEW OF OTHER WATERING ACTIVITIES DURING 2015/16

In addition to environmental water deliveries to the LMR Selected Area in 2015/16 (Figure 3), the following environmental water management events are relevant to the analyses and interpretations in this report. It is important to note with these events, for example weir pool raising (WPR), that environmental water contributed to infrastructure operation and it was the combination of management actions (i.e. environmental water delivery and infrastructure operation) that produced the outcome. Wetland pumping was not considered to influence any of the main channel indicators in the LMR.

Other watering activities in the LMR Selected Area

Raising of water levels in Weir Pools 2 and 5

Overview

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 Selected Area occurred between early September and early November 2015. Water levels within Weir Pools 2 and 5 were raised to a maximum of 0.5 and 0.45 m above the normal pool level (NPL), respectively, and returned to NPL by early December 2015 (Figure B1; Table B1). Water levels for both weir pools peaked in October 2015, resulting in an additional inundation area of 175 ha for Weir Pool 2, and 894 ha for Weir Pool 5. Approximately 5,084 ML of environmental water was delivered to account for losses (e.g. evaporation) during the raising of Weir Pools 2 and 5, of which Commonwealth environmental water contributed 100% (source, CEWO). The WPR event is described in DEWNR (2015).

Ecological outcomes

A number of targeted investigations were conducted to monitor the ecological responses to WPR in 2015. These included: channel hydraulics (Bice *et al.* 2016a), water quality (Wallace and Cummings 2016a; Cummings and Goonan 2016a), surface water-groundwater interactions (Cummings and Goonan 2016a; Gehrig *et al.* 2016), floodplain vegetation response - both canopy and understorey species (Gehrig *et al.* 2016), ecosystem metabolism (Wallace and Cummings 2016a), biofilms (Wallace and Cummings 2016b), primary consumers (Cummings and Goonan 2016b) biofilm – primary consumer interactions (Cummings *et al.* 2016), and secondary productivity via fish response (Bice *et al.* 2016b).

Key findings were:

- Increased water level variation (Bice *et al.* 2016a);
- Dissolved oxygen remained above 6 mg L^{-1} and 50% saturation throughout the event (Wallace and Cummings 2016a);
- Vegetation community composition shift – increase in the proportion of amphibious and floodplain species at higher elevation bands (0.3–0.6 m above NPL) (Gehrig *et al.* 2016);
- Increased tree condition at sites within Weir Pool 2 and 5. However, tree condition at the Weir Pool 2 site (Big Toolunka) decreased significantly after the WPR event (Gehrig *et al.* 2016);
- Increased gross primary productivity and ecosystem respiration (Wallace and Cummings 2016a);
- Increased abundance and diversity of primary consumers (e.g. macroinvertebrates) at wetlands in Weir Pools 2 and 5 (Cummings and Goonan 2016b);
- Increased abundance of the shrimp *Paratya australiensis* (including egg-bearing females) in the main channel and wetlands at the downstream end of Weir Pool 2 (Cummings and Goonan 2016b);
- Increased abundance of 'floodwater' taxa (e.g. caddisflies, mayflies, non-biting and biting midges) in Weir Pool 2 (Cummings *et al.* 2016);
- Increased body condition (weight relative to length) of Australian smelt (*Retropinna semoni*) in Weir Pool 5 compared to the unraised Weir Pool 3 during

October 2015; however, there were no differences in growth rates between raised and unraised weir pools (Bice *et al.* 2016b).

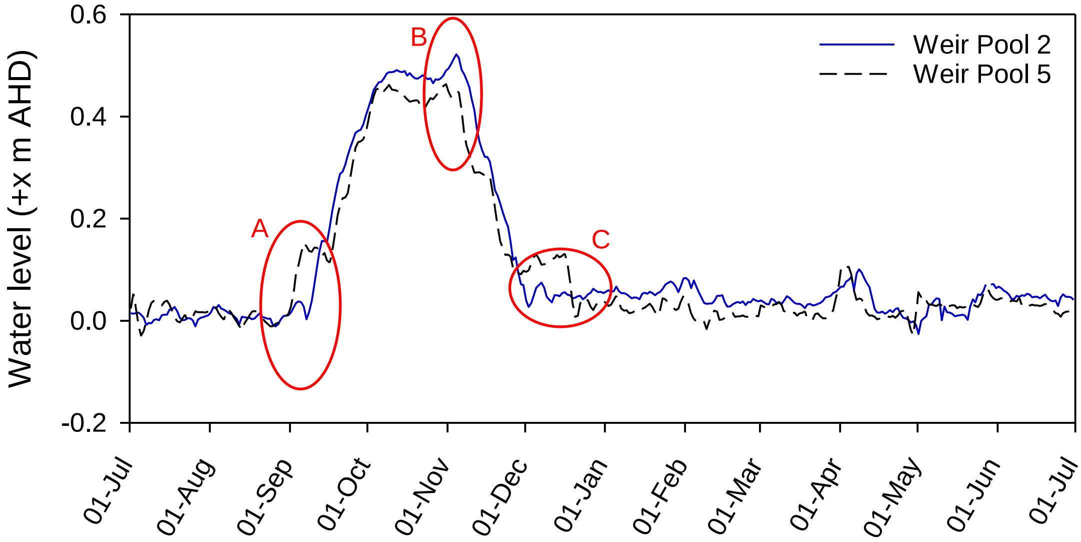

Figure B1. Water levels in the Lock 2 (US Lock 2, +6.1 m AHD) and Lock 5 (US Lock 5, +16.3 m AHD) weir pools between July 2015 and July 2016, showing weir pool raising between September 2015 and January 2016 (DEWNR). Water level is measured at Lock 2 US (A4260518) and Lock 5 US (A4260512) sites. Red circles indicate (A) the commencement of weir pool raising, (B) maximum level and (C) return to normal pool levels.

Table B1. Inundation areas for Weir Pools 2 and 5 during spring 2015 (Hanisch *et al.* 2017).

Weir pool	Height above pool level (cm)	Area (ha)	Increase compared to pool	
			Area (ha)	Area (%)
2	0	1,557	0	0%
2	50	1,732	175	11%
5	0	2,434	0	0%
5	45	3,328	894	37%

Chowilla regulator in-channel rise event

During spring 2015, water levels in the Chowilla Anabranch (Figure 7) were raised to achieve a variety of targets including: mobilisation of carbon and nutrients from fringing areas and low level wetlands to stimulate the food web; providing conditions to encourage growth of flood-dependent and aquatic vegetation in riparian zones; and providing habitat for waterbirds, amphibians and invertebrates. Commencing in early October, water levels were raised to a maximum of 1.5 m above NPL and held at ~17.6–17.8 m AHD for five weeks (Figure B2). From mid-November, stop logs in the Chowilla regulator were gradually removed to allow water levels to return to normal level by mid-December 2015.

During the regulator event and throughout summer/autumn 2016, the inlet weirs on two anabranch creeks (Pipeclay and Slaney) were managed to vary inflows to manage water quality and protect fish habitat (Figure B2). In addition, during spring/summer 2015/16, 229 ML of The Living Murray environmental water was pumped to three wetland sites (Brandy Bottle, Punkah Creek Floodrunner and Punkah Creek Depression) to achieve vegetation, frog, water quality and waterbird targets. A detailed description of the Chowilla regulator in-channel rise, operation of anabranch creek weirs and wetland watering events can be found at http://www.environment.sa.gov.au.

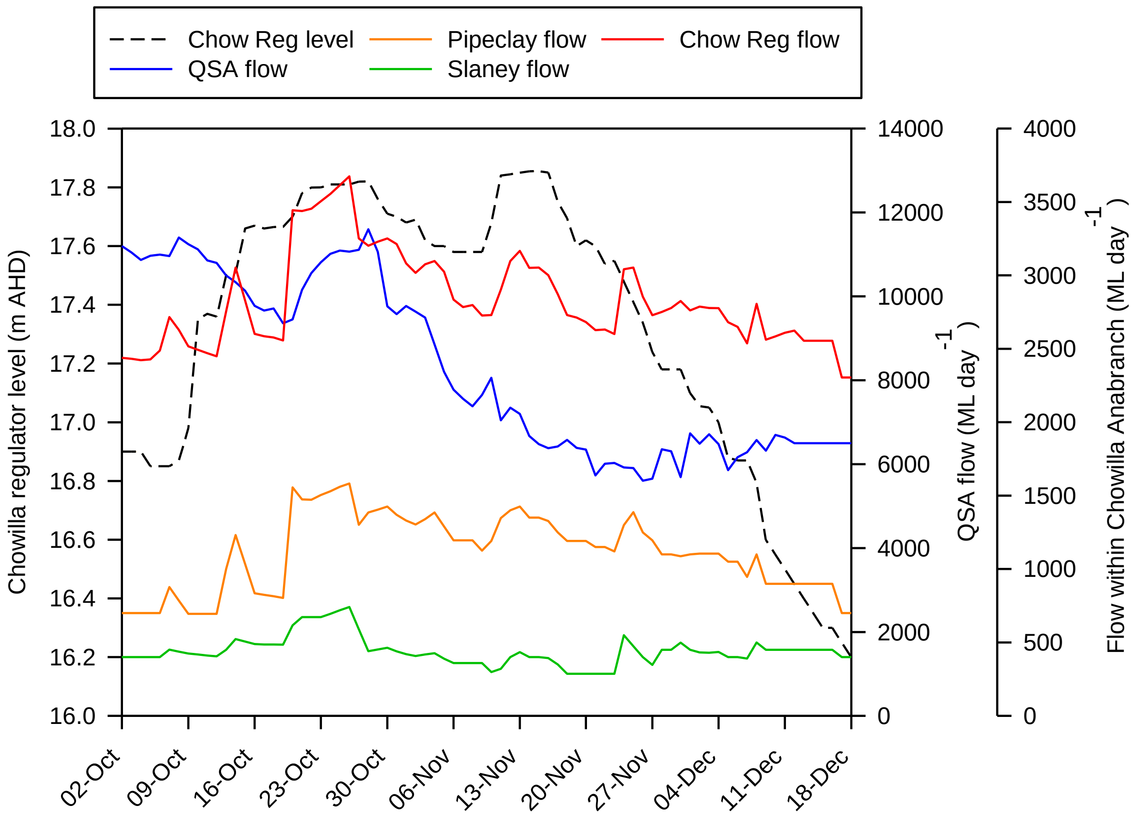

Figure B2. Water level upstream of the Chowilla regulator (m AHD) and flow (ML day^{-1}) for creeks within the Chowilla Anabranch and at the South Australian border (QSA) (DEWNR).

Watering activities above the LMR Selected Area

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

Water levels in Weir Pools 7, 8, 9 and 15 were raised and lowered, relative to their NPL, during 2015/16 (Table B2). Approximately 7,988 ML of Commonwealth environmental water was delivered to account for losses during the raising of all these weir pool (source, CEWO).

Table B2. Timing of water manipulation actions for weir pools upstream of the LMR Selected Area during 2015/16 (source, CEWO). NPL = normal pool level.

Weir pool	Action	Duration	Watering information
7	Raising of weir pool to +0.6 m NPL	August 2015 to January 2016	Flows to Lindsay River and Mullaroo Creek.
	Lowering of weir pool to -0.8 m NPL	January to May 2016	
8	Raising of weir pool to +0.8 m NPL	August 2014 to mid-December 2015	High velocity spring fresh through Potterwalkagee Creek; Low level inundation of Backwater Lagoon and other unnamed wetland sites, as well areas of river frontage and riparian vegetation; Water delivered to Wingillie Wetland.
	Lowering of weir pool to -0.8 m NPL	December 2015 to May 2016	
9	Raising of weir pool to +0.25 m NPL	July to September 2015	
	Lowering of weir pool to -0.1 m NPL (brief period of -0.2 m NPL)	October 2015 to February 2016	
15	Raising of weir pool to +0.6 m NPL	July to December 2015	Inundation of Euston Lakes, in particular Lake Caringay.
	Lowering of weir pool to -0.3m NPL	April to June 2016	

Barmah–Millewa Forest inundation

From July–September 2015, overbank flows (with two small freshes) occurred in the Barmah and Millewa Forests. This was achieved through unregulated flow releases from Hume Dam and Barmah and Millewa Floodplain regulator operation. From 11 September to 31 October 2015, The Living Murray water and Commonwealth environmental water was provided to maintain flows of ~12–13,000 ML day^{-1} to extend the inundation of Millewa Forest (source, CEWO). A gradual recession followed shortly after.

Goulburn flow pulse events

Commonwealth environmental water and environmental water from The Living Murray, Victorian Environmental Water Holder and Inter Valley Transfer were delivered to the lower Goulburn River channel during 2015/16. From 3–29 October 2015, there was a targeted spring pulse in the hydrograph that peaked at approximately 6,200 ML day^{-1} (Figure B3). This pulse was driven by Commonwealth environmental water, which contributed 99.1 GL, but was also supported by The Living Murray (4.9 GL) (source, CEWO). An autumn fresh also occurred in the Goulburn River between early March and early April 2016 (Figure B3). Autumn flow peaked at approximately 4,000 ML day^{-1}, which consisted of a combination of Commonwealth environmental water and water from the Victorian Environmental Water Holder and Inter Valley Transfer.

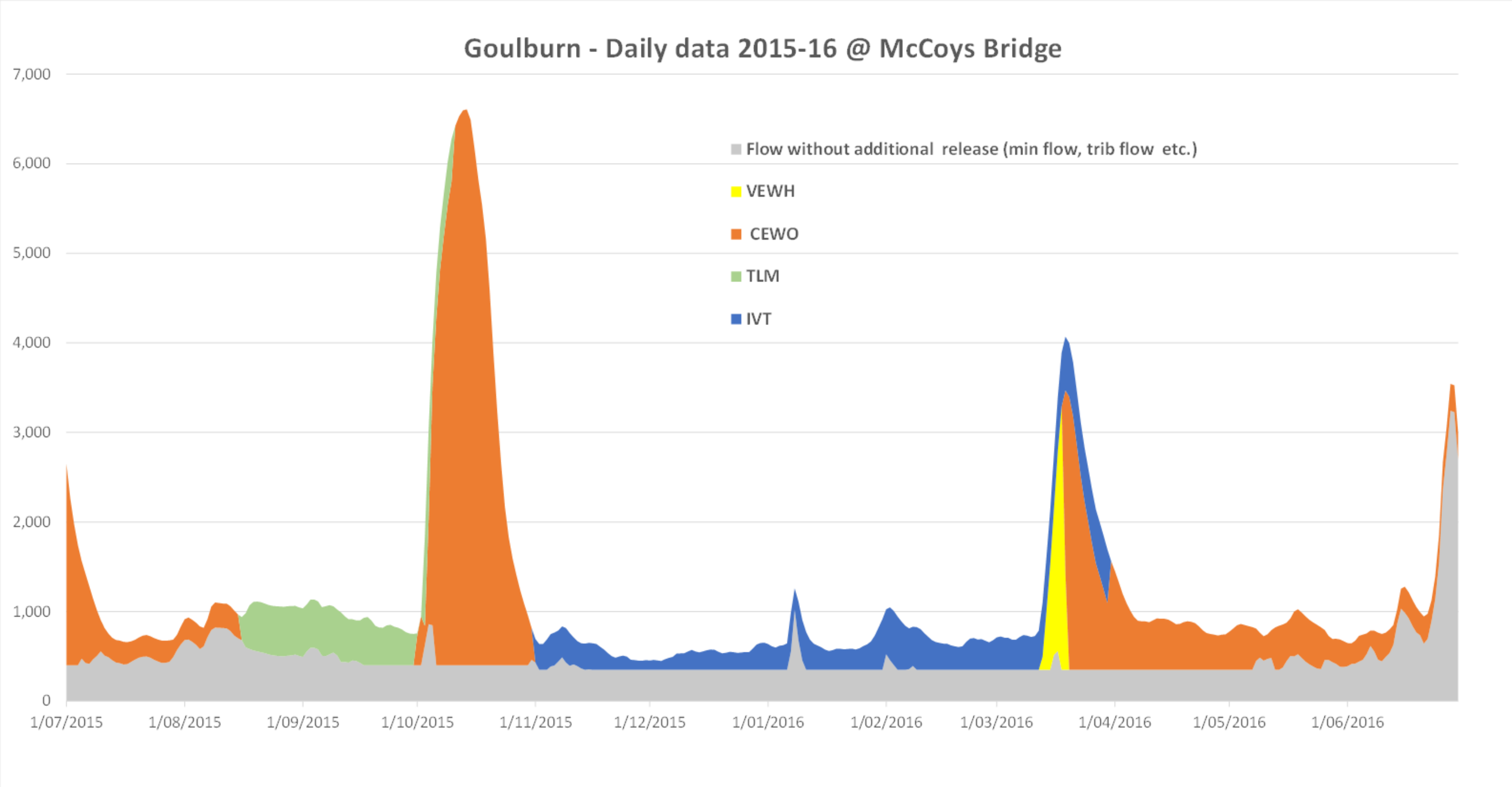

Figure B3. Hydrograph for the Goulburn River (at McCoys Bridge) from July 2015 to July 2016 (source, CEWO), showing the delivery of environmental water from the Commonwealth Environmental Water Office (CEWO), The Living Murray (TLM), Victorian Environmental Water Holder (VEWH) and Inter Valley Transfer (IVT).

APPENDIX C: STREAM METABOLISM

Background

River metabolism measurements estimate in-stream rates of photosynthesis and respiration, and provide information on the energy processed through river food webs (Odum 1956; Young and Huryn 1996; Oliver and Merrick 2006). Metabolism measurements help identify whether the sources of organic material that provide the food resources have come from within the river (autochthonous) or the surrounding landscape (allochthonous). Measurements of stream metabolism can describe the fundamental trophic energy connections that characterise different food web types (e.g. detrital, autotrophic, planktonic). They indicate the size of the food web and 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 the 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, and oxygen depletion by respiration which occurs continuously. Monitoring oxygen levels also informs on whether dissolved concentrations are suitable for aquatic organisms and provides a basis for identifying changes that result from environmental flows and the impacts these might have on the biota.

The method is based on the continuous measurement of oxygen concentrations at single river sites from which river metabolism rates are then calculated (Oliver and Merrick 2006; Oliver and Lorenz 2010; Grace and Imberger 2006). *In situ* logging of the dissolved oxygen concentration, water temperature and incident solar photosynthetically active radiation (PAR) required for estimating stream metabolism were undertaken at two sampling sites, one downstream (below) of Lock 6 and one below Lock 1. These were selected to represent the Floodplain and Gorge geomorphic zones of the LMR Selected Area, respectively. The detailed monitoring and analysis protocol described in Hale *et al.* (2014) was consistently followed but 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.

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 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), by the Australian Water Quality Centre, a registered laboratory with the National Association of Testing Authorities (NATA).

Oxygen concentration measurements were made continuously from 23 September 2015 to 2 March 2016. A complete data set was collected for the site below Lock 1 with only a few missing days due to probe maintenance and battery depletion. However, the data set collected from the site below Lock 6 was incomplete due to a probe failure from 27 December 2015 to 20 January 2016.

Refer to the LMR Selected Area SOP for Category 1 Stream Metabolism in the LMR LTIM M&E Plan 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 the Plan for timing of monitoring activities and more information on sampling sites and zones.

Results

Oxygen concentration time series

Time series of dissolved oxygen concentrations showed that the concentrations at both sites generally ranged between 7 and 10.6 mg L^{-1} over the monitoring period, with seasonal declines in oxygen concentration corresponding to increasing spring and summer temperatures (Figure C1). Exceptions occurred at the site below Lock 1 where dissolved oxygen concentrations fell to values between 5.3 and 6 on three occasions (Figure C1). These periods of reduced oxygen concentration were not found to be associated with likely environmental influences such as reductions in solar irradiance or changes in water quality, water depth or flow. It is suspected that these low oxygen concentrations resulted from biofouling of the oxygen sonde housing. The probes themselves were protected with a copper mesh resulting in minimal direct biofouling, but field observations indicated that the battery housing to which the probes are attached became heavily coated in biofilms and filamentous algae. It seems likely that these influenced the oxygen concentrations in the surrounding water.

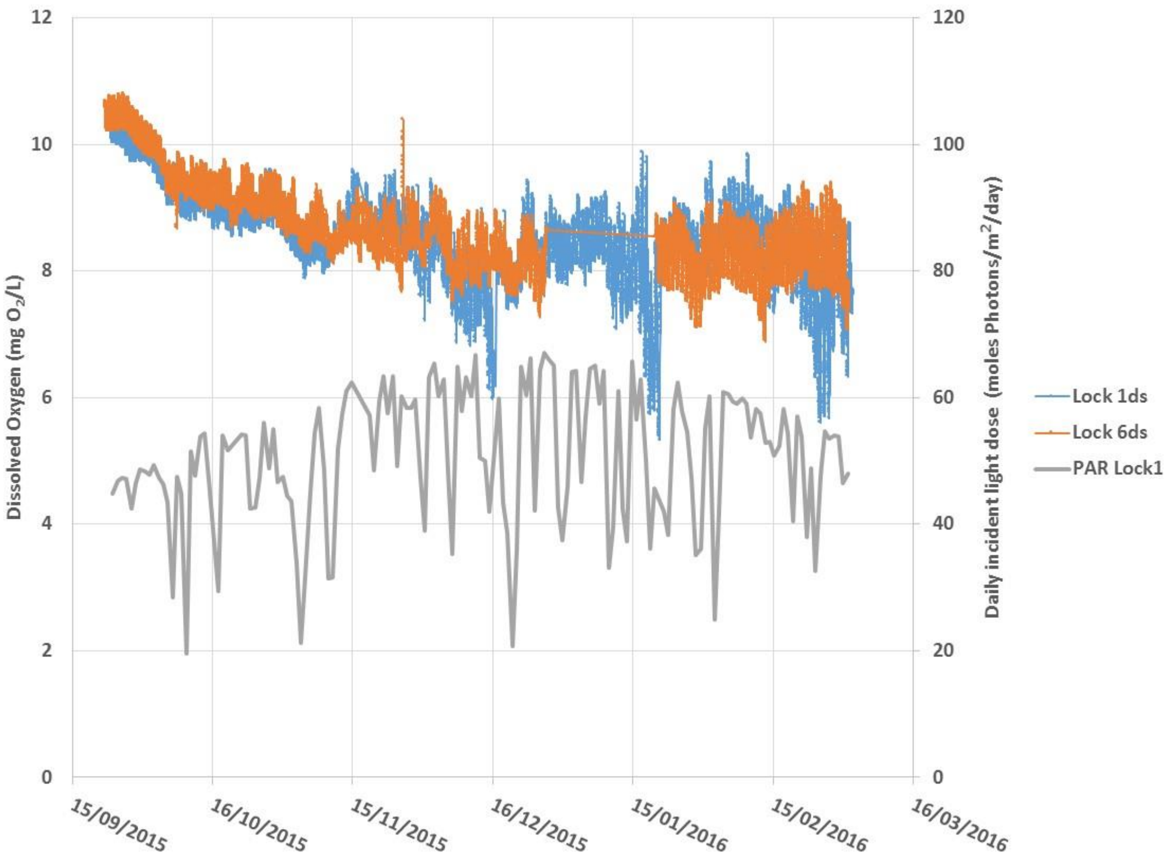

Figure C1. Time series of 10-minute interval oxygen concentrations at sites below Lock 6 and below Lock 1, and the daily solar light dose of photosynthetically active radiation (PAR) at Lock 1.

Metabolism

At both sampling sites, metabolic activity gradually increased over the sampling period, although there were clear differences in the specific patterns of metabolic activity between the two sites (Figures C2, C3 and C6).

Lock 6

Below Lock 6, GPP initially remained relatively stable averaging about 1 $mgO_2\,m^{-2}\,day^{-1}$ then slowly increased, with large oscillations, up to 2 $mgO_2\,m^{-2}\,day^{-1}$ by early November (Figure C2). There was then a steep decline in GPP to mid-November before it increased again to a peak of 2.5 $mgO_2\,m^{-2}\,day^{-1}$ by late November followed by a further precipitous fall in GPP at the end of November.

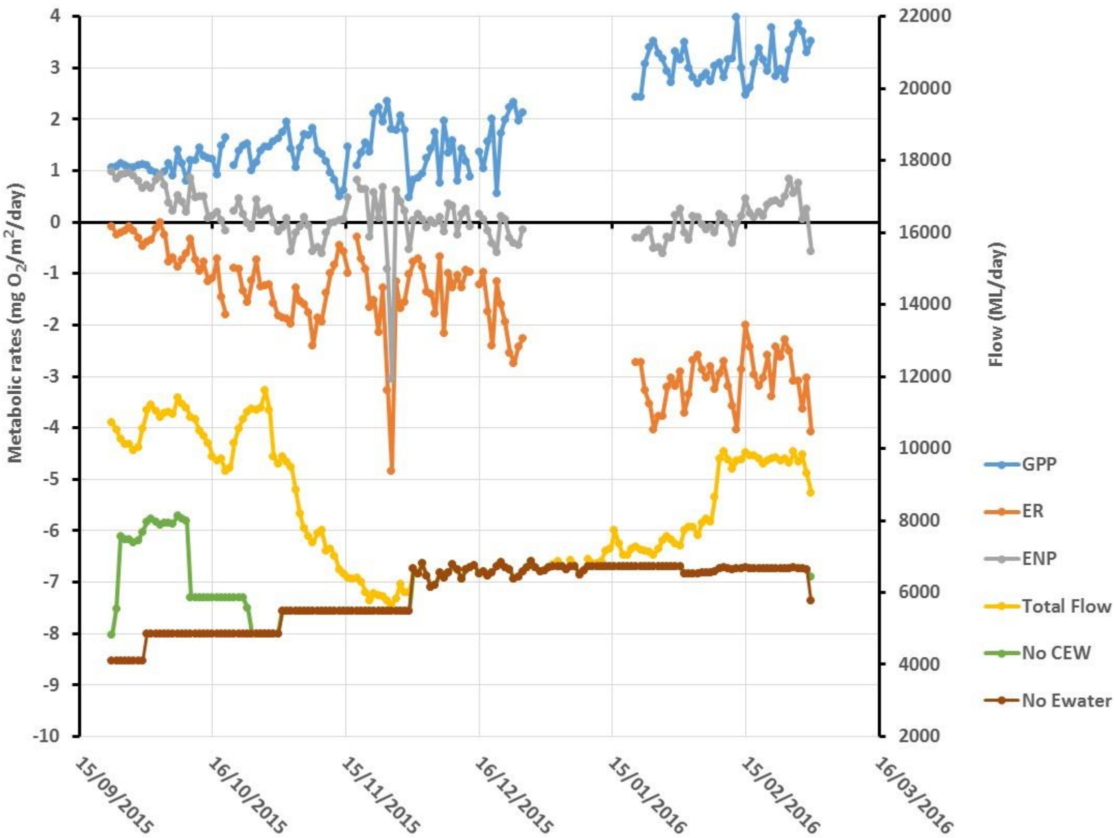

Figure C2. Time series of rates of gross primary production, ecosystem respiration and ecosystem net production with river flows including or excluding elements of environmental flows at the site below Lock 6.

During this period, respiration rates were initially particularly low resulting in positive ENP, but then ER increased at a greater rate than GPP so that ENP declined until early November (Figure C2). Following this ER mirrored the changes in GPP, declining to mid-November, increasing again, then declining to the end of November. Consequently, ENP oscillated around zero, apart from a large, but short lived increase in respiration on 25 and 26 November to 4.84 $mgO_2\,m^{-2}\,day^{-1}$ that generated a negative ENP.

This period of rapidly changing metabolism corresponded to a period when Weir Pool 5 was being raised using supplies of Commonwealth environmental water to vary the water level for environmental purposes (Figure C3a). The metabolism monitoring commenced just after the weir level had been raised 0.1 m, and then recorded the changes associated with the following 0.35 m rise and fall. The water level rise peaked around 16 October and remained at the maximum level until 8 November before being lowered back to the starting level by 23 November. It is noteworthy that the continuous increase in respiration rate observed at the site during this time continued up until 8 November, aligning with the increased connection between the floodplain and the river. Then as water level declined there was a decrease in respiration rate, but as the rate of decline slowed and stopped the respiration rate began to increase again with a peak occurring on 25 and 26 November aligned with the cessation of water level decline. The increased respiration rates after the water level returned to pool level aligned with the period that water was released from the Chowilla Regulator (Figure C3b). The return of water from the Chowilla Anabranch following an in-channel flow rise was also associated with an increase in GPP and so may be contributing to the general increased metabolism. Further detailed analyses are required but await improved flow data and further confirmation of the metabolism results. The continuing collection of data through the LTIM project should help to better understand these processes.

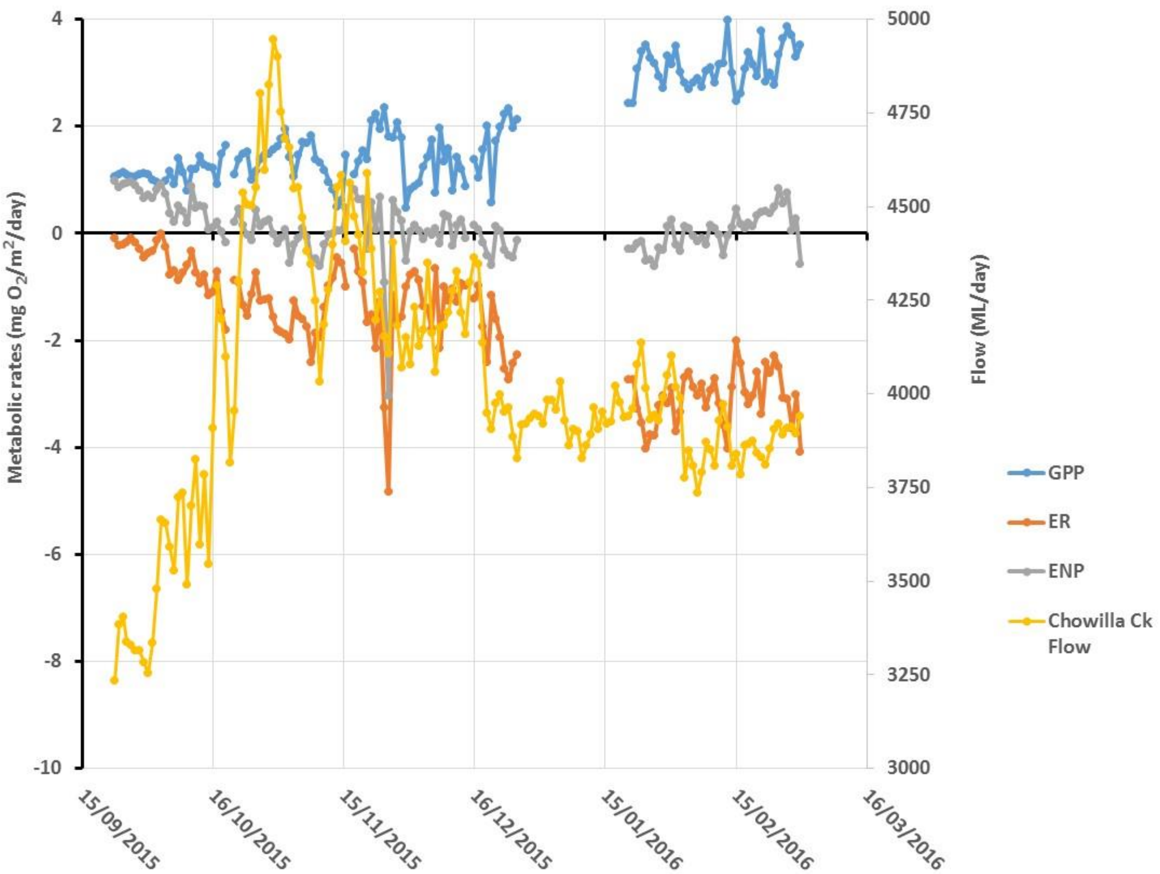

Figure C3. Comparison of time series of rates of gross primary production, ecosystem respiration and ecosystem net production at the site below Lock 6 and in Weir Pool 5 with (a - top) water levels upstream of Lock 5 and (b - bottom) flow in Chowilla Creek.

Following the period of water level manipulation in the weir, GPP and ER both increased. Due to the loss of data between mid-December and mid-January it cannot be determined whether metabolism rates increased consistently during this time or if there were step changes. However, when measurements recommenced following replacement of the faulty probe, GPP had increased by 50% and continued to slowly rise to about 4 mgO_2 m^{-2} day^{-1} in mid-February. It is suspected that the increase in metabolism just prior to and following the data loss was due to improving light conditions resulting from a continuous fall in turbidity that enhanced the extent of light penetration. ER changes mirrored those of GPP so that ENP remained close to zero, apart from a prolonged period of positive net production that occurred in the latter half of February associated with an increase in chlorophyll concentration (Figure C4).

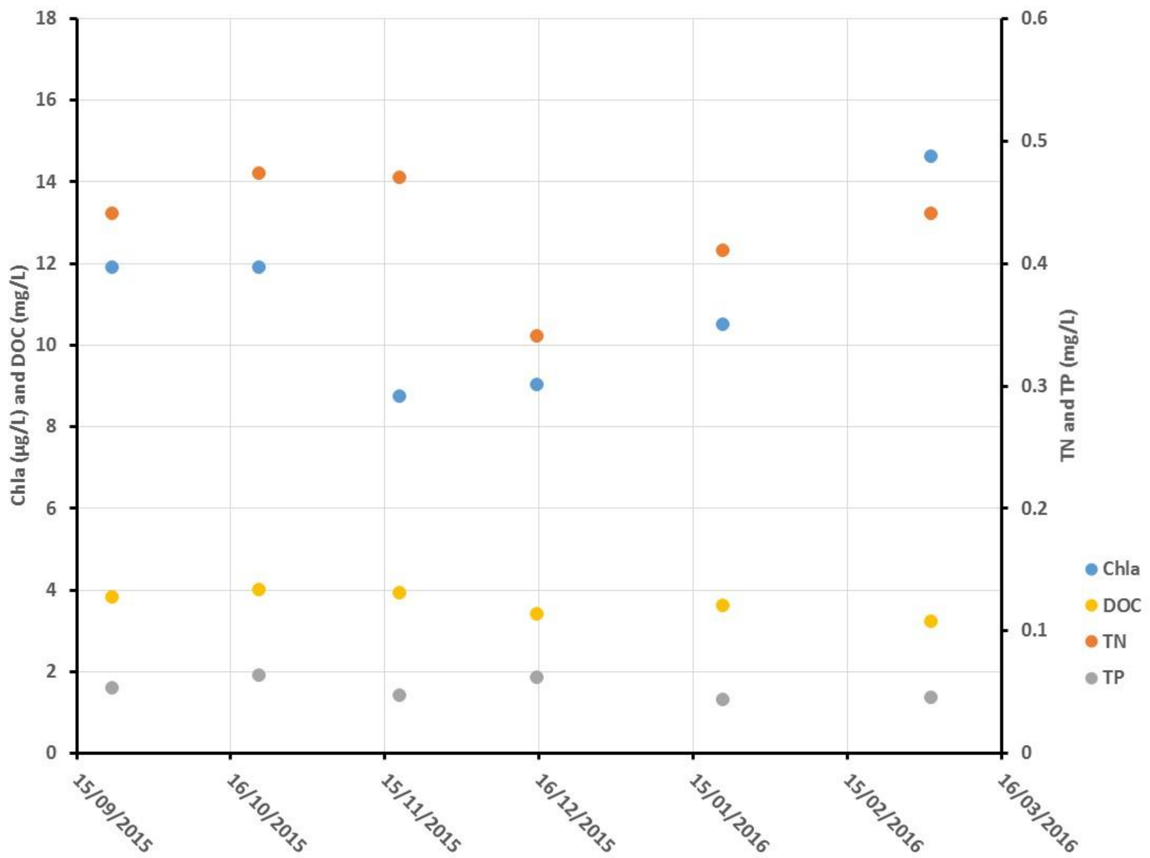

Figure C4. Time series of Chlorophyll-a (Chla), Dissolved organic carbon (DOC), Total Nitrogen (TN) and Total Phosphorus (TP) concentrations measured as part of the CEWO LTIM project at the site below Lock 6.

