Long-Term Intervention Monitoring for the Ecological Responses to Commonwealth Environmental Water Delivered to the Lower Murray River Selected Area in 2014/15

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

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Ye, Q.[1], Giatas, G.[1], Aldridge, K.[2], Busch, B.[3], Gibbs, M.[2,4], Hipsey, M.[3], Lorenz, Z.[5], Oliver, R.[5], Shiel, R.[2,6] and Zampatti, B.[1] (2016). Long-Term Intervention Monitoring for the Ecological Responses to Commonwealth Environmental Water Delivered to the Lower Murray River Selected Area in 2014/15. A report prepared for the Commonwealth Environmental Water Office. South Australian Research and Development Institute, Aquatic Sciences.

[1] SARDI Aquatics Sciences
[2] The University of Adelaide
[3] The University of Western Australia
[4] DEWNR
[5] CSIRO
[6] Wetland Research and Management

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Cover photos: Larval fish, young-of-year golden perch and Murray River (SARDI Aquatic Sciences); zooplankton (ALS, WRM); pump station (www.waterconnect.sa.gov.au).

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ACKNOWLEDGEMENTS

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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 one (2014/15) of the five-year Commonwealth Environmental Water Office (CEWO) Long-Term Intervention Monitoring (LTIM) project. In 2014/15, ~581 GL of Commonwealth environmental water was delivered to the South Australian section of the Murray River (herein, LMR). The flow releases to South Australia (SA) were coordinated through a series of watering events across the southern connected Basin to achieve multi-site environmental outcomes. Environmental watering helped to maintain river flow at 9,000–10,000 ML day^{-1} during October and November 2014, and again from mid-January to mid-March 2015 in the LMR. Environmental watering also supplemented freshwater flows to the Lower Lakes and Coorong from September 2014, with Commonwealth environmental water contributing to 100% of barrage releases between November 2014 and June 2015.

Seven indicators were selected to evaluate the effects of Commonwealth environmental water on abiotic or biotic components in the main channel habitat of the LMR Selected Area. Category 1 indicators aimed to evaluate Basin-scale objectives and outcomes, as well as local (Selected Area) objectives if appropriate, while Category 3 indicators aimed to address local evaluation questions. These seven 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 2014/15 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.

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 2014/15. 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.

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? There was no clear influence of CEW on gross primary production. Ecosystem net production summed to zero indicating a close balance between production and decomposition of organic material i.e. little external food resource supply and all food resources produced in-channel were utilised. • Patterns and rates of decomposition? Enhanced respiration rates were associated with return flows from the Chowilla Floodplain in mid-November indicating increased supplies of organic material to the river. • Dissolved oxygen levels? Reductions in oxygen concentration were associated with increased respiration rates. These were not lethal to biota but demonstrated the potential dis-benefits if water quality of supplies are not considered along with flow volumes.	Flow remained within the main channel and gross primary production (GPP) and ecosystem respiration were closely related giving an ecosystem net production near zero. This indicates that despite short-term inputs of organic material from the Chowilla Floodplain, the overall supply of externally derived organic material was small. GPP increased with higher incident light intensities but correlations were weak suggesting that other factors also influenced GPP such as water clarity modifying the underwater light intensity, or nutrient supply. Increased respiration rates were associated with the return of organic material in return water from the Chowilla Floodplain. Although a short-lived effect, it indicated the potential benefits of preconditioning flows to enhance external inputs of organic material downstream. Reductions in oxygen concentrations due to increased inputs of organic material from Chowilla Floodplain were small due to the managed flow conditions but showed the potential that exists for dis-benefit from over-supply of organic material.

Category 1: Fish (channel)	This indicator was not evaluated at the local scale (Selected Area). It was designed to provide data for Basin-scale evaluation.	Native bony herring and carp gudgeons dominated the large-bodied and small-bodied fish assemblages, respectively. Other small-bodied species sampled included the invasive Gambusia and native unspecked hardyhead and Murray rainbowfish. Other large-bodied species sampled included the invasive common carp and natives golden perch, Murray cod, freshwater catfish and silver perch.
Category 3: Hydrological Regime	What did CEW contribute to: • Help increase hydraulic diversity within weir pools? Increased median velocity up to 0.07 m s^{-1} over the year compared to without CEW, with some cross sections in the weir pool transforming into moderate-flowing habitat for large-bodied fish. • Help increase water levels within weir pools? Increases in water levels in weir pools of up to 0.2 m in the upper reaches. Periodic increases in water levels could improve the condition of riparian vegetation and increase biofilm diversity.	The increase in flow (discharge) to SA due to CEW from 5,200–6,700 ML day^{-1} to 9,000–10,000 ML day^{-1} from October to November 2014 and again from January to March 2015 resulted in an increase in water level in the order of 0.2 m in the upper reaches of each weir pool. Over the same period, CEW contributed to the increase in median velocity in the weir pool generally from ~0.1 m s^{-1} to ~0.15 m s^{-1} depending on the weir pool (lower weir pools tended to have slightly slower velocities). Velocities for some cross sections in the LMR increased into the range representing moderate-flowing habitat for large-bodied fish due to CEW.
Category 3: Matter Transport	What did CEW contribute to: • Patterns and rates of primary productivity? Increased transport of nutrients, which would likely stimulate primary and secondary productivity in the Murray River Channel, Lower Lakes, Coorong and Southern Ocean. Increased transport of phytoplankton. • Salinity regimes? Reduced salinity concentrations in the Murray Mouth.	The modelling suggests that environmental water impacted positively on the concentrations of dissolved and particulate matter. This was observed through: • A reduction in salinity in the Murray Mouth, with median salinities of 26.70 PSU with all water compared to 34.02 PSU without CEW. • Minor differences in the nutrient concentrations, with the most apparent differences being higher ammonium, silica, and particulate organic nitrogen concentrations within the Murray Mouth with CEW. This suggested that CEW provided nutrients that may have been available to support increased productivity in the Coorong.

	Increased export of salt from the Murray River Channel and Lower Lakes, and decreased net import of salt to the Coorong.	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, with CEW contributing 21% and 64% 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 157,852 tonnes with all water during 2014/15, but without CEW the modelling suggests this would have been 3,202,552 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 29%, 48% and 51% of the total silica exports from the Murray River Channel, Lower Lakes and Murray Mouth, 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 ecosystems. As such, the increased export of silica associated with CEW would be expected to support increased secondary productivity in the Murray River Channel, Lower Lakes, Coorong and near-shore environment. • Increased exports of phytoplankton biomass from the Murray River Channel, Lower Lakes and Murray Mouth. This would be expected to provide 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? Increased microinvertebrate diversity at Lock 6 in November 2014 due to the return of water from Chowilla Floodplain to the main channel. The Living Murray (TLM) environmental water was allocated for floodplain inundation, supported by CEW delivery through the main channel.	Differences in microinvertebrate diversity between all sampling events at sites downstream of 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 (including CEW) sources, thereby increasing taxonomic diversity. Population increases downstream of Lock 6 are likely to have been triggered by TLM environmental water diverted to Chowilla Floodplain, with subsequent and significant contributions to main channel microinvertebrate assemblages. Population increases downstream of Lock 1 were associated with return of water from flooded littoral margins following weir pool raising.
Category 3: Fish Spawning and Recruitment	What did CEW contribute to: • Native fish reproduction? Delivery of CEW from October to December 2014 corresponded with limited spawning of golden perch in the LMR. • Native larval fish growth and survival Following limited spawning of golden perch in the LMR in 2014/15, recruitment to young-of-year (age 0+) was negligible.	CEW contributed to low level of golden perch reproduction (spawning) in the LMR and upstream of the Selected Area. However, there was negligible recruitment of golden perch to young-of-year (age 0+). Golden perch populations in the LMR were dominated by 4+ and 5+ year old fish spawned in the Darling River in 2009/10 and the Murray and Darling Rivers in 2010/11. Moderation and fragmentation of flows between the mid and lower River Murray in spring–summer 2014 potentially mitigated spawning and recruitment of golden perch in the LMR.

Key learning and management implications

Based on insights provided by the studies through the spring/summer 2014/15 monitoring, the following points should be considered with regard to environmental water planning and management in the LMR:

- Environmental water delivery can increase hydraulic diversity (velocities and water levels), potentially leading to ecological benefits from the increased range of habitats. Based on the unregulated flow event early in 2014/15, modelling showed that increasing flows up to 18,000 ML day^{-1} resulted in velocities exceeding the level representing moderate-flowing habitat for large-bodied fish (greater than 0.18 m s^{-1}) throughout the whole LMR. Such magnitude could potentially be achieved through environmental water delivery on the top of an existing flow pulse.
- 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 input and primary productivity. Connectivity will also facilitate the transport and dispersion of aquatic biota (e.g. microinvertebrates, fish larvae) to, and throughout, the LMR, leading to increased species diversity (as observed for microinvertebates in this study) and potentially enhanced recruitment.
- The source of environmental water is important, as it influences the ecological processes and biological response. For instance, floodplain return flows were associated with reductions in dissolved oxygen concentrations. Although these occurrences in 2014/15 were not lethal, they showed potential that exists for negative impacts to aquatic biota if water quality is not considered along with flow.
- Maintaining the integrity (i.e. magnitude, variability and source) of flow from upstream (e.g. Darling 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 fish species at >14,000 ML day^{-1}).

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). Natural flow regimes play a critical role in maintaining ecological integrity of floodplain rivers (Junk *et al.* 1989; Poff *et al.* 1997; Puckridge *et al.* 1998; Lytle and Poff 2004). Therefore, ecological restoration for river systems often involves environmental flow use to re-establish key components of the natural flow regime in order to restore important ecological processes and rehabilitate the ecosystem components (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 Murray–Darling Basin (MDB) is a highly regulated river system, particularly in the southern Basin, where the natural flow regimes have been substantially modified, leading to decreased hydrological variability, increased water level stability 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 heavily modified by a series of low-level (<3 m) weirs constructed in the 1930s–1940s, changing a connected flowing river to a series of weir pools (Walker 2006). The hydrological condition has been further exacerbated by upstream diversions and increased extraction. These have had a profound impact on riverine processes and the ecological community (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 flows (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. The distribution and abundance of all aquatic biota is influenced by the flow regime which plays an overarching role in driving riverine ecosystem structure and function (Poff and Allan 1995; Sparks *et al.* 1998).

During the decadal drought in the MDB (2001–2010) (Figure 1), the ecosystem of the LMR was under severe stress; much of the biota declined and the resilience of the ecosystem was compromised (e.g. Noell *et al.* 2009; Nicol 2010; Zampatti *et al.* 2010). Since the drought broke in 2010/11, increased flow (both flood and in-channel flow) has led to some positive responses, contributing to ecological recovery (Ye *et al.* 2014; 2015a; 2015b).

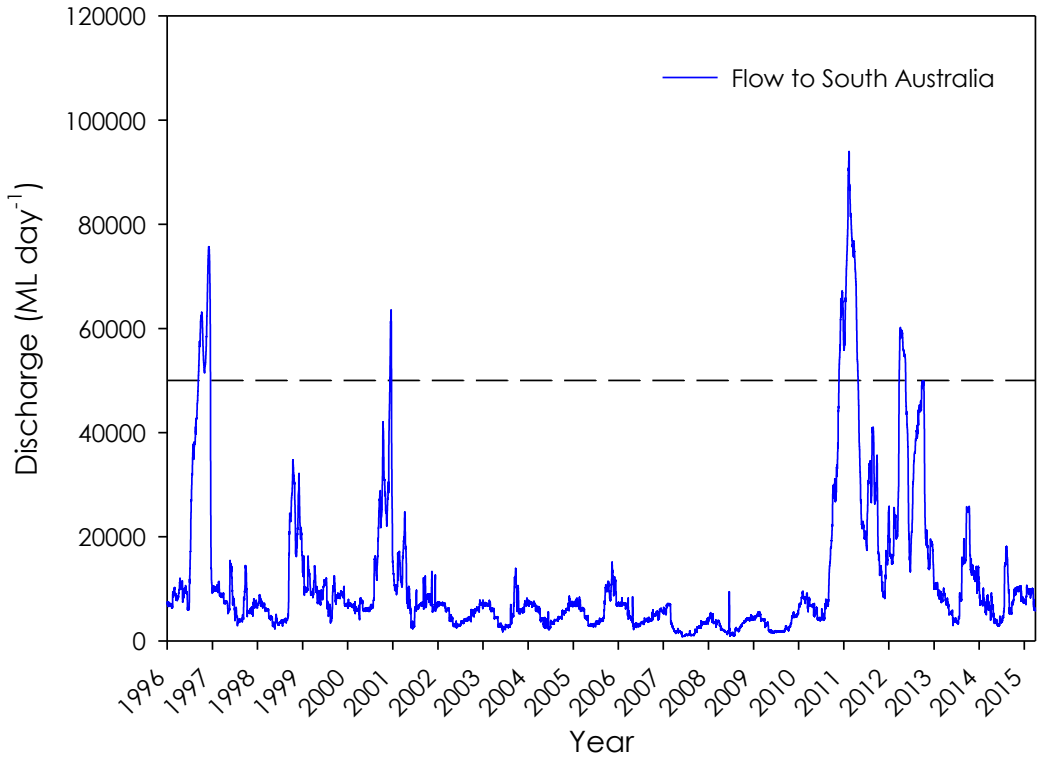

Figure 1. Daily flow (ML day^{-1}) in the LMR at the South Australian border from January 1996 to April 2015. Dotted line represents approximate bankfull flow in the main channel of the LMR.

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), to facilitate ecosystem recovery post drought and restore ecological health (www.environment.gov.au/water/cewo). Some of these flow releases to South Australia (SA) have been coordinated through a series of 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). Short-term intervention monitoring of responses to environmental flows delivered from 2011 to 2014 have demonstrated the ecological benefits of environmental water delivery in the LMR (Ye *et al.* 2015a; 2015b).

In 2014/15, ~581 GL of Commonwealth environmental water were delivered to the LMR. Following the end of unregulated flow (green peak in Figure 2), there was a series of environmental water deliveries that maintained river flow between 7,500 ML day^{-1} and 10,000 ML day^{-1} between October 2014 and March 2015. From April 2015, flows reduced from 6,500 ML day^{-1} to 4,000 ML day^{-1}. Without environmental water, flow to SA would have been at entitlement flow (which includes 119 GL of Commonwealth environmental water) of 4,500 ML day^{-1} in April and 3,000 ML day^{-1} in May and June (prior to any trade adjustment). Environmental watering also supplemented freshwater flows to the Lower Lakes and Coorong from September 2014, with Commonwealth environmental water contributing to 100% of barrage releases between November 2014 and June 2015. Commonwealth environmental water was delivered in conjunction with The Living Murray flows, return flows from Victorian Environmental Water Holder watering actions in Victorian tributaries and consumptive deliveries.

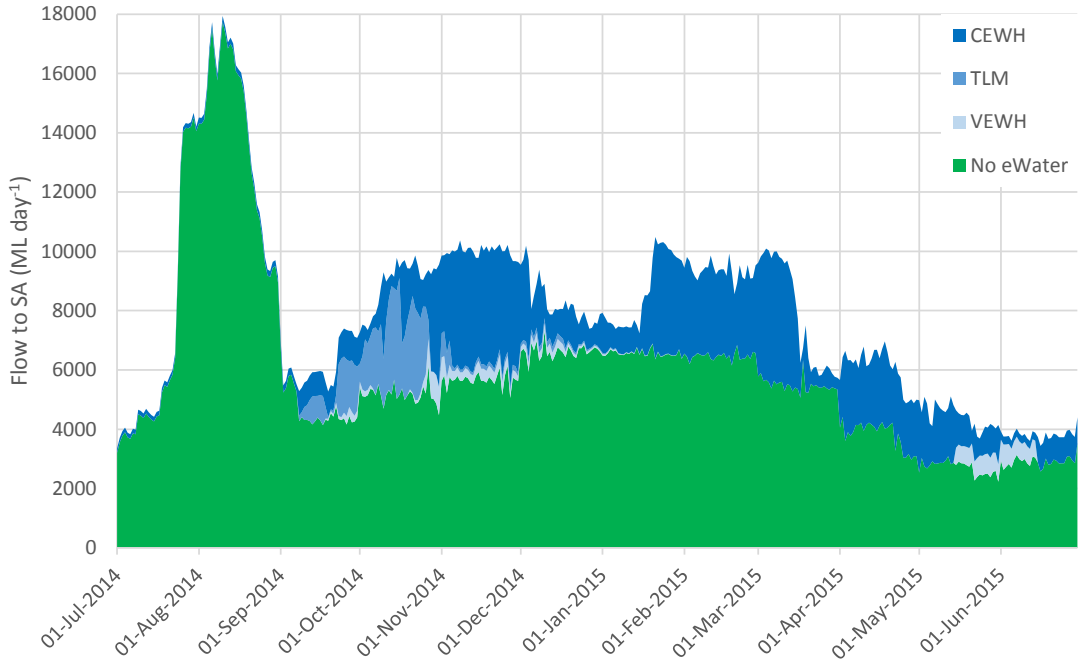

Figure 2. Flow to South Australia from July 2014 to June 2015. CEWH = Commonwealth Environmental Water Holder; TLM = The Living Murray; VEWH = Victorian Environmental Water Holder.