The alignment of changes in metabolism with the weir pool water level manipulations is suggestive of a causative link, but this could not be confirmed from the data set collected as part of this project as there were no comparative measurements from nearby reaches of the system not undergoing water level changes. However the findings of Wallace and Cummings (2016a), from a project specifically investigating the changes in metabolism associated with the water level manipulations in the weir pool, suggest that these links are likely to be causal. The enhanced rates of ER suggested delivery of metabolisable organic material due to the increases of weir pool height and the resulting improved connections with the floodplain. Direct links with flow were not obvious because flow and water level are not directly related in the weir pool, but Commonwealth environmental water was used to enable the weir pool raising (WPR) and this enhanced metabolic activity. It is also possible that increased rates of metabolism following WPR were associated with waters returning from Chowilla Creek as the water level at the Chowilla Regulator fell towards pool level, but this linkage is more difficult to isolate from the data. These responses are important as intermittent periods of increased supplies of organic material are considered critical to the food webs of the Murray River. Their decline in frequency, duration and extent has been proposed as a major cause of reductions of aquatic biota, through decreases in food supplies (Oliver and Merrick 2006; Oliver and Lorenz 2010).

Integrating metabolism over the duration of the monitoring period showed that, at the site below Lock 6 (Figure C5), GPP and ER were closely balanced and ENP was not different from zero. This suggests that the organic material enhancing respiration during the WPR was due to aquatic photoautotrophs (plants growing in the water), rather than external supplies of detrital organic material. Flooding of low lying areas can result in large expanses of shallow, warm and well illuminated waters that may enhance primary production outside of the river channel. The metabolic response then seen in the river will depend in part on the extent and timing of any exchange of water between the channel and the floodplain.

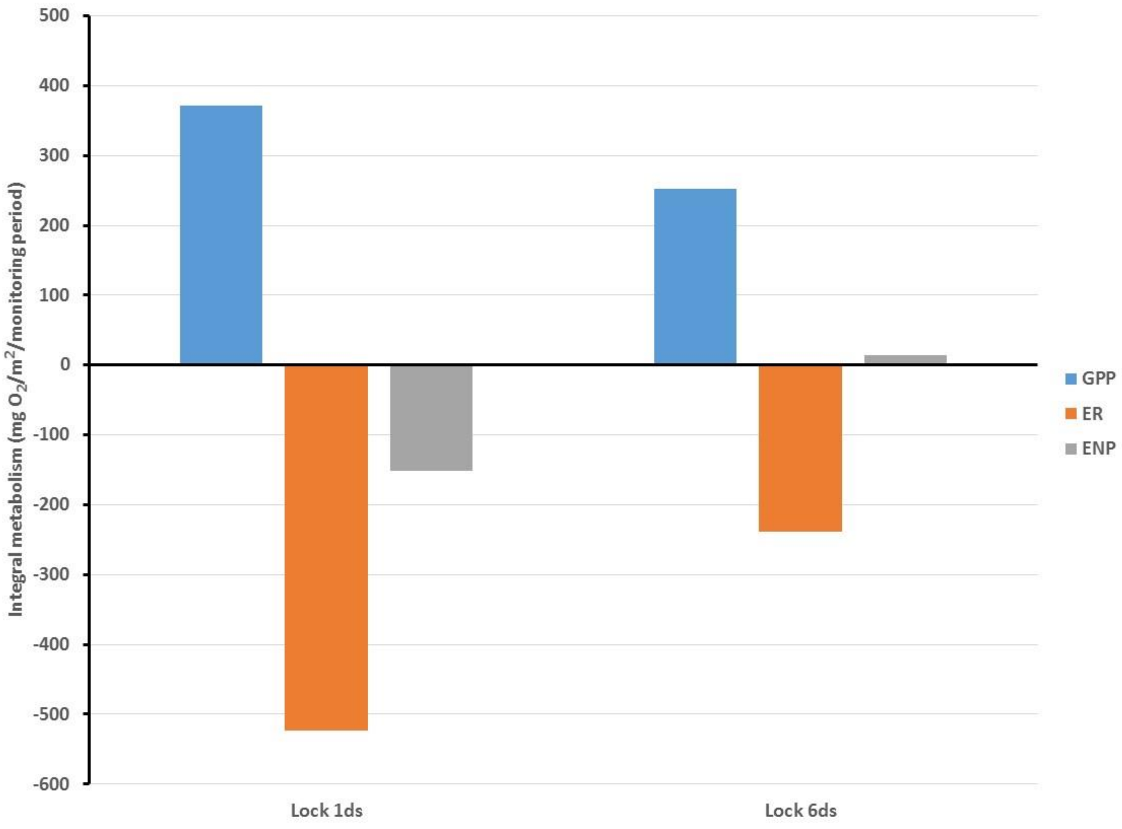

Figure C5. Total integrated gross primary production (GPP), ecosystem respiration (ER) and ecosystem net production (ENP) at the sites below Lock 6 and Lock 1 over the monitoring period between September 2015 and March 2016.

Lock 1

Below Lock 1, the rates of metabolism were initially similar to those at the site below Lock 6 (Figure C6). Average GPP remained relatively stable at around 1.5 $mgO_2\,m^{-2}\,day^{-1}$ until 26 November and then stepped up to around 2.7 $mgO_2\,m^{-2}\,day^{-1}$ before falling back in mid-December when the replacement probe was deployed.

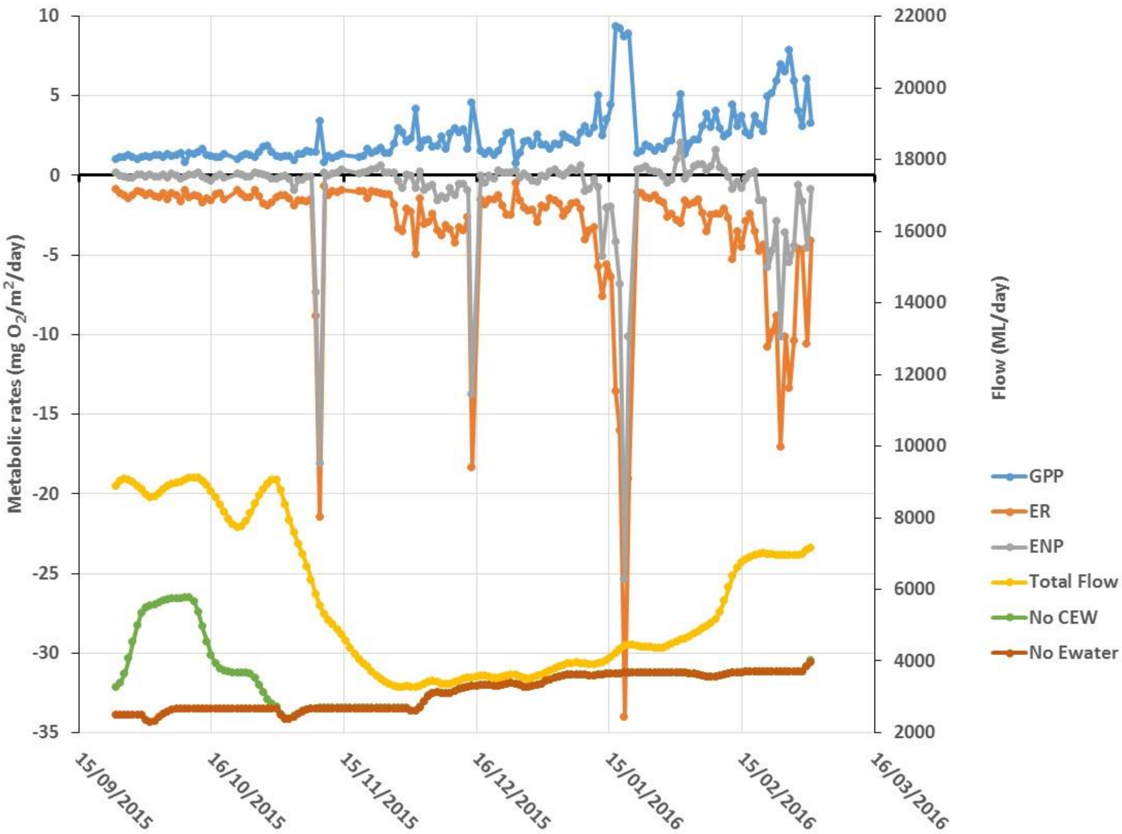

Figure C6. Comparison of time series of rates of gross primary production, ecosystem respiration and ecosystem net production with river flows including or excluding elements of environmental flows at the site below Lock 1.

Similarly, after mid-December both GPP and ER slowly increased until mid-January, mirroring each other so that ENP was close to zero. Then both GPP and ER rapidly increased, but ER increased more rapidly to a maximum of 34 $mgO_2\,m^{-2}\,day^{-1}$, driving ENP increasingly negative. This was followed by a precipitous decline in both GPP and ER between 20 and 22 January when the replacement probe was deployed. These patterns suggested that increasing biofouling influenced the oxygen probes during their deployment. The probes themselves were protected with a copper mesh resulting in minimal direct biofouling, and previous experience had indicated that this was sufficient to safeguard the probes. For example, biofouling was not observed at the site below Lock 6 over this same period. However, field observations indicated that the battery housing to which the probes are attached became heavily coated in biofilms and filamentous algae at the site below Lock 1. The sudden changes in apparent oxygen concentrations and metabolic activity on the deployment of replacement probes strongly supports the suggestion that biofouling influenced the oxygen concentrations and metabolic measurements at this site and further analysis of the data is restricted to general trends.

To further support the suggestion of biofouling, data on flow, light availability, water quality, plankton concentrations, meteorological conditions and physico-chemical attributes were all investigated, but none showed simple changes that might be responsible for the fluctuations in metabolism (Figure C7). Similarly, neither chlorophyll concentrations, that indicate phytoplankton biomass, or zooplankton numbers showed changes that might have aligned with increase metabolic rates. Basic water quality attributes collected as part of LTIM are shown in Figures C4 and C7, and a broader array of water quality data collected more regularly by SA Water from nearby sites showed similar patterns.

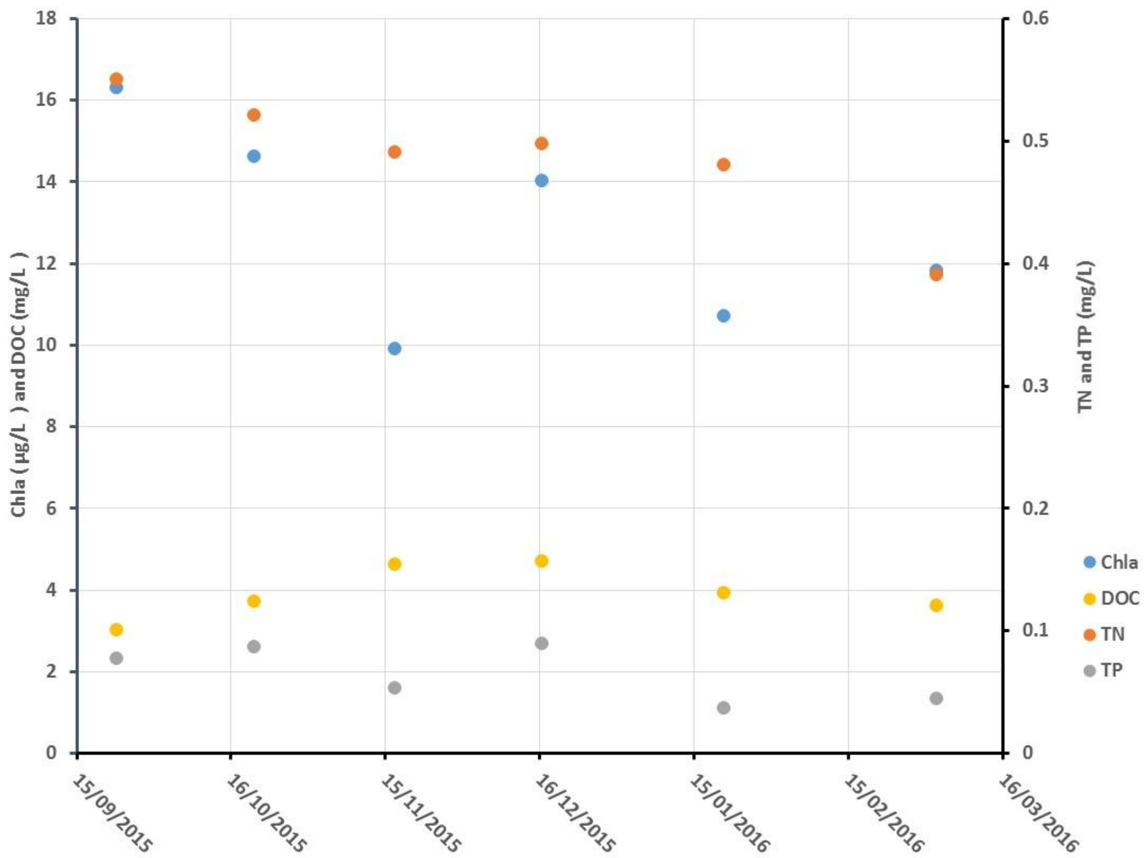

Figure C7. Time series of Chlorophyll-a (Chla), Dissolved organic carbon (DOC), Total Nitrogen (TN) and Total Phosphorus (TP) concentrations measured as part of the CEWO LTIM project at the site below Lock 1.

The two sampling sites are a considerable distance apart with several weirs inbetween and metabolic activity appeared to occur independently in each. The largest rates of GPP below Lock 1 were observed in mid-January 2016 reaching 9.4 $mgO_2\ m^{-2}\ day^{-1}$, but this was the period when data was not available for the site below Lock 6 and so direct comparisons could not be made. A second peak in GPP on the 26 February below Lock 1 was not associated with increases of GPP below Lock 6, suggesting that different processes were at play at the two sites in the flooplain and gorge geomorphic zones of the LMR Selected Area.

Conclusions

Oxygen concentrations were generally maintained at acceptable levels (>50% saturation) during the 2015/16 monitoring period. However, at the site below Lock 1, three periods of reduced oxygen concentration were recorded reaching minimum values of 5 mg L^{-1}. These responses were attributed to biofouling of the oxygen sondes at this site.

Metabolic activity was enhanced at the site below Lock 6 at the time that the weir pool was being raised facilitated by Commonwealth environmental water. It is suggested that the changes in metabolism reflect the increased connection between the river and the floodplain resulting from WPR. This was possibly augmented by the return of water down Chowilla Creek as the Chowilla Regulator was lowered following a within anabranch flow rise supported by The Living Murray and Commonwealth environmental water. Integrating the metabolic responses over the duration of the monitoring period produced an ENP close to zero suggesting that the flooded area was enhancing aquatic phototrophic production, and that this was largely returned to the river. This response is important as intermittent periods of increased supplies of organic material are critical to the food webs of the Murray River and their decline in frequency, duration and extent has been proposed as a major cause of reductions of aquatic biota due to the decline in food supplies (Oliver and Merrick 2006; Oliver and Lorenz 2010). Application of management techniques that enhance river and floodplain connectivity (e.g. WPR) to a wider range of sites more frequently might broadly enhance metabolic activity with expected benefits to food webs, although further investigations are required to identify the beneficiaries and the food web links. The continuing collection of data through the LTIM program should help to better understand these events.

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 the flow regime can influence in-channel hydraulics and structural habitat, which may influence main channel 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 Selected Area, in this report we provide commentary on the fish assemblage in the gorge geomorphic zone 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 (autumn 2015) and 2 (autumn 2016); 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 Selected Area (Figure 7) using fine-meshed (2 mm mesh) fyke nets and electrofishing, respectively. Sampling occurred during autumn 2016 (Year 2), following prescribed standard methods (Hale *et al.* 2014). 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 the LMR LTIM M&E Plan for detailed sampling design and methodology.

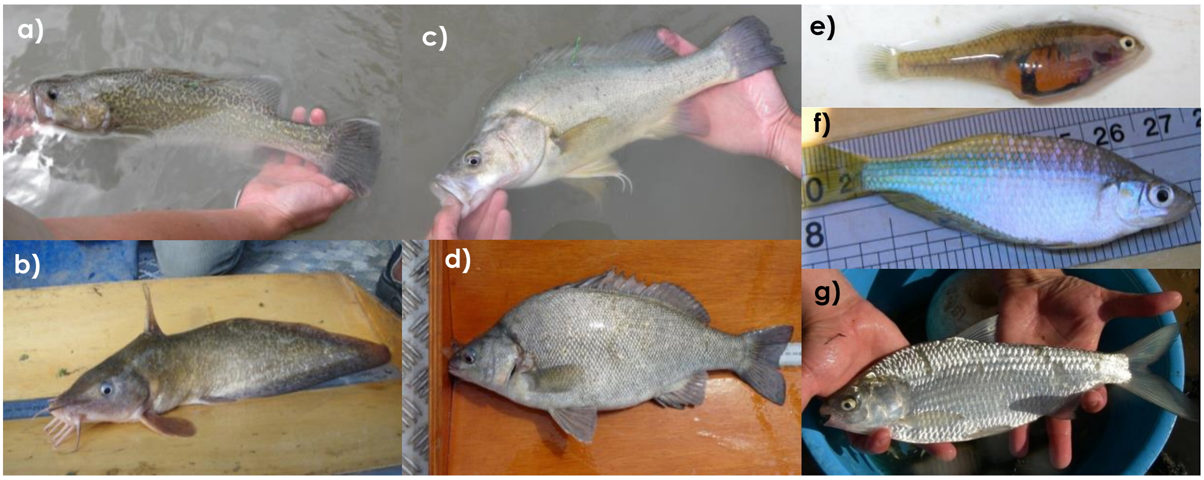

Figure D1. Target species for the LMR Selected Area: (a) Murray cod and (b) freshwater catfish (equilibrium life history); (c) golden perch and (d) silver perch (periodic life history); and (e) carp gudgeon, (f) Murray rainbowfish and (g) bony herring (opportunistic life history).

Data analysis

Temporal variation in fish assemblage structure (species composition and abundance), between sampling years (i.e. 2015 and 2016), 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 were performed on fourth-root transformed data from electrofishing (fish. 90 second electrofishing shot^{-1}) and untransformed data from 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 (i.e. 2015 and 2016).

Results

Catch summary for autumn 2016

A total of 7,992 individuals from seven large-bodied species were sampled by electrofishing from ten sites in the gorge geomorphic zone of the LMR Selected Area (Table D1). Bony herring was the most abundant species (23.2 ± 4.1 individuals per 90 second shot) and dominated electrofishing catch composition (93%) (Table D1; Figure D2a). Common carp (*Cyprinus carpio*) and golden perch (*Macquaria ambigua*) were the second and third most abundant species, respectively.

A total of 22,853 individuals from seven small-bodied species were sampled by fyke nets from ten sites in the gorge geomorphic zone of the LMR Selected Area (Table D2). Carp gudgeon (*Hypseleotris* spp.) was the most abundant species (10.4 ± 1.8 individuals per net per hour) and dominated fyke net catch composition (87%) (Table D2; Figure D2b). Gambusia (*Gambusia holbrooki*), Murray rainbowfish (*Melanotaenia fluviatilis*) and unspecked hardyhead (*Craterocephalus fulvus*) 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 Selected Area for autumn 2015 and 2016. 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											
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**

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 Selected Area for autumn 2015 and 2016. 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**
<u>2015</u>											
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	**1253**
Total	**626**	**2,365**	**430**	**850**	**535**	**875**	**3,142**	**760**	**5,892**	**5,561**	**21,036**
<u>2016</u>											
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**

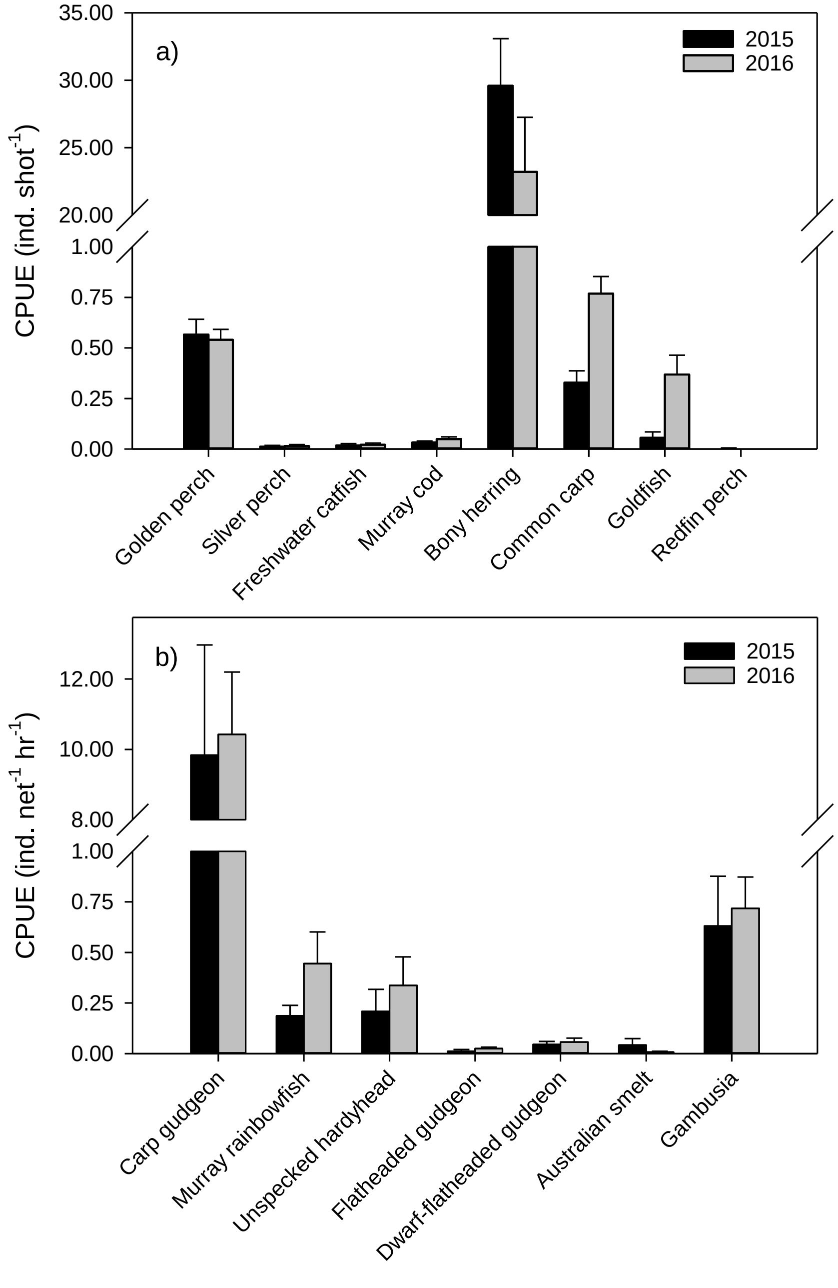

Figure D2. Mean catch-per-unit-effort (CPUE) ± standard error of (a) large-bodied fish species captured using electrofishing (individuals per 90 second shot) and (b) small-bodied fish species captured using fine-mesh fyke nets (individuals per net per hour) in the gorge geomorphic zone (10 sites) of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

Temporal variability in fish assemblage structure

MDS ordination of electrofishing data showed separation of most samples by sampling year (Figure D3a). PERMANOVA indicated that large-bodied fish assemblages were significantly different between years (*Pseudo-F*$_{1,19}$ = 5.0208, *p* = 0.002). Interspersion of samples in MDS ordination of fyke netting data (Figure D3b) was supported by PERMANOVA, which found no significant differences in small-bodied fish assemblages between years (*Pseudo-F*$_{1,19}$ = 1.6856, *p* = 0.180).

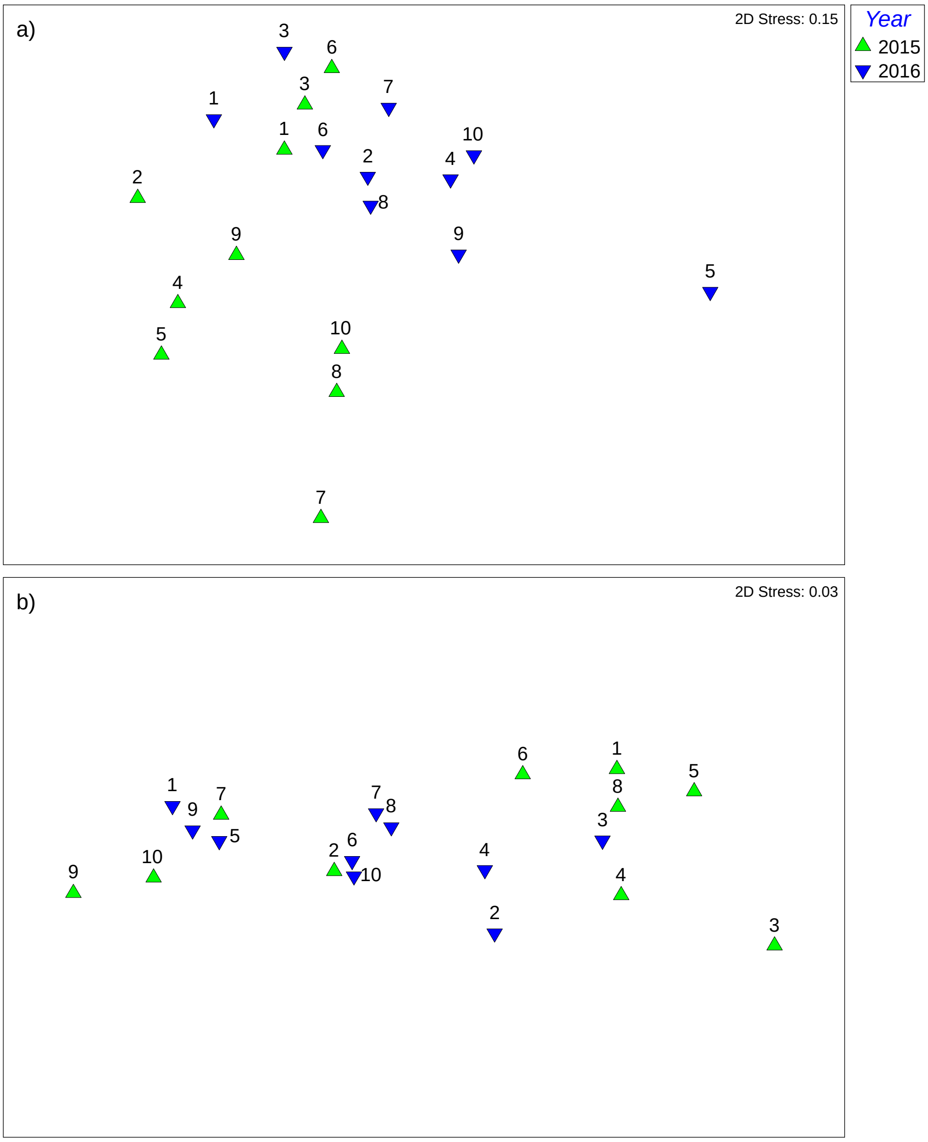

Figure D3. Non-metric multi-dimensional scaling (MDS) plot of (a) large-bodied fish assemblages sampled by electrofishing and (b) small-bodied fish assemblages sampled by fyke netting in the gorge geomorphic zone of the LMR Selected Area. Numbered sample points represent sampling sites (1–10).

SIMPER indicated that differences between years for large-bodied fish assemblages were driven by higher abundances of goldfish (*Carassius auratus*) and lower abundances of bony herring in 2016 (Figure D2; Tables D1 and D2).

Temporal variation in population structure

In 2016, length frequency distributions of most fish species sampled in the gorge geomorphic zone were similar to those in 2015. In 2016, golden perch and Murray cod (*Maccullochella peelii*) ranged in total length (TL) from 137–551 mm and 78–1270 mm, respectively (Figure D4). For golden perch, dominant TL modes at 220–240 (10%) and 320–340 mm (13%) in 2015 progressed to 240–260 (10%) and 380–400 mm (13%) in 2016. Small Murray cod (78–105 mm TL), potentially age 0+, dominated the catch composition of the species in 2016 (63%), whilst potential age 1+ (196–232 mm TL) Murray cod, which were spawned in the previous year (2014/15), represented 25% of the sampled population.

Population structure data for bony herring were not collected in 2015. In 2016, bony herring ranged in fork length (FL) from 37–330 mm and, in age, from 0+ to 6+ years (Figure D5). Age 0+ (83%) and 3+ (6%) cohorts comprised most of the catch. Based on length frequencies, there were no age 0+ golden perch, silver perch (*Bidyanus bidyanus*) or freshwater catfish (*Tandanus tandanus*) sampled in 2016 (Figure D4). Length frequencies of Murray rainbowfish and carp gudgeon indicate that the sampled populations were dominated by individuals that were age 0+, based on length-at-age data from 2014/15 (Ye *et al.* 2016).

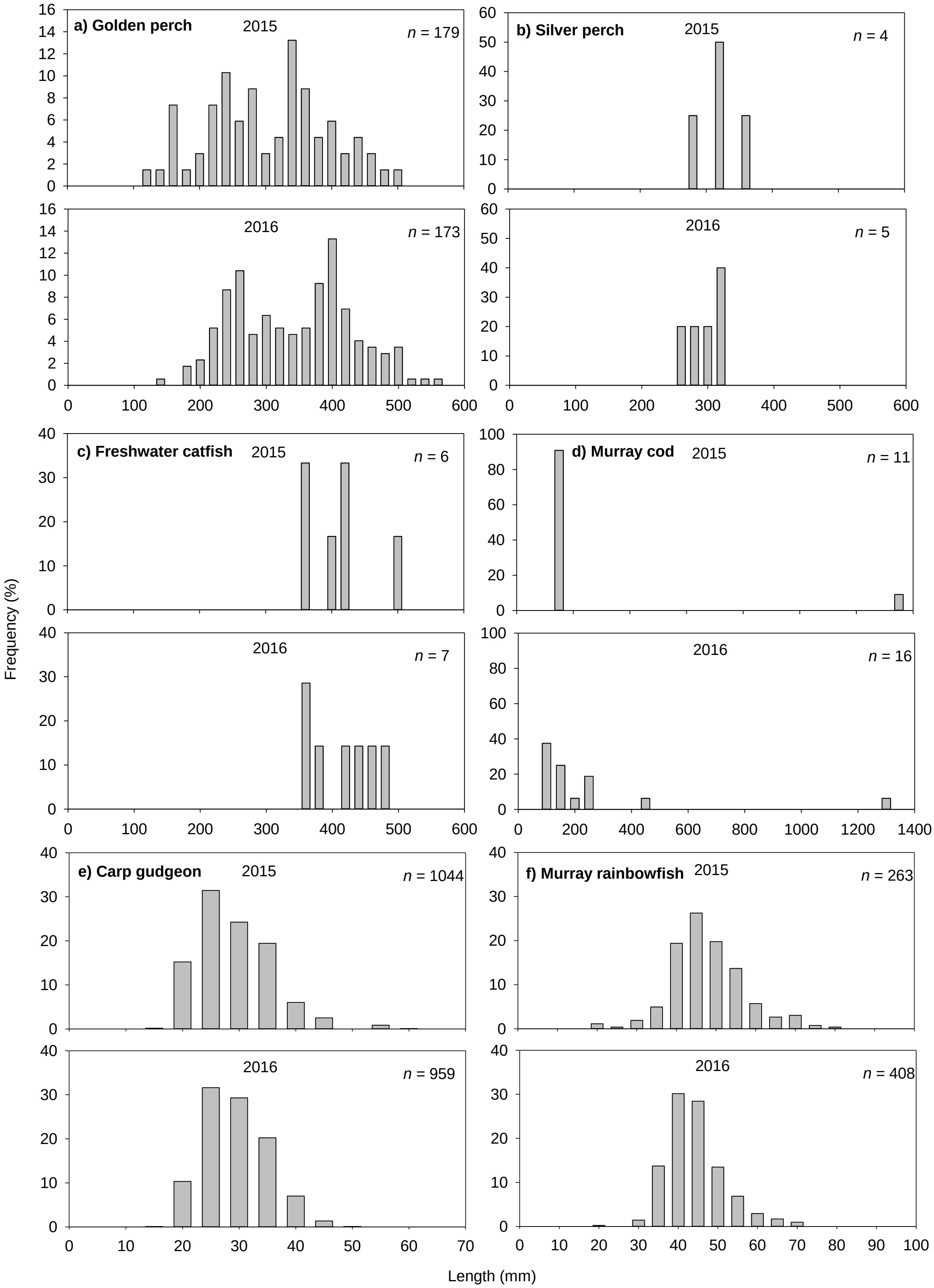

Figure D4. Length frequency distributions of periodic (a, b) equilibrium (c, d) and opportunistic (e, d) target species collected from the gorge geomorphic zone of the LMR Selected Area in autumn 2015 and 2016.

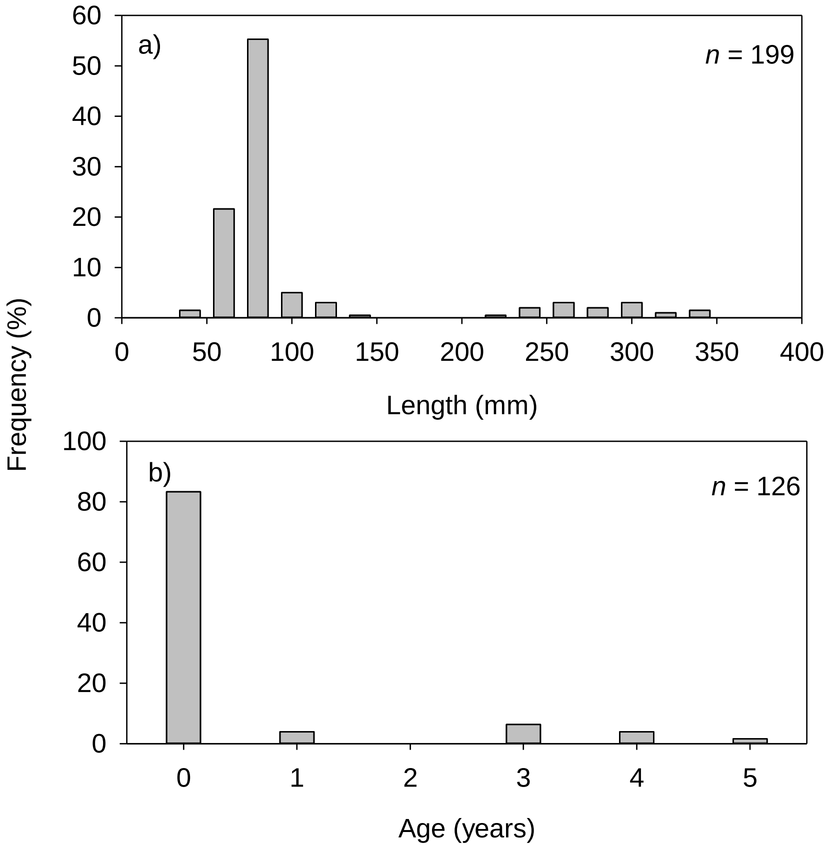

Figure D5. Fork length (a) and age (b) frequency distributions of bony herring collected from the gorge geomorphic zone of the LMR Selected Area during autumn 2016.

Discussion

Relatively low (<15,000 ML day^{-1}), stable flows predominated in the LMR Selected Area during Year 1 (2014/15) and 2 (2015/16) of the LTIM project. Consequently, small-bodied fish abundance and diversity remained high in 2015/16, and there was no significant change in small-bodied fish assemblage structure from 2014/15 to 2015/16. Abundances of flow-cued species (i.e. golden perch and silver perch) remained similar in both years; however, there was a significant change in assemblage, driven primarily by an increase in exotic goldfish, and a decrease in bony herring, in 2015/16, relative to 2014/15. Increased abundances of exotic, large-bodied species may reflect their generalist/opportunistic life histories.

Based on length frequency data, there was no recruitment to age 0+ of native, large-bodied golden perch, silver perch or freshwater catfish in 2015/16. The absence of recruitment of golden perch and silver perch is association with the 2014/15 and 2015/16 flow regimes (i.e. low, stable flows) is consistent with our contemporary understanding of the life history requirements of these flow-cued spawners (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b). For the second consecutive year, small Murray cod (<150 mm TL, likely age 0+) were sampled in the LMR Selected Area during 2015/16, indicating successful recruitment. Furthermore, there was persistence of the age 0+ cohort (100–150 mm TL) from 2014/15 as age 1+ (196–232 mm TL) in 2015/16. 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. 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 Selected Area, 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. The current fish assemblage structure is similar to that during drought (e.g. 2007–2010) (Bice *et al.* 2014) and characteristic of a low flow scenario. Persistent low flows in the main channel of the lower River Murray are likely to favour generalist/opportunistic species (e.g. small-bodied species and exotics) that prefer benign hydraulic conditions and abundant aquatic vegetation. Continued low flows are also likely to have further negative effects on the recruitment of flow-cued spawning species and, in turn, lead to a decline in their population resilience and abundance.

APPENDIX E: HYDROLOGICAL REGIME

Model calibration

The hydrodynamic models, and configuration adopted, are outlined in Ye *et al.* (2016a). Results comparing model outputs to datasets not used in the setup of the models for the 2015/16 year can be seen in Figures E1–E5. The performance of the models was deemed suitable for the purposes of evaluating the contributions of environmental water, and as such no further calibration was undertaken to that outlined in Ye *et al.* (2016a). Discussion of the model results are presented below.

Lock 1 – Lock 3 model

The water levels simulated by this model can be seen in Figure E1. It can be seen that model tends to underestimate the water level at Morgan, however the difference is typically less than 5 cm. In the same weir pool the modelled water level below Lock 2 is relatively accurate (this most distant from the downstream lock is the most responsive to flow and hence most difficult to represent accurately). Between Lock 2 and Lock 3 (Overland Corner and below Lock 3), the model generally provides a good representation of the recorded water level, however the model does slightly underestimate the recorded level in the last two months of the period. Given the good performance for the rest of the period, this may be a data issue.

A comparison between modelled and recorded cross section averaged velocity can be seen in Figure E4. The plots represent the frequency of cross section averaged velocities either modelled or recorded on each sampling date. The recorded values were undertaken by SARDI (Bice *et al.* 2016a), and a cross section average velocity was recorded for five transects 1 km apart at locations in the lower, middle and upper reaches of each weir pool. As such, there is a range in the recorded velocity values across the five transects, represented by the green shape (a density plot/histogram). The range in modelled velocities representing the same 5 km stretch of river are presented as the red shape in Figure E4. Cross sections are spaced at 250 m in this model, and as such there are approximately 20 modelled velocity values over the same reach of river. Given the extra values, the range in velocities might be expected to be greater for the model results compared to the recorded values. Keeping this factor in mind, the modelled velocity values can be seen to be in good agreement with those recorded in the river at the same time.

Katarapko model

Modelled and observed water levels from the Katarapko model from Lock 3 to Lock 5 can be seen in Figure E2. Results upstream of Lock 4 (at Berri, Lyrup and downstream of Lock 5) are typically accurate, noting that there is very little variation in the data, and as such the scale only represents 5–10 cm of variation in water level at the Lyrup and Berri sites. Below Lock 4, the model typically underestimates the water level at Loxton in the order of 10 cm, however provides a good representation of the water level below Lock 4. Given this point is the most responsive to flow within the weir pool (the water level is controlled by observed data at Lock 3) this was deemed to represent suitable accuracy.

A comparison between modelled and recorded cross section averaged velocity can be seen in Figure E5. Velocity recording were undertaken in the Lock 3–Lock 4 weir pool as a reference site for the monitoring undertaken for the WPR event (Bice *et al.* 2016a). Again, the modelled velocity values can be seen to be in good agreement with those recorded in the river at the same time, including the high velocities recorded below Lock 4.

Pike model

For this model, only the water level below Lock 6 is relevant for the model calibration. The model may be slightly under sensitive at this location (Figure E3), with the highest water levels are underestimated, and lowest water levels overestimated. This may indicate the cross section used for comparison in the model is slightly too large, however the results are considered suitable for this purpose.

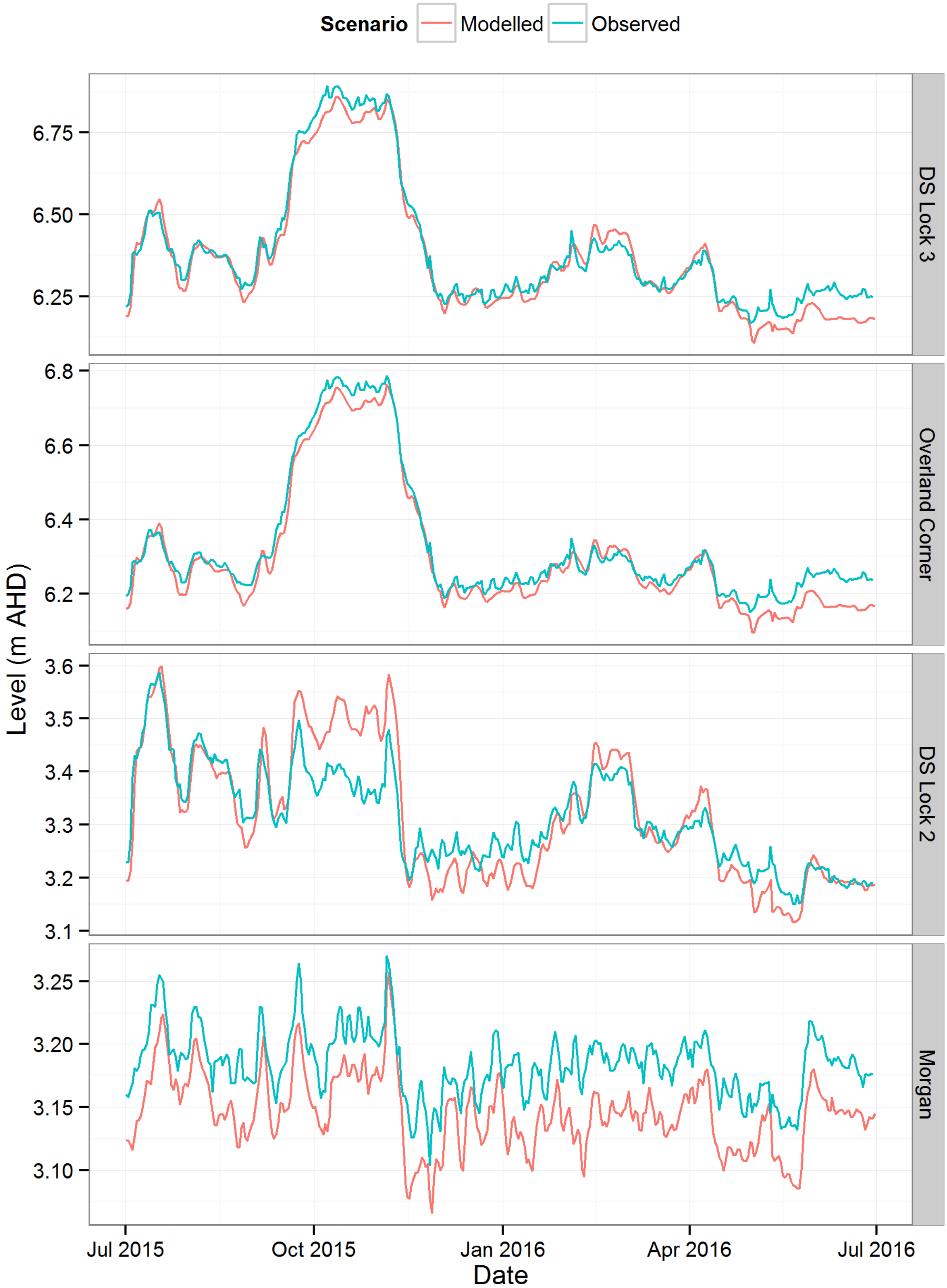

Figure E1. Water Levels used for calibration of the model from Lock 1 to Lock 3.

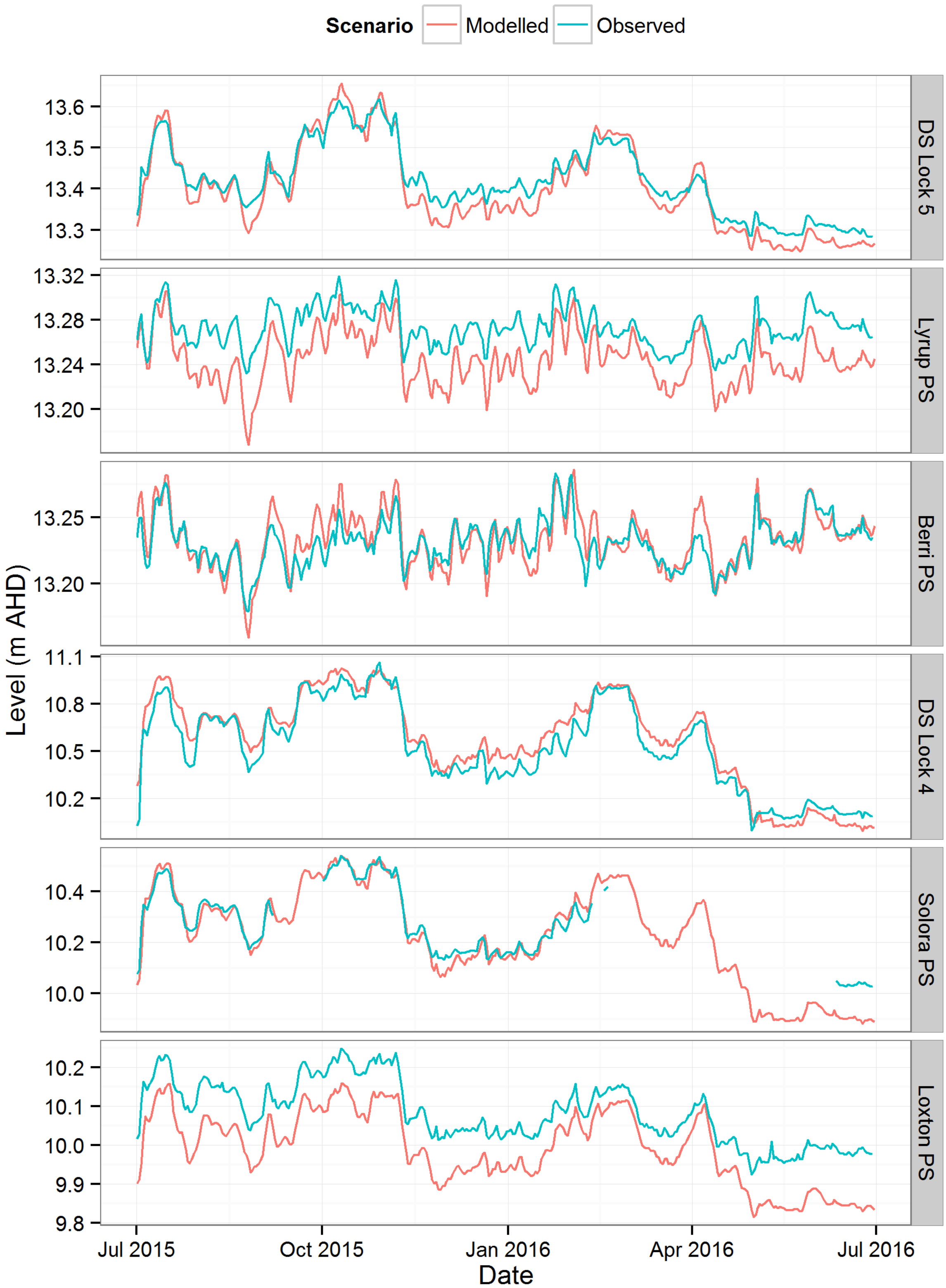

Figure E2. Water Levels used for calibration of the model from Lock 3 to Lock 5.

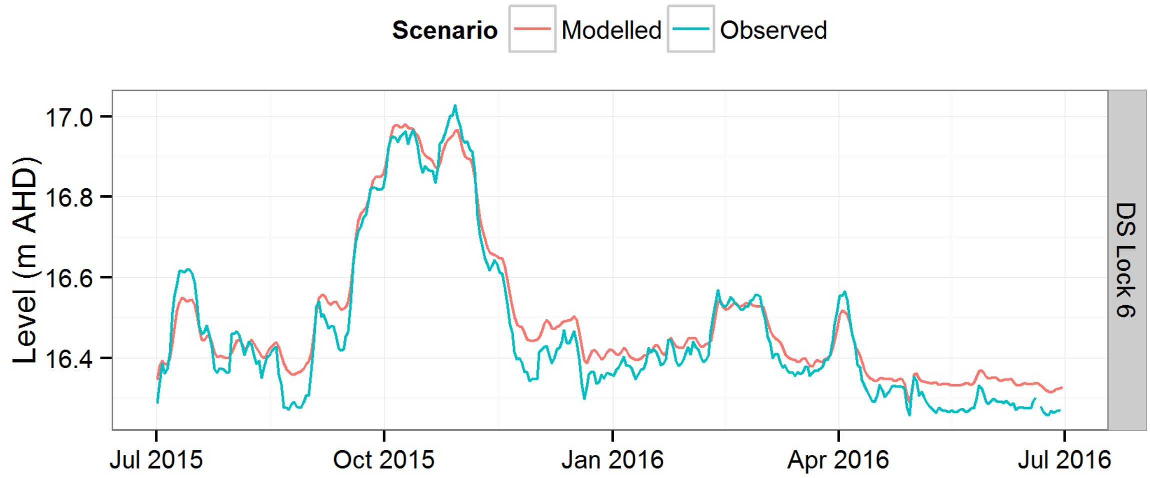

Figure E3. Water Levels used for calibration of the model from Lock 5 to Lock 6.

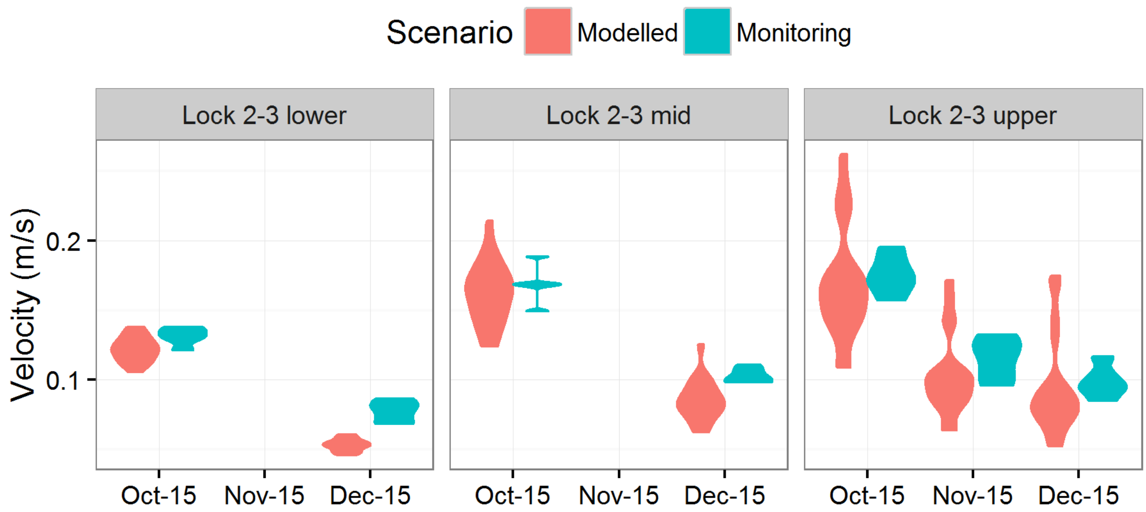

Figure E4. Modelled and measured velocity ranges between Locks 1 and 3.

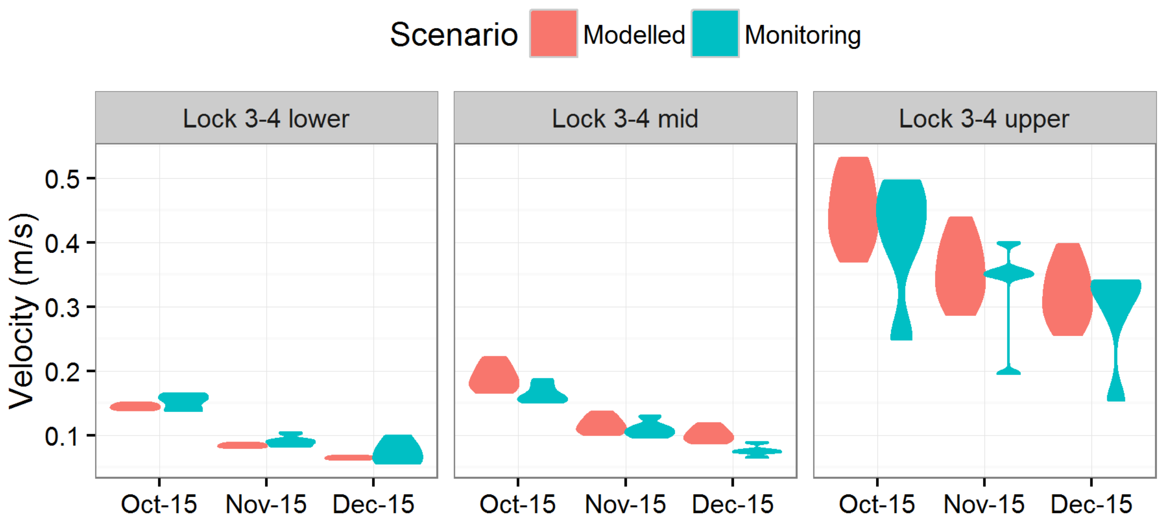

Figure E5. Modelled and measured velocity ranges between Locks 3 and 4.

Environmental water scenarios

The models used simulated 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 parameters to be quantified.
- Without any environmental water. This allows the collaborative outcomes across all environmental water holders to be quantified. There was not a case in 2015/16 where environmental water was provided without some of that water being Commonwealth environmental water.

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) at upstream boundary for each model (see 'Boundary Conditions' section above) was removed from the model, with most other settings kept the same.

There were two main changes for the without environmental water scenarios:

1. The representation of diversions within South Australia. The environmental scenarios were adjusted to account for the purchase of entitlements within South Australia by the CEWH. Calculated diversions would be expected to be reduced in the 2015/16 water year compared to the no environmental water case, where these entitlements would have been used for consumptive purposes otherwise. It was assumed that these entitlements were 100% utilised, i.e. the full 117.3 GL of entitlements (not including the 15.8 GL that was used for environmental outcomes within South Australia, e.g. pumped to higher elevation wetlands) would have been used for consumptive purposes in the no environmental water scenarios. For these scenarios, the calculated diversions were increased by the 117.3 GL, distributed temporally over the year using the same pattern as entitlement flow, and spatially across the weir pools using the same proportions as the calculated diversions in each weir pool.

2. WPR events were removed. Commonwealth environmental water was used to account for the water use due to these events in 2015/16, therefore it was assumed that these events would not have occurred without the delivery of Commonwealth environmental water. It was assumed that the water level were held at pool level during the raising events, i.e. 6.1 m AHD at Lock 2 and 16.3 m AHD at Lock 5.

Water level

Results for the three scenarios at the upper end of each weir pool can be seen in Figure E6. 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. To demonstrate this effect, the simulated water levels in the middle of each weir pool can be seen in Figure E7, where the differences in water level across the scenarios were smaller. While not represented, the water levels at each lock are assumed to be the same across all scenarios, with the exception of when WPR events were occurring at Locks 2 and 5.

It can be seen that environmental water resulted in increases in water level in winter and spring of up to 0.3 m in the upper reaches of the weir pool for Weir Pools 1 and 4. This increased up to 0.7 m in spring for Weir Pools 2 and 5, during the WPR events. Higher increases of up to 0.9 m were simulated in the upper reaches of Weir Pool 3, due to this being the longest weir pool (and therefore the upper reaches of the weir pool less influenced by Lock 3), and also the much shallower and narrower nature of the Murray River near where Katarapko Creek branches off from the Murray River. Note that this increase is limited to the upper reaches of Weir Pool 3, with much smaller increases in water level occurring near the midpoint of this weir pool (Figure E7)

Velocity

The results for the velocity in each weir pool can be seen in Figure E8. The velocities calculated by the models represent the average velocity across a river cross section at each computation point. As these points are not necessarily equality spaced along the river, a length weighted velocity was adopted to calculate the 10^{th}, 50^{th} (median) and 90^{th} 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 the models.

The median velocity in each weir pool on each day is presented as the solid lines in Figure E8, with the range represented by the 10th and 90th percentiles represented by the shaded area.

It can be seen from Figure E8 that without environmental water, the median velocity was less than 0.1 m s^{-1}across the weir pools across the year (with some exceptions in Weir Pool 5). Velocities less than 0.1 m s^{-1} represent slow-flowing habitat, and as such the without environmental water case, with entitlement flow all year, represents close to stagnant weir pools. In contrast, the with Commonwealth environmental water case represents an increase in weir pool median velocity of ~ 0.1 m s^{-1} in winter and spring across the weir pools. The shaded blue areas in Figure E8 indicate that, with Commonwealth environmental water, there were some cross sections in each weir pool (typically the upper reaches) greater than 0.17 m s^{-1}, between July and November 2015 as well as in February 2016, coinciding with the delivery of Commonwealth environmental water increasing QSA to over 9,000 ML day^{-1}.

Effects of weir pool raising

Results with and without Commonwealth environmental water, and with and without WPR, on the day corresponding to the largest raising and largest Commonwealth environmental water contribution are presented in Figure E9 and E10 for Weir Pools 5 and 2, respectively. The 0.5 m WPR can be seen to increase the water level by this amount at Lock 2, at the downstream end of the weir pool (right hand side of the upper plot of Figure E10), with similar but slightly smaller increases for Weir Pool 5 (Figure E9).

One concern with WPR is the negative impact on velocity, due to increasing the cross sectional area to flow for the same discharge. This can be seen to occur in Figures E9 and E10, with lower velocities for the with WPR case compared to without. However, this reduction can be seen to be relatively small in 2015/16, with the decrease in the velocity typically less than 0.05 m s^{-1}, and much smaller than the corresponding increase in velocity due to the delivery of Commonwealth environmental water in this water year.

There will always be a trade-off between in-channel velocity and inundation area when using structures to inundate larger areas with the same flow.

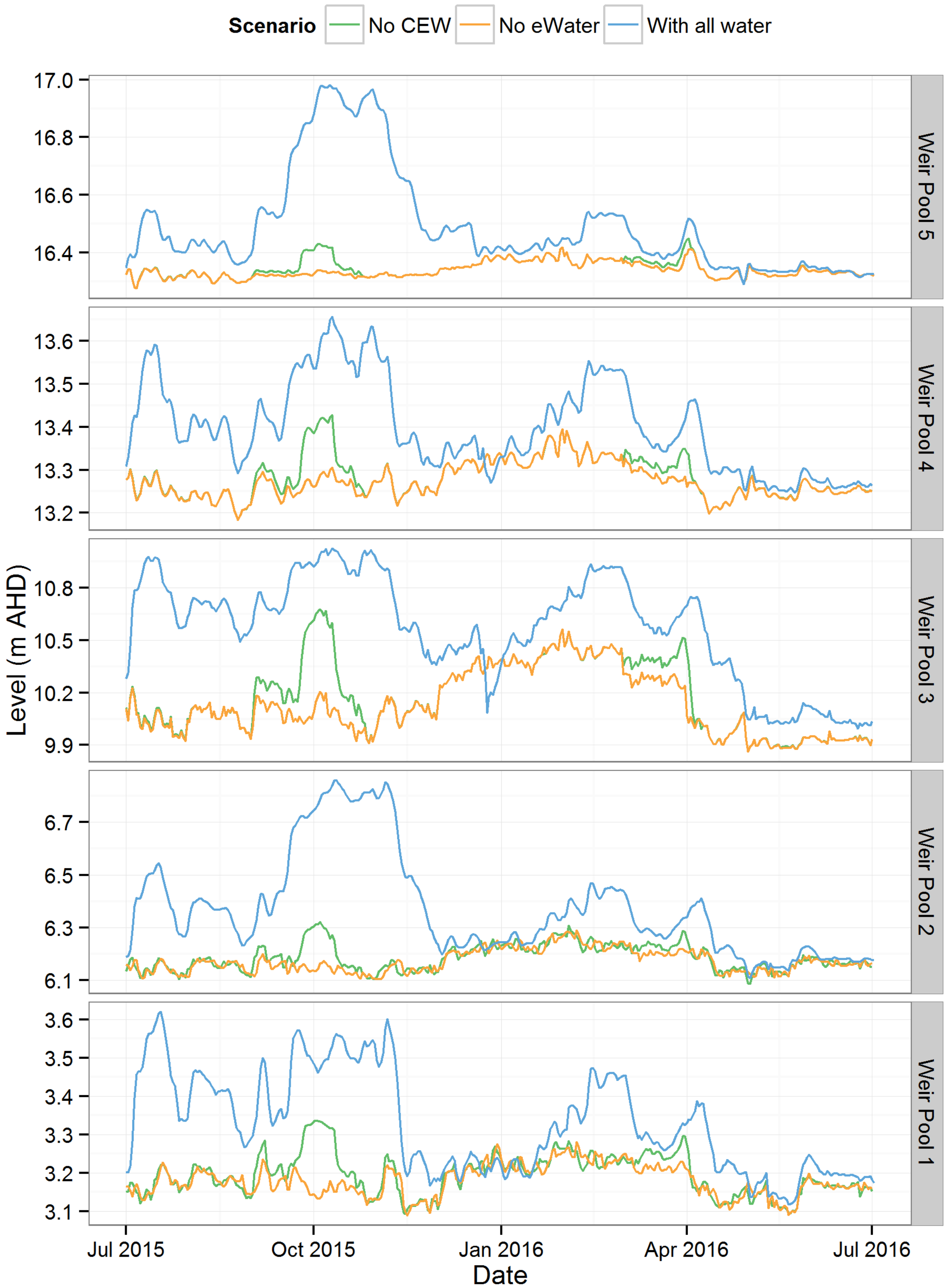

Figure E6. Modelled water level at the upstream end of each weir pool without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

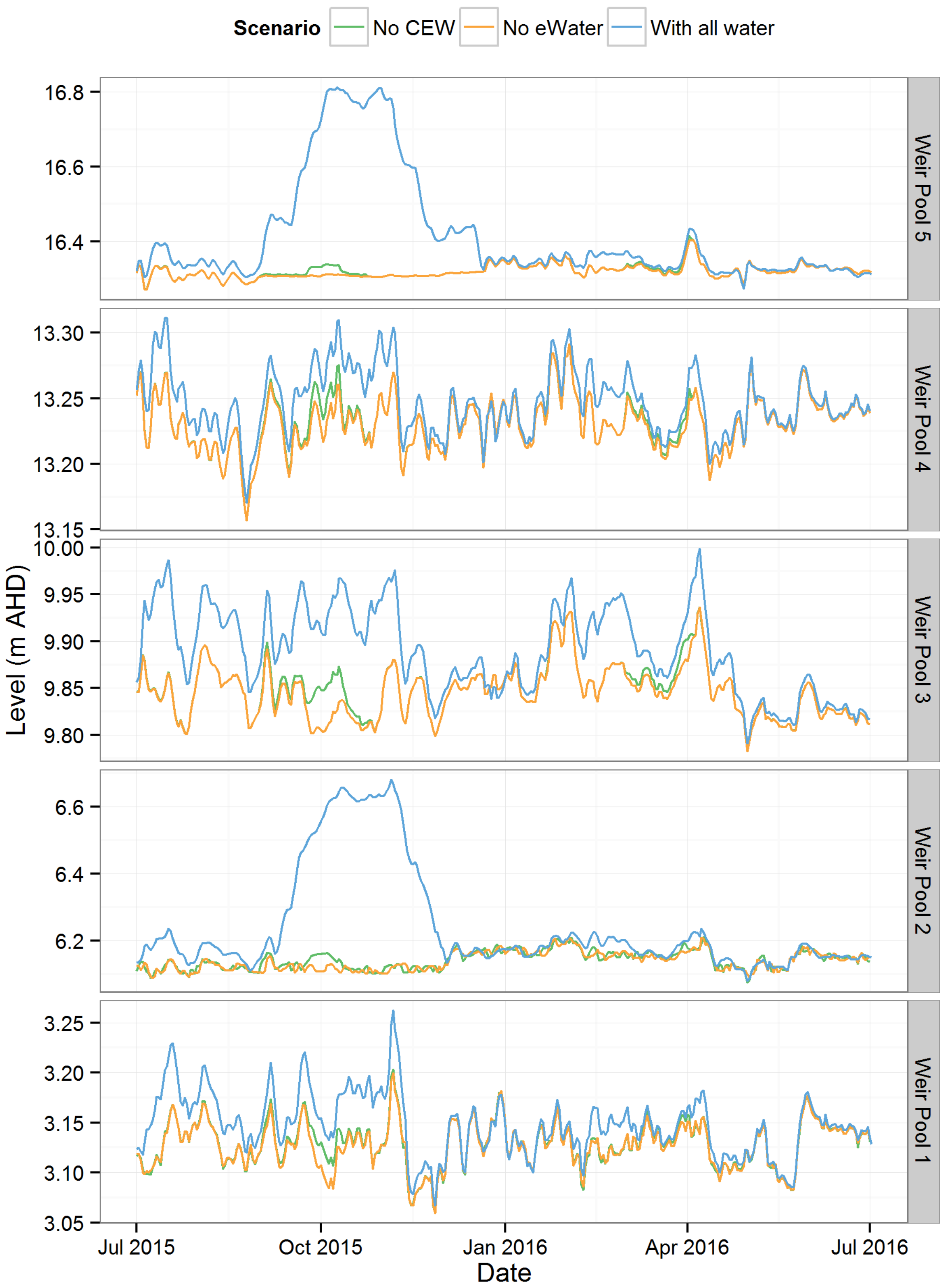

Figure E7. Modelled water level in the midpoint of each weir pool without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

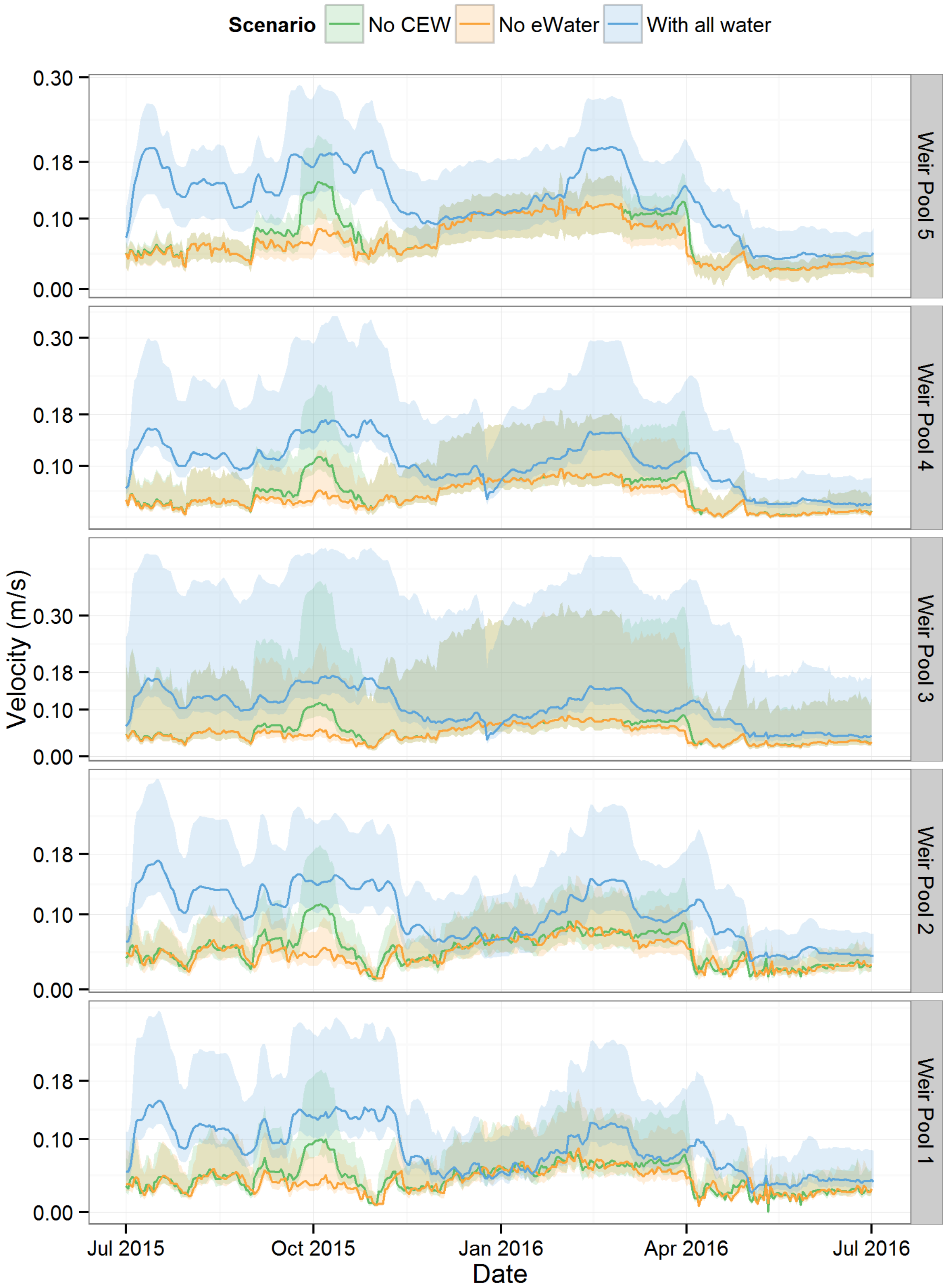

Figure E8. Median modelled velocity in each weir pool (line), with the 10th and 90th percentile 1D cross section velocities the shaded band. Scenarios presented are without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

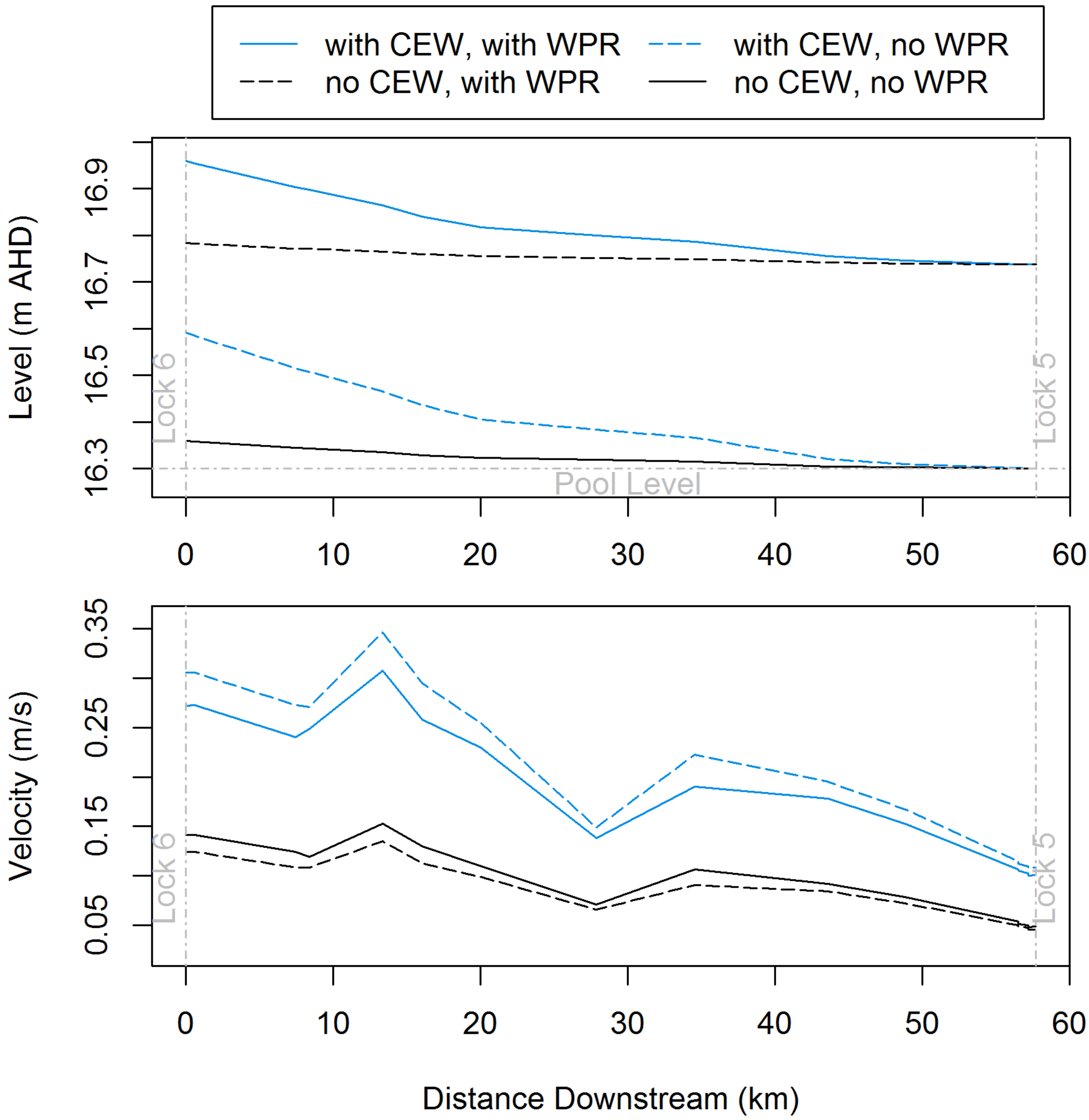

Figure E9. Change in water level and velocity along the weir pool with and without CEW and with and without the weir pool raising for Weir Pool 5 on 13 October 2015. This day represents the largest difference between the scenarios, with the weir pool at the maximum raising, and the largest volume of CEW delivered at the time of the raising.