While environmental water delivery occurred throughout the year, there were three main watering events during 2014/15, including: 1) September to mid-January: 357 GL of environmental water delivered, of which the Commonwealth Environmental Water Holder (CEWH) contributed 67%; 2) Mid-January to March: 198 GL of environmental water delivered, of which CEWH contributed 100%; and 3) April to June: 153 GL of environmental water delivered, of which CEWH contributed 87% (Figure 3).

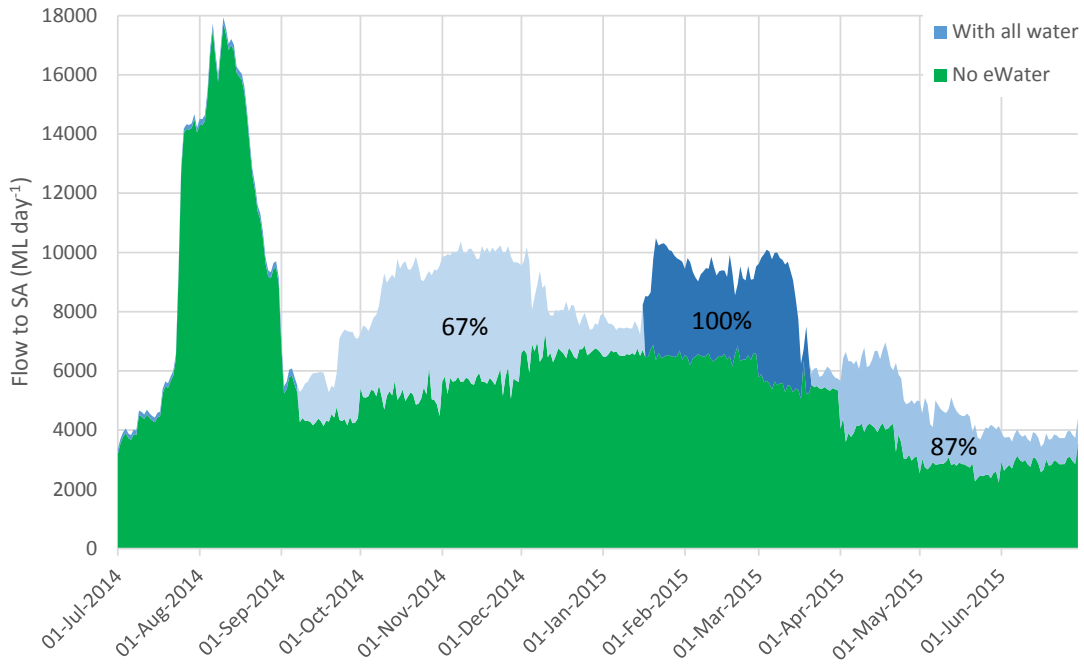

Figure 3. Commonwealth environmental water contribution to main watering events in 2014/15. Shading of the blue environmental water area represents the proportion of Commonwealth environmental water.

The original source of the water arriving in SA can also affect the environmental response. For instance, changing the source of the water can alter the amount and form of nutrients that enter a water body, which can be further influenced by river operation through altering nutrient cycling processes. As such, the amount and composition of primary producers (e.g. phytoplankton) could be affected, thus leading to potential changes in secondary productivity and the structure of the aquatic food web. Managing source flow and river operation to improve connectivity could also facilitate biological dispersion (e.g. larval fish transport and juvenile dispersion); thus, enhance recruitment in the LMR (*Ye et al.* 2015b). The sources of flow to SA in 2014/15 can be seen in Figure 4. The components of flow

were estimated by the MDBA by assessing flow contributions upstream from the main channel, tributaries and in some instances change in water in storage. The upstream contributions, appropriately lagged, were aggregated to give the total of all the upstream components. These lags are based on observed changes in flow along the main channel, and it should be noted that the travel time of actual water molecules and other substances within flow from upstream locations could be expected to arrive substantially later and more attenuated than presented in Figure 4.

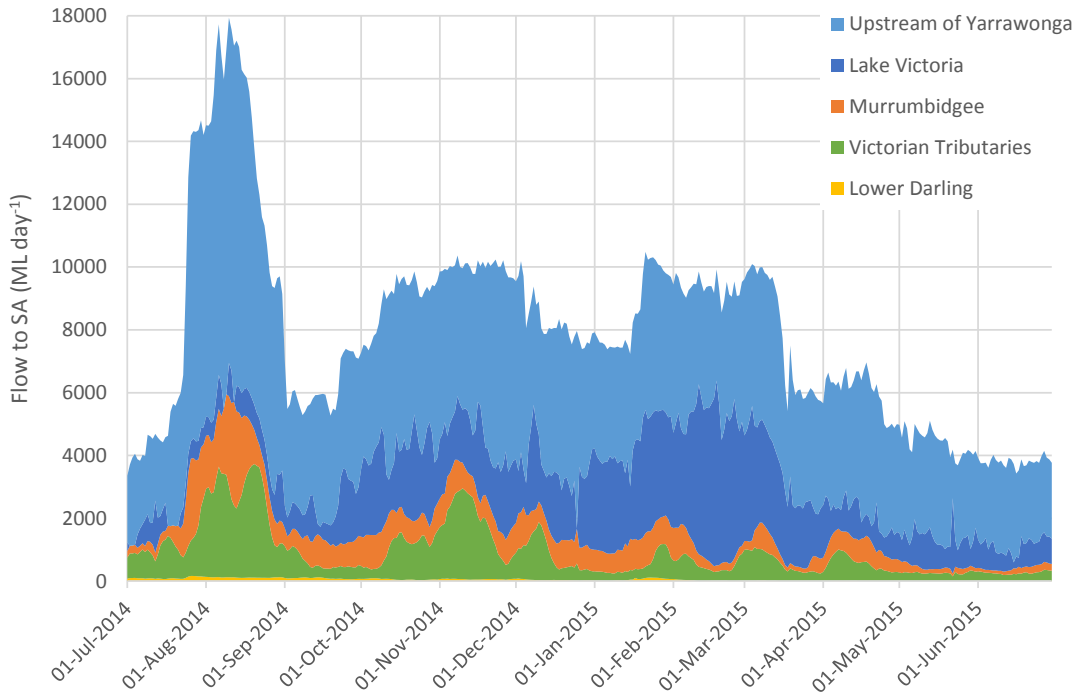

Figure 4. Source of all (environmental and consumptive) water delivered to the South Australian border (MDBA). Caveats for estimated water delivery time are mentioned above.

Concurrently with these environmental water deliveries, there were other flow management interventions in SA between late winter and early summer 2014/15, which may have affected ecological responses in the LMR Selected Area. These included raising of Weir Pools 1 (between Lock 1 and 2) and 2 (between Lock 2 and 3), and the artificial inundation of the Chowilla Floodplain (~2,300 ha) (refer to Appendix B for more detail).

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 project aims to demonstrate the ecological outcomes of Commonwealth environmental water delivery and support adaptive management.

CEWO LTIM 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 5). 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);

To assess ecological responses to environmental water delivery, a number of indicators were identified for the LMR, including:

Category 1

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

Category 3

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

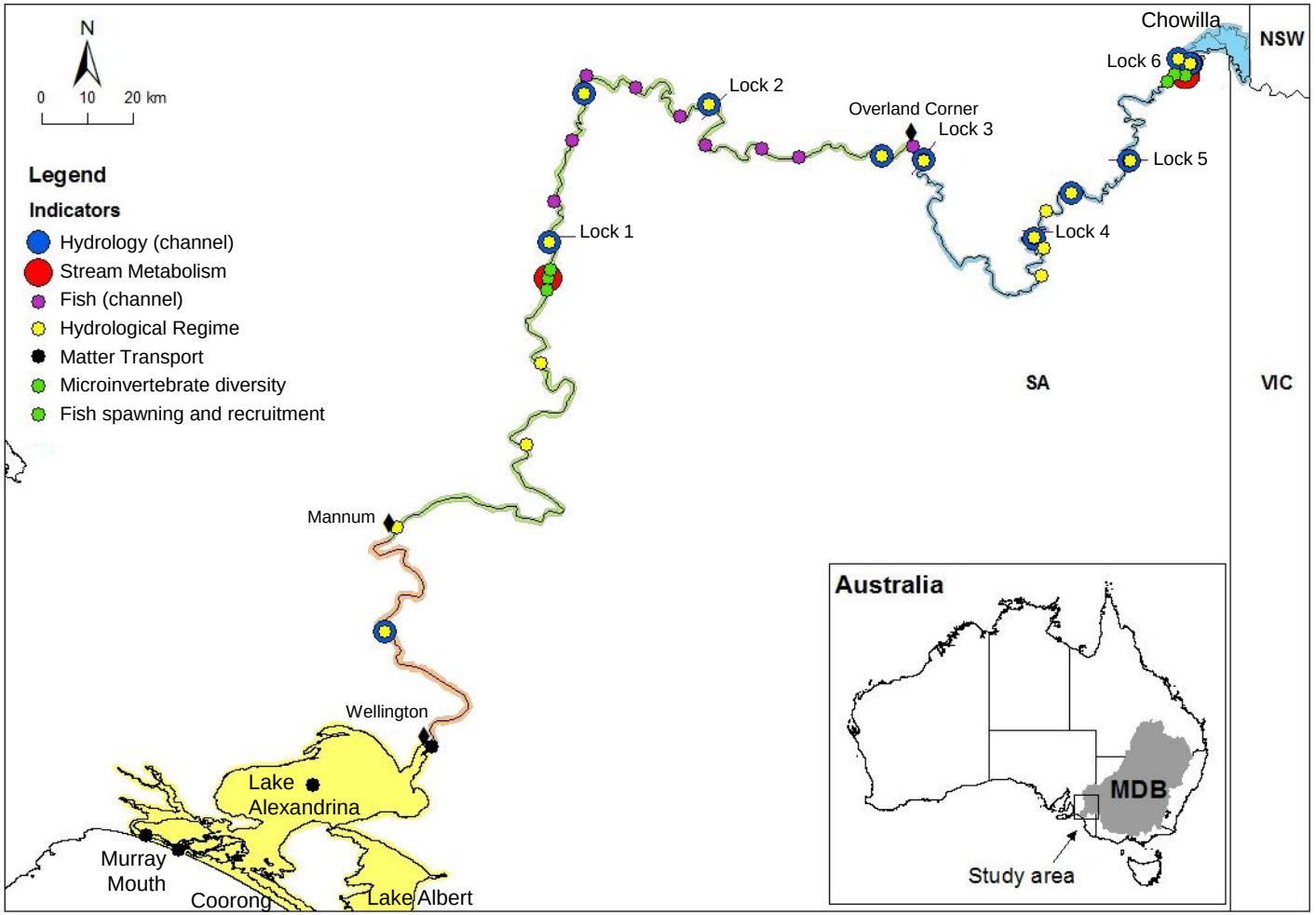

Figure 5. Map of the LMR Selected Area showing the floodplain (blue), gorge (green) and swamplands (orange) geomorphic zones, and the Lower Lakes, Coorong and Murray Mouth (yellow). Sampling sites are indicated by coloured circles. Fish spawning and recruitment sites represent larval sampling only.

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 http://www.environment.gov.au/water/cewo/publications/cewo-ltim-lower-murray. 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 the selected indicators contribute toward a holistic understanding of ecosystem responses to flow management and ecological benefits (Figure 6).

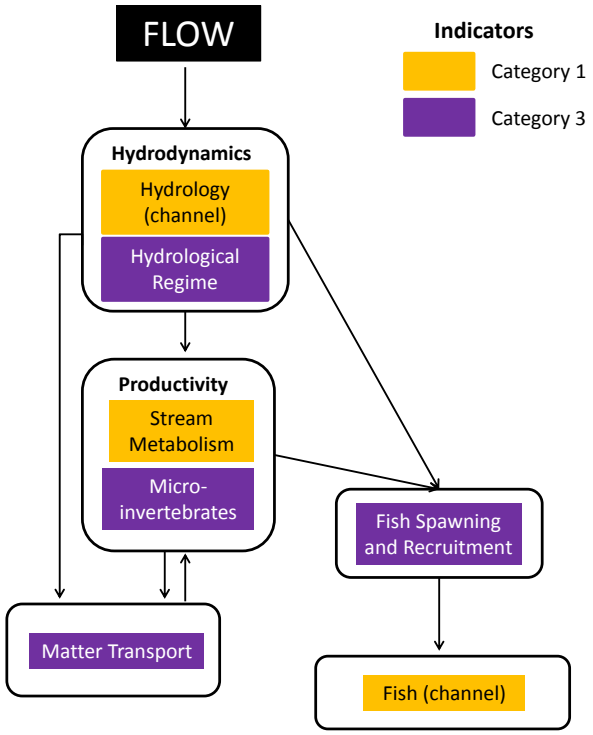

Figure 6. Conceptual diagram of how the main channel of river systems are affected by the key ecosystem driver (flow regime), subject to flow management and climate effects, and how complementary monitoring components (indicators) contribute toward a holistic understanding of ecosystem responses to flow management and ecological benefits in the LMR Selected Area. Magnitude, timing and duration are factors of flow (in black).

This synthesis report presents a summary of the key findings of each indicator for the LMR Selected Area (Section 2), with regards to CEWO short-term (one-year) evaluation questions (Section 3). Category 1 Hydrology (channel) does not directly address specific evaluation questions, 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. There is no Selected Area evaluation for the Category 1 Fish (channel) indictor. Basic summary statistics of the catch rates and population demographics for this indicator are presented in Section 2.1, while the basin-scale evaluation for fish community responses to Commonwealth environmental water will be carried out by the 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 time. 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 photosynthesis and respiration 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. Net production, 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).

In situ logging of the dissolved oxygen concentration, water temperature and incident irradiance required for estimating stream metabolism were undertaken at single river sites in the gorge (downstream of Lock 1) and floodplain (downstream of Lock 6) geomorphic zones of the LMR Selected Area in 2014/15 (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).

Oxygen concentration time series

Over the monitoring period the dissolved oxygen concentration ranged between 7 and 10 mg L^{-1}; the higher values indicating that at times significant photosynthetic production increased the oxygen concentration above saturation levels, reflecting an enhanced biomass of phytoplankton. Conversely, for a period of a few days between 3–6 February 2015, the oxygen concentration at the site downstream of Lock 6 appeared to reduce considerably with levels overnight falling to 5.2 mg L^{-1} (Figure C1 in Appendix C). This period was not associated with alterations in the incident irradiance (light intensity) that might have reduced photosynthetic oxygen production and caused a shift in the oxygen balance. There was a fall in temperature of ca. 2 °C from 31 January to 4 February 2015 and then an increase of 2 °C up to 11 February 2015, but these would not account for the large reductions in the dissolved oxygen concentration. The mean oxygen concentrations from an upstream site maintained by DEWNR (Custom's House, A4261022) over this period were of similar magnitude to the mean oxygen concentrations measured downstream of Lock 1, indicating that the low oxygen concentrations observed at the intermediate sampling site at Lock 6 (Figure C2 in Appendix C) were due to local effects if real, or alternatively due to fouling of the sensor or a fault with the probe. Water quality data from sites upstream and downstream of Lock 6 were scrutinised, but no explanation for a fall in oxygen to these low levels could be identified.

Oxygen concentrations at the site downstream of Lock 6 also declined rapidly from 12–18 November 2014, although concentrations did not fall below 7 mg L^{-1}. This event aligned with the return of water from the Chowilla Floodplain at the end of the testing period for the new regulator (Appendix B). This low-oxygen water was measured passing the site downstream of Lock 1 about two weeks later (Figure C1 in Appendix C). Although the dissolved oxygen concentrations did not fall to levels that might be considered harmful to the aquatic biota, the data indicates the potential for transfer of poorly oxygenated environmental water that in severe circumstances could impact the biota. This highlights the need to manage water quality, as well as flow.