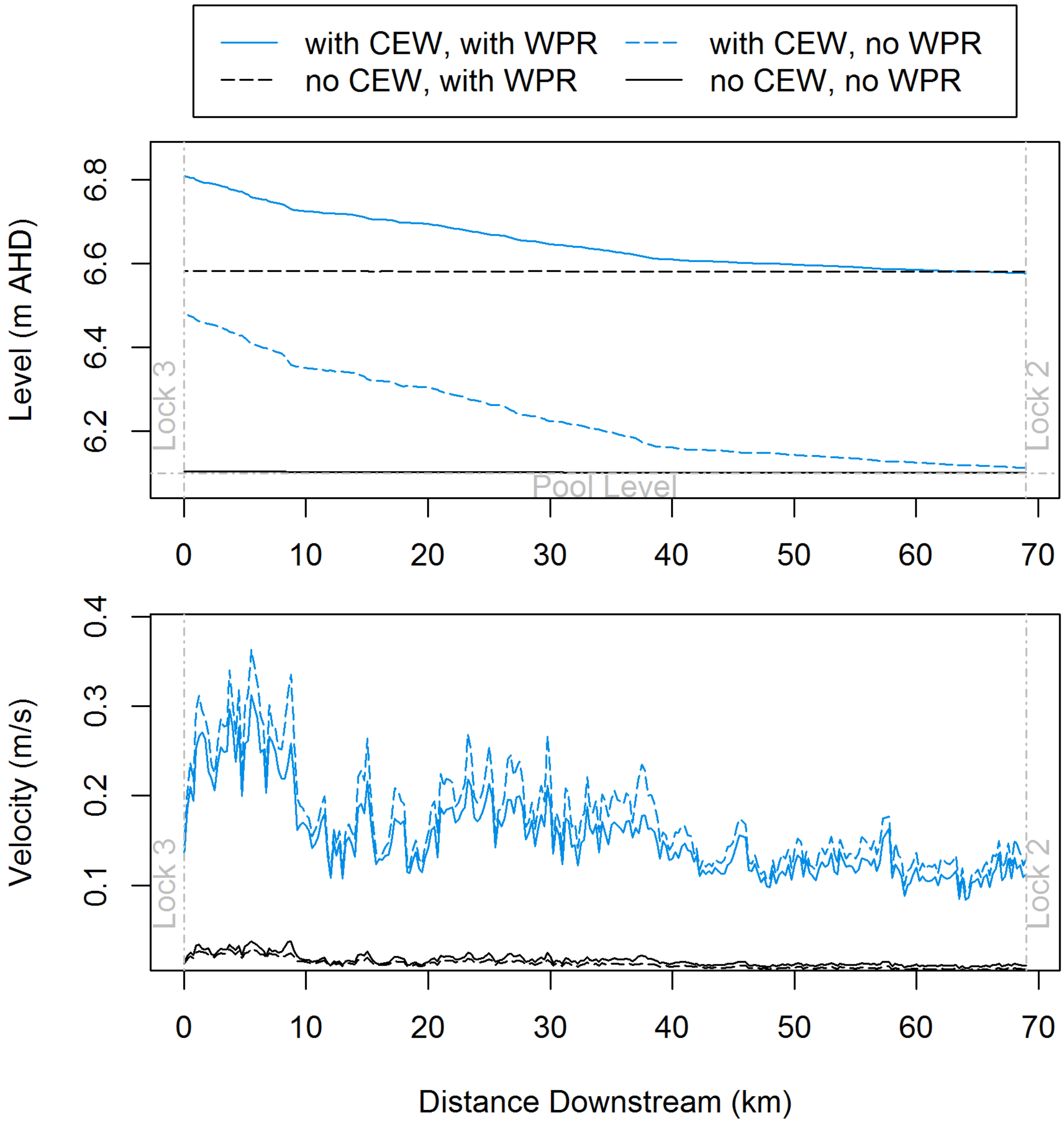

Figure E10. Change in water level and velocity along the weir pool with and without CEW and with and without the weir pool raising for Weir Pool 2 on 31 October 2015. This day represents the largest difference between the scenarios, with the weir pool at the maximum raising, and the largest volume of CEW delivered at the time of the raising.

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 2015/16, 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 between July 2015 and June 2016 (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 (herein 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 01/07/2015 and 30/06/16	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 and 2014/15 (Ye *et al*. 2016a; 2016b). A single model domain was applied spanning Lock 1 to the Southern Ocean, including the Coorong (Figure 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.

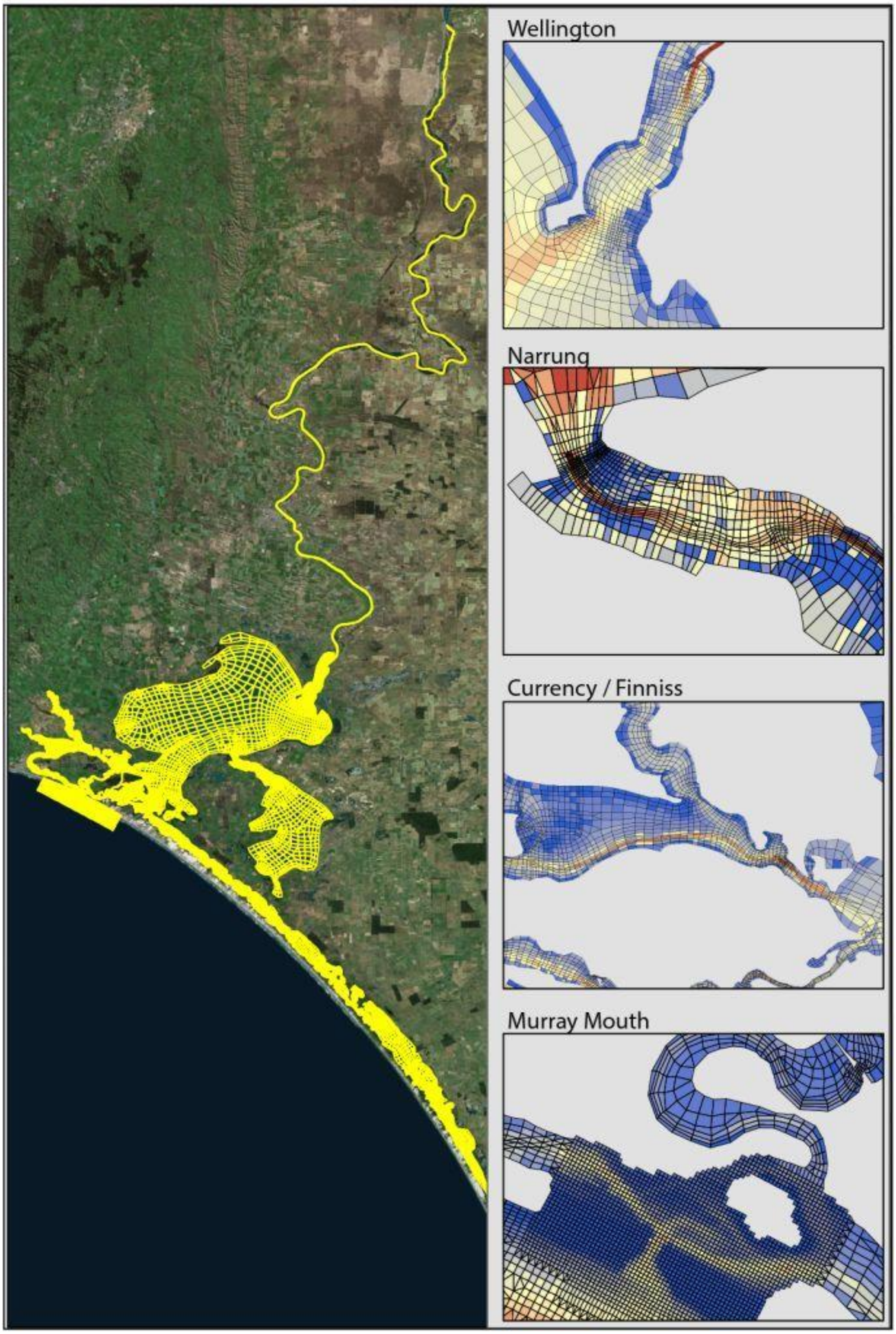

Figure F1. Overview of model domain applied in this study using TUFLOW-FV. Grid provided courtesy of Department of Environment, Water and Natural Resources.

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 2015 and June 2016.

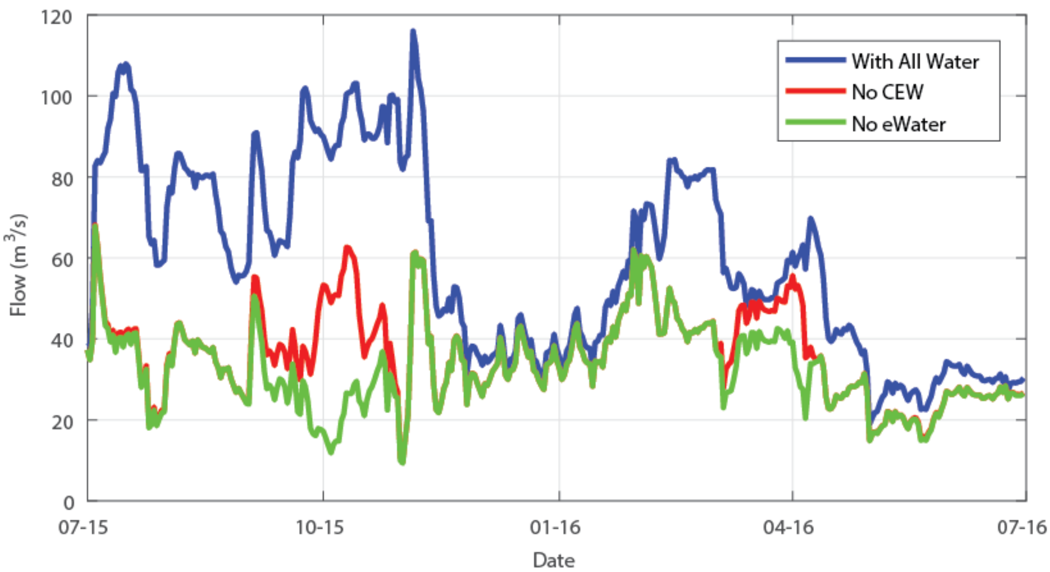

Figure F2. Overview of the three flow scenarios assessed by the model simulations. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater). Flows were applied to the model at the upstream Lock 1 boundary.

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 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.

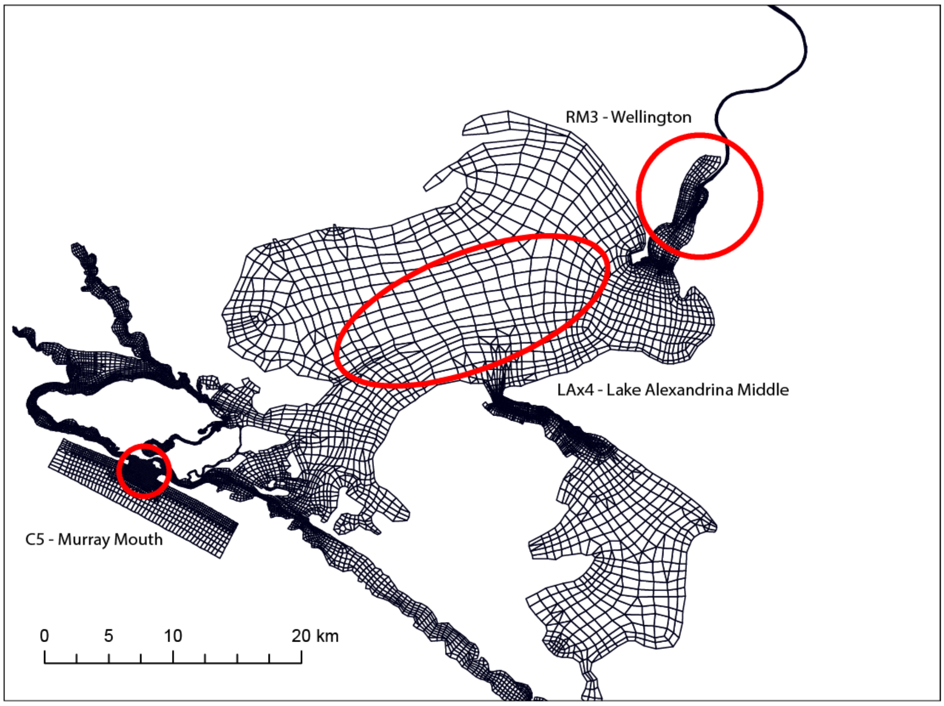

Figure F3. Modelled cells (circled) used for calculating the modelled concentration of sites (Wellington, Lake Alexandrina Middle and Murray Mouth).

The transport of matter was assessed through modelled exports from the Murray River Channel (Wellington), Lower Lakes (Barrages) and 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 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 2.2 Matter Transport. Here, more detailed presentation of data is included (Figures F4–11) than in Section 2.2 Matter Transport, including field collected data used for model validation.

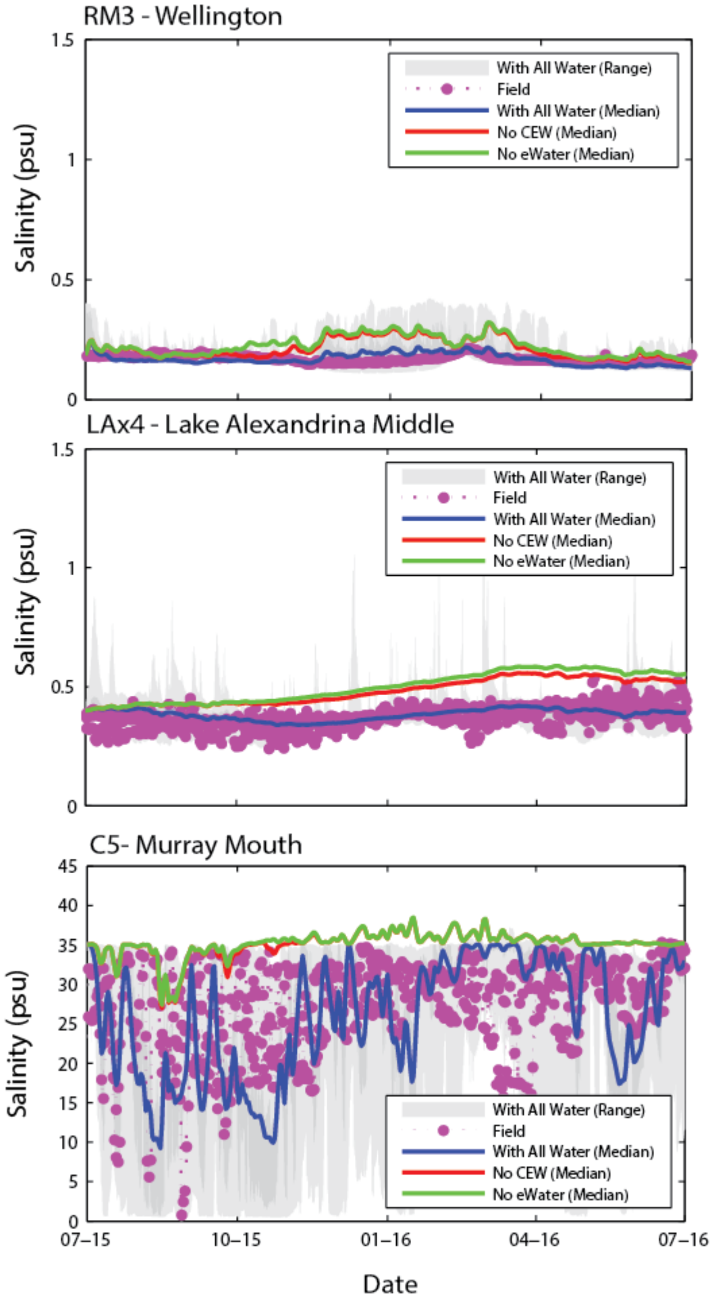

Figure F4. Observed and modelled practical salinity units (PSU) at selected sites. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater). Median values represent that of selected modelled cells surrounding sampling sites.

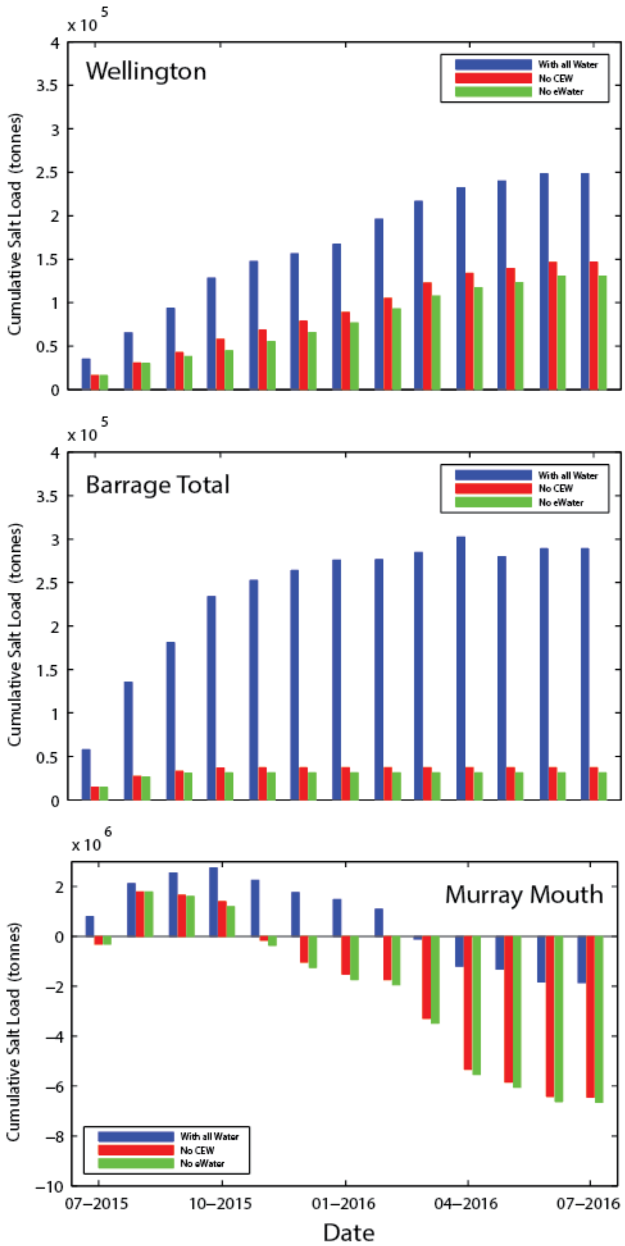

Figure F5. Modelled cumulative salt exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

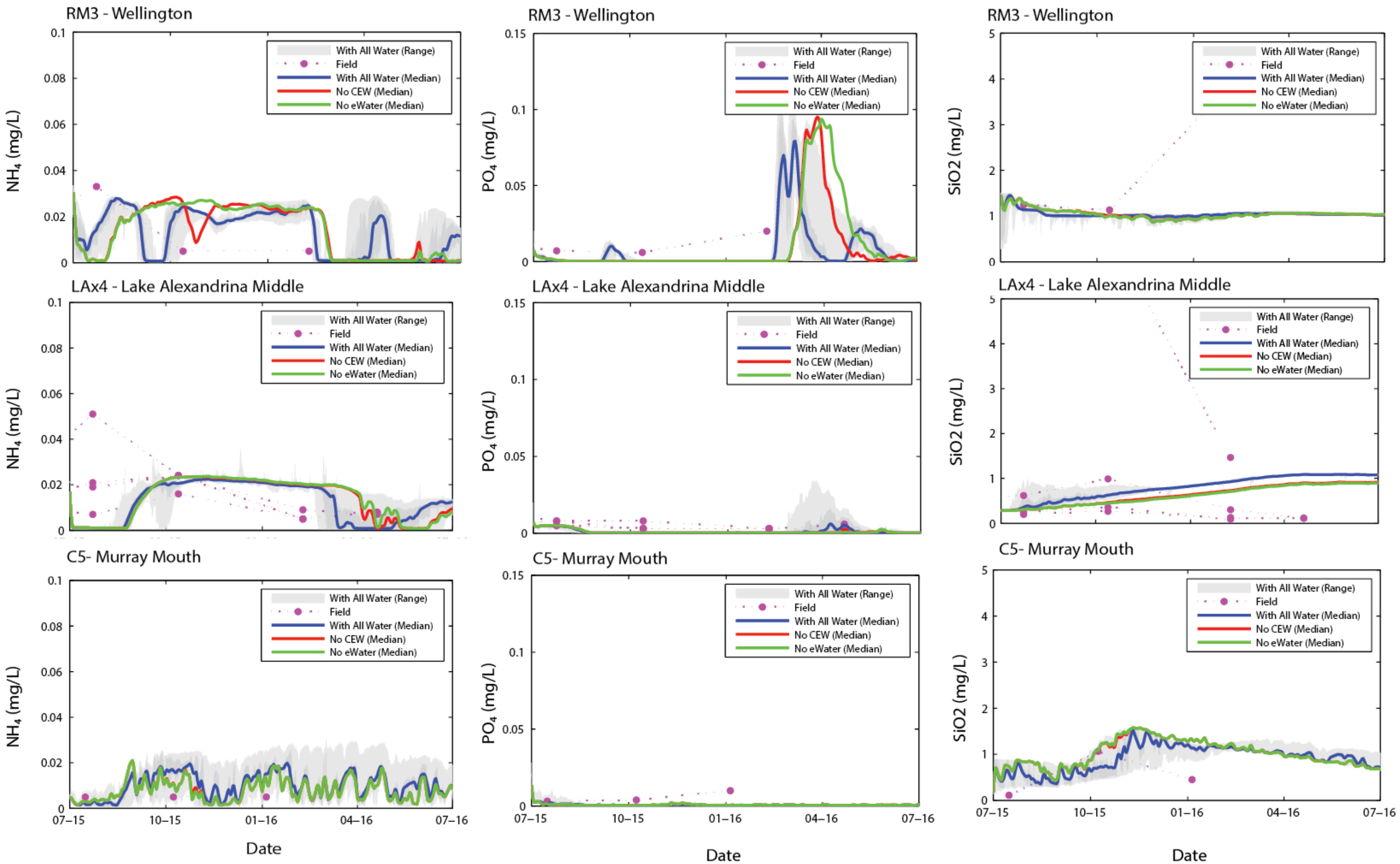

Figure F6. Observed and modelled ammonium (NH4), phosphate (PO4) and silica concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater). Median values represent that of selected modelled cells surrounding sampling sites.

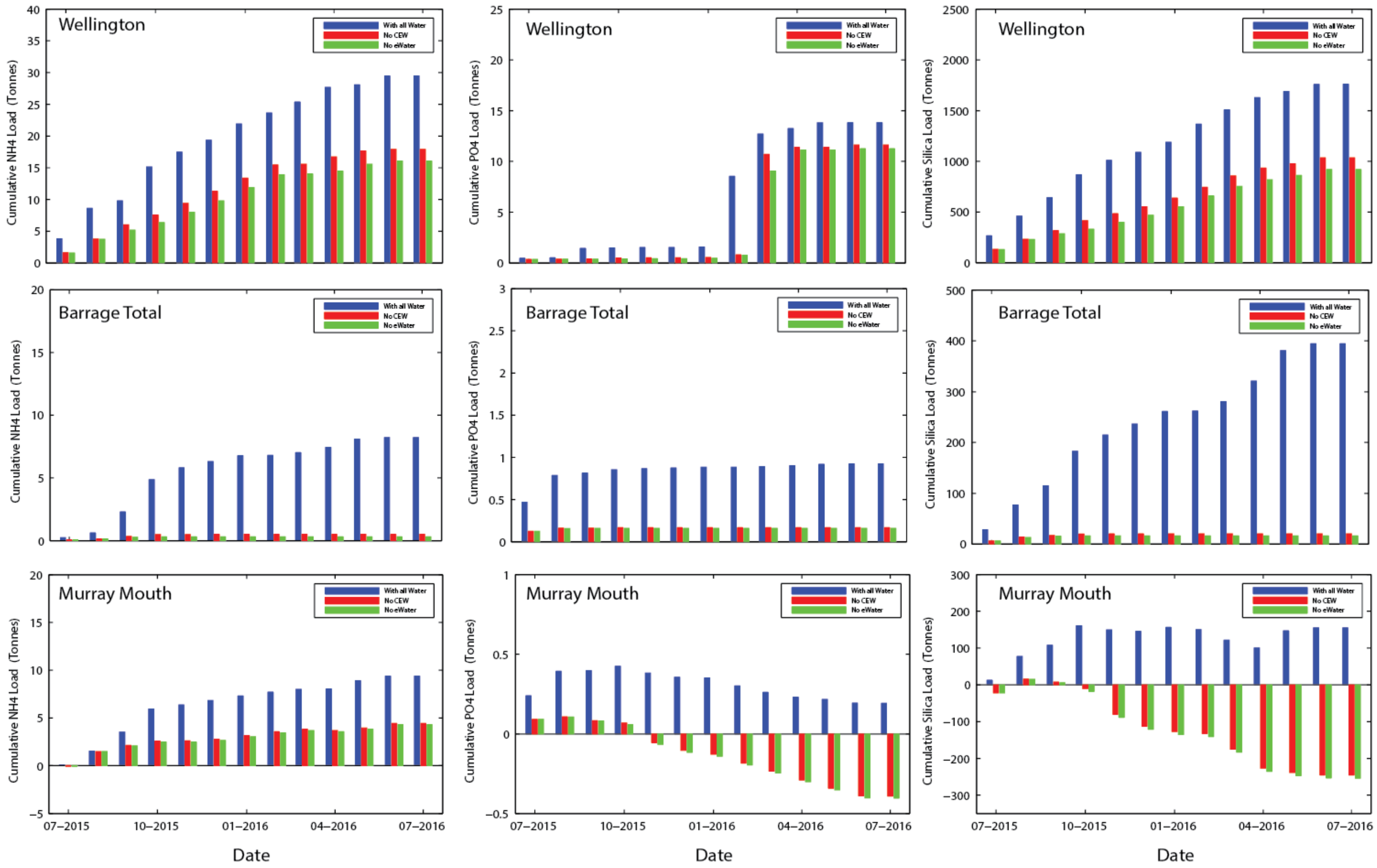

Figure F7. Modelled cumulative ammonium (NH4), phosphate (PO4) and silica exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

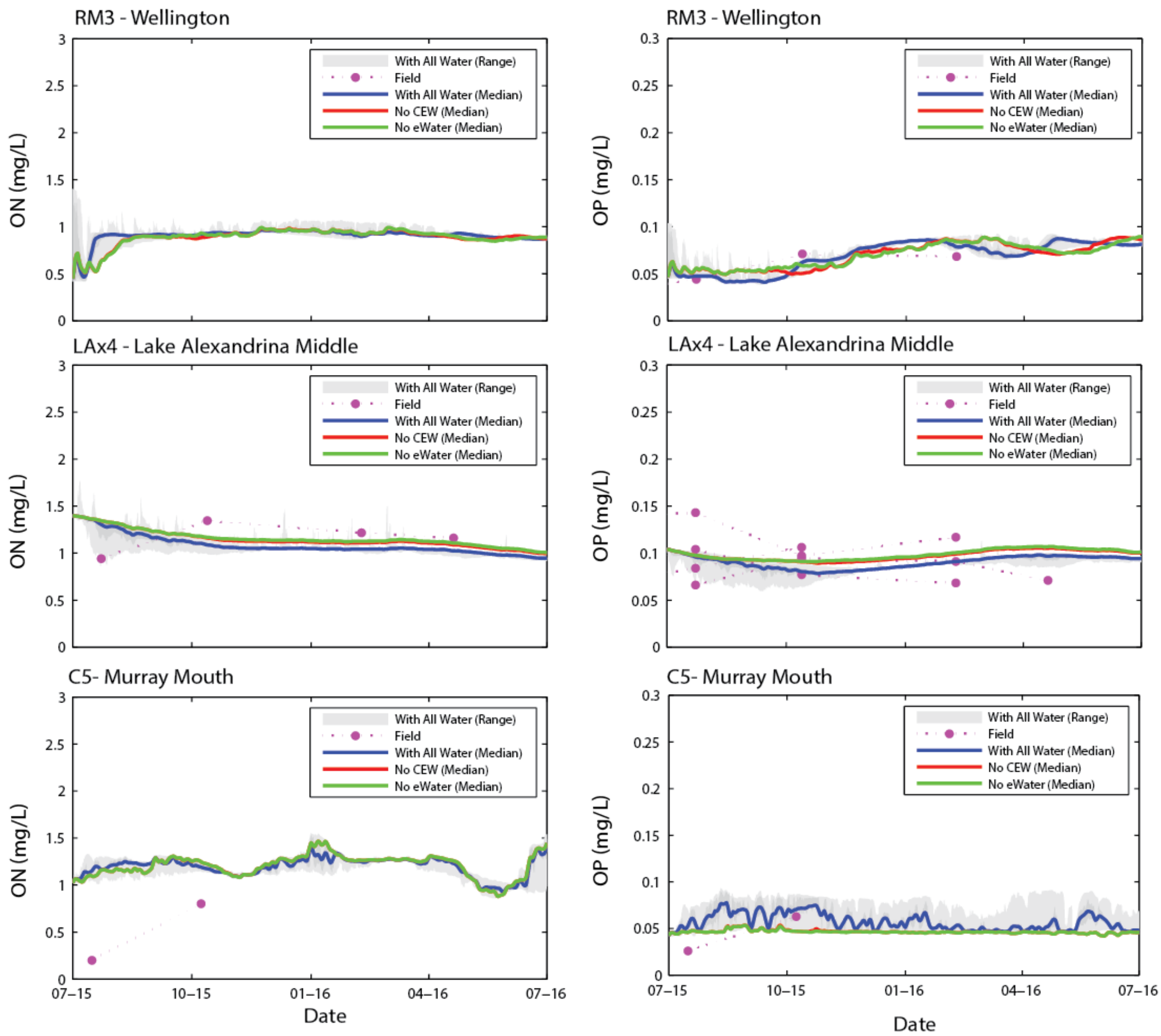

Figure F8. Observed and modelled particulate organic nitrogen (ON) and phosphorus concentrations (OP) at selected sites. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater). Median values represent that of selected modelled cells surrounding sampling sites.