Metabolism

The general patterns of metabolic activity were similar at both sampling sites (Figures C3 and C4 in Appendix C), with steady rates until about 14 January 2015 when both gross primary production (GPP) and ecosystem respiration (ER) increased in magnitude. At the site downstream of Lock 6, the increases in GPP and ER continued until 8 February and then rates began to decline. At the site downstream of Lock 1, increases in GPP and ER continued until about the 20 February and then declined. These patterns reflected a complex function of seasonal changes in incident sunlight and temperature, decreases in river turbidity, and changing phytoplankton concentrations. Despite fluctuations in GPP and ER the metabolic rates were virtually mirror images of each other so that ecosystem net production (ENP) oscillated around zero (Figures C3 and C4 in Appendix C), indicating that virtually all energy was produced and consumed within the river main channel.

No simple relationships between metabolic activity and river flow were evident in the data with flow sometimes undergoing major changes without influencing metabolism (Figures C6 and C7 in Appendix C). At both sampling sites, flow and velocity were closely correlated due to the simple channel shape. Velocity at the site downstream of Lock 6 was always less than 0.25 m s^{-1} which is in the range where direct effects of velocity are small and the reduced turbulence results in physico-chemical structuring of the water column that influences phytoplankton production (Oliver and Merrick 2006; Oliver and Lorenz 2010). Downstream of Lock 1 velocities were an order of magnitude lower and their variation due to flow was unlikely to directly influence metabolic responses. The low velocities at both sites provided conditions for enhanced accumulation of microbial populations and increased metabolic activity compared to conditions at higher velocities.

At the site downstream of Lock 6, two periods of enhanced ER resulted in large negative rates of ENP; one in mid-November 2014 that was associated with the water being released back from Chowilla Floodplain to the river channel through the Chowilla regulator, and a second in early February that coincided with the period of reduced oxygen concentrations likely associated with probe malfunctioning.

Despite these occasional disruptions to the opposing patterns of GPP and ER, the integrated values over the monitoring period were similar between sites with ENP close to zero (Figure C8 in Appendix C). These findings are similar to those previously reported for flowing sections of the river (Oliver and Merrick 2006), including downstream of weirs (Oliver and Lorenz 2010). The zero ENP suggests that food resources were largely produced in-stream and fully utilised.

The enhanced rates of ER and large negative values for ENP observed downstream of Lock 6 in mid-November indicated transport of metabolisable organic material back into the river in return water that had been pre-conditioned by its slow passage across the Chowilla Floodplain. Intermittent periods of increased supplies of organic material like this are thought to be critical to the food webs of rivers and their decline in frequency, duration and extent has been proposed as a major cause of reductions in populations of aquatic biota due to the decline in food supplies (Oliver and Merrick 2006; Oliver and Lorenz 2010).

Fish (channel)

Small-bodied and large-bodied fish assemblages in the gorge geomorphic zone of the LMR Selected Area (Figure 5) were sampled using fine-meshed fyke nets and electrofishing, respectively. Sampling occurred during March/April 2015, following standard methods outlined in Hale *et al.* (2014). Population structure (i.e. length and age) data were obtained for six target species (Figure 7). Refer to the LMR LTIM M&E Plan for detailed sampling design and methodology.

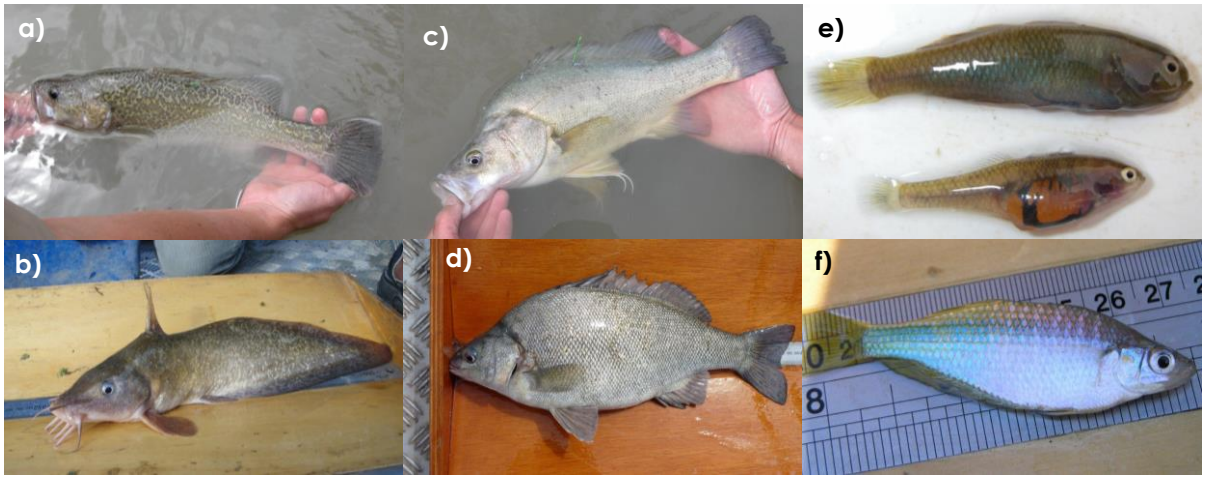

Figure 7. Target species for the LMR Selected Area: (a) Murray cod and (b) freshwater catfish (equilibrium life history); (c) golden perch and (d) silver perch (periodic life history); and (e) carp gudgeon and (f) Murray rainbowfish (opportunistic life history).

Catch summary

A total of 9,797 individuals from eight large-bodied species were sampled by electrofishing from ten sites in the gorge geomorphic zone (Table C1). Bony herring was the most abundant species (29.6 ± 3.5 individuals per 90 second shot) and dominated electrofishing catch composition (97%) (Figure 8a). Golden perch and common carp were the second and third most abundant species, respectively.

A total of 21,036 individuals from seven small-bodied species were sampled by fyke nets from ten sites in the gorge geomorphic zone (Table C1). Carp gudgeon was the most abundant species (9.8 ± 3.1 individuals per net per hour) and dominated fyke net catch composition (89%) (Figure 8b). Gambusia, unspecked hardyhead and Murray rainbowfish were the second, third and fourth most abundant species, respectively.

Population structure

Golden perch sampled in the gorge geomorphic zone ranged in total length (TL) from 112–525 mm and, in age, from 2+ to 18+ years (Figure D1 in Appendix D). Age 4+ (35%), 5+ (25%) and 18+ (13%) cohorts comprised most of the catch. Silver perch and freshwater catfish were sampled in low numbers (*n* = 4 and 6, respectively). Silver perch ranged in age from 2+ to 5+ years, whilst freshwater catfish ranged in age from 5+ to 9+ years. Young-of-year (0+ year) Murray cod (103–145 mm TL) dominated the catch composition of the species (*n* = 10), with one large individual

(1310 mm TL) also captured. Carp gudgeons and Murray rainbowfish ranged in TL from 14–55 mm and 16–75 mm, respectively, with all aged individuals aged 0+.

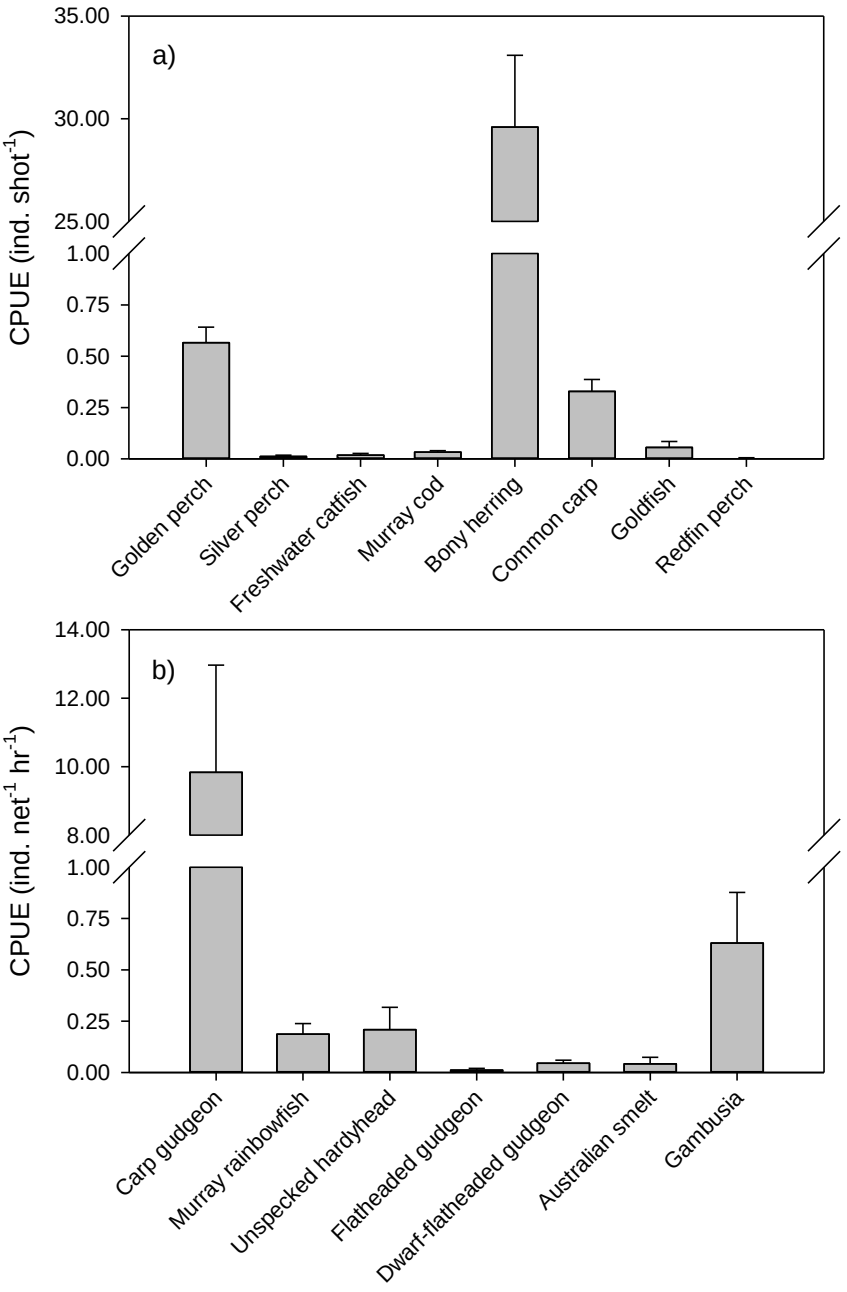

Figure 8. Mean catch-per-unit-effort (CPUE) ± standard error of a) large-bodied fish species captured using electrofishing (individuals per 90 second shot) and b) small-bodied fish species captured using fine-mesh fyke nets (individuals per net per hour) in the gorge geomorphic zone (10 sites) of the LMR Selected Area.

2.2 Category 3

Hydrological Regime

Increase in discharge alone can be difficult to relate directly to ecological response, as it is the corresponding change in hydraulic variables such as velocity and water level that trigger the observed response. The Hydrological Regime indicator used models to convert the discharge delivered to the LMR Selected Area in 2014/15 to water levels and velocities. Such information was provided for the observed (with all water, including environmental water) case, as well as allowing the without environmental water case to be simulated. The models were calibrated to observed discharge, water level and velocity measurements, to ensure they provide an accurate representation of reality. Details of the models and calibration are presented in Appendix E.

Water level and velocity results for a weir pool in the gorge (Weir Pool 1, Lock 1 – Lock 2) and floodplain (Weir Pool 4, Lock 4 – Lock 5) zones (Figure 5) are presented in Figures 9 and 10. The increase 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). It has been assumed that lock operations would not have changed in 2014/15 due to the provision of environmental water, and the observed lock levels have been used as inputs to the models. This observed data includes the weir pool raisings undertaken at Locks 1 and 2 in 2014/15.

The water level at the upper end of the weir pool (e.g. directly downstream of 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.

As noted above, it has been assumed that lock operations would not have changed due to the delivery of environmental water, and as such there is no difference in water level in the environmental water scenarios at the most downstream end of the weir pool. However, a higher discharge with environmental water, flowing through the same cross-sectional area, results in increased velocities

due to the environmental water. Results for other locations are presented in Appendix E.

The increase in water levels and velocities due to the unregulated flow event (up to 18,000 ML day^{-1}) in August can be seen in Figures 9 and 10, where there was almost no difference between the scenarios presented. This resulted in the highest water levels that occurred during 2014/15, and an increase in velocity greater than that representative of moderate-flowing habitat for large-bodied fish all along the LMR (greater than 0.18 m s^{-1}, Mallen-Cooper *et al.* 2011).

The increase in discharge due to Commonwealth environmental water delivery from 5,200 – 6,700 ML day^{-1} to 9,000 – 10,000 ML day^{-1} between October and November 2014 and between January and March 2015 (Figure 2) resulted in an increase in water level in the order of 0.2 m. Over the same period, the Commonwealth environmental water can be seen to increase the median velocity in the weir pool, generally from ~0.1 m s^{-1} to ~0.15 m s^{-1}; the maximum increase was by up to 0.07 m s^{-1}, depending on the weir pool (lower weir pools tended to have slightly slower velocities). Some cross sections in the weir pool increasing into the range representing moderate-flowing habitat for large-bodied fish (Mallen-Cooper *et al.* 2011), shown as the blue shaded area between 0.18 and 0.3 m s^{-1}.

Given that variability in hydraulics is how many biota perceive changes in flow volumes, hydraulic changes, such as increased velocities, may stimulate ecological responses that lead to ecological benefits. For example, increased velocities may provide cues for reproductive activity in flow-cued spawning fish species (e.g. golden perch), facilitate downstream drift and transportation of larvae to favourable nursery habitats, and provide more suitable hydraulic habitats for certain species (e.g. Murray cod) (Zampatti *et al.* 2014), all of which are likely to be integral to the population dynamics and recruitment success of these species.

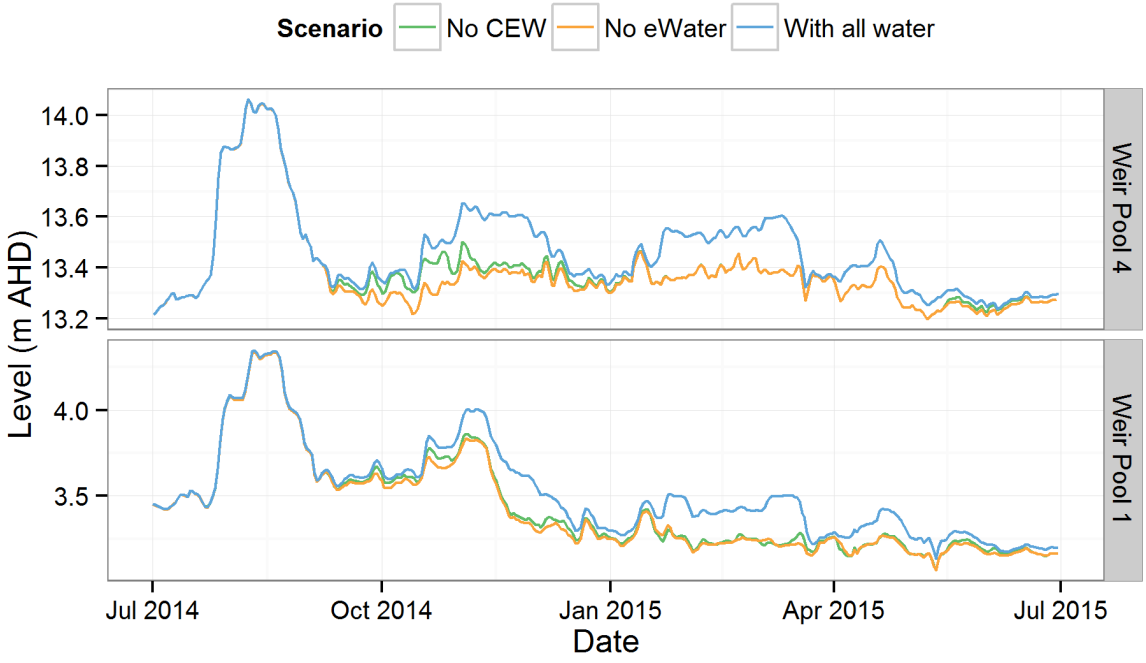

Figure 9. Water levels at the upper end of the weir pool for a weir pool in the floodplain zone (Weir Pool 4) and gorge zone (Weir Pool 1) representing observed conditions (With all water), without Commonwealth environmental water (No CEW) and without any environmental water (No eWater) for 2014/15. The upper end of the weir pool is most responsive to changes in discharge.

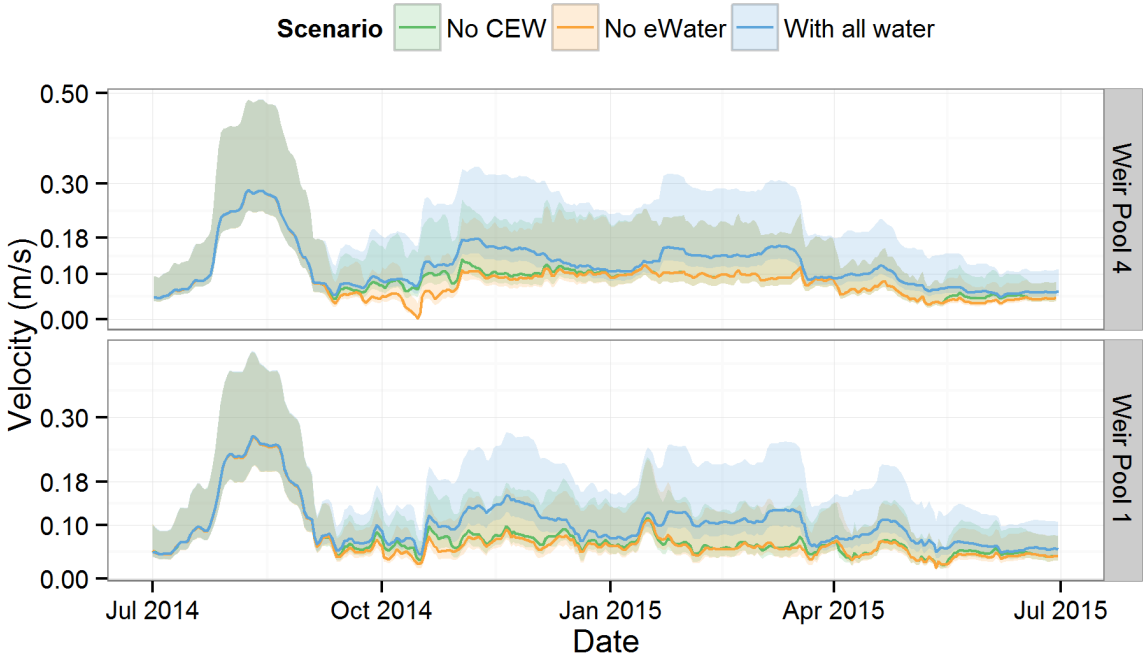

Figure 10. Cross section averaged velocities in a weir pool in the floodplain zone (Weir Pool 4) and gorge zone (Weir Pool 1) representing observed conditions (With all water), without Commonwealth environmental water (No CEW) and without any environmental water (No eWater) for 2014/15. The median velocity 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 had significant consequences for 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 concentrations 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 concentrations 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 set-up and 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 are not treated as 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 2014 to 30 June 2015:

- With all water (i.e. observed, including all environmental and consumptive water);
- Without Commonwealth environmental water; and
- Without any environmental water.

Salinity

The modelling suggests that environmental water had no effect on salinity levels in the Murray River Channel (i.e. Wellington) in 2014/15 (Table 2). Similarly, there was only a minor effect within Lake Alexandrina, with median salinities over 2014/15 of 0.30 PSU with all water compared to 0.34 PSU without any environmental water. However, within the Murray Mouth, salinity was reduced significantly as a result of environmental water delivery, with median salinities of 26.70 PSU with all water

compared to 34.02 PSU without Commonwealth environmental water and 35.02 PSU without any environmental water.

Based on the modelling outputs, environmental water increased salt exports from the Murray River Channel and Lower Lakes (Table 3). Environmental water contributed to 96,284 tonnes (27%) and 294,449 tonnes (66%) to the total modelled export from the Murray River Channel and Lower Lakes, respectively. Commonwealth environmental water contributed to 21% and 64% of the total modelled export from the Murray River Channel and Lower Lakes, respectively. There was a modelled import of 157,852 tonnes of salt to the Coorong during 2014/15 with all water. However, without any environmental water there was a modelled import of 5,048,511 tonnes of salt to the Coorong and without Commonwealth environmental water there was a modelled import of 3,202,552 tonnes.

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 ammonium and silica concentrations within the Murray Mouth, suggesting that Commonwealth environmental water provided nutrients that may have been available to support increased productivity within the Coorong. Furthermore, there were lower silica concentrations in the Lower Lakes with environmental water, which may have been associated with increased evapo-concentration of silica within the Lower Lakes without environmental water.

Since there were only small differences in concentrations of dissolved nutrients within the Murray River Channel, differences in modelled exports between the scenarios were largely a result of differences in discharge (Table 3). Environmental water increased exports of all dissolved nutrients from the Murray River Channel and Lower Lakes and this was also the case from Murray Mouth for ammonium and silica. For phosphate, environmental water decreased the import to the Coorong from the Southern Ocean. The most apparent differences in exports associated with environmental water were for silica, with all environmental water contributing to 34%, 59% and 61% of total exports from the Murray River Channel, Lower Lakes and Murray Mouth, respectively. Commonwealth environmental water contributed to

29%, 48% and 51% of total silica exports from the Murray River Channel, Lower Lakes and Murray Mouth, 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 would be expected to support increased secondary productivity along the Murray River Channel, Lower Lakes, Coorong and near-shore environment.

Particulate organic nutrients

There were only small differences in the modelled particulate nutrient concentrations with all water and without environmental water, particularly given uncertainties associated with the modelled outputs (Table 2; Appendix F). The most apparent difference was for particulate organic nitrogen concentrations, with higher concentrations in the Murray Mouth associated with environmental water. This was associated with the increased inputs of water (including environmental water) from the Lower Lakes, which had higher concentrations of particulate organic matter relative to the Murray Mouth. Similar responses were observed for other nutrients but to a lesser degree. Within the Murray River Channel, Lower Lakes and Coorong, particulate organic nitrogen and phosphorus exports were also higher with environmental water and increased proportionally with discharge (Table 3). Over the study period, the Commonwealth environmental water contributed to 26%, 31% and 34% of the total exports of particulate organic nitrogen from the Murray River Channel, Lower Lakes and Murray Mouth, respectively. For particulate organic phosphorus, the contribution was 26%, 38% and 40%. The increased export of organic nutrients associated with environmental water would be expected to provide benefits for the Lower Lakes, Coorong and near-shore environment by providing energy to support secondary productivity.

Chlorophyll a and suspended solids

There were only minor differences in the modelled chlorophyll *a* concentrations and turbidity levels between scenarios (Table 2; Appendix F). As a result, differences in modelled exports reflected that of discharge, with the additional environmental water delivered resulting in additional exports (Table 3). Overall, Commonwealth environmental water contributed to 35%, 22% and 20% 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 would be expected to provide 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). For total suspended solids, Commonwealth environmental water contributed to 41% and 10% of the total exports from the Murray River Channel and Lower Lakes, respectively, and reduced suspended solid inputs to the Coorong.

Table 2. Median concentration of salinity, nutrients, chlorophyll *a* and turbidity during 2014/15 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)	Turbidity (TSS)
Wellington	With all water	0.14	BDL	BDL	1.08	0.95	0.10	17.4	43.2
	No CEW	0.14	0.008	BDL	1.12	0.98	0.10	17.2	36.4
	No eWater	0.14	0.009	BDL	1.13	0.99	0.10	18.1	34.6
Lake Alexandrina Middle	With all water	0.30	0.035	BDL	1.73	1.30	0.13	12.7	10.1
	No CEW	0.32	0.040	BDL	1.82	1.34	0.13	10.9	7.5
	No eWater	0.34	0.041	BDL	1.87	1.37	0.13	10.7	6.3
Murray Mouth	With all water	26.70	0.020	BDL	1.19	0.82	0.07	3.7	20.4
	No CEW	34.02	0.009	BDL	1.11	0.69	0.06	0.9	22.4
	No eWater	35.02	0.008	BDL	1.09	0.66	0.06	0.5	23.0

Table 3. Net cumulative load (tonnes) of salt, nutrients, chlorophyll a and total suspended solids during 2014/15 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	Chlorophyll *a*	Total suspended solids
Wellington	With all water	362,631	13	6.9	3,081	1,983	189	43	35,658
	No CEW	286,907	11	4.9	2,199	1,471	139	28	24,541
	No eWater	266,347	10	4.3	2,024	1,330	125	25	21,206
Barrage	With all water	446,855	81	0.3	2,401	2,144	170	27	4,323
	No CEW	161,791	48	0.2	1,253	1,488	106	21	3,941
	No eWater	152,406	38	0.2	989	1,283	92	19	3,911
Murray Mouth	With all water	-157,852	78	-0.1	2,115	1,873	147	25	947
	No CEW	-320,2552	53	-0.5	1,036	1,227	88	20	-760
	No eWater	-504,8511	48	-0.6	825	1,095	77	19	-1,403

Microinvertebrates

The importance of aquatic microinvertebrates (protists, rotifers and microcrustaceans) as a major food source for larger organisms in freshwater systems is well recognised (Schmid-Araya and Schmid 2000; Pernthaler and Posch 2009). Availability of suitable microinvertebrate prey for early life stages of fish larvae (i.e. during the switch from endogenous (yolk sac absorption) to exogenous feeding) can determine fish survival and the level of recruitment success (year-class strength). 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). To assess the responses of microinvertebrates to Commonwealth environmental water delivery in the LMR, mid-channel microinvertebrate assemblages were sampled during spring/summer 2014/15 using a Haney trap at sites downstream of Lock 1 and Lock 6, in the gorge and floodplain geomorphic zones, respectively (Appendix G, LMR LTIM M&E Plan).

To preface the comments below on the marked changes in microinvertebrate assemblages downstream of Lock 6/Lock 1, it should be noted that there was little input into the LMR Selected Area from the Darling catchment (Figure 4) during the 2014/15 sampling period. Sources of Commonwealth environmental water were directly traded to SA from upstream of Yarrawonga (ca. 58%), with the balance made up from the Victorian tributaries, and a small proportion of return flows from Hattah Lakes. The occurrence of 'tropical' taxa, i.e. known warm stenotherms, could be from northern environmental water sources, e.g. Murrumbidgee, or represent flood-transported populations maintained since the 2010/11 Darling River floods in off-channel standing waters, e.g. Lake Victoria, Chowilla Floodplain.

Over the 2014/15 sampling period, 183 microinvertebrate taxa were discriminated from 144 trap samples from the gorge and floodplain geomorphic zones of the LMR, including 74 Protista (largely testate rhizopods), 84 Rotifera, 13 Cladocera, 4 Copepoda, 2 Ostracoda, 6 juvenile macroinvertebrates (gastrotrichs, turbellarians, tardigrades, mussel glochidia, chironomids and mites). These are underestimates of species numbers in view of the single aliquot subsampling. Microinvertebrate

diversity and densities are summarised by sampling event by geomorphic zone in Figure 11, averaged across the three sampling sites at each lock. The protist/rotifer-dominated assemblage recorded through the sampling period was typical for the lower River Murray as reported in earlier studies (Shiel *et al.* 1982; Cheshire 2010; Furst *et al.* 2014). Approximately 30% of taxa identified were also listed from the LMR at Mannum (swamplands zone, Figure 5) by Shiel *et al.* (1982).

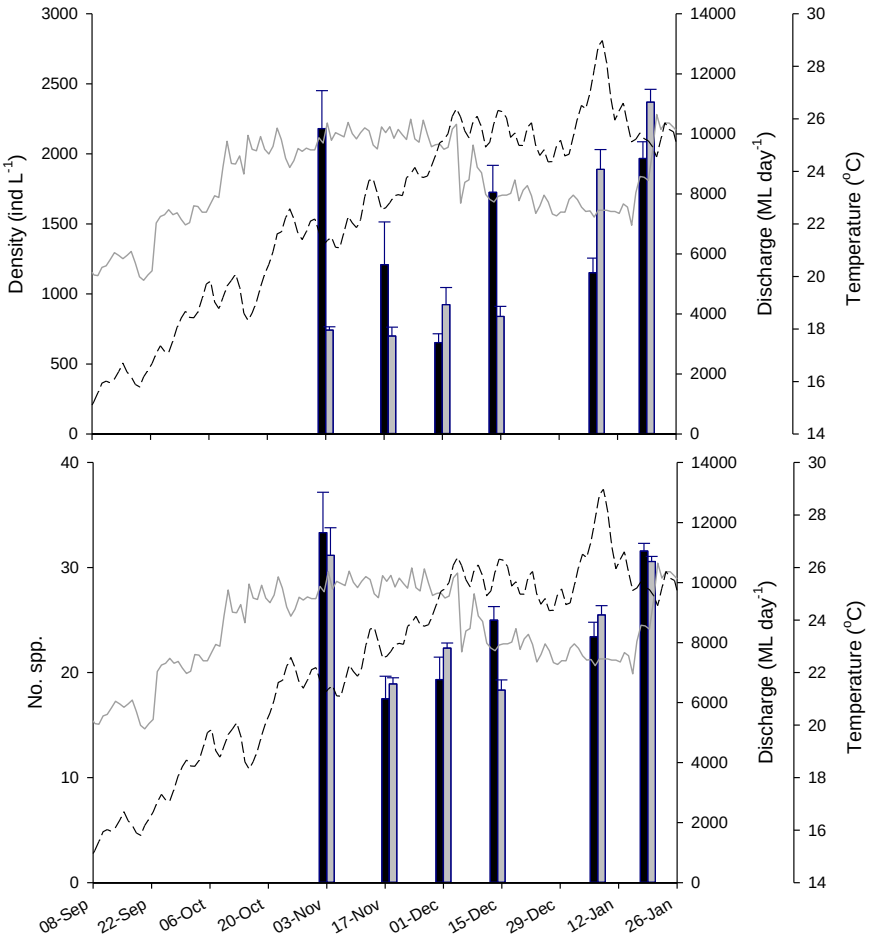

Figure 11. Mean (±S.E.) (a) density and (b) species richness of microinvertebrates collected in the LMR Selected Area at sites downstream of Lock 1 (light bars) and Lock 6 (dark bars) in 2014/15, plotted against discharge (ML day^{-1}) in the LMR at the South Australian border (solid grey line) and water temperature (°C) (dashed black line). Sampling was undertaken approximately fortnightly from 3 November 2014 to 20 January 2015.

Higher densities of testate ciliates, diverse rhizopods, and the presence of standing-water microcrustaceans (cladocerans, including littoral chydorids, and copepods) at Lock 6 (more specifically 7 km below Lock 6) in November were indicative of environmental water-derived floodplain returns to the main channel. As this was the first sampling event it is not possible to determine if the early November peak density

and diversity below Lock 6 represent a recession of environmental water from the Chowilla Floodplain to the main channel (Appendix B), or a declining input to the main channel after an earlier floodplain return. Thereafter, there were reduced microinvertebrate density/diversity at sites below Lock 6 through November to December despite continued above-entitlement flows, which may represent dilution effects (Figure 11). Further Commonwealth environmental water releases in late January 2015 (Figure 2) were associated with marked increases in microinvertebrate density and diversity, with changes in species composition maintaining the separation of sampling events. Microinvertebrate assemblages were more similar in mid-December 2014, early January and mid-January 2014 events than in the first three sampling events (Figure G6 in Appendix G). The later events were dominated by a suite of warm-water taxa, for example brachionid rotifers *Anuraeopsis coelata, Brachionus budapestinensis, B. diversicornis, B. falcatus, K. lenzi,* the trochosphaerid rotifer *Filinia opoliensis,* among others, suggesting seasonal succession with rising water temperature, or water releases (including Commonwealth environmental water) from a northern (NSW) source. Of the disparate microinvertebrate assemblages identified in both geomorphic zones, significant differences (early November) were notable between the site 5 km below Lock 6 and the sites 7 km (Lock 6A) and 9 km (Lock 6B) below Lock 6, which were downstream of Chowilla Creek.