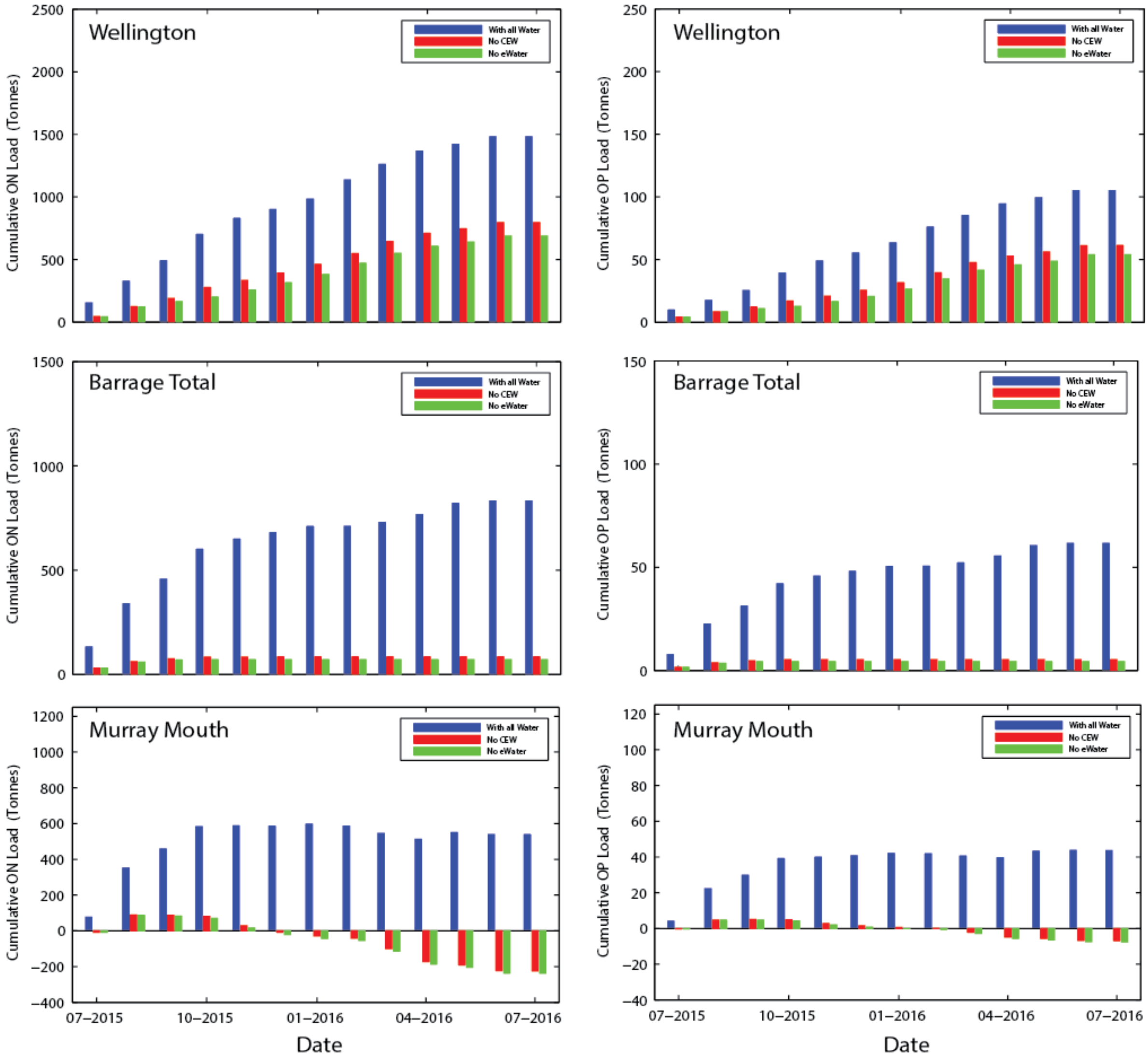

Figure F9. Modelled cumulative particulate organic nitrogen (ON) and phosphorus (OP) exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

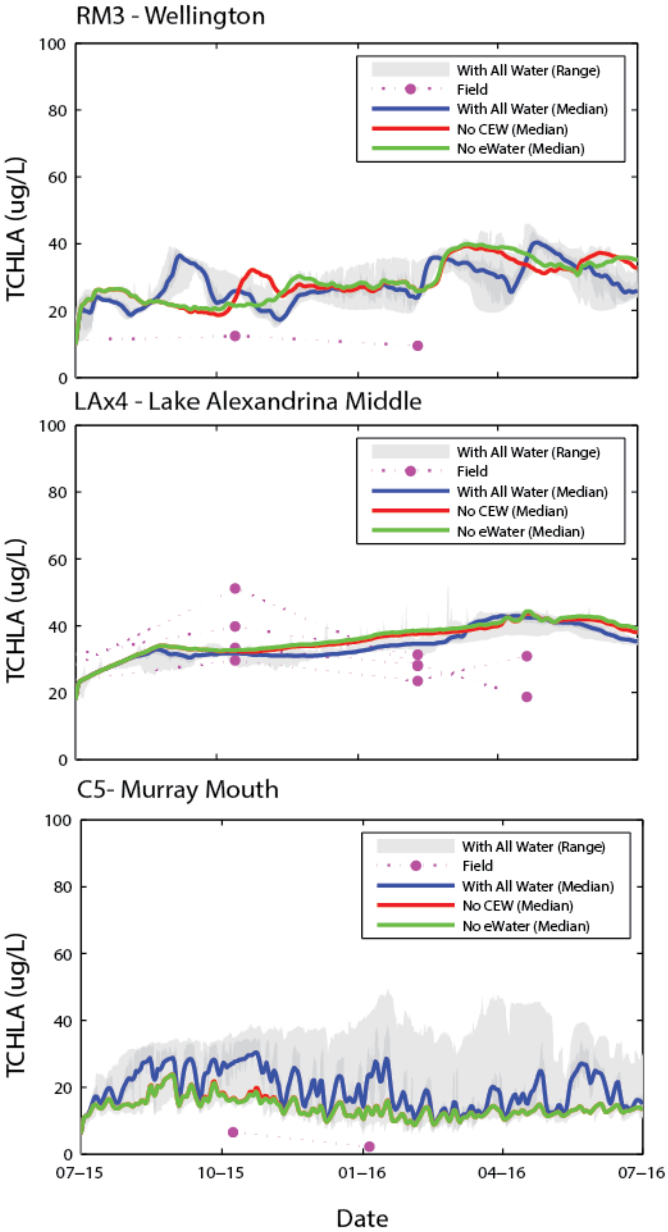

Figure F10. Observed and modelled (with and without environmental watering) chlorophyll a concentrations with and without environmental flows. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater). Median values represent that of selected modelled cells surrounding sampling sites.

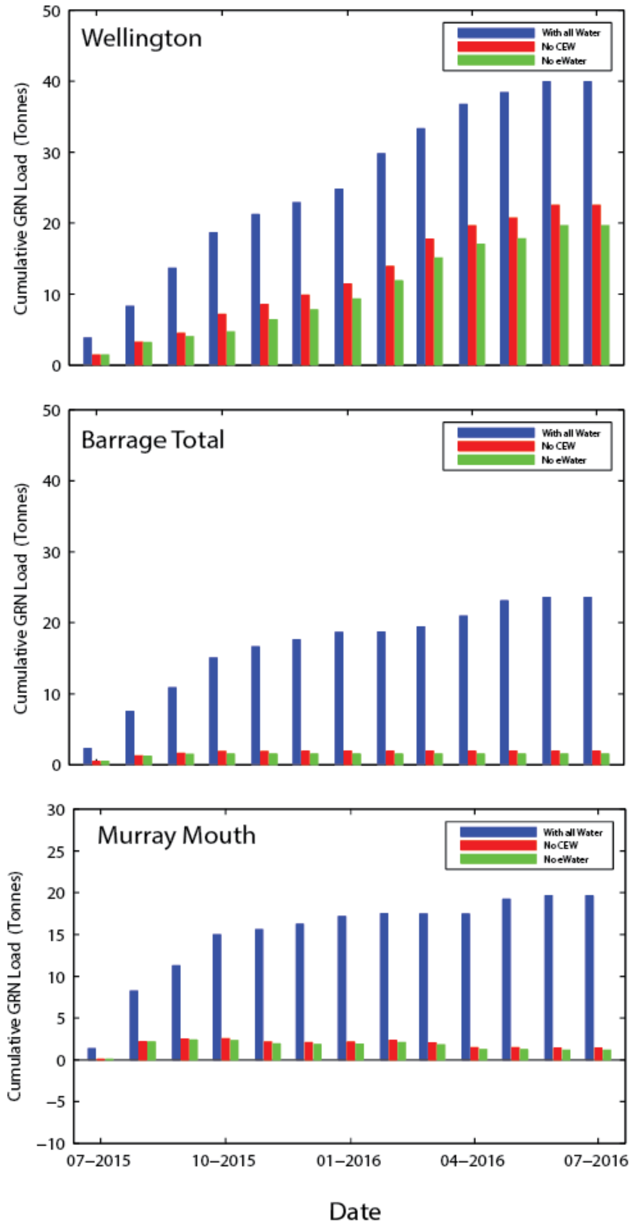

Figure F11. Modelled cumulative phytoplankton (GRN, as measured by carbon) exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

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 overbank 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). In 2014/15, marked changes in microinvertebrate species composition below Lock 1 in the LMR during January were attributed to flooded littoral margins from the raising of upstream weir pools (Ye *et al.* 2016a). Similarly, changes in the microinvertebrate community below Lock 6 in the LMR during November was associated with the regulated inundation of a large floodplain (Ye *et al.* 2016a).

To assess the responses of microinvertebrates in the LMR Selected Area to delivery of Commonwealth environmental water in the LMR Selected Area during 2015/16, 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 Selected Area?
- to the timing of microinvertebrate productivity and presence of key species in relation to diet of golden perch larvae?

Methods

<u>*Sampling sites and procedure*</u>

Microinvertebrate sampling was conducted approximately fortnightly between 6 October 2015 and 21 January 2016 at three sites within the floodplain and gorge geomorphic zones of the LMR Selected Area (Figure 7; 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.

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

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 ca. 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. Triplicate aliquots were taken from the early November series, however time constraints precluded triplicates thereafter, and later counts were based on a single subsample.

To get a better representation of microinvertebrate taxa richness, a surface plankton tow net (37 µm-mesh) was used, which samples a greater volume than the Haney trap. The net was towed mid-channel (by boat) for 3 hauls of a 5-metre line. The catch was decanted through the net to reduce the filtrate volume to approximately 30–40 ml in the PET bottle, then topped up and preserved with 100% ethanol. In the laboratory, the settled filtrate was extracted by a wide-bore glass 10 ml pipette, decanted into a 125 mm gridded Greiner tray, agitated to disperse the contents, and the tray scanned by grid row, with the first 200–250 zooplankters encountered identified and enumerated. Having established proportional composition, the remainder of the tray was then scanned for missed species, generally small numbers or singletons, which were recorded as 'present' by an asterisk in the relevant cell of the spreadsheet.

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

Statistical analyses

All statistical analyses were conducted on Haney trap data only. Temporal variation (between sampling events) in microinvertebrate densities and taxa richness were analysed qualitatively using graphical plots of mean values ± standard error. Temporal variation in microinvertebrate assemblage structure was investigated using a two-factor (i.e. sampling event 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). Significance levels for all comparisons were set to $\alpha = 0.05$. Night samples at sites 5 km below each lock were included in statistical analyses as preliminary analyses in PERMANOVA determined there were no significant differences between night and day-time samples (two-factor (sampling event x lock) PERMANOVA; Pseudo $F_{1,31} = 0.7074$, $p = 0.7795$). 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. Non-metric Multi-Dimensional Scaling (MDS), generated from the same matrices, was used to visualise microinvertebrate assemblages from different sampling events. Groupings of similarity (40 and 60%) from SIMPROF cluster analysis was overlaid on MDS ordinations to show similarity between sampling events. When differences in microinvertebrate assemblages occurred between sampling events for PERMANOVA, Similarity Percentages (SIMPER) analysis was used to determine the zooplankton 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

<u>*Microinvertebrate catch summary and novel taxa*</u>

Over the 2015/16 sampling period, 177 microinvertebrate taxa were discriminated from 192 trap samples from the gorge (below Lock 1) and floodplain (below Lock 6) geomorphic zones of the LMR (vs. 185 during 2014/15). The 2015/16 assemblage included 59 Protista (largely testate rhizopods) (74 in 2014/15), 95 Rotifera [84], 11 Cladocera [13], 7 Copepoda [6], 2 Ostracoda [2] and 5 juvenile macroinvertebrates [6] (gastrotrichs, nematode, oligochaetes, mussel glochidia and chironomids). Notably, 102 taxa (57.6%) of the assemblage were littoral, epiphytic or epibenthic in habit, incursion species in the riverine plankton.

Among the diverse brachionids recorded below Lock 1, *Keratella* cf. *americana* (Figure G2) was recorded 21 October 2015, then in increasing numbers in subsequent samples at densities of 60–120 ind. L^{-1} to the end of sampling period (late January 2016). This rotifer is new to the continent, has previously been recorded from North and South America (Amazonia), at temperatures to 31 °C, i.e. it is a warm stenotherm. Also new for the continent, and also initially from Lock 1, another warm-water species, *Hexarthra braziliensis* was noted in early November, then in increasing numbers through to the end of January. It was recorded at Lock 6 on 20 January. It co-occurred with a smaller congener, *H. intermedia.* The marked size difference of the congeners suggests that they are grazing on a different component of the available algae (they are herbivorous). *H. braziliensis* was described from tropical South America.

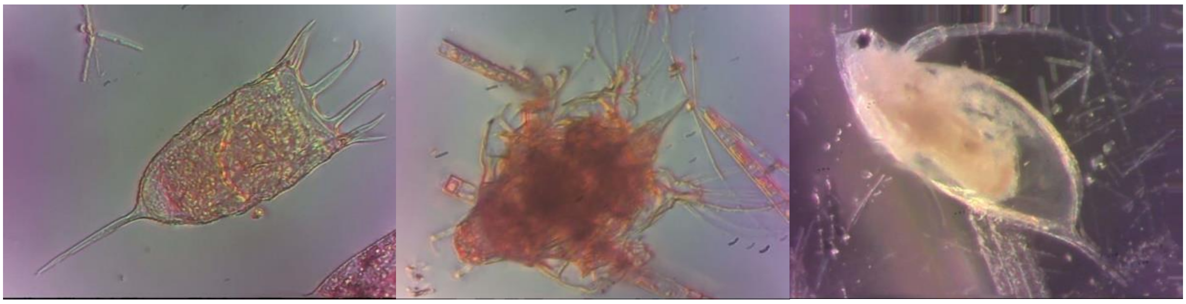

Figure G2. Novel microinvertebrate taxa sampled in the LMR Selected Area. *Keratella cf americana* (left, 178 µm) from Lock 1 during 21 January 2016, and *Hexarthra braziliensis* (centre) from Lock 6 on 20 January 2016, and (right) *Daphnia galeata* (1.1 mm) from a night net tow in Lock 6A on 6 October 2015.

Another first record from the continent was the daphniid *Daphnia galeata* (Figure G2), a Eurasian species. It was recorded only once, in a night net tow from Lock 6A (7 km below Lock 6), below the Chowilla junction, and may have come from a shallow floodplain source, or in the main channel. This species was not recorded from the Lock 6 site that was above the Chowilla junction.

The origin of these two Neotropical taxa must be speculative. Given the intensity of plankton sampling In the LMR over >30 years (Shiel *et al*. 1982, Furst *et al.* 2014) it is likely they would have been recognised. An upstream origin is likely on this occasion. The rotifers may well be Gondwanan, of restricted distribution, uncollected/unrecognised until now. The *Daphnia* is presently regarded as an invasive species, and is spreading in North America after introduction to the Great Lakes (Benzie 2005). Importation with tropical fish and subsequent release is possible (e.g. Duggan 2010).

<u>*Densities and taxa richness*</u>

At sites below Lock 6, microinvertebrate density gradually increased throughout the sampling period (Figure G3). Density was lowest during mid-October 2015 (mean ± S.E. = 312.5 ± 9.2 ind.L^{-1}) and greatest in late January 2016 (2227.3 ± 179.0 ind.L^{-1}). At sites downstream of Lock 1, densities peaked early, in mid-October 2015 (2,320.0 ±

116.0 ind.L⁻¹), before sharply falling to 115.8 ± 8.7 ind.L⁻¹ in mid-November (Figure G3). Densities below Lock 1 increased after mid-December to 2,248.0 ± 553.5 ind.L⁻¹ by late January 2016.

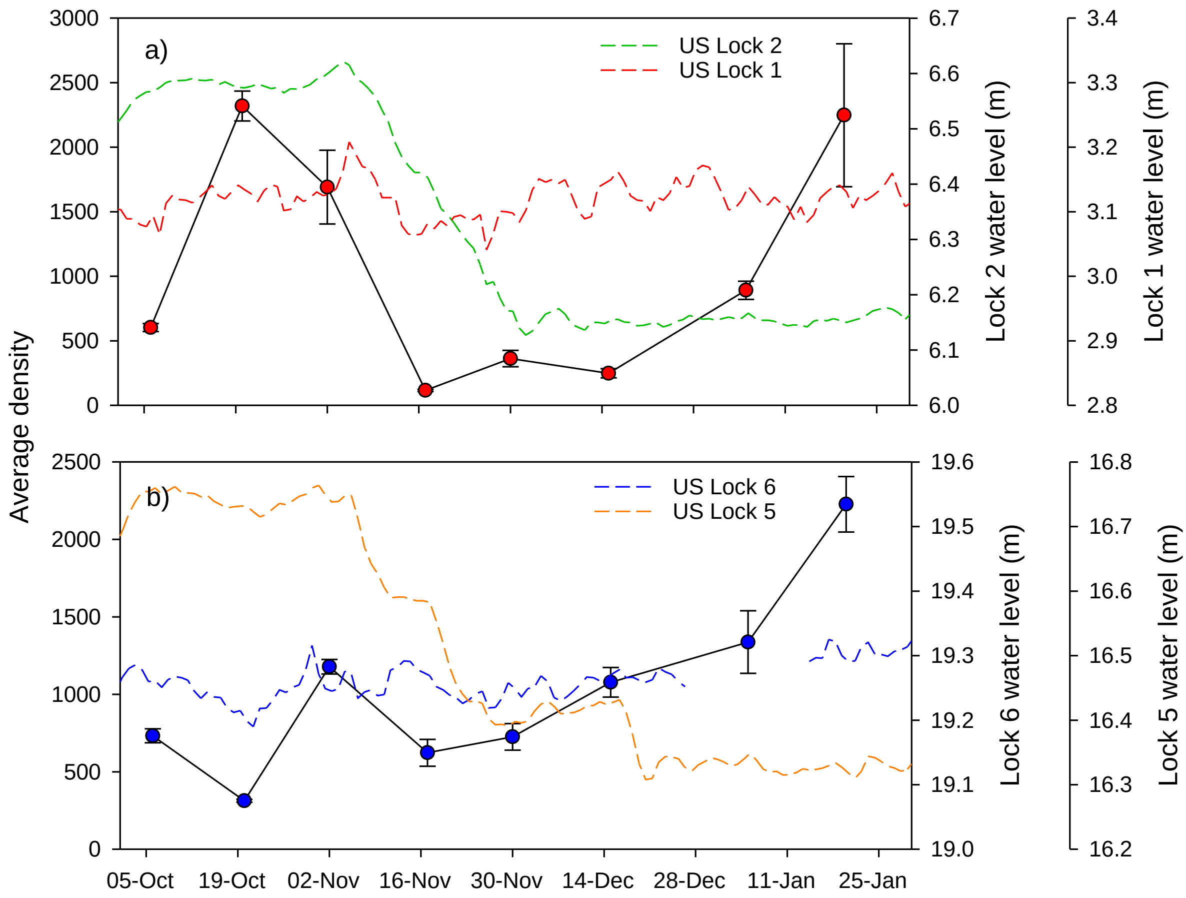

Figure G3. Average microinvertebrate density (ind.L⁻¹ ±S.E.) at sites below a) Lock 1 (red circles) and b) Lock 6 (blue circles) in each sampling event. Dotted lines show water level (m AHD) variations from weir pool raising within Weir Pools 2 (upstream (US) Lock 2) and 5 (US Lock 5). Water levels are also presented for locks that are immediately upstream of the sampling sites.

At sites below Lock 6, microinvertebrate taxa richness followed a similar trend to density below Lock 6, and gradually increased throughout the sampling period (Figure G4). Taxa richness was lowest during mid-October 2015 (mean ± S.E. = 24.3 ± 2.7 spp.) and greatest in late January 2016 (50.3 ± 3.4 spp.). During the other sampling months, taxa richness was relatively similar (31–40 spp.). Below Lock 1, taxa richness sharply rose from 36.3 ± 3.3 spp. in early October 2015 to 52.8 ± 0.8 spp. in early November, before dramatically falling to ~18–24 spp. from mid-November to mid-December 2015 (Figure G4). Taxa richness then increased throughout January 2016.

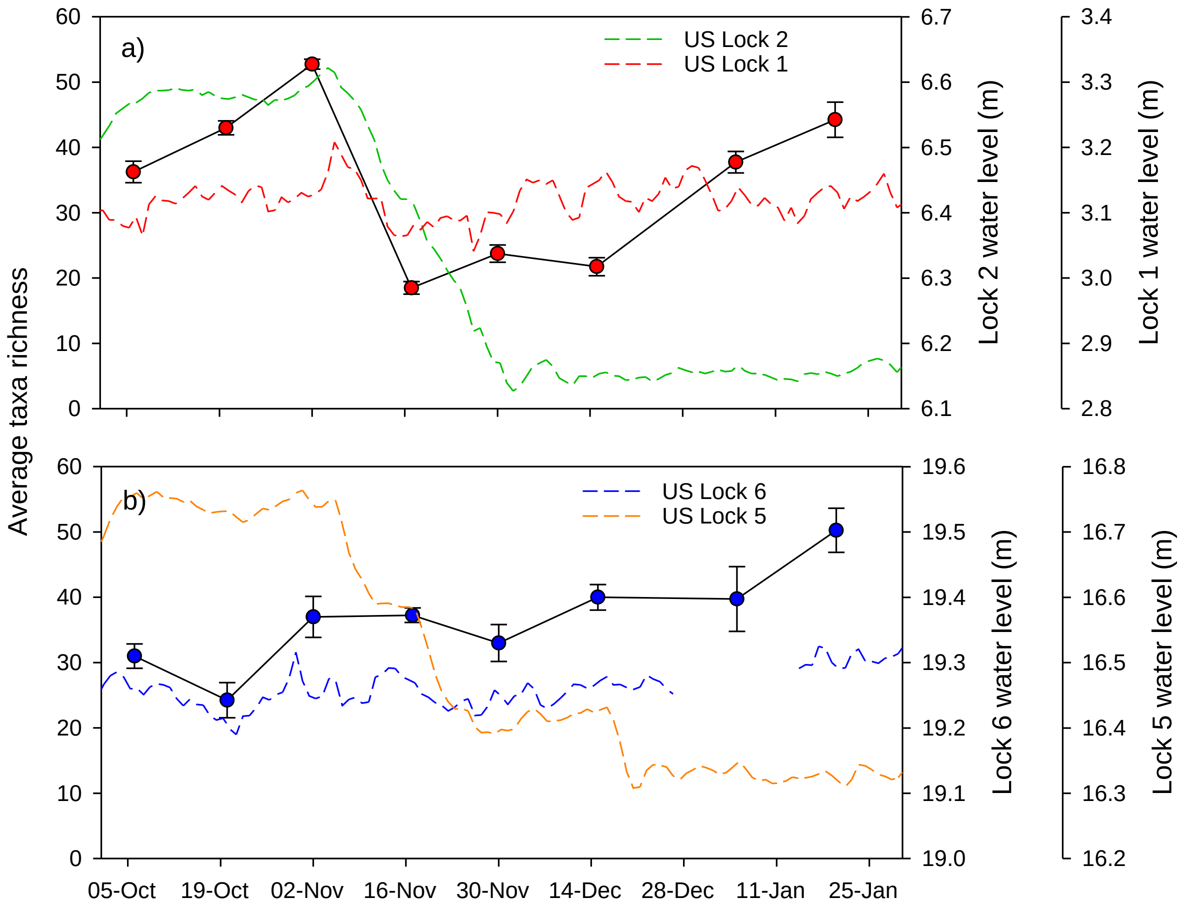

Figure G4. Average microinvertebrate taxa richness (±S.E.) at sites below a) Lock 1 (red circles) and b) Lock 6 (blue circles) in each sampling event. Dotted lines show water level (m AHD) variations from weir pool raising within Weir Pools 2 (upstream (US) Lock 2) and 5 (US Lock 5). Water levels are also presented for locks that are immediately upstream of the sampling sites.

Microinvertebrate assemblage structure

Microinvertebrate assemblages appeared to separate well based on sampling event, with individual events forming relatively tight groups and a temporal sequence noticeable across the MDS ordination (Figure G5). Within each of the sampling events, within-lock assemblage similarity was high, such that individual locks tended to group together (Figure G5). Separation of locks were less apparent in the later sampling events (early and mid-January 2016) (Figure G5). The microinvertebrate assemblage below Lock 1 during mid-November 2015, which was characterised by low density and taxa richness (Figures G3 and G4), showed a high degree of separation from other sampling events (Figure G5).

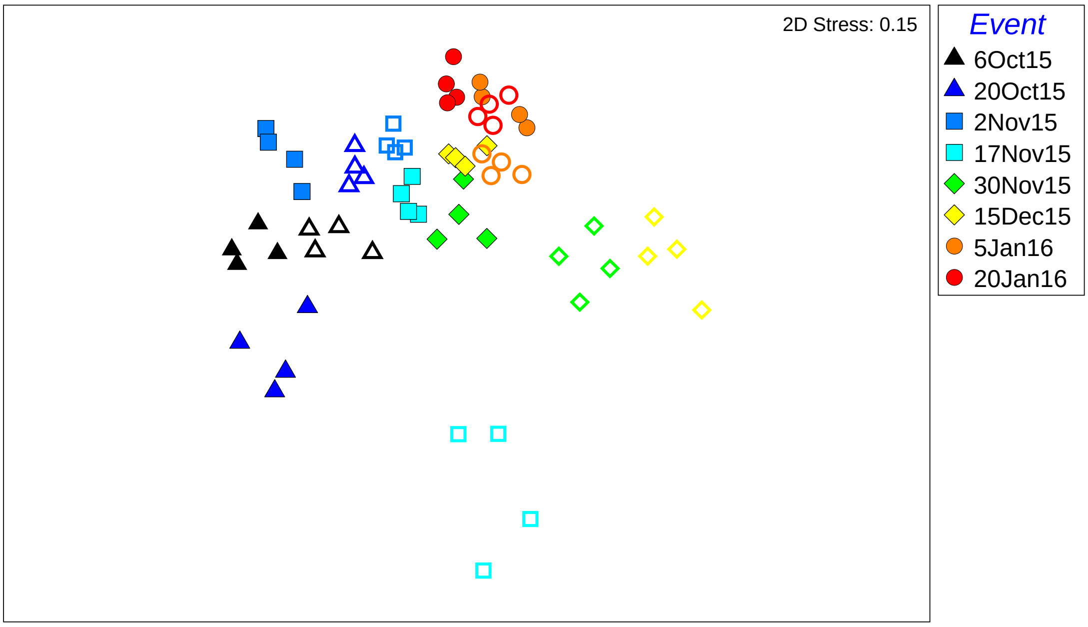

Figure G5. MDS ordination of microinvertebrate assemblage data (log transformed) for sites below Lock 6 (closed symbols) and Lock 1 (open symbols).

A significant interaction was detected between locks and sampling events (two-factor PERMANOVA; Pseudo-$F_{7,63}$ = 5.9127, p < 0.0001), suggesting inconsistent spatio-temporal variation among sampling events between the two locks. Pairwise tests were conducted separately for each lock to examine differences over time (i.e. between sampling events) (see Tables G6 and G7).

Lock 6

Within sites below Lock 6, all sampling events were significantly different from one another (Table G2 and Figure G6). Generally, separation between groups was high, with the exception of the mid-December and early January events, which were not as well separated, although still significant (Figure G6).

Table G2. Within sites below Lock 6 pair-wise results of microinvertebrate log(x+1) abundance data amongst sampling events, showing p-values. * = groups significantly different.

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
6-Oct							
20-Oct	0.0294*						
2-Nov	0.0287*	0.0299*					
17-Nov	0.0279*	0.0273*	0.0312*				
30-Nov	0.0312*	0.0304*	0.0289*	0.0282*			
15-Dec	0.0303*	0.0315*	0.0318*	0.0291*	0.0312*		
5-Jan	0.0316*	0.0285*	0.0288*	0.0268*	0.027*	0.0287*	
20-Jan	0.0288*	0.0263*	0.0270*	0.0273*	0.0283*	0.0276*	0.0287*

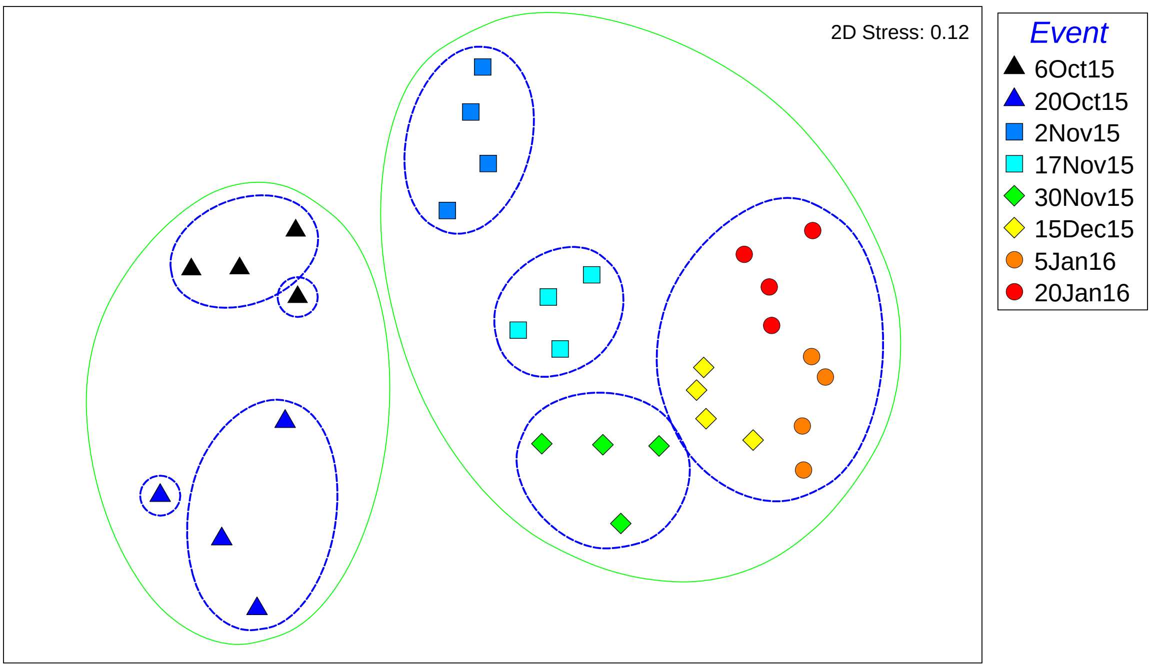

Figure G6. MDS ordination of microinvertebrate assemblage data (log transformed) from sites below Lock 6, with samples identified by sampling event. Samples are grouped at a Bray-Curtis similarity of 40% (green circles) and 60% (blue circles) (SIMPROF).

SIMPER analysis was used to determine which taxa were driving the apparent differences between sampling events (all significant). Results are provided below in Table G3. Dissimilarly between groups was primarily driven by lower abundances of rotifers *Trichocerca* sp. c and *Polyarthra* sp. a and sp. b during the October sampling events, higher abundance of the rotifer *Brachionus* [*angularis*] *bidens* during the mid- and late November sampling events, and higher abundance of the rotifer *Keratella lenzi* in the last two January sampling events (Table G3).

Table G3. Microinvertebrate taxa responsible for the dissimilarity between sampling events for sites below Lock 6 (SIMPER). Bold taxa were more abundant during the sampling event in the respective column, while unbolded taxa were those more abundant during the sampling event in the respective row. Average dissimilarity (%) between sampling events is provided for each comparison.

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
6-Oct							
20-Oct	52.65% **Indet. glob. ciliate [sm], *Filinia pejleri*, *Synchaeta* sp. c [tiny], *Polyarthra* sp. a [sm], *Trichocerca similis*** and *Conochilus* sp. a [sm].						
2-Nov	53.40% ***Filinia pejleri***, *Trichocerca* sp. c [long toe, med], *Brachionus calyciflorus amphiceros*, *Cephalodella catellina*, *Conochilus* sp. b [lg], **indet. glob. ciliate [sm]** and ***Bosmina meridionalis***.	56.61% *Trichocerca* sp. c [long toe, med], *Cephalodella catellina*, *Brachionus calyciflorus amphiceros*, *Synchaeta* sp. b [sm., cf. oblonga], *Trichocerca similis* and *Conochilus* sp. b [lg].					

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
17-Nov	50.65% *Brachionus* [*angularis*] *bidens*, ***Filinia pejleri***, *Keratella tropica*, *Polyarthra* sp. b [lg], *Hexarthra* sp. [?spp.], *Difflugia gramen*, ***Bosmina meridionalis*** and **indet. glob. ciliate [sm]**.	59.14% *Brachionus* [*angularis*] *bidens*, *Polyarthra* sp. a [sm], *Trichocerca* sp. c [long toe, med], *Keratella tropica*, indet. glob. ciliate [sm], *Polyarthra* sp. b [lg] and *Hexarthra* sp. [?spp.].	45.62% *Brachionus* [*angularis*] *bidens*, *Polyarthra* sp. a [sm], ***Brachionus calyciflorus amphiceros***, *Keratella tropica*, ***Cephalodella catellina***, *Hexarthra* sp. [?spp.] and ***Codonaria* sp.**				
30-Nov	56.67% ***Filinia pejleri***, *Brachionus* [*angularis*] *bidens*, **indet. glob. ciliate [sm]**, *Polyarthra* sp. b [lg], *Trichocerca* sp. c [long toe, med], *Keratella tropica* and ***Synchaeta* sp. c [tiny]**.	59.16% *Trichocerca* sp. c [long toe, med], *Brachionus* [*angularis*] *bidens*, *Polyarthra* sp. a [sm], *Polyarthra* sp. b [lg], *Keratella tropica* and *Filinia terminalis*.	56.06% **Synchaeta sp. b [sm., cf. oblonga]**, *Brachionus* [*angularis*] *bidens*, ***Cephalodella catellina***, *Polyarthra* sp. a [sm], ***Conochilus* sp. b [lg]**, *Keratella tropica* and ***Synchaeta pectinata* [med-lg]**.	43.35% ***Synchaeta* sp. b [sm., cf. oblonga]**, ***Synchaeta* sp. c [tiny]**, *Filinia terminalis*, ***Hexarthra* sp. [?spp.]**, cyclopoid nauplii, ***Trichocerca similis***, **indet. glob. ciliate [sm]**, ***Trichocerca* sp. d [gracile, med toe(s)]** and ***Asplanchna priodonta***.			