As for downstream of Lock 6, the first three sampling events for sites downstream of Lock 1 were more different than the last three (Figure G8 in Appendix G). Species driving the disparate assemblages between sampling events are detailed in Appendix G. A similar suite of warm-water rotifers dominated the December to January sampling events below Lock 1, with additional taxa possibly derived from weir pools or marginal habitats as the Commonwealth environmental water moved downstream. Population density increases at sites below Lock 1 during January were attributable to downstream passage of the below Lock 6 assemblage, additional taxa collected *en route*, and instream reproduction.

Testates are normally epibenthic/epiphytic, rarely recorded in the plankton. More than 30 testate species were present in trap samples during early November, and, together with a suite of bacterivorous ciliates (*Condonaria, Stenosemella* and others), accounting for the high diversity recorded from Lock 1 and Lock 6 during

the first sampling trip in early November. The 0.4–0.5 m increase in water level of both Lock 1 and Lock 6 weir pools prior to the early November sampling (Appendix B) flooded riparian margins and flushed fringing reedbeds, accounting for the diverse suite of testate amoebae recorded at both Locks. Similarly, rare cladocerans, e.g. *Leberis diaphanus, Pseudomonospilus diporus,* are not plankters, but epiphytic/epibenthic in habit, and their presence in plankton samples from Lock 1 in January and Lock 6 in November, respectively, suggests littoral or riparian source. For Lock 1, it could have resulted from weir pool raising (at Weir Pools 1 and 2), and for Lock 6, it was more likely associated with the regulated inundation of Chowilla Floodplain (Appendix B).

Cool water taxa, e.g. *Keratella quadrata, Filinia terminalis,* recorded at both Locks from December to January were likely derived from an Upper Murray or southern (Victoria) sources, including Commonwealth environmental water release. Rare rotifers, including *Brachionus caudatus* and *Filinia brachiata*, were recorded only as singletons below Lock 1 in January. These species are plankters and would have been transported downstream from upstream sources, potentially associated with environmental water.

Fish Spawning and Recruitment

Spawning and recruitment of golden perch (*Macquaria ambigua ambigua*) in the southern MDB corresponds with overbank flooding and increased discharge that remains in-channel (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b). As such, golden perch is considered a candidate species for measuring ecological response to environmental water. Understanding the influence of hydrology on the population dynamics of golden 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 2014/15, 581 GL of Commonwealth environmental water was allocated to the LMR Selected Area with environmental water delivery peaking at 4,400 ML day^{-1} in November 2014 and March 2015 (Figure 2). To evaluate the contribution of Commonwealth environmental water to the spawning and recruitment of flow-dependent fishes in the LMR Selected Area, we sampled larval and young-of-year

(YOY) golden perch (Figure 12) at sites in the gorge and floodplain geomorphic zones (Figure 5); used otolith microstructure and geochemistry, specifically strontium (Sr) isotope ratios ($^{87}Sr/^{86}Sr$), to retrospectively determine the time and place of spawning of larval and YOY golden perch; and used electrofishing to collect a representative subsample of the golden perch population in the LMR Selected Area to enable determination of population demographics.

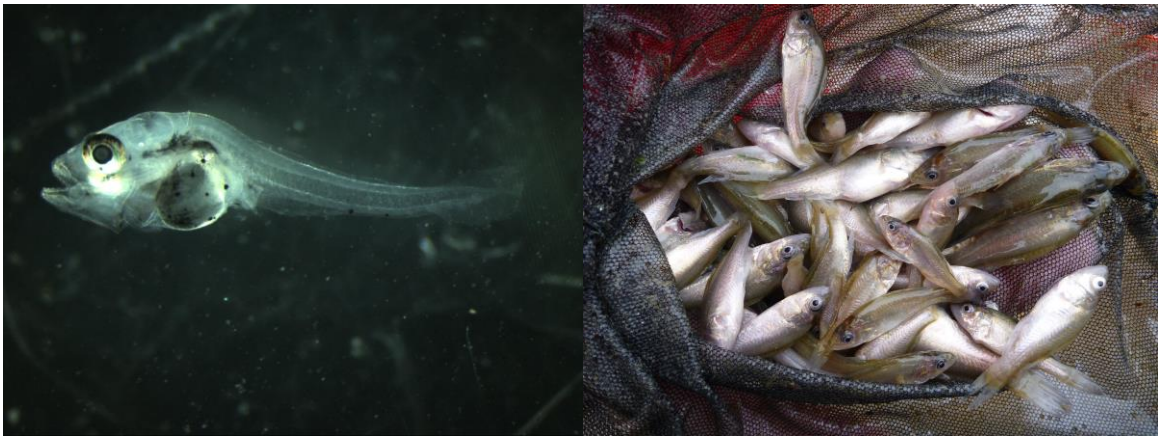

Figure 12. Larval (left) and young-of-year (right) golden perch were sampled as indicators for spawning and recruitment of the species.

In 2014/15, golden 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 November to mid-December in the lower River Murray downstream of the Darling River junction, including the LMR Selected Area. Larval abundances were low with a total of 9 golden perch larvae collected over six fortnightly sampling events between November 2014 and January 2015. Spawning in the LMR Selected Area coincided with water temperatures ≥22 ˚C and relatively stable discharge (QSA ~9,000–10,000 ML day^{-1}) in November 2014 or decreasing discharge (~9,000–7,000 ML day^{-1}) in December 2014.

Assessment of the resilience of golden perch populations requires an understanding of survivorship and population demographics. Sampling of golden perch populations in the LMR Selected Area in 2015 revealed an absence of age 0+ and 1+ fish, indicating that recruitment to YOY, following spawning from November to December 2014, was poor. In conjunction, spawning and recruitment data indicate that the flow regime in the lower River Murray in spring–summer 2014/15 (including

Commonwealth environmental water) led to minimal spawning and recruitment of golden 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 in the LMR Selected Area in 2015, with fish ranging from age 2+ to 18+ years. Throughout the LMR, however, populations were dominated by age 5+ and 4+ fish, spawned in 2009/10 and 2010/11, respectively, in both the Murray and Darling rivers. Sequential year classes from 2010–2013, conferred resilience on the golden perch population in the LMR Selected Area following episodic recruitment throughout the Millennium drought (2001–2010) (Zampatti and Leigh 2013b). Nevertheless, negligible recruitment in 2014 and 2015, and the prospect of ongoing low flows in the lower River Murray in 2015/16, reveal the vulnerability of this species to flow regulation.

3 SYNTHESIS AND EVALUATION

Over the long-term, the delivery of Commonwealth environmental water to increase the magnitude or duration of natural freshes and overbank flows is expected to make a significant contribution to achieving ecological outcomes in the LMR Selected Area, through restoring ecological processes and improving habitats in the main channel and floodplain/wetlands (see Figure 6). To assess ecological response, five-year evaluation questions (LMR LTIM M&E Plan) will be used, as well as evaluation questions relevant to the Long-term Watering Plan of the SA River Murray. In this first year's report of the five-year monitoring and evaluation project, the ecological outcomes of the 2014/15 Commonwealth environmental water delivery are focused on addressing CEWO short-term (one-year) evaluation questions (Table 4).

During 2014/15, ~581 GL of Commonwealth environmental water were delivered to the LMR Selected Area, in conjunction with other sources of environmental water (e.g. MDBA The Living Murray), through a series of watering events targeted to the LMR (along with return flows from Victorian tributaries) to achieve multi-site environmental outcomes. 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. The environmental water delivery contributed to a number of short-term ecological outcomes in the LMR Selected Area (Table 4), and the key outcomes are described in more detail below.

Commonwealth environmental water contributed to an increased hydraulic diversity in the LMR Selected Area. This was 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 slow (0.11–0.17 m s^{-1}) to moderate-flowing (0.18–0.3 m s^{-1}) habitat. These increased water velocities may have provided additional habitat for fishes with life histories adapted to lotic (flowing water) habitats (e.g. Murray cod). There were also increased water levels of up to 0.2 m in the upper reaches weir pools due to Commonwealth environmental water delivery, which would have increased the inundated area of the riparian zone of the river channel.

Additional environmental/ecological outcomes, associated with Commonwealth environmental water delivery to the LMR Selected Area in 2014/15, included

- Increased transport of nutrients and phytoplankton, which would likely stimulate primary productivity in downstream ecosystems.
- Intermittent increases in supplies of organic material, which are deemed critical to the food webs of rivers. The increases were linked to return flows from the inundated Chowilla Floodplain using The Living Murray environmental water, supported by the concurrent delivery of Commonwealth environmental water through the main channel.
- Increased microinvertebrate diversity and abundance, likely triggered by the return flows from Chowilla Floodplain, which increased nutrient and plankton input to the main channel.
- 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 golden perch spawning, with low numbers of larval fish collected during 2014/15. Accordingly, recruitment to YOY (age 0+) was negligible, and the populations of golden perch in the LMR were dominated by 4+ and 5+ year old fish spawned in the Darling River in 2009/10 and the Murray and Darling Rivers in 2010/11. These findings support contemporary conceptual models of the flow-related ecology of golden perch in the lower River Murray, with spawning and recruitment being associated with spring–summer in-channel flow variability (nominally greater than 14,000 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 2014/15. Moderation and fragmentation of flow between the mid and lower Murray River in 2014 by re-regulation (through the operation of Lake Victoria) potentially mitigated spawning and recruitment of golden 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 from Gawne *et al.* (2013). Category 1 Hydrology (channel) and Category 1 Fish (channel) did not directly address specific 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.

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?	There was no clear influence of CEW on gross primary production. Ecosystem net production summed to zero indicating a close balance between production and decomposition of organic material i.e. little external food resource supply and all food resources produced in-channel were utilised.
	What did CEW contribute to patterns and rates of decomposition?	Enhanced respiration rates were associated with return flows from the Chowilla Floodplain in mid-November indicating increased supplies of organic material to the river.
	What did CEW contribute to dissolved oxygen levels?	Reductions in oxygen concentration were associated with increased respiration rates. These were not lethal to biota but demonstrated the potential dis-benefits if water quality of supplies are not considered along with flow volumes.
Category 3. Hydrological regime	What did CEW contribute to hydraulic diversity within weir pools?	Increased median velocity up to 0.07 m s^{-1} over the year compared to without CEW, with some cross sections in the weir pool transforming into moderate-flowing habitat for large-bodied fish.

Indicator	CEWO key one-year evaluation questions	Outcomes of Commonwealth environmental water delivery
Category 3. Hydrological regime cont.	What did CEW contribute to water levels within weir pools?	Increases in water levels in weir pools of up to 0.2 m in the upper reaches. 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 patterns and rates of primary productivity?	Increased transport of nutrients, which would likely stimulate primary and secondary productivity in the Murray River Channel, Lower Lakes, Coorong and Southern Ocean. Increased transport of phytoplankton.
	What did CEW contribute to salinity regimes?	Reduced salinity concentrations in the Murray Mouth. Increased export of salt from the Murray River Channel and Lower Lakes, and decreased net import of salt to the Coorong.
Category 3. Micro-invertebrate diversity	What did CEW contribute to microinvertebrate diversity?	Increased microinvertebrate diversity at Lock 6 in November 2014 due to the return of water from Chowilla Floodplain to the main channel. The Living Murray (TLM) environmental water was allocated for floodplain inundation, supported by CEW delivery through the main channel.
Category 3. Fish spawning and recruitment	What did CEW contribute to native fish reproduction?	Delivery of CEW from October to December 2014 corresponded with limited spawning of golden perch in the LMR.
	What did CEW contribute to native larval fish growth and survival?	Following limited spawning of golden perch in the LMR in 2014/15, recruitment to young-of-year (age 0+) was negligible.

4 MANAGEMENT IMPLICATIONS AND RECOMMENDATIONS

Monitoring and evaluating outcomes through the current project will underpin the adaptive management of Commonwealth environmental water and river operation to maximise ecological benefits from available water in the LMR Selected Area. 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.

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 input and primary productivity. Connectivity will also facilitate the transport and dispersion of aquatic biota (e.g. microinvertebrates, fish larvae) to, and throughout, the LMR, leading to increased species diversity (as observed for microinvertebates in this study) and potentially enhanced recruitment of fish. Also important is the source of water, which can influence the ecological processes and biological response. For instance, floodplain return flows were associated with reductions in dissolved oxygen concentrations. Although these occurrences in 2014/15 were not lethal in the LMR, they showed the potential that exists for negative impacts to aquatic biota if water quality is not considered along with flow. Furthermore, maintaining the integrity (i.e. magnitude, variability and source) of flow from upstream (e.g. Darling 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 fish species). Specific management considerations from indicators are provided below, which relate to the key findings in Section 2.

Hydrology and Hydrological Regime

The increases in water levels and velocities due to the unregulated flow event in August peaking at 18,000 ML day^{-1} were the highest that occurred during 2014/15. This increased velocities greater than that representing moderate-flowing habitat for

large-bodied fish across the whole LMR, with some areas increasing toward values representative of fast-flowing habitat for large-bodied fish (greater than 0.3 m s^{-1}, Mallen-Cooper *et al.* 2011). The increase in water level during this unregulated flow event led to increases in the upper reaches of the weir pools in the order of 0.7–1 m.

In contrast, the increase in discharge due to Commonwealth environmental water from 5,200–6,700 ML day^{-1} to 9,000–10,000 ML day^{-1} between October and November and between January and March resulted in an increase in water level in the order of 0.2 m and a general increase in the median velocity in the weir pool from ~0.1 m s^{-1} to ~0.15 m s^{-1}. At these flows, some areas of moderate-flowing habitat for large-bodied fish were created, although large sections of the LMR still remained as slow-flowing habitat.

Comparing the unregulated flow event of 18,000 ML day^{-1} to the later 10,000 ML day^{-1} events, it can be seen that the larger flow rate resulted in substantially higher velocities and water levels. Increases in velocity from slow to moderate and fast-flowing habitat may provide cues for reproductive activity in flow-cued spawning fish species (e.g. golden perch), facilitate downstream drift and transportation of larvae to favourable nursery habitats, and provide more suitable hydraulic habitats for certain species (e.g. Murray cod) (Zampatti *et al.* 2014), all of which are likely to be integral to the population dynamics and recruitment success of these species. However, it is important to note that discharge or velocity is not the only factor to ensure an ecological response. For example the timing of freshes is critical when meeting temperature thresholds for the spawning of flow-cued fish species or biofilm growth.

Stream Metabolism

The close matching of GPP and ER suggested that supplies of organic materials were largely restricted to those formed by photosynthesis in the river channel during 2014/15 which in this system is predominantly due to phytoplankton (Oliver and Merrick 2006). As there is a constant loss of phytoplankton through grazing, sedimentation and death, the close matching of the GPP and ER indicates that the captured energy is dissipated or used in the system with little accumulation, suggesting a limiting food supply.

Low flows maintained in-channel can lead to increased autochthonous production but do not greatly influence overall metabolism compared with the effect of additional supplies of external organic material. During the 2014/15 monitoring, floodplain return waters had a larger influence on metabolism than did changes in flow. This suggests that there is value in pre-conditioning source water through floodplain interactions to enhance the transport of organic material. This leads to greater metabolic activity, but the levels and types of organic material need to be carefully managed to maintain suitable oxygen levels.

It is likely that longer periods of in-channel flow will be more beneficial if they are structured to support the development of a range of habitats. In particular, they should enable the establishment of aquatic macrophytes that create complex habitats suitable for a variety of organisms. This requires better matching of seasonal flows and rates of change in water level to the requirements of macrophytes. Macrophytes themselves provide organic materials to the food webs, but more importantly they create a large surface area in the illuminated zone that supports biofilms and epiphytes and an associated complex community of microbiota that can greatly enhance system productivity.

Environmental flows aimed at improving the food webs of the river channel should be focussed on improving hydrological connectivity longitudinally and with the floodplain, and also supporting the establishment of in-channel habitats.

Matter Transport

The significant contributions of environmental water appear to have significantly increased the exchange of dissolved and particulate matter through the LMR to the Southern Ocean. Nevertheless, 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, including Aldridge *et al*. (2013) and Ye *et al*. (2015b), the following points could be used to help guide future environmental water use:

- Environmental flow delivery can significantly influence salinity concentrations within the Murray Mouth and Coorong;

- Environmental flow deliveries during extended low flow periods are likely to have greater impacts on concentrations of dissolved and particulate matter than periods with antecedent moderate flow conditions;
- 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

Overbank flows inundate 'new' habitat, trigger emergence of egg bank propagules, leading to population increases. Diversity is also enhanced; taxa such as cladocerans and copepods, which tend to avoid flowing water, are able to develop on the still or slow-flowing inundated floodplain. These taxa may still be found under in-channel flows, washed in from sheltered backwaters or riparian margins of weir pools, however usually only as singletons or in small numbers. In contrast, high population densities can develop rapidly on inundated floodplains (Tan and Shiel 1993). The importance of lateral connectivity of the floodplain with the main river channel in the transfer of organic material and biotic production back to riverine food webs was clearly demonstrated by Furst *et al.* (2014). They reported up to 6.3 tonnes day^{-1} of zooplankton (dry weight) exported from the Chowilla Floodplain back to the LMR at the height of the 2010/11 floods. Results from the 2014/15 The Living Murray environmental water diversions across Chowilla (supported by Commonwealth environmental deliveries within the river channel), while not to the levels of production of the flood, suggest at least an order-of-magnitude increase in zooplankton densities relative to pre-environmental watering assemblages. Diversity also appeared to be enhanced from Chowilla returns and by transport of upstream taxa from different water sources (including Commonwealth environmental water deliveries). Some of these are known to persist in the LMR to Lake Alexandrina (Shiel and Tan 2013a; 2013b), but it is not yet clear whether they can establish populations there, i.e. they have only been recorded during Darling or other floods.

Environmental water delivered to the floodplain will enhance microinvertebrate productivity, which may contribute to the productivity increase in river channel. Flow provided at any time of the year will stimulate productivity of some component of the egg bank, however to maximise productivity, Commonwealth environmental water delivery at times when the dormant biota was historically cued to 'expect' it, e.g. late winter through to early summer, is likely to be of more benefit to riverine food webs.

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 2014/15, freshes present in the mid-Murray River were regulated out of the flow regime of the lower River Murray, primarily through the operation of Lake Victoria. This tempering of the flow regime, in conjunction with artificial inundation of the Chowilla Floodplain, potentially mitigated the spawning and recruitment of golden perch in the lower River, including the LMR Selected Area, whilst concurrently promoting the spawning and recruitment of 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 through the delivery of Commonwealth environmental water will require consideration of maintaining the longitudinal integrity of flows and the potentially discordant outcomes of alternative flow management scenarios, including engineered artificial floodplain inundation.

5 CONCLUSION

In 2014/15, the delivery of Commonwealth environmental water (~581 GL), in conjunction with other environmental water, helped in particular to maintain river flow at 9,000–10,000 ML day^{-1} during spring–summer in the LMR Selected Area. This led to an increase in hydraulic diversity (velocity and water levels) in the river channel; 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, environmental water delivery and the resulting flow regime led to insubstantial golden perch spawning and recruitment (to age 0+) in the LMR during 2014/15. This outcome concurs with contemporary flow-related ecological models for golden perch.

The LMR is heavily regulated with substantially altered hydrology compared to historical natural flow regime. Environmental water delivery to improve hydraulic and habitat diversity can provide benefits for ecological restoration in the LMR. We suggest that the timing of flow delivery should continue to align with ecological objectives and consider biological requirements that occur 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 (within operational limitations) will increase the productivity of the LMR 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 environmental water is also important, which can influence the ecological process and biological response. Furthermore, environmental flow delivery should, where possible, maintain longitudinal 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 suspended solids 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)
- Suspended solid loads (including phytoplankton biomass) 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 IN THE LMR SELECTED AREA

In addition to Commonwealth environmental water deliveries, The Living Murray in-channel flows and return flows from Victorian Environmental Water Holder watering actions in Victorian tributaries (Figure 2), the following environmental water management events in the LMR are relevant to the analyses in this report.

Weir pool raising

Raising of Weir Pool 1 (between Lock 1 and 2) and Weir Pool 2 (between Lock 2 and 3) in the gorge geomorphic zone of the LMR Selected Area occurred between late August and mid-November 2014, whereby water levels within weir pools were raised to a maximum of 0.5 m above the normal pool level (Figure B1; Table B1). Water levels returned to normal pool levels by mid-December 2014, with the exception of Weir Pool 1, which fell an additional 0.1 m below normal pool level (Figure B1). The weir pool raising event is described in the 'Riverine Recovery Lock 1 Spring 2014 Weir Pool Raising Event Plan' and the 'Riverine Recovery Lock 2 Spring 2014 Weir Pool Raising Event Plan'.

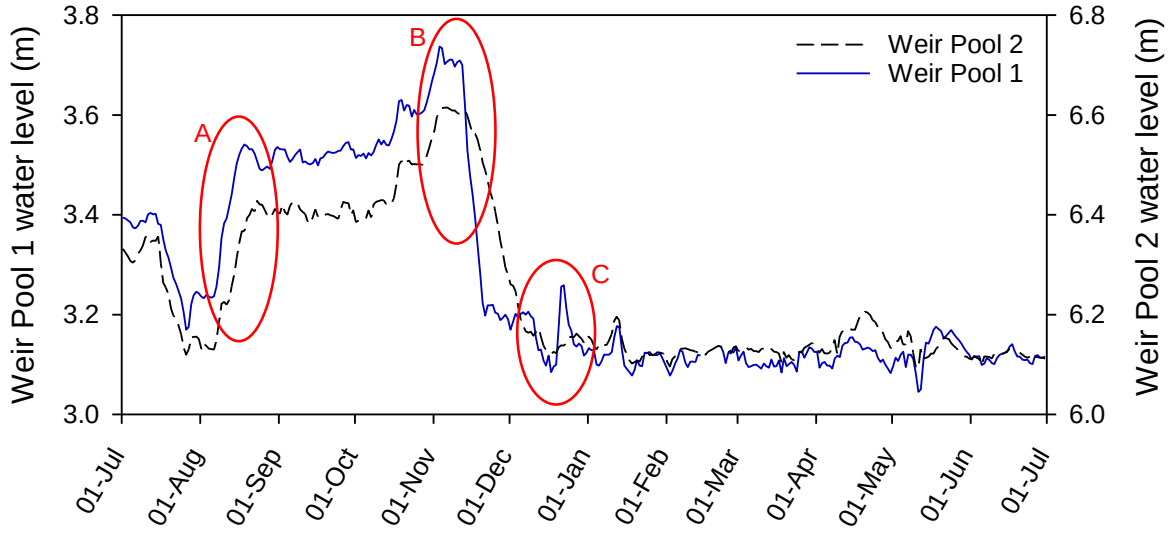

Figure B1. Water level for Lock 1 and 2 weir pools between July 2014 and June 2015. Water level is measured at Lock 1 US (A4260902) and Lock 2 US (A4260518) sites. Red circles indicate (A) the commencement of weir pool raising, (B) maximum level and (C) return to normal pool levels. Water levels in Weir Pool 1 were lowered earlier due to structural problems with the weir.

Table B1. Inundation areas for Weir Pools 1 and 2 during spring 2014 (Macky and Bloss 2012).

Weir pool	Height above pool level (cm)	Area (ha)	Increase compared to Pool	
			Area (ha)	Area (%)
1	0	2,275	0	0
1	15	2,333	58	3%
1	50	2,712	437	19%
2	0	1,557	0	0%
2	20	1,620	63	4%
2	50	1,766	209	13%

Chowilla Floodplain inundation

During spring 2014, 104 GL of The Living Murray environmental water was allocated to support the operation of the Chowilla regulator, which achieved an inundation of 2,300 ha of the Chowilla Floodplain (floodplain geomorphic zone of the LMR Selected Area) (MDBA). The peak water level (19.1 m AHD at the Chowilla regulator) was reached in mid-October 2014 and water recession occurred between early November and early December 2014. In conjunction with the operation of the Chowilla regulator, the water level directly upstream of Lock 6 was raised by 0.4 m between late September and mid-November 2014 in order to achieve Chowilla Floodplain event targets. Lock and regulator heights were managed to ensure flow through the Chowilla Anabranch exceeded a 20% turnover rate (calculated as flow over the regulator divided by volume stored behind the regulator) and minimum velocities representing core fish habitat were maintained. Consequently, there was water passing off the Chowilla Floodplain downstream into the LMR main channel throughout the regulation event.

The Chowilla regulator (floodplain inundation) event was achieved primarily using The Living Murray water, however, the concurrent passing of Commonwealth environmental water along the system during the Chowilla regulator event supported maintenance of the required flow to SA. This enabled the event to occur at the desired magnitude, while also diluting floodplain flows returning to the river channel. A detailed description of the Chowilla Floodplain inundation event can be found at http://www.environment.sa.gov.au.

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 from 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 rates of river metabolism 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 irradiance required for estimating stream metabolism were undertaken at two sampling sites, one downstream of Lock 6 and one downstream of 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. The first field deployments were of Clarke style oxygen electrodes rather than the preferred fluorescence probes due to unavoidable delays in their purchase. The fluorescence

probes were deployed in place of the Clarke probes as soon as they became available. Also, 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. Barometric pressure is measured every 30 minutes at these sites and the 10 minute interval data required for the metabolism analyses were determined by interpolation. Daily incident irradiance data were also obtained from the BOM during a period when the project irradiance sensor failed. Although these BOM light data are less satisfactory than the direct site measurements that are made at 10 minute intervals, they provided a useful contingency in the circumstances.

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 (the sum of all forms of nitrogen), nitrate and nitrite the oxides of nitrogen (NO_X), ammonium (NH_4), total phosphorus (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 5 November 2014 to 24 February 2015 with only a few missing days due to probe maintenance and battery depletion. Due to unavoidable delays in initial probe deployment, prior Commonwealth environmental water flows that commenced in mid-September were not captured in the metabolism data. The monitoring was completed at the end of February as arranged in the LMR LTIM M&E Plan; however, Commonwealth environmental water delivery continued through March to June and may have influenced river metabolism.

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

Results

Oxygen concentration time series

Time series of oxygen concentrations showed that over the monitoring period the dissolved oxygen concentration at both sites generally ranged between 7 and 10 mg L^{-1}, the higher values indicating that at times significant photosynthetic production increased the dissolved oxygen concentration above saturation levels (Figure C1).

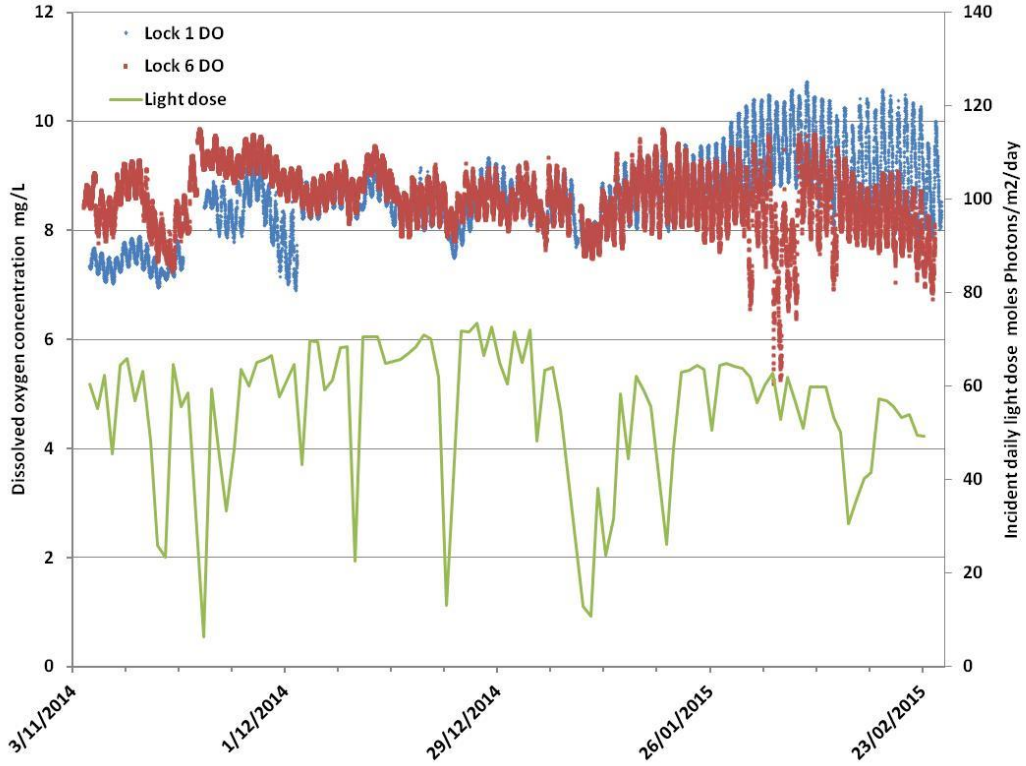

Figure C1. Time series of 10 minute interval oxygen concentrations and the total daily incident light dose at the site downstream of Lock 6.

Conversely, for a short period of a few days from 3–6 February 2015, the oxygen concentration at the site downstream of Lock 6 reduced overnight to 5.2 mg L^{-1} and at the same time the maximum day time oxygen concentrations were also reduced compared to the preceding and following days (Figure C1). Similar events also occurred on 31 January and 11 February. This period was not associated with alterations in the total summed daily incident irradiance (daily light dose, Figure C1), that might have reduced photosynthetic oxygen production and caused a shift in the oxygen balance. There was a fall in temperature of ca. 2 °C between 31

January and 4 February and then an increase of 2 °C up to 11 February but these would not account for the large reductions in the dissolved oxygen concentration. Initially it was hypothesised that these changes were associated with a shift in the sources of environmental water as they aligned with reducing flows from the mid-Murray and Murrumbidgee rivers and increasing releases from Lake Victoria. However, the availability of continuous oxygen measurements from an upstream site maintained by DEWNR at Custom's House (A4261022) showed that the decline in oxygen concentration was not transported from upstream (Figure C2). The mean oxygen concentrations at Customs House over this time were of similar magnitude to the mean oxygen concentrations measured downstream of Lock 1, indicating that the low oxygen concentrations at the intermediate sampling site at Lock 6 were due to local effects if real, or alternatively due to fouling of the sensor or a fault with the probe. Interpretation of such events is made difficult when only a single oxygen probe is monitoring each station. Water quality data from sites upstream and downstream of Lock 6 were scrutinised but no explanation for a fall in oxygen to these low levels could be identified.

Oxygen concentrations at the site downstream of Lock 6 also declined rapidly during the period 12–18 November 2014, although not to levels below 7 mg L^{-1}. This event aligned with the return of water from the Chowilla Floodplain at the end of the testing period for the new regulator. This low oxygen water was measured passing the downstream Lock 1 site about two weeks later (Figure C1). Although the dissolved oxygen concentrations did not fall to levels that might be considered harmful to the aquatic biota, the data indicates the potential for transfer of poorly oxygenated environmental water that in severe circumstances could impact the biota.

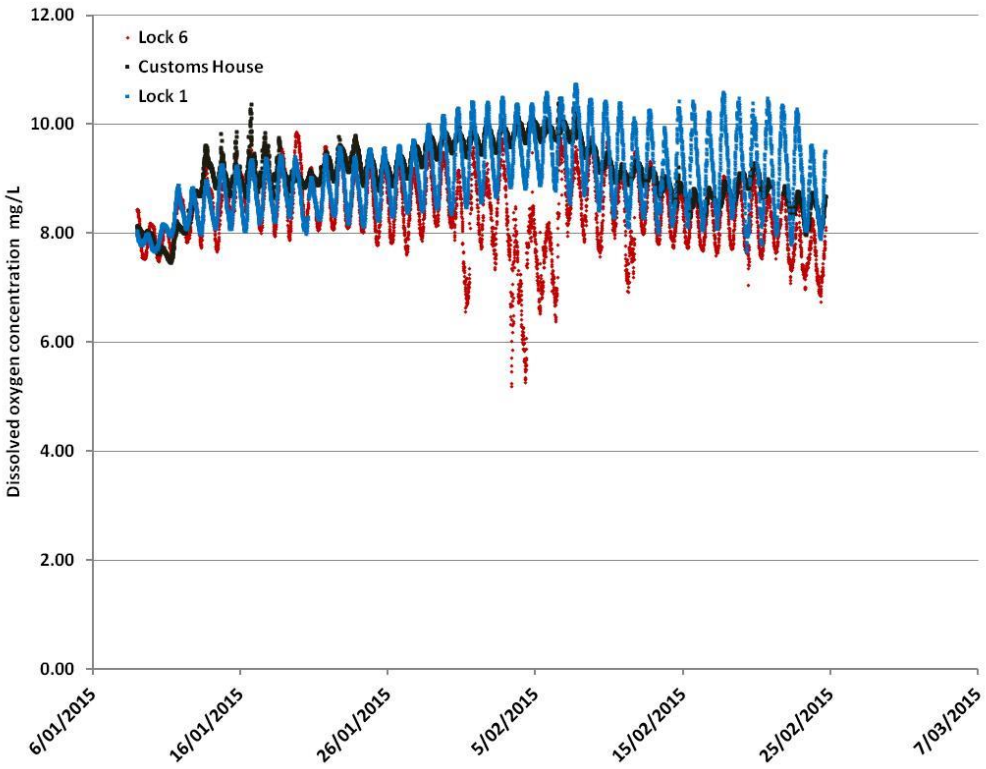

Figure C2. Time series of 10 minute interval oxygen concentrations at sampling sites downstream of Lock 6, and Lock 1 and at Customs House during early 2015.