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
15-Dec	62.05% *Filinia terminalis*, ***Filinia pejleri***, *Hexarthra* sp. [?spp.], **indet. glob. ciliate [sm]**, *Keratella tropica*, *Keratella javana*, *Polyarthra* sp. b [lg] and *Polyarthra* sp. a [sm].	62.81% *Polyarthra* sp. a [sm], *Trichocerca* sp. c [long toe, med], *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Keratella tropica* and *Keratella javana*.	55.87% *Polyarthra* sp. a [sm], *Filinia terminalis*, *Keratella tropica*, ***Cephalodella catellina***, ***Synchaeta pectinata* [med-lg]**, *Hexarthra* sp. [?spp.] and ***Brachionus calyciflorus amphiceros***.	44.52% *Filinia terminalis*, *Keratella javana*, *Keratella lenzi*, cyclopoid nauplii, ***Synchaeta* sp. c [tiny]**, ***Trichocerca similis***, **indet. glob. ciliate [sm]**, ***Asplanchna priodonta*** and ***Synchaeta pectinata* [med-lg]**.	39.33% *Hexarthra* sp. [?spp.], *Keratella javana*, *Conochilus* sp. b [lg], *Keratella lenzi*, ***Filinia terminalis***, *Trichocerca* sp. d [gracile, med toe(s)], *Filinia australiensis*, ***Synchaeta pectinata* [med-lg]** and *Trichocerca similis grandis*.		
5-Jan	67.63% *Keratella lenzi*, **indet. glob. ciliate [sm]**, ***Filinia pejleri***, *Filinia terminalis*, *Keratella javana*, *Trichocerca* sp. c [long toe, med], *Hexarthra* sp. [?spp. and *Keratella tropica*.	69.74% *Trichocerca* sp. c [long toe, med], *Keratella lenzi*, *Polyarthra* sp. a [sm], *Filinia terminalis*, *Keratella javana* and *Hexarthra* sp. [?spp.].	60.18% *Keratella lenzi*, ***Synchaeta* sp. b [sm., cf. oblonga]**, *Filinia terminalis*, *Polyarthra* sp. a [sm], ***Cephalodella catellina***, *Keratella javana* and ***Brachionus calyciflorus amphiceros***.	48.64% *Keratella lenzi*, *Filinia terminalis*, *Keratella javana*, ***Synchaeta* sp. b [sm., cf. oblonga]**, ***Synchaeta* sp. c [tiny]**, **indet. glob. ciliate [sm]** and ***Trichocerca similis***.	46.63% *Keratella lenzi*, *Keratella javana*, *Trichocerca* sp. d [gracile, med toe(s)], *Hexarthra* sp. [?spp.], ***Trichocerca pusilla***, *Filinia opoliensis*, indet. glob. ciliate [lg] and ***Filinia terminalis***.	35.81% *Keratella lenzi*, ***Trichocerca pusilla***, **cyclopoid nauplii**, **cyclopoid copepodite**, ***Filinia australiensis***, *Trichocerca* sp. d [gracile, med toe(s)], ***Stenosemella* sp., indet. glob. ciliate [sm]**, *Brachionus falcatus*, indet. glob. ciliate [lg] and ***Trichocerca similis grandis***.	

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
20-Jan	63.82% *Keratella lenzi*, *Filinia terminalis*, *Keratella tropica*, **indet. glob. ciliate [sm], *Filinia pejleri***, *Trichocerca* sp. d [gracile, med toe(s)], *Difflugia gramen* and ***Polyarthra* sp. a [sm]**.	68.25% *Keratella lenzi*, *Polyarthra* sp. a [sm], *Filinia terminalis*, *Trichocerca* sp. c [long toe, med], *Keratella tropica* and *Trichocerca* sp. d [gracile, med toe(s)].	55.80% *Keratella lenzi*, *Filinia terminalis*, *Polyarthra* sp. a [sm], *Keratella tropica*, ***Synchaeta* sp. b [sm., cf. *oblonga*]**, ***Brachionus calyciflorus amphiceros*** and *Difflugia gramen*.	48.24% *Keratella lenzi*, *Filinia terminalis*, *Brachionus* [*angularis*] *bidens*, *Synchaeta* sp. c [tiny], *Anuraeopsis coelata*, *Hexarthra braziliensis*, indet. glob. ciliate [sm] and *Codonaria* sp.	48.39% *Keratella lenzi*, *Trichocerca* sp. d [gracile, med toe(s)], ***Brachionus* [*angularis*] *bidens***, ***Filinia terminalis***, *Anuraeopsis coelata*, *Hexarthra braziliensis*, *Trichocerca similis* and *Asplanchna priodonta*.	38.77% *Keratella lenzi*, *Asplanchna priodonta*, *Trichocerca similis*, *Anuraeopsis coelata*, *Hexarthra braziliensis*, ***Hexarthra* sp. [?spp.]**, *Keratella* cf. *americana*, ***Brachionus* [*angularis*] *bidens***, *Synchaeta pectinata* [med-lg] and ***Keratella javana***.	41.31% *Trichocerca similis*, *Asplanchna priodonta*, *Hexarthra braziliensis*, ***Keratella javana***, ***Brachionus* [*angularis*] *bidens***, ***Hexarthra* sp. [?spp.]**, *Difflugia* cf. *fallax*, *Filinia australiensis*, *Anuraeopsis coelata*, *Filinia longiseta* and *Keratella* cf. *americana*.

All environmental predictor variables for the microinvertebrate assemblage structure below Lock 6 were significant (Table G4). However, the best combination of environmental predictor variables was pH, water temperature and river flow, which collectively explained 35.4% of the variation (Table G4). River flow was the best environmental variable to explain the horizontal separation of the data cloud, while pH and water temperature best explained the vertical separation (Figure G7).

Table G4. DistLM sequential 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.	Cumul.
pH	6.3253	0.0001*	0.15197	0.15197
Water temperature	4.8226	0.0001*	0.10195	0.25392
Mean QSA flow	5.8548	0.0001*	0.10051	0.35443
Dissolved oxygen	3.2231	0.0029*	0.097014	0.45144
Electrical conductivity	2.7532	0.0095*	0.078294	0.52974
Turbidity	2.0159	0.0218*	0.04107	0.57081

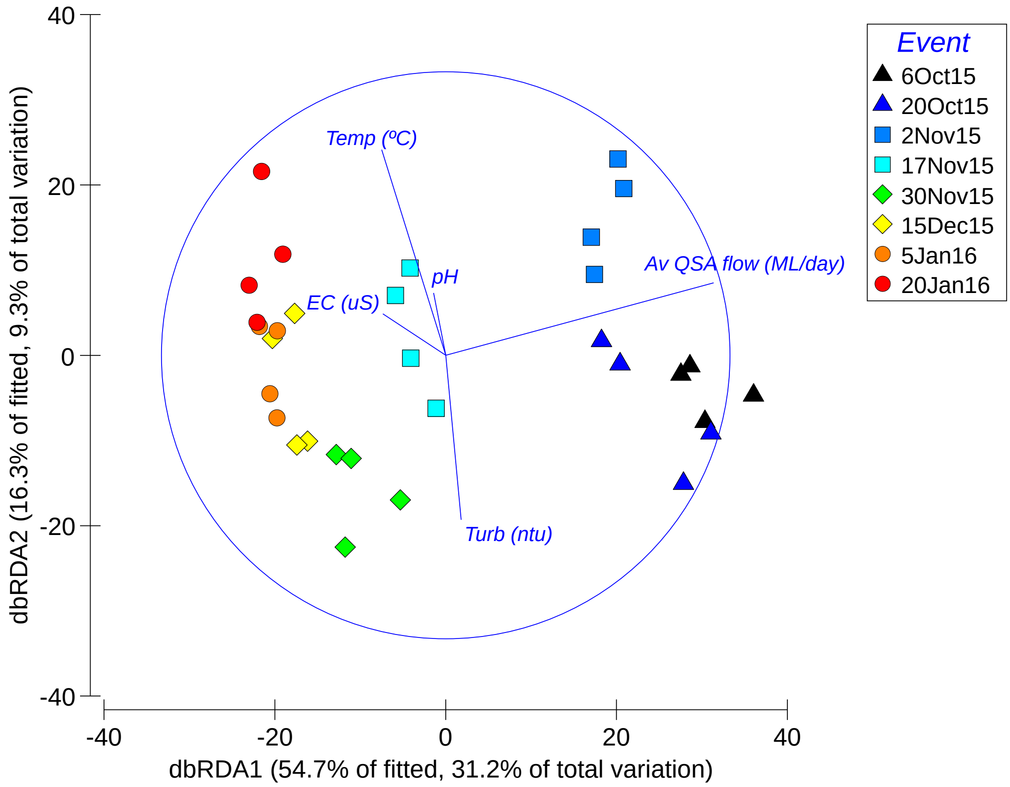

Figure G7. dbRDA ordination of the fitted model of microinvertebrate assemblage data from below Lock 6 (based on Bray-Curtis measure of log transformed data) versus the predictor variables. The vector overlay indicates multiple partial correlations (correlation coefficient > 0.2) between the predictor variables and dbRDA axes 1 and 2.

Lock 1

For sites below Lock 1, all sampling events were significantly different from one another (Table G5 and Figure G8). Generally, separation between groups was high, with the exception of the late October and early November events, and the January events, which were not as well separated although still significant (Table G5 and Figure G8). The microinvertebrate assemblage during mid-November 2015 was very distinct from other sampling events and had high within-sample variability (Figure G8).

Table G5. Within sites below Lock 1 pair-wise results of microinvertebrate log(x+1) abundance data amongst sampling events, showing p-values. * = groups significantly different.

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
6-Oct							
20-Oct	0.0294*						
2-Nov	0.0267*	0.0321*					
17-Nov	0.0280*	0.0282*	0.0275*				
30-Nov	0.0294*	0.0277*	0.0281*	0.0278*			
15-Dec	0.0274*	0.0263*	0.0304*	0.0286*	0.0273*		
5-Jan	0.0303*	0.0297*	0.0277*	0.0281*	0.0304*	0.0302*	
20-Jan	0.0287*	0.0315*	0.0310*	0.0304*	0.0243*	0.0306*	0.0307*

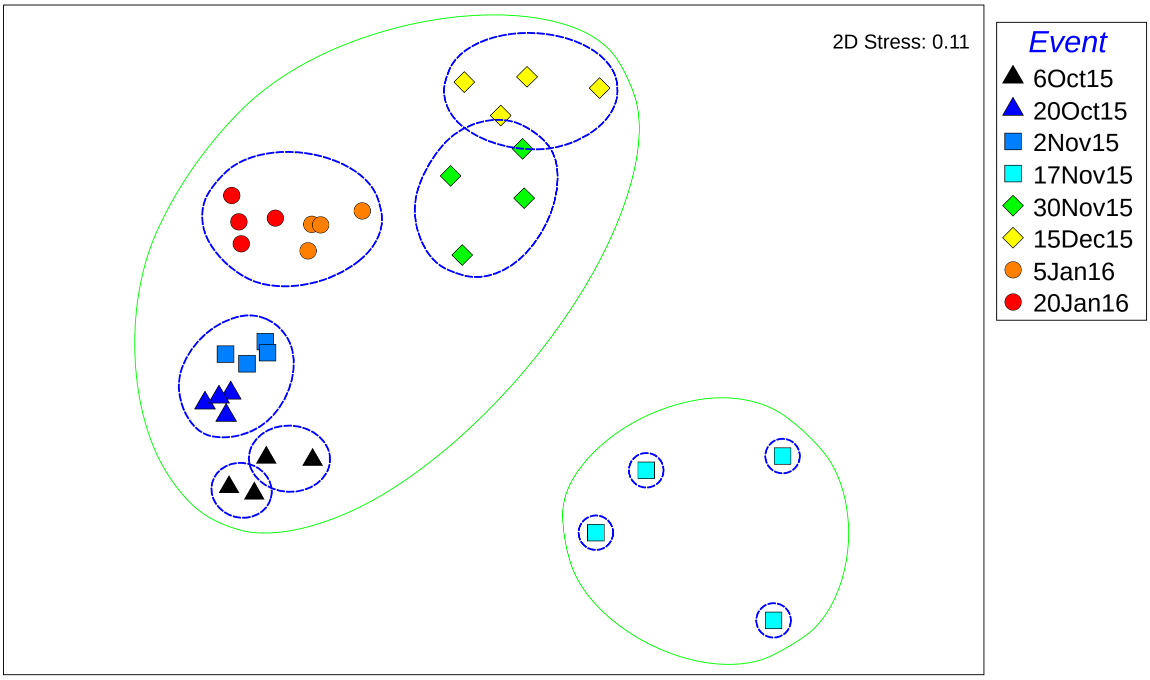

Figure G8. MDS ordination of microinvertebrate assemblage data (log transformed) from Lock 1, with samples identified by sampling event. nMDS was based on Bray-Curtis Similarities. Samples are grouped at a Bray-Curtis similarity of 40% (green circles) and 60% (blue circles) (SIMPROF).

Results from the SIMPER analysis comparing below Lock 1microinvertebrate assemblages between sampling events (all significant) is provided below in Table G6. Dissimilarly between groups was primarily driven by higher abundance of the cladoceran *Bosmina meridionalis* during early October, higher abundance of the rotifer *Brachionus* [*angularis*] *bidens* during early November, lower abundances of rotifers *Polyarthra* sp. a and sp. b during mid-November, and higher abundances of the rotifers *Keratella lenzi* and *K.* cf. *americana* during late January (Table G6).

Table G6. Microinvertebrate taxa responsible for the dissimilarity between sampling events for sites below Lock 1 (SIMPER). Bold taxa were more abundant during the sampling event in the respective column, while unbolded taxa were those more abundant during the sampling event in the respective row. Average dissimilarity (%) between sampling events is provided for each comparison.

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
6-Oct							
20-Oct	41.47% indet. glob. ciliate [sm], indet. glob. ciliate [lg], *Conochilus* sp. a [sm], ***Filinia pejleri***, *Polyarthra* sp. b [lg], *Conochilus* sp. b [lg], *Codonaria* sp., ***Keratella cochlearis*** and *Trichocerca pusilla*.						
2-Nov	45.22% *Brachionus* [*angularis*] *bidens*, indet. glob. ciliate [sm], *Keratella tropica*, *Brachionus angularis*, *Polyarthra* sp. b [lg], *Brachionus calyciflorus amphiceros*, indet. glob. ciliate [lg] and *Conochilus* sp. a [sm].	35.08% *Brachionus* [*angularis*] *bidens*, *Filinia pejleri*, *Brachionus angularis*, *Brachionus calyciflorus amphiceros*, *Filinia opoliensis*, ***Synchaeta pectinata* [med-lg]**, *Keratella tropica*, *Keratella cochlearis* and ***Synchaeta* sp. b [sm., cf. oblonga]**.					

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
17-Nov	72.31% ***Bosmina meridionalis***, ***Keratella javana***, ***Synchaeta* sp. b [sm., cf. oblonga]**, ***Keratella cochlearis***, ***Keratella australis***, ***Trichocerca similis***, ***Synchaeta* sp. c [tiny]** and ***Filinia pejleri***.	73.17% **indet. glob. ciliate [sm]**, ***Conochilus* sp. a [sm]**, ***Conochilus* sp. b [lg]**, ***Codonaria* sp.**, ***Trichocerca pusilla***, ***Polyarthra* sp. b [lg]** and ***Trichocerca similis***.	70.81% **indet. glob. ciliate [sm]**, ***Brachionus* [*angularis*] *bidens***, ***Conochilus* sp. a [sm]**, ***Keratella tropica***, ***Polyarthra* sp. b [lg]**, ***Polyarthra* sp. a [sm]**, ***Keratella australis*** and ***Brachionus angularis***.				
30-Nov	60.71% ***Codonaria* sp.**, ***Synchaeta* sp. b [sm., cf. oblonga]**, ***Keratella javana***, ***Synchaeta* sp. c [tiny]**, *Keratella tropica*, *Polyarthra* sp. b [lg], ***Trichocerca similis*** and *Polyarthra* sp. a [sm].	59.94% **Indet. glob. ciliate [sm]**, ***Codonaria* sp.**, ***Conochilus* sp. b [lg]**, ***Trichocerca* sp. c [long toe, med]**, ***Trichocerca pusilla***, ***Trichocerca similis*** and ***Synchaeta* sp. b [sm., cf. oblonga]**.	56.24% ***Brachionus* [*angularis*] *bidens***, **indet. glob. ciliate [sm]**, ***Codonaria* sp.**, ***Trichocerca* sp. c [long toe, med]**, ***Conochilus* sp. b [lg]**, ***Brachionus angularis***, ***Trichocerca similis*** and ***Filinia pejleri***.	68.63% *Polyarthra* sp. b [lg], *Polyarthra* sp. a [sm], *Keratella tropica*, ***Trichocerca* sp. c [long toe, med]** and calanoid nauplii.			

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
15-Dec	67.42% ***Bosmina meridionalis*, *Synchaeta* sp. c [tiny], *Conochilus* sp. b [lg], *Synchaeta* sp. b [sm., cf. oblonga], *Trichocerca* sp. c [long toe, med], *Trichocerca pusilla*, *Trichocerca similis*,** *Keratella tropica* and ***Filinia pejleri*.**	68.71% **indet. glob. ciliate [sm], *Conochilus* sp. b [lg], *Codonaria* sp., *Trichocerca pusilla*, *Trichocerca* sp. c [long toe, med], *Conochilus* sp. a [sm]** and ***Trichocerca similis***.	65.47% **indet. glob. ciliate [sm], *Brachionus [angularis] bidens*, *Trichocerca* sp. c [long toe, med], *Conochilus* sp. b [lg], *Trichocerca pusilla*, *Conochilus* sp. a [sm], *Brachionus angularis*** and ***Filinia pejleri***.	72.24% ***Trichocerca* sp. c [long toe, med]**, *Polyarthra* sp. b [lg], *Keratella tropica*, *Polyarthra* sp. a [sm], *Hexarthra intermedia* and ***Brachionus calyciflorus amphiceros***.	44.92% *Filinia opoliensis*, cyclopoid copepodite, *Hexarthra intermedia*, ***Bosmina meridionalis***, *Keratella procurva*, ***Proalides tentaculatus***, *Keratella javana* and ***Conochilus* sp. a [sm]**.		
5-Jan	56.90% *Hexarthra braziliensis*, *Polyarthra* sp. a [sm], *Polyarthra* sp. b [lg], *Filinia opoliensis*, *Filinia terminalis*, *Hexarthra intermedia*, ***Keratella cochlearis***, ***Keratella australis*** and ***Bosmina meridionalis***.	51.21% **indet. glob. ciliate [sm]**, ***Conochilus* sp. b [lg]**, *Hexarthra braziliensis*, ***Trichocerca pusilla***, ***Trichocerca similis***, *Filinia opoliensis*, *Hexarthra intermedia*, ***Filinia australiensis*** and ***Codonaria* sp.**	48.11% **indet. glob. ciliate [sm]**, *Synchaeta pectinata* [med-lg], *Hexarthra braziliensis*, ***Brachionus [angularis] bidens***, *Hexarthra intermedia*, ***Keratella australis***, ***Conochilus* sp. b [lg]**, ***Brachionus angularis*** and ***Filinia pejleri***.	68.13% *Polyarthra* sp. a [sm], *Polyarthra* sp. b [lg], *Synchaeta* sp. b [sm., cf. oblonga], *Synchaeta pectinata* [med-lg], *Filinia terminalis* and *Hexarthra braziliensis*.	52.98% *Synchaeta* sp. b [sm., cf. oblonga], *Synchaeta pectinata* [med-lg], *Filinia terminalis*, *Trichocerca* sp. c [long toe, med], *Synchaeta* sp. c [tiny] and *Filinia opoliensis*.	53.40% *Trichocerca* sp. c [long toe, med], *Synchaeta pectinata* [med-lg,], *Synchaeta* sp. b [sm., cf. oblonga], *Filinia terminalis*, *Synchaeta* sp. c [tiny] and *Keratella lenzi*.	

Sampling Event	6-Oct	20-Oct	2-Nov	17-Nov	30-Nov	15-Dec	5-Jan
20-Jan	57.57% *Keratella lenzi*, *Keratella* cf. *americana*, *Hexarthra braziliensis*, *Polyarthra* sp. a [sm], *Filinia terminalis*, *Difflugia gramen*, *Difflugia* cf. *fallax* and *Keratella tropica*.	51.32% *Keratella lenzi*, **indet. glob. ciliate [sm]**, *Keratella* cf. *americana*, *Hexarthra braziliensis*, *Difflugia gramen*, *Filinia terminalis*, ***Trichocerca pusilla*** and *Difflugia* cf. *fallax*.	44.90% **indet. glob. ciliate [sm]**, *Hexarthra braziliensis*, *Keratella lenzi*, ***Keratella australis***, *Keratella* cf. *americana*, *Brachionus falcatus*, *Difflugia gramen*, ***Brachionus* [*angularis*] *bidens***, *Filinia terminalis* and ***Brachionus calyciflorus amphiceros***.	72.54% *Polyarthra* sp. a [sm], *Keratella lenzi*, *Filinia terminalis*, *Keratella* cf. *americana*, *Hexarthra braziliensis* and *Polyarthra* sp. b [lg].	57.49% *Keratella lenzi*, *Codonaria* sp., *Filinia terminalis*, *Keratella* cf. *americana*, *Trichocerca similis*, *Trichocerca* sp. c [long toe, med] and *Hexarthra braziliensis*.	59.79% *Keratella lenzi*, *Filinia terminalis*, *Codonaria* sp., *Keratella* cf. *americana*, *Trichocerca* sp. c [long toe, med], *Trichocerca similis* and *Difflugia* cf. *fallax*.	35.45% *Keratella* cf. *americana*, *Trichocerca similis*, ***Synchaeta pectinata* [med-lg]**, ***Synchaeta* sp. b [sm., cf. *oblonga*]**, *Codonaria* sp., *Keratella lenzi* and ***Synchaeta* sp. c [tiny]**.

All environmental predictor variables for the microinvertebrate assemblage structure below Lock 1 were significant (Table G7). However, electrical conductivity explained most (23.0%) of the variation (Table G7). Electrical conductivity and river flow were the best environmental variables to explain the horizontal separation of the data cloud, while water temperature best explained the vertical separation (Figure G9).

Table G7. DistLM sequential 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.	Cumul.
Electrical conductivity	9.4699	0.0001*	0.22986	0.22986
pH	4.43	0.0002*	0.096154	0.32601
Water temperature	4.4247	0.0005*	0.085574	0.41159
Turbidity	4.2126	0.0003*	0.072807	0.48440
Dissolved oxygen	2.1282	0.0311*	0.066239	0.55063
Mean DS Lock1 flow	4.1981	0.0001*	0.06461	0.61524

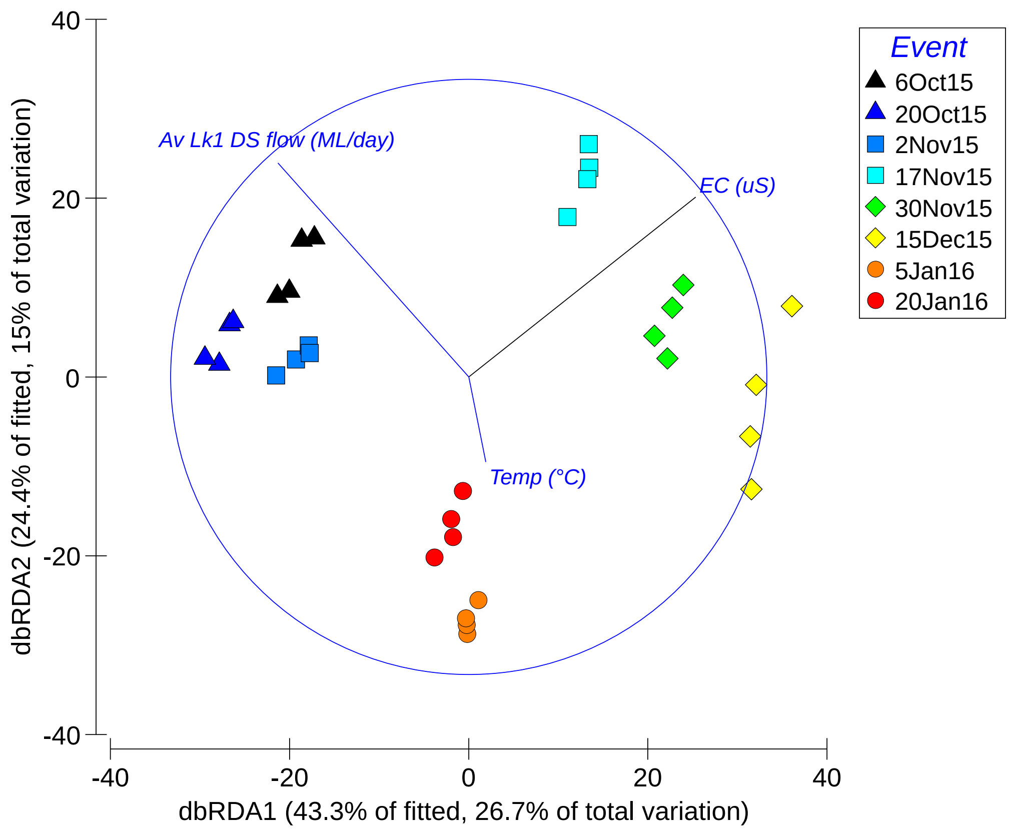

Figure G9. dbRDA ordination of the fitted model of microinvertebrate assemblage data from below Lock 1 (based on Bray-Curtis measure of log transformed data) versus the predictor variables. The vector overlay indicates multiple partial correlations (correlation coefficient > 0.2) between the predictor variables and dbRDA axes 1 and 2.

Qualitative (net tow) vs. quantitative sampling (Haney trap)

In view of the relatively small volume represented by 9 L Haney traps, a concurrent 37 µm-mesh net tow was taken on each sampling date to determine if the larger volume collected would sample more 'rare' species, and therefore provide higher taxa richness estimates. Taxa richness from the first and last sampling events are shown in Figure G10, as an example only.

Higher diversities of microinvertebrate taxa were expected for net tow samples compared to trap samples, because of a larger volume that was sampled with the tows. They consistently collected more species at every site during the early October trip (Figure G10). However, during the late January trip, traps consistently collected more species. A possible explanation for this is turbidity, which was high in October and low in January (Figure G11), and the known vertical migration of the microinvertebrate assemblage. To avoid predation, microinvertebrates may have occurred deeper in the water column during clearer conditions in January, relative to October. Surface sampling with the net tow during January likely underestimated diversity, while the trap sampling aggregated both surficial and deeper assemblages.

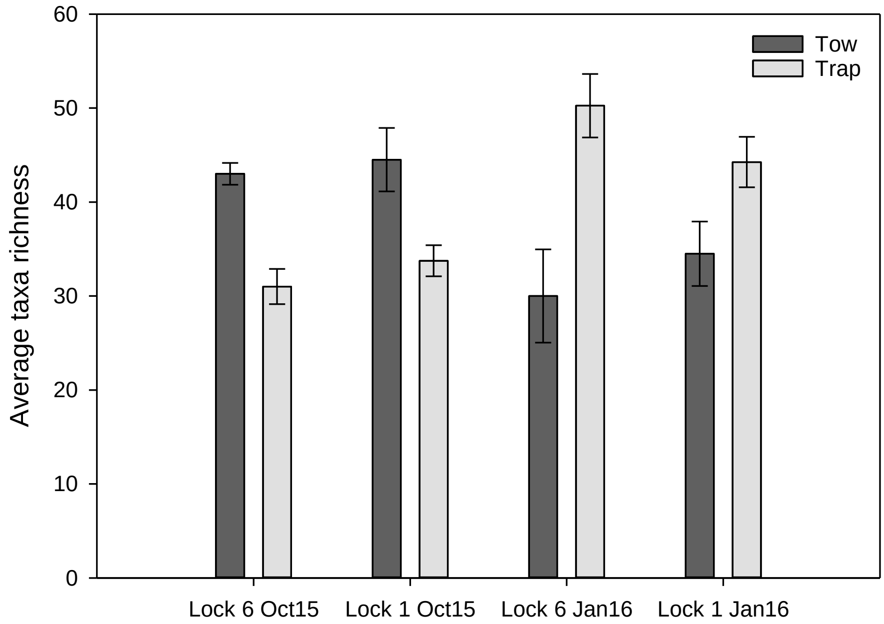

Figure G10. Mean taxa richness (± S.E.) of net tows vs Haney trap volumes from sites below Lock 6 and Lock 1 on the first trip (6–7 October 2015) and last trip (20–21 January 2016).

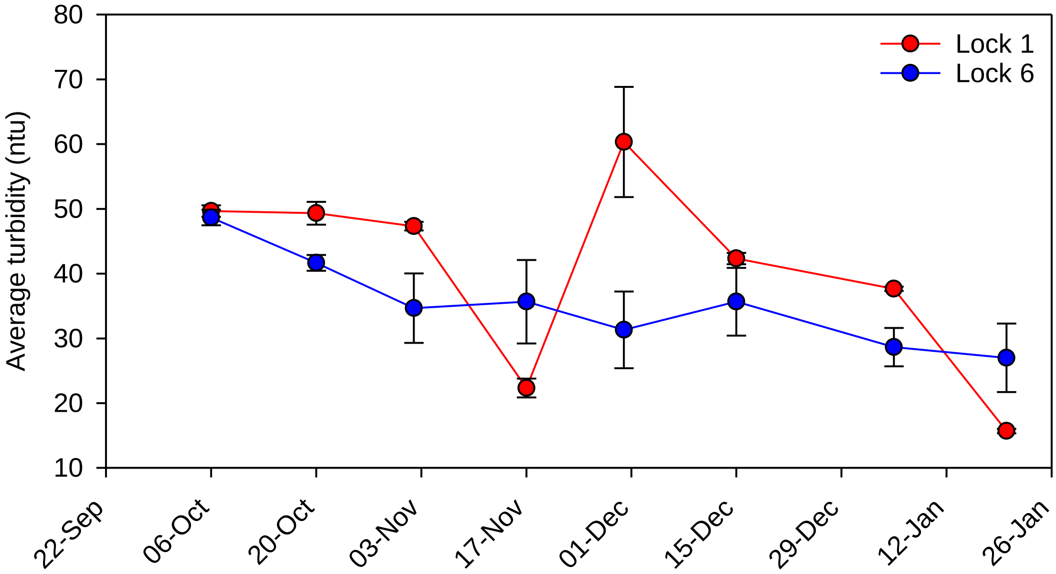

Figure G11. Mean turbidity (± S.E.) at sites below Lock 6 (blue symbols) and Lock 1 (red symbols) from October 2015 to January 2016.

Larval gut-content

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 golden perch larvae. Due to low sample sizes of golden perch post-larvae (*n* = 1), larvae of other large-bodied species were included in the gut-content analysis. Gut contents of golden perch (*n* = 1), silver perch (*n* = 1), Murray cod (*n* = 29) and freshwater catfish (*n* = 14) post-larvae collected opportunistically through larval fish sampling as part of Category 3 Fish Spawning and Recruitment (Table G8) were analysed using traditional taxonomic methods. Unlike the other three species, most Murray cod (21/29) individuals had empty guts (Table G8). The calanoid copepod, *Boeckella triarticulata*, was the only prey that was consumed by all four species (Table G9). There were 13 individuals of this species present in the only post-larval golden perch collected, during early December 2015. By number, calanoid and cyclopoid copepods collectively made up 81.3% of the diet composition for larval Murray cod, while chydorid cladocerans made up almost half (46.9%) of the diet of larval freshwater catfish.

Based on qualitative investigation, there was no clear association of larval diet composition with sampling events or location. Similar to the previous year, low sample sizes of larvae and patchiness of samples at temporal and spatial scales in 2015/16 (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. However, most prey in diet were typical of those from littoral margins, which could have been flushed into the main channel following a suite of management interventions that occurred at the time (e.g. weir pool raising, Appendix B).

Table G8. Catch details for post-larval fish that were analysed for gut-content. Lock 1, 1A and 1B sites are situated 5, 7 and 9 km below Lock 1. Similarly, Lock 6, 6A and 6B sites are situated 5, 7 and 9 km below Lock 6. Two pre-larval fish (i.e. 4.7 mm golden perch and 4.8 mm Murray cod) were not analysed for gut-content. The presence of food in guts is indicated by x. Total lengths (TL) were rounded to the nearest mm.

Species	TL (mm)	Site	Date	Gut contents
Silver perch	30	1	01/12/2015	x
Golden perch	13	1	01/12/2015	x
Murray cod	10	6	20/10/2015	
Murray cod	11	6	20/10/2015	
Murray cod	10	6B	20/10/2015	
Murray cod	10	1	21/10/2015	
Murray cod	10	6	02/11/2015	
Murray cod	11	6	02/11/2015	x
Murray cod	11	6	02/11/2015	x
Murray cod	11	6	02/11/2015	
Murray cod	11	6	02/11/2015	
Murray cod	11	6	02/11/2015	
Murray cod	11	6	02/11/2015	
Murray cod	12	6	02/11/2015	x
Murray cod	11	1	03/11/2015	
Murray cod	11	1	03/11/2015	
Murray cod	11	1	03/11/2015	
Murray cod	11	1	03/11/2015	
Murray cod	12	1	03/11/2015	
Murray cod	12	1	03/11/2015	x
Murray cod	15	1	03/11/2015	x
Murray cod	11	1A	03/11/2015	
Murray cod	12	1A	03/11/2015	
Murray cod	11	6	17/11/2015	
Murray cod	10	1	16/11/2015	x
Murray cod	10	1	16/11/2015	
Murray cod	10	1	16/11/2015	
Murray cod	10	1	16/11/2015	x
Murray cod	10	1	16/11/2015	
Murray cod	10	1	16/11/2015	x
Murray cod	11	1	16/11/2015	
Freshwater catfish	15	6	17/11/2015	
Freshwater catfish	16	6	17/11/2015	x
Freshwater catfish	16	6	17/11/2015	x
Freshwater catfish	12	1	16/11/2015	
Freshwater catfish	15	1	18/11/2015	x
Freshwater catfish	16	1	16/11/2015	x
Freshwater catfish	16	1	16/11/2015	
Freshwater catfish	16	1	16/11/2015	x
Freshwater catfish	16	1	18/11/2015	x
Freshwater catfish	16	1A	18/11/2015	x
Freshwater catfish	16	1B	18/11/2015	
Freshwater catfish	21	6	30/11/2015	x
Freshwater catfish	15	6	15/12/2015	
Freshwater catfish	16	6	15/12/2015	x

Table G9. Summary of gut content analysis of post-larval silver perch (*n* = 1; total length (TL) = 30 mm) golden perch (*n* = 1; TL = 13 mm), Murray cod (*n* = 8; TL = 10–12 mm) and freshwater catfish (*n* = 9; TL = 15–21 mm). %N represents the numerical proportion of a prey item towards the total within each species.