Metabolism

The general patterns of metabolic activity were similar at both sampling sites (Figures C3 and C4), with steady rates until about 14 January when both GPP and ER increased in magnitude. At the site downstream of Lock 6 the increases continued until 8 February and then rates began to decline again. At the site downstream of Lock 1 increases continued until about 20 February and then declined. Despite fluctuations in GPP and ER the metabolic rates were virtually mirror images of each other so that ENP generally oscillated around zero (Figures C3 and C4). These patterns were a complex function of seasonal changes in incident sunlight and temperature, decreases in river turbidity, and changing phytoplankton concentrations. Data on phytoplankton cell counts provided by SA Water showed increases in cell concentrations, largely cyanobacteria, during the period of increasing metabolism (Figure C5), although increased cell counts were not consistently observed at all sites along the river with no peak in cyanobacteria at Waikerie. A significant proportion of the increase in cell concentration appeared to

have been transported from upstream of Lock 9 and associated with increases in CEW flows.

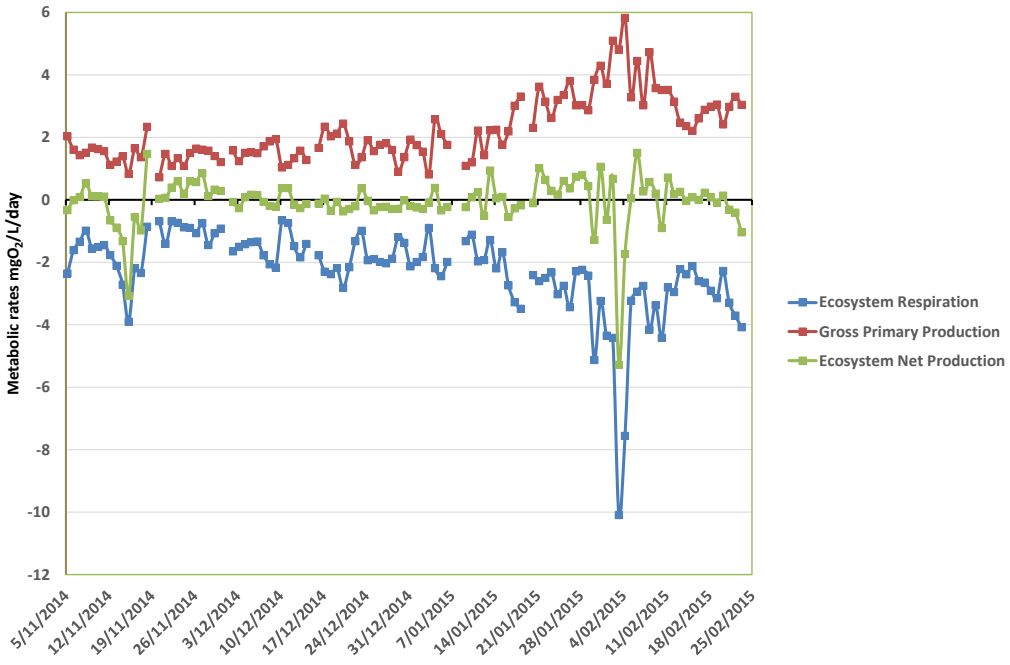

Figure C3. Time series of rates of gross primary production, ecosystem respiration and ecosystem net production at the site downstream of Lock 6.

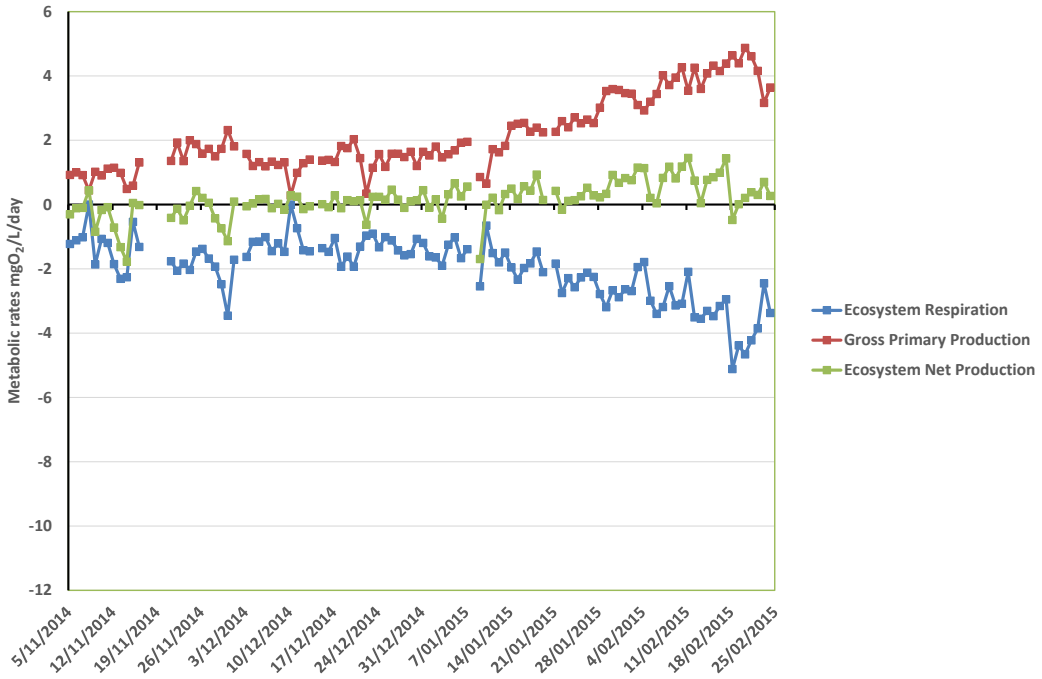

Figure C4. Time series of rates of gross primary production, ecosystem respiration and ecosystem net production at the site downstream of Lock 1.

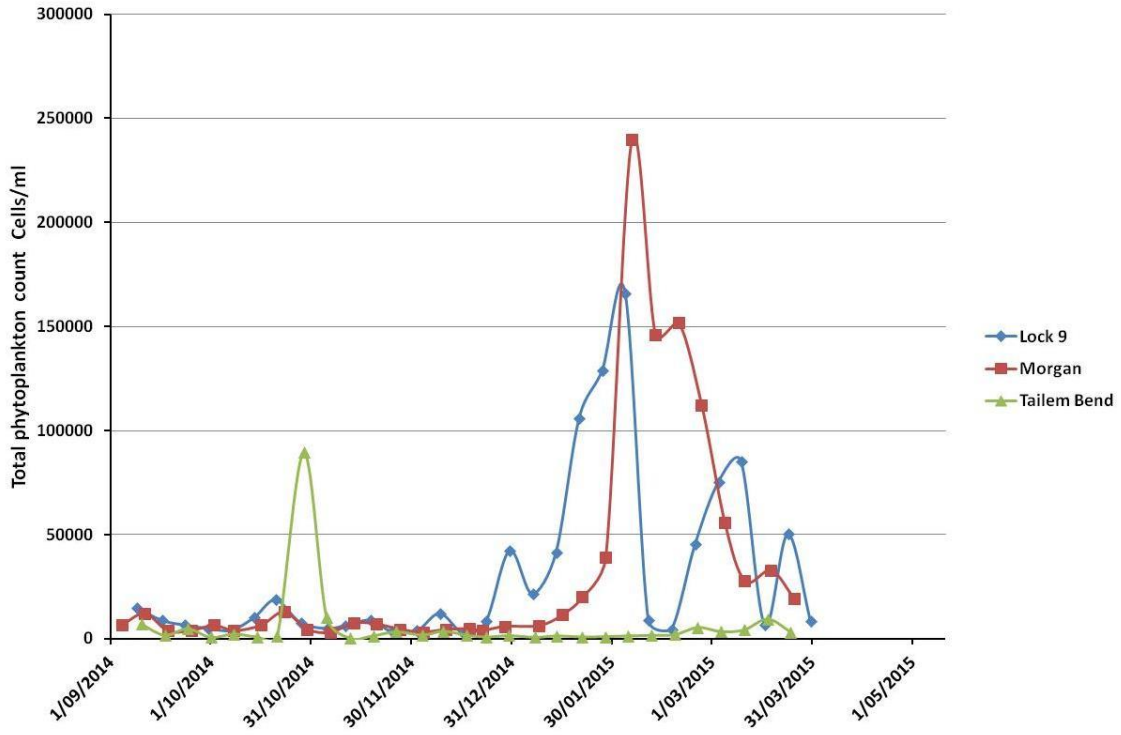

Figure C5. Time series of changes in total phytoplankton cell concentrations at three sites along the Murray River, upstream (Lock 9, Victoria), downstream (Tailem Bend, below Lock1), and between (Morgan, Lock 1 to 2) the two monitored zones.

No simple relationships between metabolic activity and river flow were evident in the data with flow sometimes undergoing major changes without influencing metabolism (Figures C6 and C7). At both the sites flow and velocity were closely correlated due to the simple channel shape. Velocity at the site downstream of Lock 6 was always less than 0.25 m s^{-1}. Previous studies suggest that such velocity is in the range where direct effects of velocity are small and the reduced turbulence results in phyco-chemical structuring of the water column that influences phytoplankton production (Oliver and Merrick 2006; Oliver and Lorenz 2010). Downstream of Lock 1 velocities were an order of magnitude lower and their variation unlikely to influence metabolic responses. The low velocities at both sites provide conditions for enhanced microbial populations and increased metabolic activity compared to conditions at higher velocities.

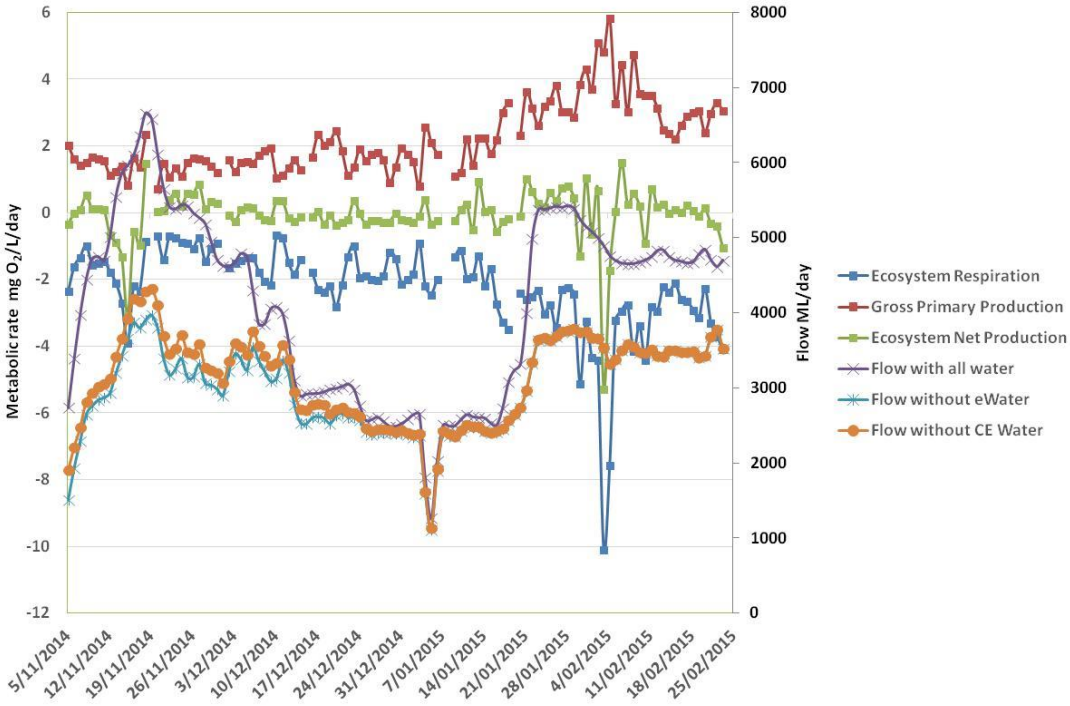

Figure C6. Comparison of time series of rates of gross primary production, ecosystem respiration and ecosystem net production with river flows including or excluding elements of environmental flows at the site downstream of Lock 6.

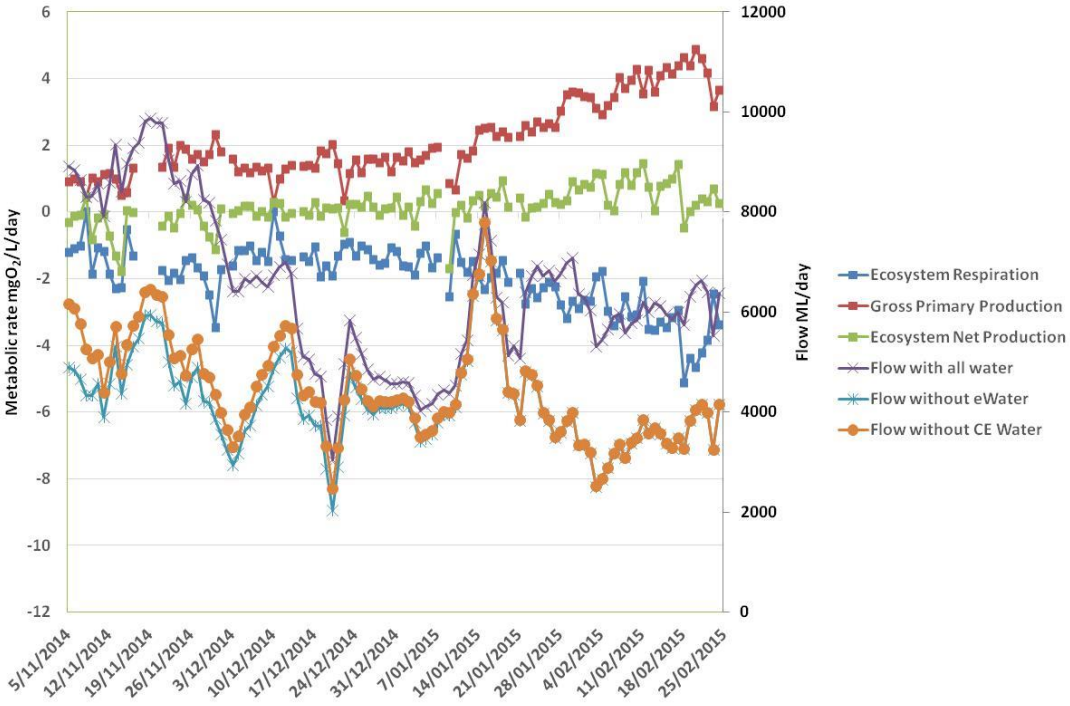

Figure C7. Comparison of time series of rates of gross primary production, ecosystem respiration and ecosystem net production with river flows including or excluding elements of environmental flows at the site downstream of Lock 1.

At the site downstream of Lock 6 two periods of enhanced ER resulted in large negative rates of ENP, one in mid-November 2014 was associated with the release back to the river of floodplain water from the Chowilla regulator event, and a second in early February that coincided with the period of reduced oxygen concentrations likely associated with probe malfunctioning. Despite these occasional disruptions to the patterns of metabolism, the integrated values of GPP, ER and ENP over the monitoring period were very similar between sites with ENP close to zero (Figure C8). These findings are similar to those previously reported for flowing sections of the river (Oliver and Merrick 2006), including flowing sections downstream of weirs (Oliver and Lorenz 2010). The zero ENP suggests that food resources were largely produced in-stream and all were fully utilised.

Figure C8. Total integrated gross primary production (GPP), ecosystem respiration (ER) and ecosystem net production at the sites downstream of Lock 6 and Lock 1 over the monitoring period.