	Silver perch		**Golden perch**		**Murray cod**		**Freshwater catfish**	
Prey	Presence	%N	Presence	%N	Presence	%N	Presence	%N
Copepoda								
Copepoda unid.							1/9	2.0
Calanoida								
Boeckella sp.							2/9	16.3
Boeckella triarticulata	1/1	30.0	1/1	100.0	2/8	12.5	2/9	6.1
Calamoecia sp.	1/1	10.0						
copepods					1/8	6.25		
copepodites					1/8	37.5		
eggs							2/9	4.1
Cyclopoida								
Australocyclops australis					1/8	6.25		
copepods	1/1	30.0			1/8	6.25		
copepodites					2/8	12.5	1/9	2.0
Cladocera								
Bosmina meridionalis					1/8	6.25		
Moina micrura					1/8	6.25		
Macrothrix sp.					1/8	6.25		
Ceriodaphnia sp.	1/1	10.0						
Chydoridae							1/9	2.0
Chydorus sp.							2/9	4.1
Picripleuroxus quasidenticulatus							1/9	40.8
Ostracoda								
Newnhamia sp.							1/9	2.0
Decapoda								
Atyidae							4/9	16.3
Insecta								
Diptera							1/9	2.0
Chironomidae	1/1	20.0					1/9	2.0

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 2012; 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 ambigua*) in the southern MDB has been associated with overbank flooding 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 (~1,000 GL) of environmental water to augment flow regimes in the MDB to rehabilitate the health of aquatic ecosystems. In the LMR Selected Area, 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 (LMR M&E Plan). 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 Selected Area.

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 Selected Area 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 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 Selected Area. 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 2015/16, 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	15/09/15–29/02/16	13
Murray	Lock 6	15/09/15–16/02/16	12
Murray	Lock 9	15/09/15–16/02/16	12
Murray	Lock 11	15/09/15–15/02/16	12
Murray	Torrumbarry	14/09/15–15/02/16	10
Murray	Barmah	14/10/15–25/11/15	6
Darling	Weir 32	14/09/15–22/02/16	11
Edward–Wakool	Deniliquin	17/09/15–18/02/16	12
Murrumbidgee	Narrandera	16/09/15–19/02/16	11
Goulburn	Yambuna	05/10/15–08/12/15	6
Goulburn	Pyke Road	05/10/15–07/12/15	7

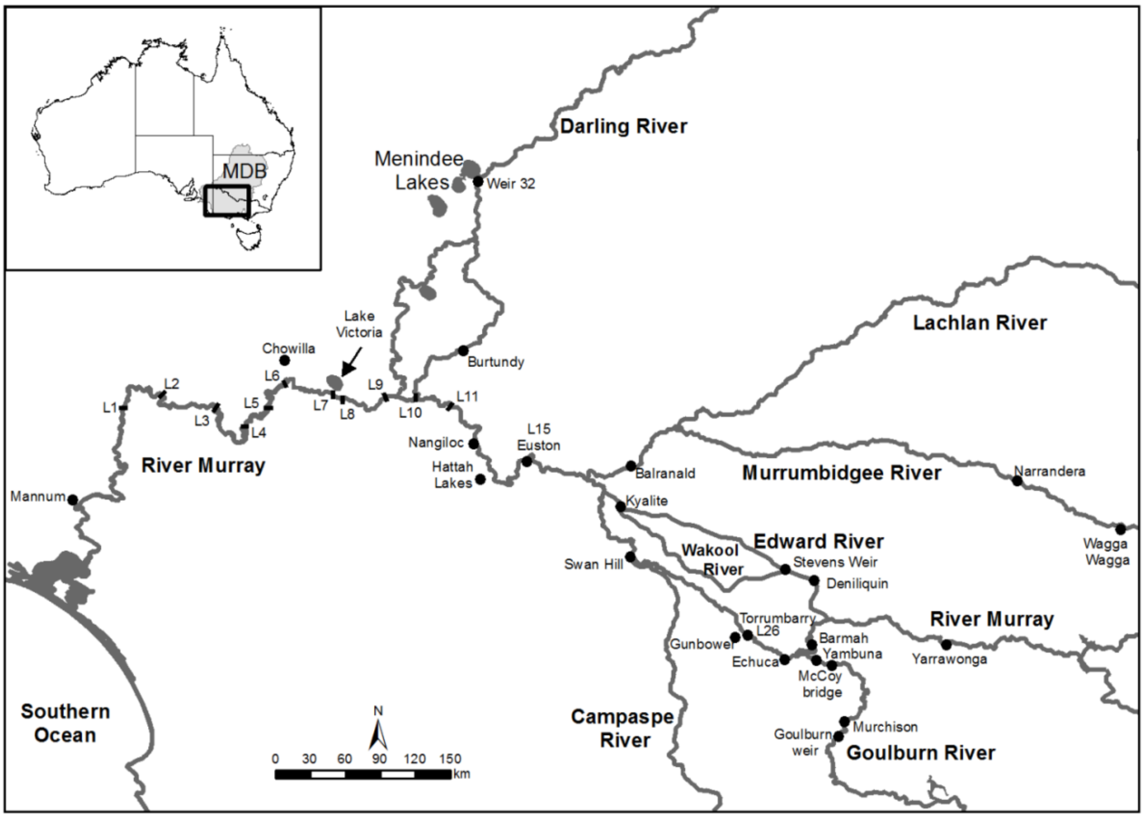

Figure H1. Map showing the location of the Murray–Darling Basin and the major rivers that comprise the southern Murray-Darling Basin, the numbered Locks and Weirs (up to Lock 26, Torrumbarry), the Darling, Lachlan, Murrumbidgee, Edward–Wakool, Campaspe and Goulburn rivers and Lake Victoria, an off-stream 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 Selected Area (Figure 7; Table H2).

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

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 regions of the LMR Selected Area, respectively, (Table H3).

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

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 work (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 EICHROM 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 6 October 2015 and 21 January 2016. 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 2016 to maximise the chance of collecting YOY spawned in the spring–summer 2015/16 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 879 to 2880 seconds. All individuals were measured to the nearest mm (total length, TL) and a subsample of golden perch (n = 55–74) proportionally representing the length-frequency of golden perch collected from the gorge and floodplain geomorphic zones of the LMR Selected Area was retained for ageing. All silver perch (n = 9) collected from floodplain geomorphic zone were retained for ageing.

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 to the primordium using a graded series of wetted lapping films (15, 9 and 3 μm). Sections were then polished using 0.3 μm alumina slurry to a thickness of 40–80 μm.

Sections were examined using a compound microscope (x 600) 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* = 129) and silver perch (*n* = 9) retained for ageing were euthanized 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 downwards, on an acid-washed glass slide and polished down to the primordium 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 or 10 um 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 mid-September 2015 and extended, at the majority of sites, through until mid-February 2016. Overall, $^{87}Sr/^{86}Sr$ at most locations remained reasonably stable throughout the period of collection, with the highest ratios (>0.7185) measured in the Murray River at Barmah and the Edward River, and the lowest (<0.7080) in the Darling River (Figure H4). Water $^{87}Sr/^{86}Sr$ 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 Goulburn River was similar to $^{87}Sr/^{86}Sr$ at Lock 11 in the mid-Murray River and Lock 9 in the lower River Murray from early October to mid-November, and $^{87}Sr/^{86}Sr$ of the Murrumbidgee River showed overlap with $^{87}Sr/^{86}Sr$ at Lock 6 in the LMR from mid-October. Water $^{87}Sr/^{86}Sr$ was most variable in the Murrumbidgee River (0.7145–0.7161), particularly between mid-September and early December 2015 (Figure H4).

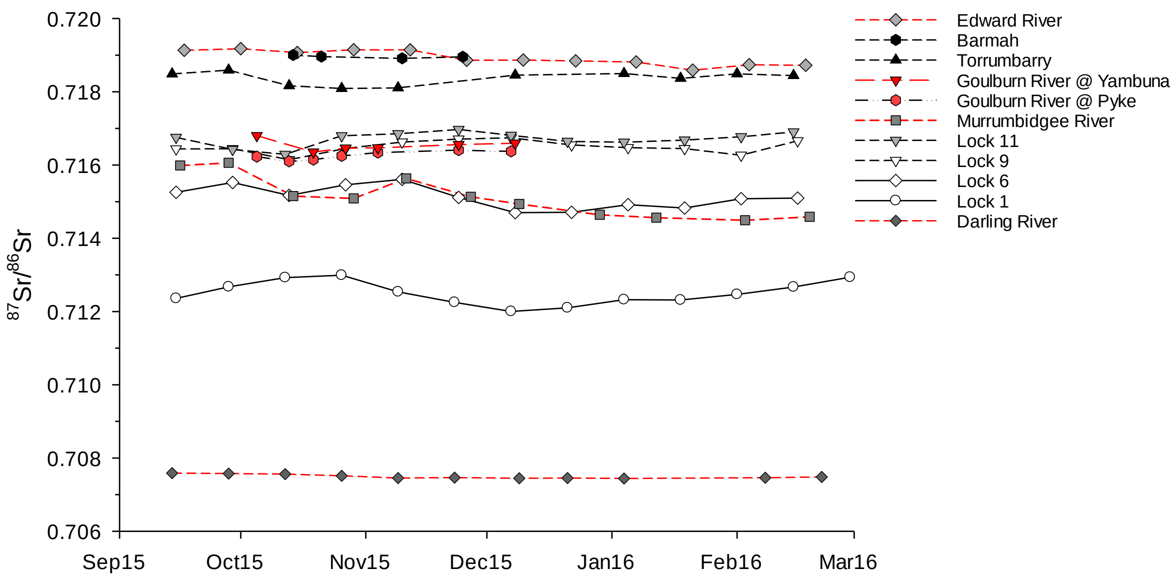

Figure H4. $^{87}Sr/^{86}Sr$ ratios in water samples collected from mid-September 2015 to late February 2016 in the Murray (Lock 1, 6, 9, 11 Torrumbarry and Barmah), Darling, Goulburn, Edward and Murrumbidgee rivers.

From mid-September 2015 to March 2016, flow in the LMR (discharge at the South Australian border, QSA) ranged approximately 5,600–11,700 ML day^{-1} (Figure H5). From mid-September to late October 2015, flow ranged 9,600–11,700 ML day^{-1} before steadily decreasing to 5,600 ML day^{-1} by late November 2015. From late November, flow gradually increased to 10,000 ML day^{-1} in early February 2016, before declining to 6,500 ML day^{-1} in mid-March 2016. QSA was mainly comprised of flow from the upper Murray River, Murrumbidgee River and Victorian tributaries of the Murray River (Figure 6).

Flow in the mid-reaches of the Murray River at Euston peaked at 17,600 ML day^{-1} in mid-September 2015 then steadily decreased to 7,500 ML day^{-1} in early October 2015, before rising again to peaks of 14,400 ML day^{-1} and 14,300 ML day^{-1} in late October and mid-November 2015, respectively (Figure H5). Flow then decreased to approximately 7,000 ML day^{-1} in early December 2015 before gradually rising to 11,800 ML day^{-1} in mid-February 2016. Flow in the Darling River at Burtundy was mostly absent (<20 ML day) from early September 2015 to March 2016 (Figure H5).

From early September 2015 to early March 2016, the contribution of Commonwealth environmental water to flow at the South Australian border ranged 0–6,700 ML day^{-1}, peaking initially at ~5,900 ML day^{-1} on 21 September and then at ~6,700 ML day^{-1} on 28 October 2015 (Figure 3). Environmental water from the MDBA's The Living Murray program was delivered 30 August to 22 October 2015, and 28 February to 29 March 2016, peaking at ~3,000–3,500 ML day^{-1} 23 September to 8 October 2015 (Figure 3).

Throughout the sampling period, $^{87}Sr/^{86}Sr$ in water samples collected from Lock 9, 6 and 1 in the lower River Murray, reflected water delivery from the mid-Murray River, and negligible input from the Darling River (Figures H4 and H5).

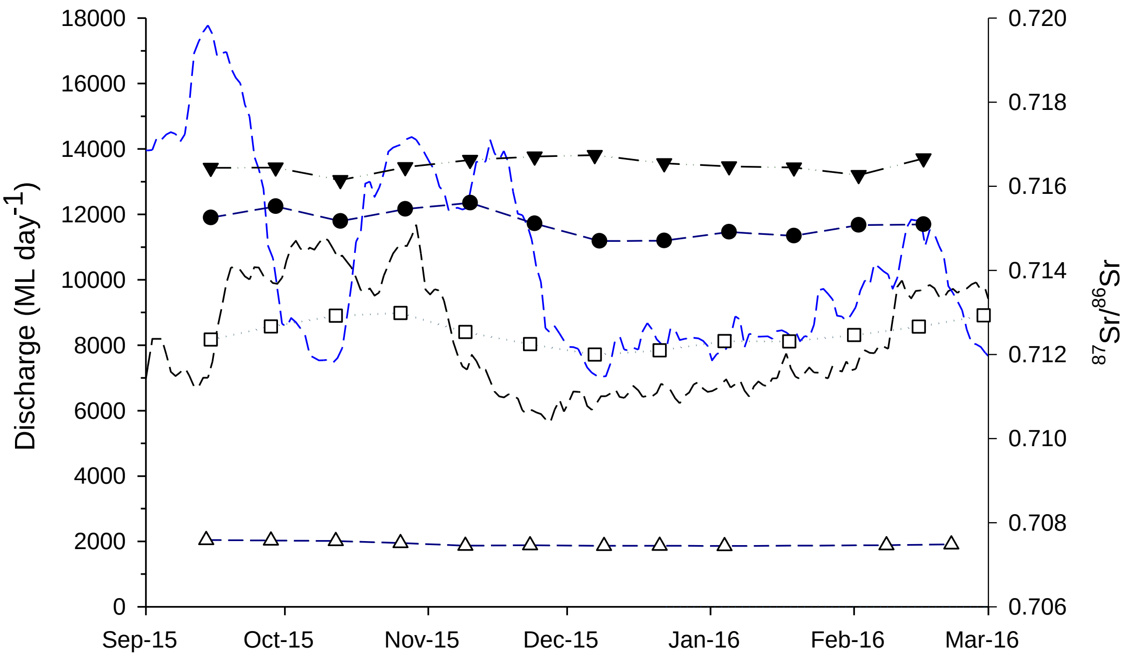

Figure H5. Mean daily discharge (ML day^{-1}) in the Murray River at the South Australian border (dashed black line) and Euston (dashed blue line). Mean daily discharge for the Darling River at Burtundy was <20 ML day^{-1} during sampling. $^{87}Sr/^{86}Sr$ in water samples collected from mid-September 2015 to late February 2016 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 18,499 larvae from five small-bodied species and 3,736 larvae from six large-bodied species were sampled by plankton tows from three sites in each of the gorge and floodplain geomorphic zones (combined) of the LMR Selected Area (Tables H2 and H4). Flathead gudgeons (*Philypnodon* spp.), carp gudgeon and Australian smelt (*Retropinna semoni*) were the most abundant small-bodied species, while bony herring was the most abundant large-bodied species. Silver perch, golden perch and Murray rainbowfish were sampled in low abundance (<5 individuals per species).

Table H4. Total catches from larval fish sampling conducted between 6 October 2015 and 21 January 2016. 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#	2,444	288	442	**3,174**	2,653	521	291	**3,465**	**6,639**
Carp gudgeon	2,088	774	569	**3,431**	1,984	489	389	**2,862**	**6,293**
Australian smelt	2,632	198	292	**3,122**	1,950	373	106	**2,429**	**5,551**
Unspecked hardyhead	0	0	0	**0**	12	0	0	**12**	**12**
Murray rainbowfish	0	0	0	**0**	4	0	0	**4**	**4**
Large-bodied									
Bony herring	1,507	268	453	**2,228**	1,200	120	30	**1,350**	**3,578**
Common carp	22	0	0	**22**	83	5	2	**90**	**112**
Murray cod	15	2	0	**17**	11	0	1	**12**	**29**
Freshwater catfish	6	1	1	**8**	6	0	0	**6**	**14**
Golden perch	2	0	0	**2**	0	0	0	**0**	**2**
Silver perch	1	0	0	**1**	0	0	0	**0**	**1**
Perch eggs*	*			*****	*	*		*****	*****
Perch hatchlings*					*			*****	*****

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

* Perch eggs and 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 2015/16, two golden perch larvae were collected below Lock 1, one on 21 October and one on 1 December 2015. Ages of these larvae were 2 (pre-flexion) and 18 days (post-flexion), corresponding to spawn dates of 19 October and 13 November 2015, respectively (Table H5; Figure H7). One silver perch larvae was collected below Lock 1 on 1 December 2015. This larvae was 47 days old (post-flexion), which corresponded to a spawn date of 15 October 2015 (Table H5; Figure H7). No golden perch or silver perch larvae were collected below Lock 6.

Table H5. Capture location and date, length (mm), age (days), spawn date and otolith core $^{87}Sr/^{86}Sr$ values for larval golden perch and silver perch collected from the gorge geomorphic zone of the LMR Selected Area. Daily age and spawn date for the smallest golden perch (*) 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	Gorge	Lock 1	21/10/2015	4.7	2*	19/10/2015	-
Golden perch	Gorge	Lock 1	1/12/2015	12.9	19	13/11/2015	0.7120
Silver perch	Gorge	Lock 1	1/12/2015	29.5	47	15/10/2015	0.7129

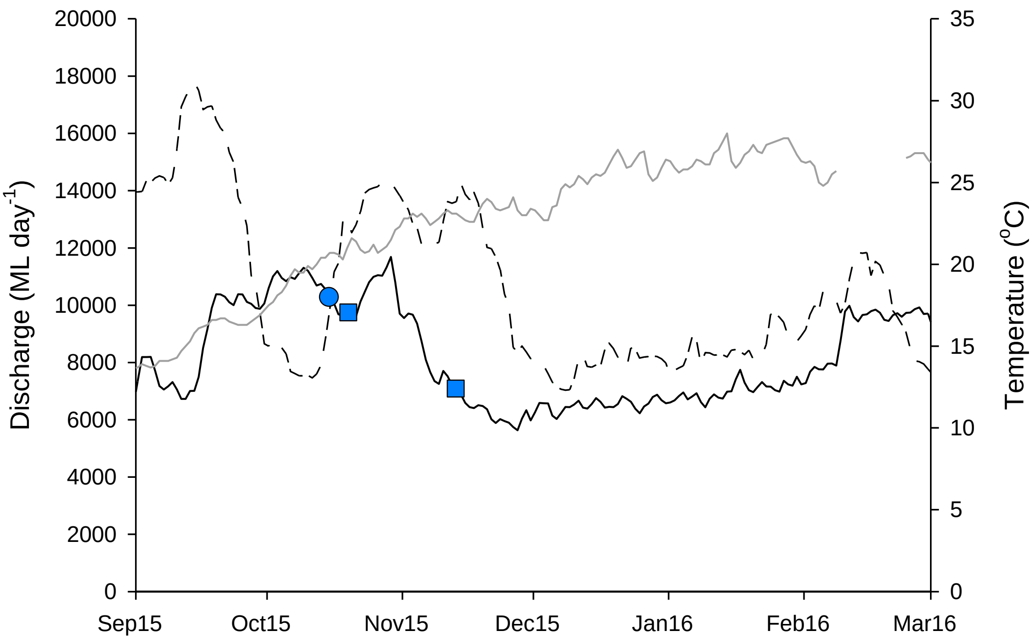

Figure H7. Back-calculated spawn dates for larval golden perch (*n* = 2, blue squares) and silver perch (*n* = 1, blue circle) captured from the LMR Selected Area during 2015/16, plotted against discharge (ML day^{-1}) in the Lower Murray River at the South Australian border (solid black line) and Euston (dashed black line), and water temperature (°C) (grey line).

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

Otoliths from the largest golden perch (12.9 mm) and the silver perch (29.5 mm) larvae were analysed for $^{87}Sr/^{86}Sr$ (Table H5). The otoliths of the remaining larval golden perch was too small for LA-ICPMS analysis. Both larvae had otolith core $^{87}Sr/^{86}Sr$ indicative of their capture location in the LMR, below Lock 1 (i.e. 0.7120–0.7130) (Table H5; Figure H8).

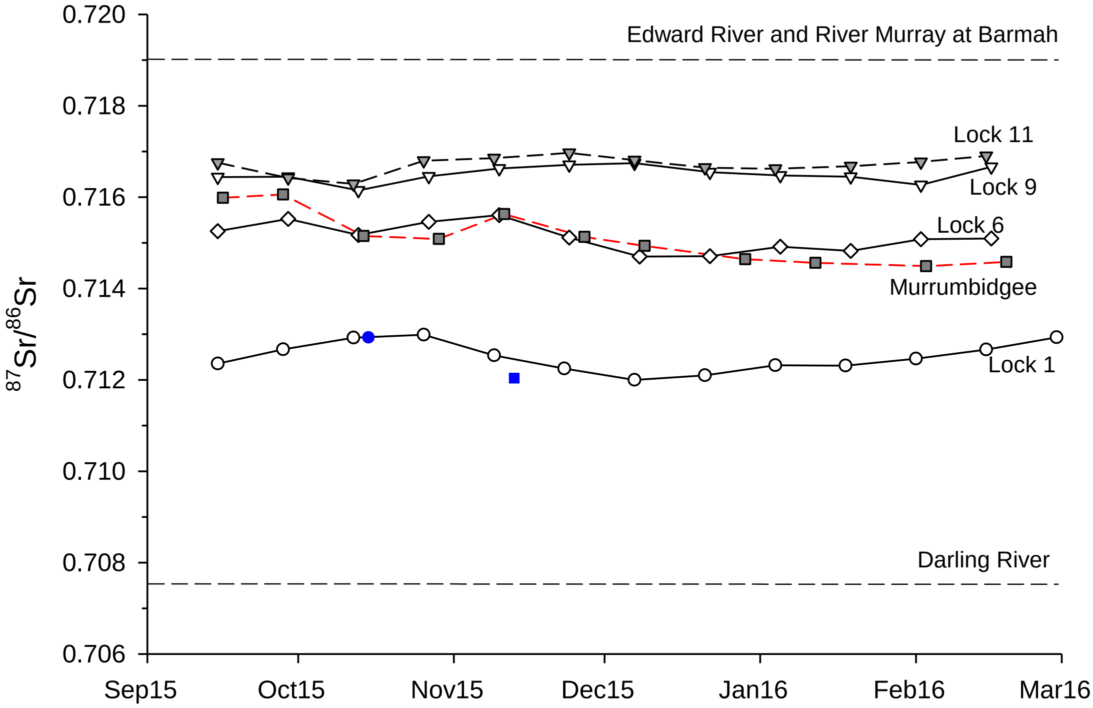

Figure H8. $^{87}Sr/^{86}Sr$ in water samples collected from late September 2015 to late February 2016 at sites in the southern MDB. $^{87}Sr/^{86}Sr$ in the Darling River and Edward River/Murray River at Barmah are presented as dashed straight lines as these were temporally stable and represent the maximum and minimum $^{87}Sr/^{86}Sr$ measured in water samples in the southern MDB in 2015/16. Closed blue symbols represent spawn date and otolith core $^{87}Sr/^{86}Sr$ of larval golden perch (square) and silver perch (circle) collected in the LMR Selected Area from October to December 2015.

Transects of $^{87}Sr/^{86}Sr$ from the otolith core to edge can elucidate the movement history of golden perch but may also reflect temporal variability in ambient $^{87}Sr/^{86}Sr$ in water. Transects of otolith $^{87}Sr/^{86}Sr$ for golden perch and silver perch larvae captured below Lock 1 indicated that both individuals were spawned in the LMR Selected Area, likely below Lock 1, and remained in this region throughout their early life (Figure H9a).

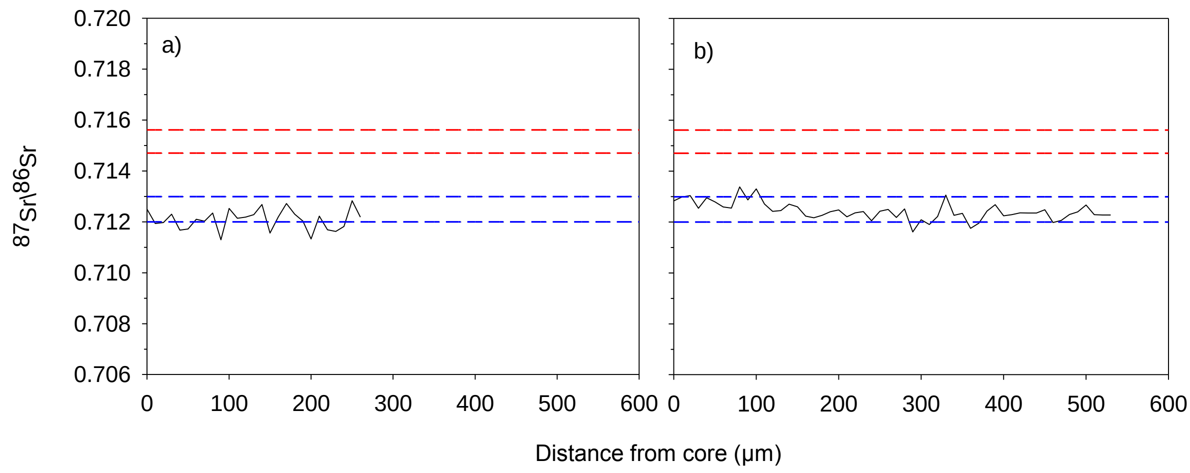

Figure H9. Individual life history profiles based on otolith Sr isotope transects (core to edge) for a (a) golden perch larvae aged 19 days and (b) silver perch larvae aged 47 days, collected below Lock 1 in the gorge zone of the LMR Selected Area. Dashed lines denote minimum and maximum $^{87}Sr/^{86}Sr$ ratios in the Murray River at Lock 1 (blue) and Murray River at Lock 6 (red).

Golden perch and silver perch length and age structure

In 2016, no YOY golden perch or silver perch were collected during Category 1 and 3 Fish LTIM electrofishing sampling in the LMR Selected Area. Golden perch sampled in the gorge and floodplain geomorphic zones of the LMR Selected Area ranged in age from age 2+ to 19+, with dominant cohorts of age 6+ and 5+ fish, spawned in 2009/10 and 2010/11, respectively. Age 6+ fish comprised 55 and 33% of the sampled population in the floodplain and gorge geomorphic zones, respectively, whilst age 5+ fish comprised 25 and 27% of the population in the floodplain and gorge zones, respectively (Figure H10). In the gorge geomorphic zone, age 15+ and 19+ fish spawned in 2000/01 and 1996–97 comprised 5 and 14% of the sampled population, respectively (Figure H10).

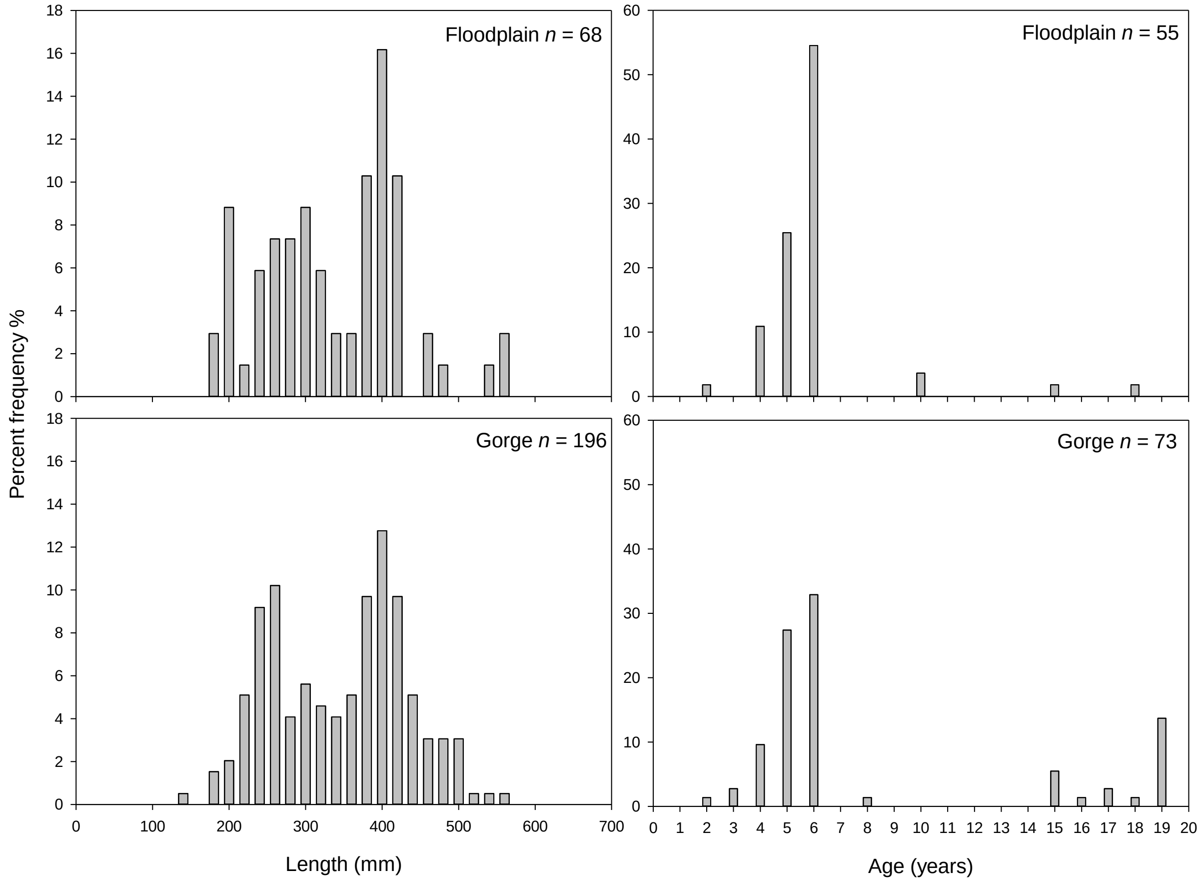

Figure H10. Total length (left column) and age (right column) frequency distribution of golden perch collected by boat electrofishing from the floodplain (top) and gorge (bottom) geomorphic zones of the LMR Selected Area in April 2016.

In 2016, low numbers of silver perch were sampled from the gorge (*n* = 5) and floodplain (*n* = 9) geomorphic zones of the LMR Selected Area. Silver perch sampled in the floodplain geomorphic zone ranged from age 2+ to 6+ (Figure H11), with age 2+, 4+ and 6+ fish the most abundant cohorts (Figure H11). No ageing was conducted for samples from the gorge geomorphic zone for this species.

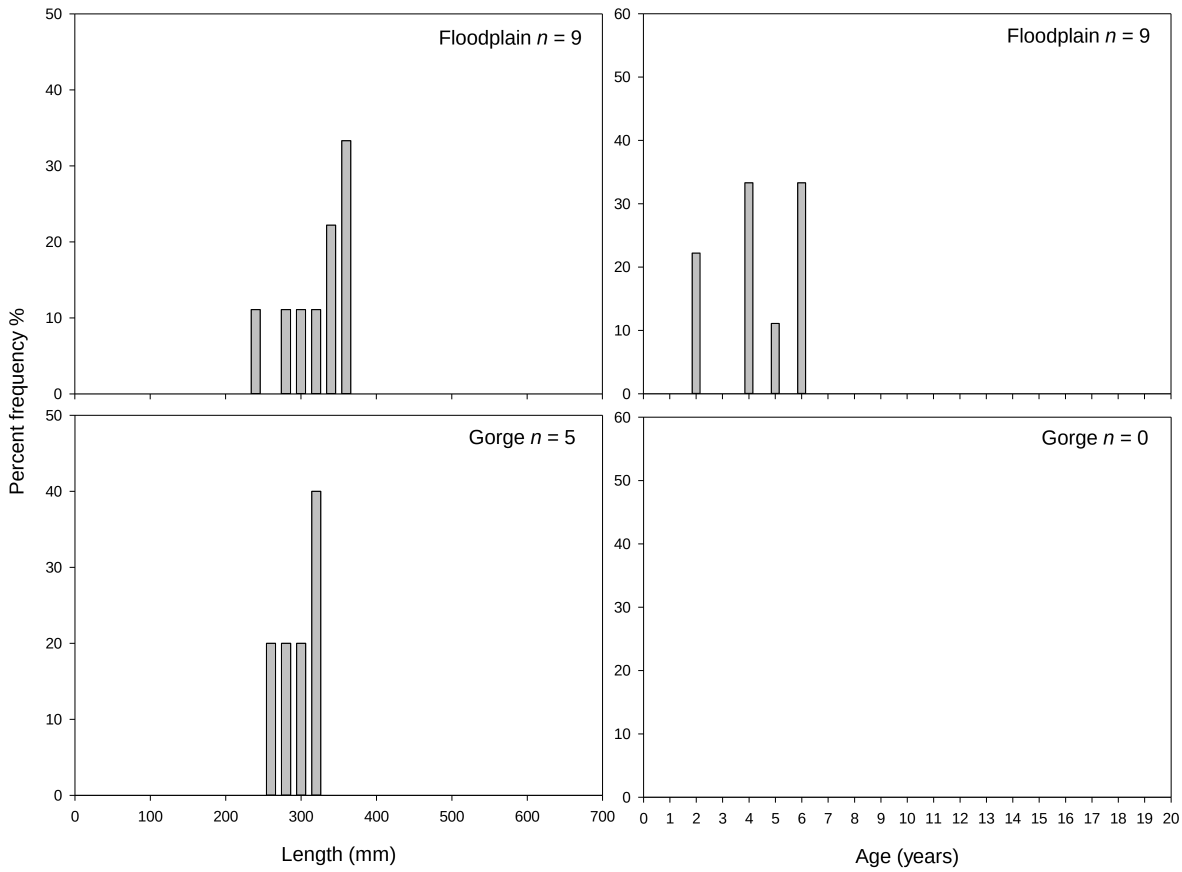

Figure H11. Fork length (left column) and age (right column) frequency distribution of silver perch collected by boat electrofishing from the floodplain (top) and gorge (bottom) geomorphic zones of the LMR Selected Area in April 2016.

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 H10) of golden perch in the lower River Murray (gorge and floodplain geomorphic regions) in 2015/16, we analysed $^{87}Sr/^{86}Sr$ from the otolith core to edge in a subsample of fish from age 5+ (*n* = 10), 6+ (*n* = 10) and 19+ (*n* = 5) cohorts (Table H6; Figures H13–16). We compared these transects to water $^{87}Sr/^{86}Sr$ measured at sites across the southern MDB from 2011–2016 (this report; Zampatti *et al*. 2015; SARDI unpublished data) (Figure H12).

Table H6. Capture location and region, length (mm), age (years) and spawn year, and otolith core $^{87}Sr/^{86}Sr$ of 25 golden perch collected from the lower River Murray in April 2016. Life history profiles are shown for individuals marked with *.

Region	Capture location	Length (mm)	Age (years)	Spawn year	Core $^{87}Sr/^{86}Sr$
Gorge	Swan Reach	253	5*	2010/11	0.707375
Gorge	Caurnamont	239	5	2010/11	0.707304
Gorge	Waikerie	335	5	2010/11	0.707722
Gorge	Morgan	307	5	2010/11	0.707515
Gorge	Blanchetown	275	5*	2010/11	0.707359
Floodplain	Murtho Forest	261	5*	2010/11	0.712130
Floodplain	Murtho Forest	213	5	2010/11	0.709026
Floodplain	Rili Island	250	5*	2010/11	0.710623
Floodplain	Plushes Bend	244	5	2010/11	0.711023
Floodplain	Plushes Bend	281	5	2010/11	0.709863
Gorge	Swan Reach	361	6	2009/10	0.707497
Gorge	Caurnamont	409	6*	2009/10	0.707277
Gorge	Cadell	272	6*	2009/10	0.708859
Gorge	Blanchetown	350	6	2009/10	0.708269
Gorge	Scott's Creek	347	6	2009/10	0.707678
Floodplain	Murtho Forest	298	6*	2009/10	0.707702
Floodplain	Murtho Forest	369	6	2009/10	0.709794
Floodplain	Rili Island	297	6	2009/10	0.707500
Floodplain	Plushes Bend	372	6	2009/10	0.707585
Floodplain	Plushes Bend	393	6	2009/10	0.707620
Gorge	Swan Reach	362	19*	1996/97	0.713399
Gorge	Caurnamont	404	19*	1996/97	0.713035
Gorge	Overland Corner	373	19	1996/97	0.713269
Gorge	Cadell	379	19	1996/97	0.713561
Gorge	Blanchetown	413	19	1996/97	0.713213

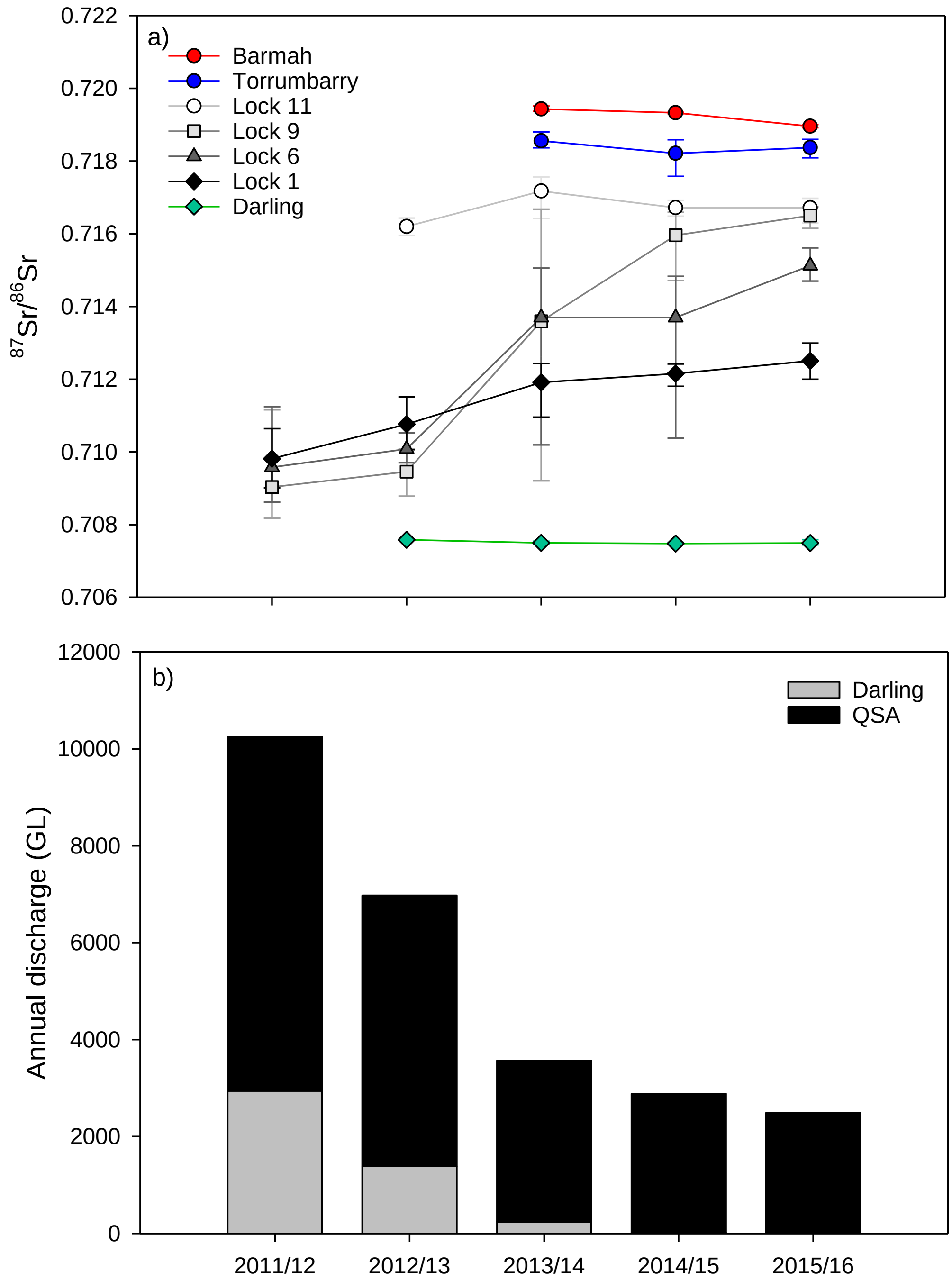

Figure H12. (a) Mean $^{87}Sr/^{86}Sr$ (with minimum and maximum values as error bars) in water samples collected from spring/summer in the mid-Murray (Barmah, Torrumbarry and Lock 11), lower Murray (Lock 9, 6 and 1) and Darling Rivers from 2011 to 2016, and (b) annual discharge (GL) in the Murray River at the South Australian border (QSA) and the proportion of discharge from the Darling River at Burtundy that contributed to QSA.

Of the age 5+ golden perch (spawned 2010/11), all fish analysed from the gorge geomorphic region (n = 5) exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) 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 all of these fish transitioned from the Darling River in their first year of life (i.e. age 0+), but potentially at different times in this first year (Figure H13a and Figure H13b)).

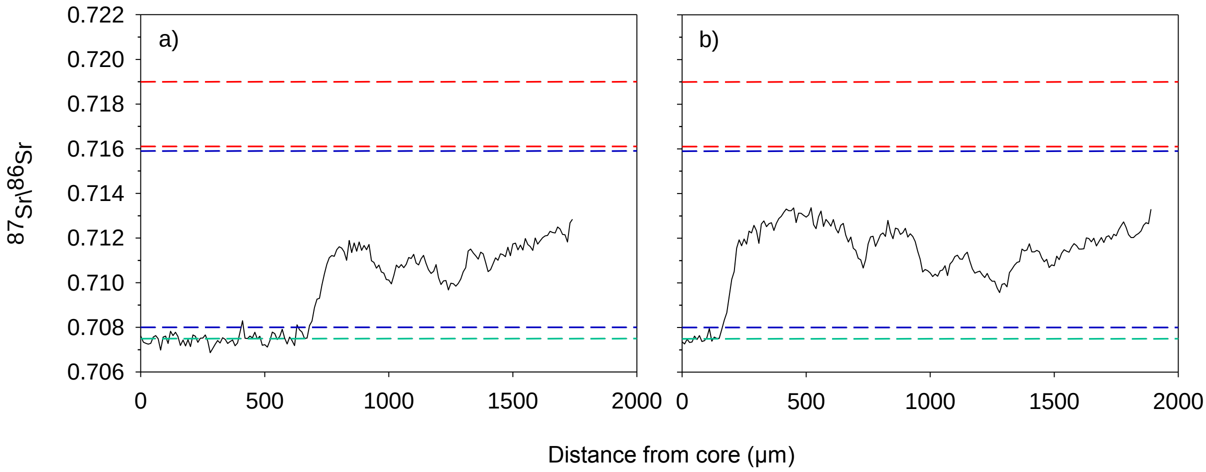

Figure H13. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from two age 5+ golden perch collected from (a) Swan Reach and (b) Blanchetown in the gorge geomorphic region of the lower River Murray. Green dashed line indicates the temporally 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).

In contrast to the age 5+ golden perch from the gorge geomorphic region, all age 5+ fish analysed from the floodplain geomorphic region (n = 5) had higher core $^{87}Sr/^{86}Sr$ (Table H6), comparable to water $^{87}Sr/^{86}Sr$ in the lower Murray River (~0.7085–0.7140) (Figure H12), indicating these fish were potentially spawned in various locations in the lower River Murray. Transects of otolith $^{87}Sr/^{86}Sr$, indicate all five fish had spent their entire lives in the lower Murray River (Figure H14).

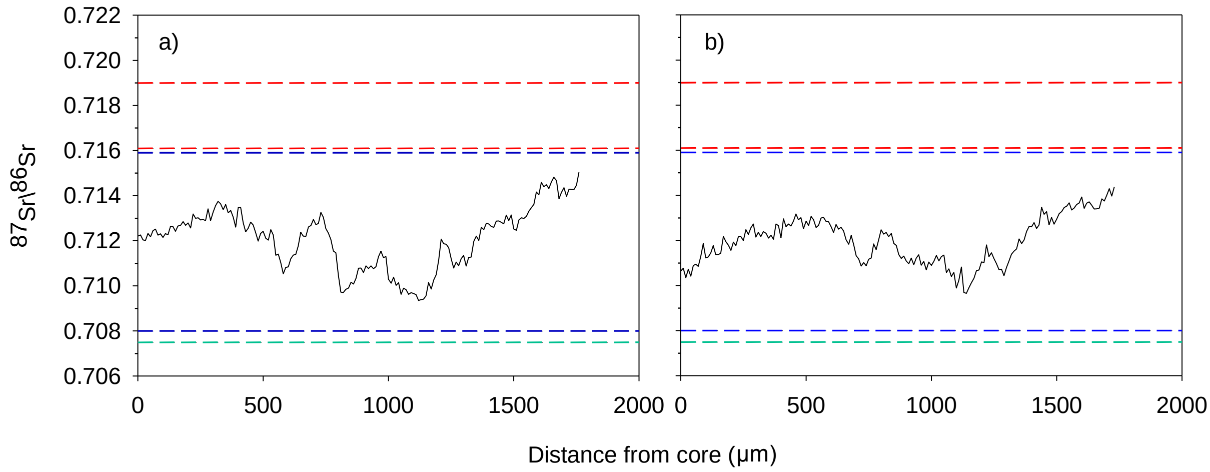

Figure H14. An individual life history profile based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of an otolith from an age 5+ golden perch collected from (a) Murtho Forest and (b) Rilli Island in the floodplain geomorphic region of the lower River Murray. Green dashed line indicates 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 2009/10), 70% (*n* = 7) exhibited otolith core $^{87}Sr/^{86}Sr$ (Table H6) comparable to the distinct Darling River water $^{87}Sr/^{86}Sr$ of ~0.7075 (Figure H12), indicating these fish were spawned in the Darling River. The remaining three age 6+ fish exhibit otolith core $^{87}Sr/^{86}Sr$ slightly greater than the Darling River, but generally lower than most Murray River water $^{87}Sr/^{86}Sr$ values (Figure H12), suggesting these fish may have been spawned in the Murray River close to the Darling confluence. Transects of otolith $^{87}Sr/^{86}Sr$, indicate that all age 6+ spawned in the Darling River transitioned into the lower River Murray as age 0+ (Figure H15b) or 1+ (Figure H15c) (approximately 320 µm of otolith growth) and remained in this region until capture in 2016 (Figure H15).

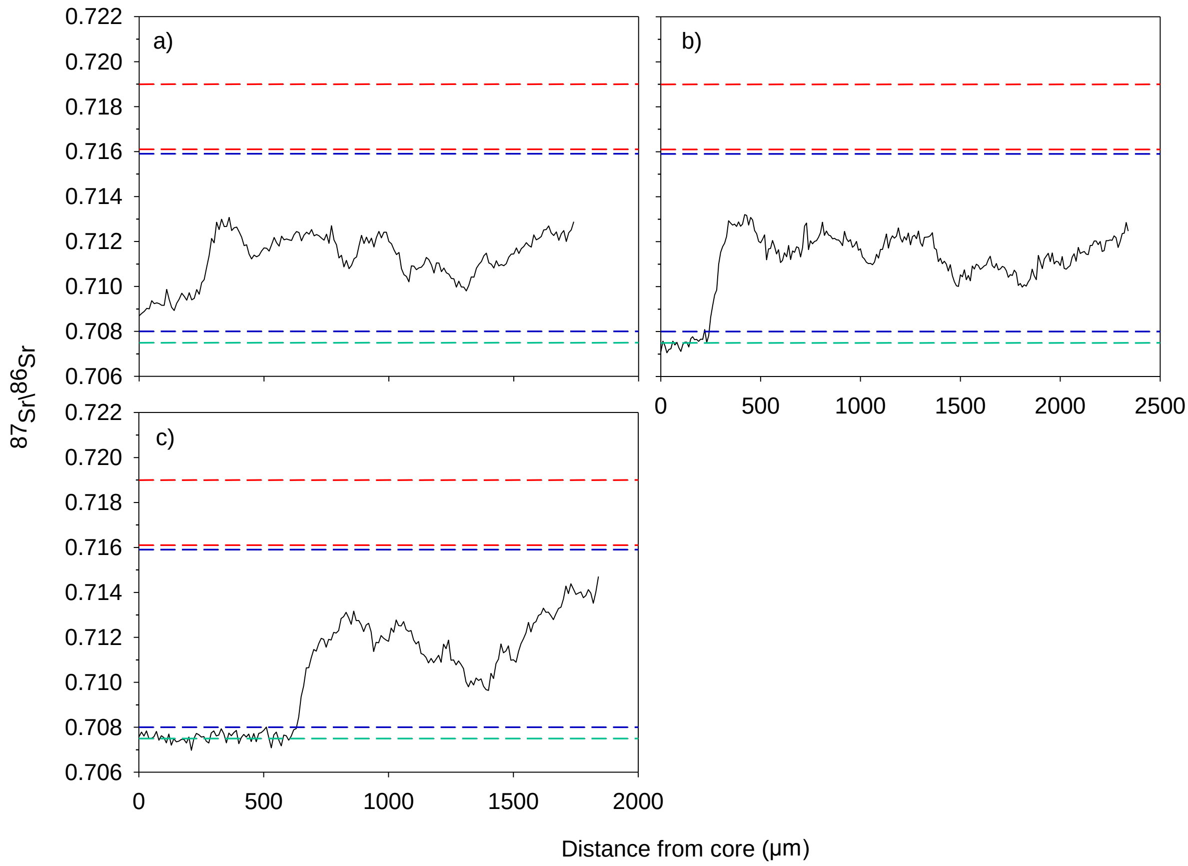

Figure H15. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from two age 6+ golden perch collected from (a) Cadell, (b) Caurnamont and (c) Murtho Forest in the lower River Murray. Green dashed line indicates the temporally stable water $^{87}Sr/^{86}Sr$ 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).

Age 19+ golden perch (spawned in 1996/97) comprised 14% of the sampled population in the gorge geomorphic region of the lower Murray River (Figure H10). Five age 19+ golden perch were analysed for otolith $^{87}Sr/^{86}Sr$ and all exhibited similar otolith core $^{87}Sr/^{86}Sr$ values (0.7130–0.7136, Table H6), indicative of a lower River Murray spawning origin (Figure H12). Transects of otolith $^{87}Sr/^{86}Sr$, indicate all five fish had spent their entire lives in the lower Murray River (Figure H16) with variability in $^{87}Sr/^{86}Sr$ a result of fish moving within the lower Murray or reflecting temporal variability in water $^{87}Sr/^{86}Sr$ in this region.

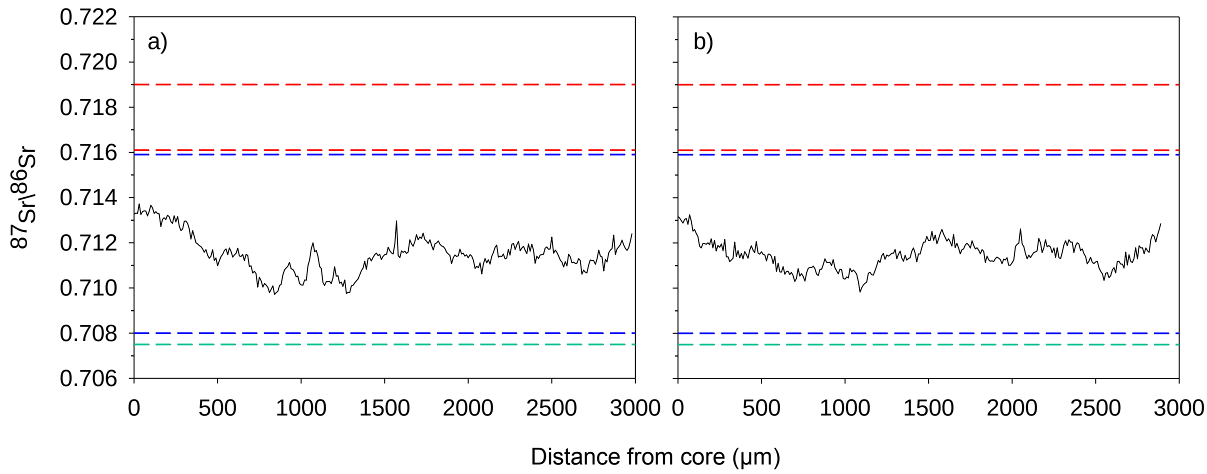

Figure H16. An individual life history profile based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of an otolith from an age 19+ golden perch collected from (a) Swan Reach and (b) Caurnamont in the gorge geomorphic region of the lower River Murray. Green dashed line indicates the temporally 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).

<u>Silver perch</u>

Like golden perch, distinct age cohorts in the sampled silver perch population exhibited a range of natal origins and migration histories. Two age 2+ silver perch (spawned in 2013/14) 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 H17).

Table H7. Capture location and region, length (mm), age (years) and spawn year, and otolith core $^{87}Sr/^{86}Sr$ of 8 silver perch collected from the floodplain geomorphic region of lower River Murray in April 2016. Life history profiles are shown for individuals marked with *.

Region	Capture location	Length (mm)	Age (years)	Spawn year	Core $^{87}Sr/^{86}Sr$
Floodplain	Rilli Island	263	2*	2013/14	0.710252
Floodplain	Plushes Bend	226	2*	2013/14	0.710591
Floodplain	Rilli Island	332	4*	2011/12	0.710633
Floodplain	Rilli Island	283	4*	2011/12	0.707364
Floodplain	Plushes Bend	333	4	2011/12	0.709051
Floodplain	Plushes Bend	312	5	2010/11	0.712255
Floodplain	Rilli Island	362	6*	2009/10	0.716889
Floodplain	Plushes Bend	337	6*	2009/10	0.716644

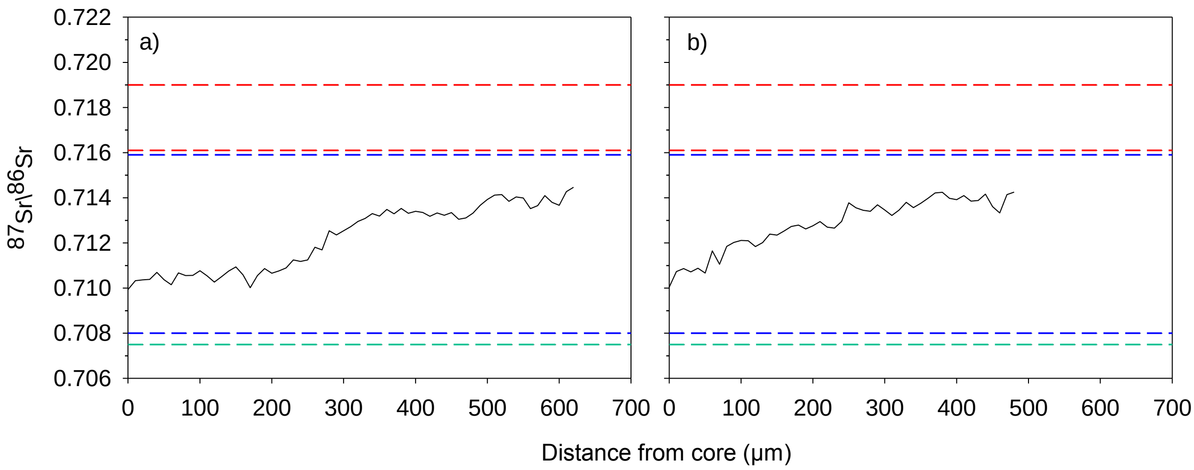

Figure H17. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from two age 2+ silver perch collected from (a) Rilli Island and (b) Plushes Bend in the floodplain geomorphic region of the lower River Murray. Green dashed line indicates the 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).

In contrast, three age 4+ silver perch (spawned 2011/12) exhibit a range of core $^{87}Sr/^{86}Sr$ (Table H7), indicative of spawning origins in the Darling River and lower River Murray (Figure H12). Transects of otolith $^{87}Sr/^{86}Sr$ indicate that age 4+ silver perch spawned in the lower Murray remained in this region (Figure H18a), whilst the fish spawned in the Darling River transitioned into the lower Murray at age 0+ (Figure H18b).

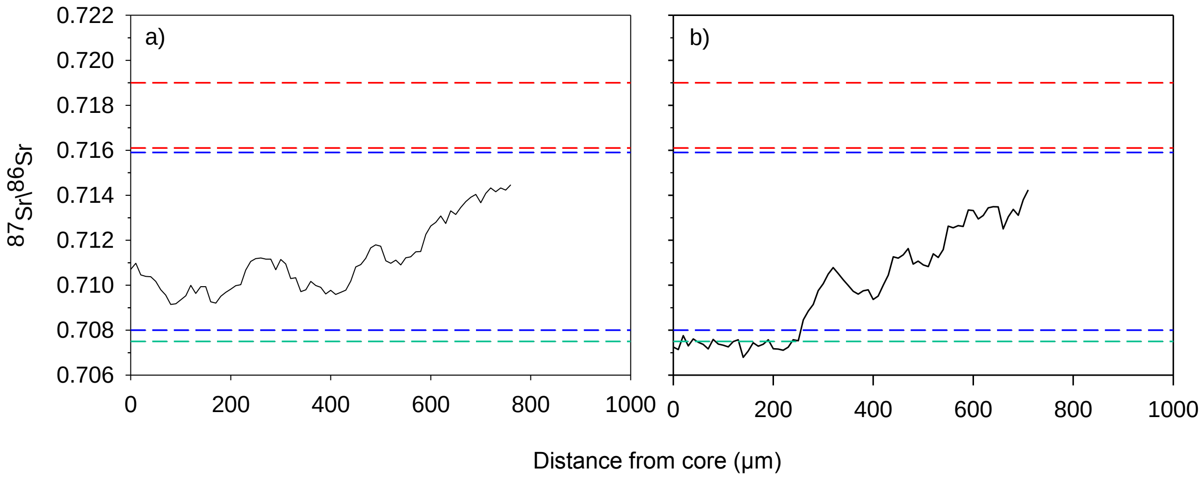

Figure H18. Individual life history profiles based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of otoliths from two age 4+ silver perch collected from Rilli Island in the floodplain geomorphic region of the lower River Murray. Green dashed line indicates the temporally stable water $^{87}Sr/^{86}Sr$ 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).

Two age 6+ silver perch (spawned 2009/10) were analysed for otolith $^{87}Sr/^{86}Sr$ and both 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 H12). Transects of otolith $^{87}Sr/^{86}Sr$ indicate that both these fish transitioned into the lower River Murray as age 0+, but just prior to their first birthday (Figure H19).

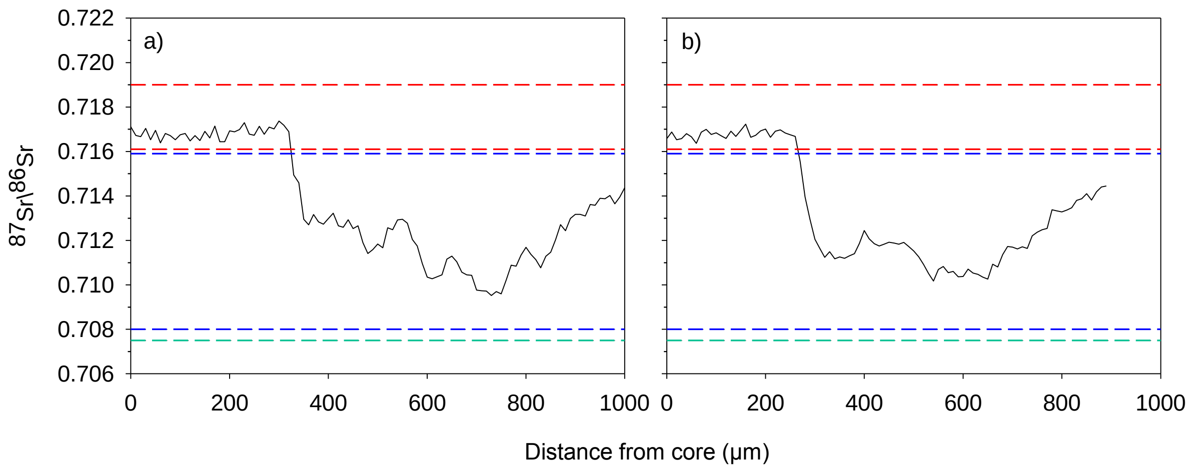

Figure H19. An individual life history profile based on transect analysis of $^{87}Sr/^{86}Sr$ from the core to edge of an otolith from an age 6+ silver perch collected from (a) Rilli Island and (b) Plushes Bend in the floodplain geomorphic region of the lower River Murray. Green dashed line indicates 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 2015/16, flow in the LMR Selected Area was maintained at a reasonably stable 9,600–11,700 ML day^{-1} in mid-September and late October 2015 before steadily decreasing to 5,600 ML day^{-1} by late November 2015 and then gradually increasing to 10,000 ML day^{-1} in early February 2016. Through this period, Commonwealth environmental water compromised a maximum of ~5,900 ML day^{-1} on 21 September and ~6,700 ML day^{-1} on 28 October 2015.

Sampling for golden perch and silver perch eggs and larvae from early October 2015 to end January 2016 revealed low numbers of golden perch (*n* = 2) and silver perch larvae (n = 1) in the LMR Selected area from mid-October to early December 2015. The age of these larvae (2–19 days) and/or otolith $^{87}Sr/^{86}Sr$ indicate these fish were spawned from 15 October–13 November in the LMR Selected Area, below Lock 1. Consequently there was a low level of golden perch and silver perch spawning in the LMR Selected Area in conjunction with the delivery of Commonwealth environmental water in October–November 2015.

In 2016, the golden perch population in the floodplain and gorge geomorphic zones of the LMR was dominated by age 6+ and 5+ fish, representing 80% and 60% of the sampled fish, respectively. In the gorge geomorphic zone, the remainder of the population was comprised of generally older fish (i.e. age 19+, 14%, and 15+, 5%). No age 0+ or 1+ golden perch were collected in either region indicating negligible recruitment from spawning in 2014/15 and 2015/16. Overall, these data demonstrate episodic recruitment of golden perch during the period of the Millennium drought (2001–2010), but more consistent recruitment from 2010 to 2013.

In 2016, the sampled silver perch population in the LMR was comprised of age 2+–6+ 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+ or 1+ silver perch were collected indicating negligible recruitment from spawning in 2014/15 and 2015/16.

Consecutive year-classes of golden perch (i.e. age 2+–6+) and silver perch (i.e. age 4+–6+) from 2010–2014, were spawned in association with in-channel and overbank increases in flow in the lower River Murray and the Darling River and, uniquely for silver perch, in the mid-Murray River (age 6+ cohort). The addition of these year classes improved the resilience of golden perch and silver perch populations in the lower River Murray and reinforces the premise that water management, or unregulated flows, that promote flow variability (in-channel and overbank) above regulated entitlement flows, may stimulate golden perch spawning in the lower River Murray and Darling River, and silver perch spawning in the lower and mid-Murray River, and Darling River, and subsequently promote golden perch and silver perch recruitment in the LMR Selected Area.

Conclusions

These findings support and expand contemporary conceptual models of the flow-related ecology of golden perch and silver perch in the River Murray. In the LMR, golden perch recruitment is promoted by spawning associated with spring–summer increases in flow (in-channel and overbank) in the lower River Murray and lower Darling River. Likewise, silver perch recruitment in the LMR is promoted by spawning associated with spring–summer increases in flow in the lower River Murray and Darling River, but also the mid-Murray River. The absence of these hydrological characteristics in the LMR and lower Darling River in 2015/16 led to limited spawning and negligible recruitment of golden perch and silver perch to age 0+ in the LMR Selected Area. Hence the delivery of Commonwealth environmental water did not support the CEWO objective of contributing to increased spawning and/or recruitment of flow-dependent fish species in the LMR Selected Area.

In spring–early summer 2015/16, flow between the mid- and lower River Murray was fragmented and homogenised through the operation of Lake Victoria. In the LMR, this moderated distinct spring pulses in flow (present in the mid-Murray River) that may stimulate golden perch and silver perch spawning and promote recruitment. Indeed, age 0+ silver perch were collected in the mid-Murray River (where these pulses were intact) in autumn 2016 (SARDI unpublished data). Consequently, in the LMR Selected Area, achieving positive outcomes for native fishes through the delivery of Commonwealth environmental water may require the longitudinal maintenance of flow characteristics (e.g. magnitude and shape of the hydrograph) present in the mid-Murray River.

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, WPR = weir pool raising.

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?	CEW supported the water level manipulations in Weir Pool 5 and contributed to increased water levels in the Chowilla Creek Anabranch. Increased water levels led to increased rates of ecosystem respiration (ER) and increased heterotrophy. However, integrated responses at the weir pool site gave an ecosystem net production (ENP) close to zero, suggesting that the periods of enhanced respiration were related to increased autotrophic production. These periods were considered to have enhanced energy supplies for food webs.
	What did CEW contribute to increased nutrients and DOC levels?	The data suggested that CEW contributed little to increased nutrients or DOC concentrations. Turbidity consistently declined over the monitoring period, improving the sunlight available to phytoplankton and increasing metabolic activity, but it is yet to be determined whether the turbidity reductions were a result of different supply sources for the CEW.
	What did CEW contribute to maintaining dissolved oxygen levels above 50% saturation throughout the water column at all times?	Dissolved oxygen levels were always above 50% at the two sampling sites over the monitoring period. This suggests that the quality of the environmental water contributing to the flow was adequate to avoid any major deoxygenation processes.

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?	Yes. During autumn 2016, recent recruits (i.e. <300 mm TL, 88%) sub-adults (i.e. 300–600 mm TL, 6%) and adults (>600 mm TL, 6%) were sampled in the Gorge zone of the LMR Selected Area.
	Did a YOY cohort represent >50% of the Murray cod population from the Gorge zone?	Yes. During autumn 2016, a YOY cohort (i.e. <150 mm TL) of Murray cod represented 62.5% of the population in the Gorge zone of the LMR Selected Area.
	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 2016, 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 ten sites in 2015/16, relative to the previous year. During 2014/15 the mean site ratio was 0.58 carp (± 0.36 S.E.) to every 1 flow-dependant, native species. In 2015/16, this ratio increased to 1.65 carp (± 0.09) 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?*	There was an increase in the ratio (total biomass) of common carp to flow-dependant, native species (golden perch and silver perch) at six of the ten sites in 2015/16, relative to the previous year. In 2015/16, the sites where the estimated biomass of carp did not increase at a greater rate than flow-dependant, native species were Qualco, Waikerie, Lowbank B and Overland Corner A (Figure 7; Table H3 in Appendix H). During 2014/15, the mean site ratio was 1.32 kg of carp (± 0.32 S.E.) to every 1 kg of flow-dependant, native species. In 2015/16, this ratio increased to 2.01 kg of carp (± 0.39) 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?	Without CEW, 2015/16 would have been at entitlement flow. As such, CEW contributed to most of the variation in discharge (and hence water level and velocity) over the year. Weir pool raising at Locks 2 and 5 also increased variation in water levels in these reaches.
Category 3. Hydrological Regime	What did CEW contribute to providing diverse hydraulic conditions and complex habitat for flow dependant biota and processes?	With the exception of Weir Pool 3, without environmental water the median velocities in each weir pool each day would not have exceeded 0.17 m s^{-1} during 2015/16. However, CEW did increase the median velocities in each weir pool in the LMR to exceed this threshold from July to November, and again in February.

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 2015/16, CEW contributed to create these conditions for short periods in late September and October. 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 minor reduction in salinities in the Murray River Channel and Lower Lakes, and a considerable reduction in salinity in the Murray Mouth. Within the Murray Mouth, the modelling suggest that there was median salinity of 27.73 practical salinity units (PSU) with all water during 2015/16, compared to 35.23 PSU without CEW. Salinity is known to have a significant impact upon biogeochemical processes and so maintaining salinities at the Murray Mouth within that of normal estuarine conditions may have maintained normal biogeochemical processes for this region. Furthermore, reduced salinity concentrations in the Lower Lakes and Murray Mouth, may have improved habitat for estuarine biota.

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 exports from the Murray River Channel and Lower Lakes, and a decreased net import of salt to the Coorong, with CEW contributing 41% and 87% of the total modelled export from the Murray River Channel and Lower Lakes, respectively. There was a net modelled import of salt to the Coorong of 1,850,028 tonnes with all water during 2015/16, but without CEW the modelling suggests this would have been 6,441,297 tonnes. • Increased exports of nutrients from the Murray River Channel, Lower Lakes and Murray Mouth. The most apparent differences in exports associated with environmental water were for silica, with CEW contributing to 41% and 95% of the total silica exports from the Murray River Channel and Lower, respectively. • Increased exports of phytoplankton biomass from the Murray River Channel, Lower Lakes and Murray Mouth. This increased transport of dissolved and particulate matter may have provided benefits for the Lower Lakes, Coorong and near-shore 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?	CEW returns (potentially from Barmah–Millewa Forest, also The Living Murray), and flooded littoral/riparian margins (e.g. Chowilla Anabranch and Weir Pools 5, 2, 7, 8 and 9) likely translocated 102 non-riverine microinvertebrate taxa (57.6% of the identified taxa in trap samples mid-stream), some in appreciable numbers, into the main channel. These included epibenthic testate rhizopods, epiphytic rotifers, surface-associated or floc-dwelling chydorid and other cladocerans, and heloplanktonic cyclopoid and benthic harpacticoid copepods not considered part of the riverine potamoplankton, although they may occur in shallow 'slackwaters', billabongs, or flooded riparian margins. Notably, 44 taxa (25% of the total assemblage) were not recorded during similar sampling in 2014/15, including several new records for the continent (Rotifera), or new to the lower River Murray and South Australia (Chydoridae).
	What did CEW contribute to increased dispersal of organisms between river and wetlands?	No wetland samples were collected in 2015/16 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 Selected Area?	CEW delivery in 2015/16 did not result in the presence of any new cohorts (age 0+) of golden perch in the LMR Selected Area.
	What did CEW contribute to the population age structure of silver perch in the LMR Selected Area?	CEW delivery in 2015/16 did not result in the presence of any new cohorts (age 0+) of silver perch in the LMR Selected Area.

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 Selected Area?	No. No age 0+ (2015/16 cohort) or 1+ (2014/15 cohort) golden perch were detected during electrofishing in Autumn 2016.
	Did CEW contribute to a YOY or age 1+ cohort that represented >30% of the silver perch population in the LMR Selected Area?	No. No age 0+ (2015/16 cohort) or 1+ (2014/15 cohort) silver perch were detected during electrofishing in Autumn 2016.

*Site ratios of common carp to flow-dependant, native species was 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 ten 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).

Acronyms

AHD	Australian Height Datum
CEW	Commonwealth environmental water
CEWH	Commonwealth Environmental Water Holder
CEWO	Commonwealth Environmental Water Office
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEWNR	Department of Environment, Water and Natural Resources
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
NPL	Normal pool level
PSU	Practical salinity units
TL	Total length
TLM	The Living Murray
VEWH	Victorian Environmental Water Holder
WPR	Weir pool raising
YOY	Young-of-year