The enhanced rates of ER that were observed at the site downstream of Lock 6 in mid-November and that led to large negative values for ENP suggest delivery to the site of metabolisable organic material. This event was associated with the release back into the river of the environmental water that had been pre-conditioned by its slow passage across the Chowilla Floodplain. The LTIM sampling site is 1220m upstream of the major outlet from the floodplain but there may be smaller creeks

returning to the river nearer the site, or perhaps the inflow was large enough to cause mixing back up the river channel in this low flow region. Intermittent periods of increased supplies of organic material like these are thought to be critical to the food webs of rivers and their decline in frequency, duration and extent has been proposed as a major cause of reductions in populations of aquatic biota due to the decline in food supplies (Oliver and Merrick 2006; Oliver and Lorenz 2010).

Conclusions

The close matching of GPP and ER suggest that supplies of organic materials are largely restricted to those formed by photosynthesis in the river channel which in this system is predominantly due to phytoplankton (Oliver and Merrick 2006). As there is a constant loss of phytoplankton through grazing, sedimentation and death, the close matching of the GPP and ER indicates that the captured energy is dissipated or used in the system with little accumulation, suggesting a limiting food supply.

Low flows maintained in-channel can lead to increased autochthonous production but do not greatly influence overall metabolism compared with the effect of additional supplies of external organic material. During the 2014/15 monitoring season the source of water had a larger influence on metabolism than did changes in flow. This suggests that there is value in pre-conditioning source water to enhance the transport of organic material as this leads to greater metabolic activity, but the levels and types of organic material need to be carefully managed to maintain oxygen concentrations at levels suitable for the biota.

It is likely that long periods of in-channel flow could be more beneficial if they were structured to support the development of a broader range of habitats. In particular, they should enable the establishment of aquatic macrophytes that create complex habitats suitable for a range of organisms. Macrophytes themselves provide organic materials to the food webs, but more importantly they create a large surface area in the illuminated zone that supports biofilms and epiphytes and an associated complex community of microbiota that can greatly enhance system productivity.

In summary, environmental flows aimed at improving the food webs of the river channel should be focussed on improving hydrological connectivity longitudinally and with the floodplain, and also supporting the establishment of in-channel habitats.

APPENDIX D: FISH (CHANNEL)

Results

Table D1. Catch summary (total catch) for small-bodied (fyke netting, 10 nets) and large-bodied (electrofishing, 2880 electrofishing seconds) fish species in the gorge geomorphic zone of the LMR Selected Area. Site numbering increases with distance upstream.

Site No.	1	2	3	4	5	6	7	8	9	10	**Total**
Site Name	Blanchetown	Scotts Creek	Morgan	Cadell	Qualco	Waikerie	Lowbank B	Lowbank A	Overland Corner B	Overland Corner A	**species catch**
Fyke netting											
Carp gudgeon	577	2,003	275	550	480	860	3,080	655	5,649	4,697	**18,826**
Murray rainbowfish	6	59	68	91	29	8	17	37	3	32	**350**
Unspecked hardyhead	18	87	2	23	7	5	2	20	13	248	**425**
Flatheaded gudgeon	15	1		1					1	2	**20**
Dwarf-flatheaded gudgeon	5	4	2	2	11	1	9	5	29	18	**86**
Australian smelt		5		58				7	4	2	**76**
Gambusia	5	206	83	125	8	1	34	36	193	562	**1,253**
Total fyke catch	**626**	**2,365**	**430**	**850**	**535**	**875**	**3,142**	**760**	**5,892**	**5,561**	**21,036**
Electrofishing											
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 e-fishing catch	**1,003**	**938**	**1,265**	**996**	**697**	**1,860**	**694**	**677**	**850**	**817**	**9,797**

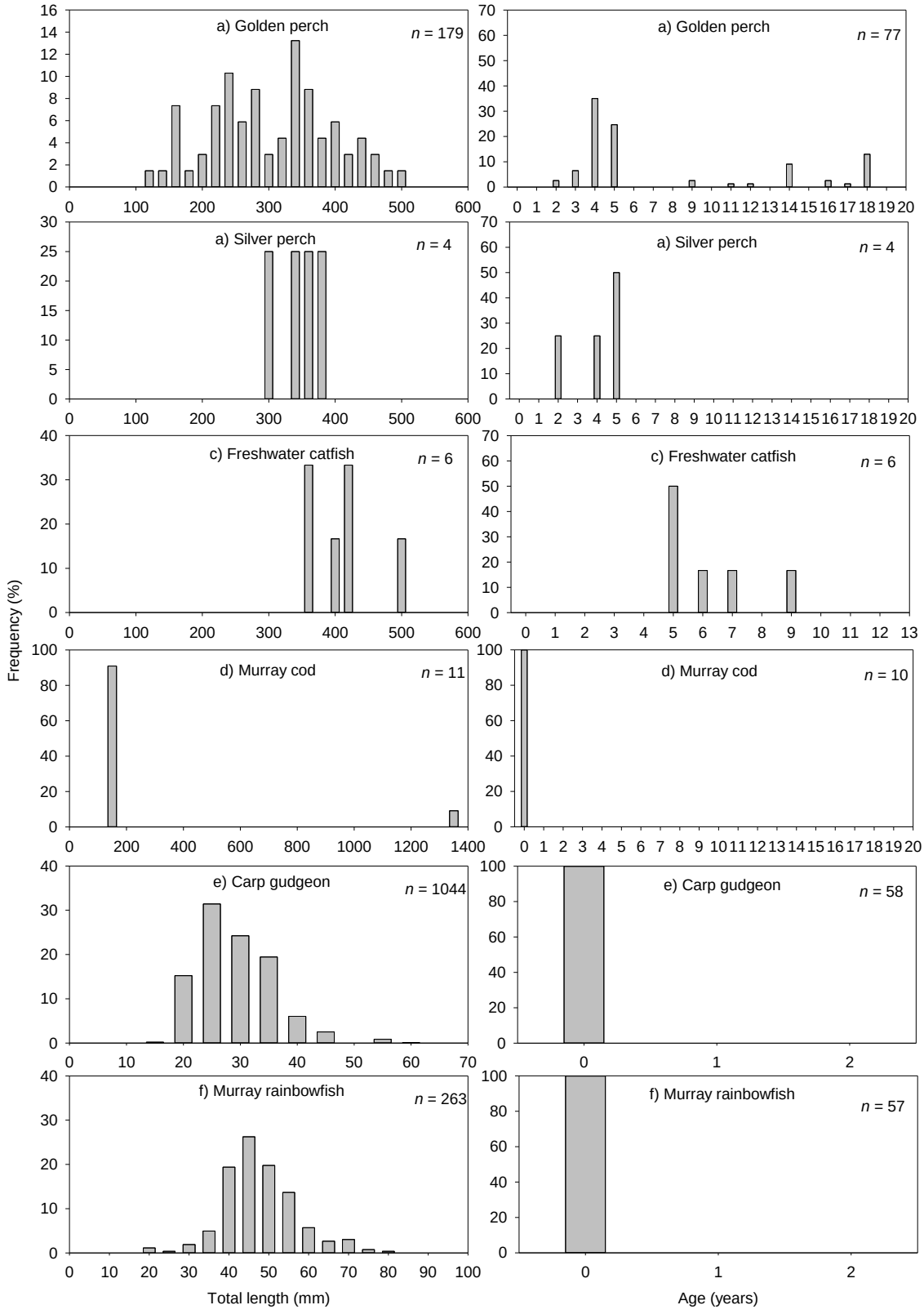

Figure D1. Length (left column) and age (right column) frequency distributions of periodic (a, b) equilibrium (c, d) and opportunistic (e, d) target species collected from the gorge geomorphic zone of the LMR Selected Area in March/April 2015. Note that the large Murray cod (1310 mm) collected during electrofishing was not retained for ageing.

APPENDIX E: HYDROLOGICAL REGIME

Hydrodynamic models have been adopted to provide outputs of interest for ecological response (e.g. discharge, water level and velocity) in locations were these parameters were not recorded, or were not recorded at a high temporal scale (for the case of velocity). The hydrodynamic models also allow the without environmental water scenarios to be represented.

This appendix outlines the hydrodynamic models used for the hydrological regime indicator, the calibration of these models for the 2014/15 water year, and presents further results from the scenarios considered.

Overview of Models

A number of MIKE FLOOD models exist for the LMR Selected Area. For example, 2D models exist for all the weir pools (Macky and Bloss 2012) a flexible mesh model from Overland Corner to Wellington (DHI 2014) and coupled 1D and 2D models for the Chowilla (DHI 2006), Pike (McCullough 2013), and Katarapko (McCullough 2014) floodplains. For the purpose of evaluating the benefit of environmental water delivery, long simulation periods (i.e. one-year) are required. These long simulation periods, combined with the approximately 600 km of river to be considered in the LMR Selected Area, means that adopting 2D and flexible mesh models is not practical. Also, there is limited need to adopt 2D models when flows are below that expected to result in substantial overbank inundation. As such, 1D model have been used to represent the river for this purpose.

1D models for the main channel from Lock 6 to Lock 3 have been developed as part of the MIKE FLOOD models for the Pike and Katarapko floodplains. The river above Lock 6 has not been included as part of the LTIM project, as the Chowilla Icon Site is outside the scope of the project. Also, the river below Lock 1 is modelled as part of the Matter Transport indicators. A new model was created to fill the gap in suitable 1D models of the river from Lock 3 to Lock 1.

Weir Pools 1 & 2 Model

The configuration of boundary conditions and calibration of the model is outlined in the following sections, as this approach is more generic and was adopted for all

models used for the hydrological regime indicator. The main task required to develop the model from Lock 3 to Lock 1 was to extract the cross sections representing the river.

A combined DEM was created from the LiDAR derived DEM and boat based survey data previously, for the purposes of developing the flexible mesh model of the same area (DHI 2014). Based on this DEM, cross sections were extracted every 250 m along the river centreline using MIKE Hydro. The LMR has a number of large and sometimes severe meanders. This resulted in some overlapping of the automatically generated cross sections. Any overlapping cross sections were removed from the model. Bends such as these will also increase the resistance to flow through the MIKE 11 model. As such, for cross sections corresponding to these bends the Manning's n roughness coefficient was increased by a factor of 1.15 (Chow 1959). Bank markers were set using the levee bank option to keep flow within the main channel. If higher flows were to be modelled, it is likely that the cross sections would need to be modified to account for overbank flow.

Modelling Methodology

Boundary Conditions

Upstream flow boundary

The upstream flow boundary was set to the daily recorded flow. The specific data used can be seen in Table E1. Based on the modelled and recorded flow within the model, an extra inflow was added if necessary to account for anabranches around the data used for the upstream boundary (Pike River for Lock 5 and Banrock wetland for Lock 3). See the Diversions section for detail on this approach.

Table E1. Recorded data used for upstream boundary conditions

Model	Inflow
Pike Floodplain	Lock 6 (A4250511)Separate point source inflow for Chowilla Creek (A4261091)
Katarapko Floodplain	Lock 5 (A4260513)
Lock 3 – Lock 1	Lock 3 (A4260517)

Downstream water level boundary

The downstream water level boundary for each model was the daily water level recorded at the relevant lock (Table E2). This allows the actual conditions in the river to be simulated, and allows different weir pool raising scenarios to be considered in a dynamic manner.

Table E2. Recorded data used for downstream boundary conditions.

Model	Water Level
Pike Floodplain	Lock 4 (A4250514)
Katarapko Floodplain	Lock 3 (A4260516)
Lock 3 – Lock 1	Lock 1 (A4260902)

Net evaporation from the river

SILO climate data (Jeffrey *et al.* 2001) was used to determine the loss due to evaporation, with one representative station used each weir pool. Stations were selected based on providing observed (as opposed to interpolated) data, and secondly being located near the middle of the weir pool, with the stations adopted seen in Table E3. Net evaporation was calculated as the Morton's Lake evaporation minus the rainfall for that day and applied as a global boundary condition.

Table E3. SILO climate stations used for net evaporation.

Model	Climate station
Pike Floodplain	Lock 6 – Lock 5: 24037 Lock 5 Lock 5 - Lock 4: 24008 Lyrup
Katarapko Floodplain	Lock 5 - Lock 4: 24008 Lyrup Lock 4 - Lock 3: 24013 Loxton (Pyap)
Lock 3 – Lock 1	Lock 3 – Lock 2: 24029 Waikerie (Eremophila Park) Lock 2 – Lock 1: 24578 Morgan (Brenda Park Station)

Control structure for internal Locks

Each model represents two weir pools, with a Lock near the model of the model. The influence of the lock on the river has been represented using a control structure. These control structures were already configured in the Pike and Katarapko models, and for the Lock 3 – Lock 1 model the representation of Lock 2 was adopted from the flexible mesh model of the same area (DHI 2014). Each control structure was

configured to target the recorded daily water level at the lock within 1 cm of the observed value, by increasing or decreasing the discharge over the structure as necessary. The data used can be seen in Table E4.

Table E4. Target water level data for internal locks.

Model	Water Level
Pike Floodplain	Lock 5 upstream (A4260512)
Katarapko Floodplain	Lock 4 upstream (A4260514)
Lock 3 – Lock 1	Lock 2 upstream (A4260518)

Diversions

Excluding SA Water diversions for the Morgan to Whyalla pipeline, there is limited information regarding diversions from the river at a short time scale, e.g. days or weeks. In order to represent this loss in flow along the river, diversions necessary to match the recorded discharge at internal and downstream locks in each model have been back calculated as a weekly average diversion. The weekly time step was adopted to aggregate errors that may propagate due to travel time or other inputs (calculated discharge or net evaporation), but still account for shorter term increases in diversions, such as a very hot period.

Based on the above configuration, each model could be run with the relevant inflow, target water levels and evaporation loss. The difference between the modelled and observed discharge was calculated for the internal lock and downstream lock for each model. The resulting daily time series were smoothed to a weekly average and applied as a negative point discharge in the centre of the weirpool.

If the model underestimated the Lock 4 flow in the Katarapko model this was assumed to be due to discharge from the Pike River not accounted for in the inflow recorded at Lock 5, and an extra inflow equal to the underestimation was added at Lock 5. The same approach was used to account for Banrock wetlands at Lock 3 in the Lock 3 – Lock 1 model.

Model Calibration

The modelling methodology outlined above makes use of as much recorded data about the 2014/15 watering year as possible, and forces the model to match the water level and discharge recorded at each lock through a combination of boundary conditions, control structures and back calculated water balances.

The data that has not been used in this approach that is available for model calibration are the water levels recorded within the river between the locks. The main calibration parameter is the Manning's roughness n value. Within bank flow occurred in 2014/15, and one global parameter has been adopted for each model, as the influence of different vegetation due to overbank flows is expected to be minimal. On meanders the roughness value is multiplied by a factor of 1.15 to account for the increased head losses that would occur (see 'Overview of Models' section above).

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 less than 10 cm. In the same weir pool the modelled water level downstream of 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), as such the Manning's value was determined to be suitable. Between Lock 2 and Lock 3 (Overland Corner and downstream of Lock 3) there is no obvious over or underestimation in the model outputs compared to the observed water level. The manning's value adopted was 0.027 s $m^{-1/3}$.

A comparison between modelled and recorded cross section averaged velocity can be seen in Figure E2. 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 and Zampatti 2015), 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 E2. 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, and noting the possibly slightly underestimated velocity in the lower reaches of weir pool 2, 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 E4. Results upstream of Lock 4 (at Berri and Lyrup pump stations) look to have some inconsistencies between the model and the observed results. This is in part due to the very constant water level maintained, and the range on the y axis is much smaller when compared to other plots of water level (i.e. 0.2 m compared to ~1 m for other plots). Also, the recorded water level used to control Lock 4 in both this model and the Pike model includes periods below the normal pool level of 13.2 m AHD, which is not obvious in the upstream gauges at Berri and Lyrup. It is possible that this is an inconsistency in the data at Lock 4.

A comparison between modelled and recorded cross section averaged velocity can be seen in Figure E3. Velocity recording were undertaken in the Lock 3 –Lock 4 weir pool as a reference site for the monitoring undertaken for the weir pool raising event (Bice and Zampatti 2015). 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 downstream of Lock 4. The same manning's value was adopted of 0.027 s $m^{-1/3}$, providing increased confidence this value is representative of the LMR.

Pike Model

Both the Pike and Katarapko models represent weir pool 4 (Lock 4 – Lock 5). The Katarapko model was selected to model this weir pool, as it includes a much closer spacing of cross sections within the main channel of the LMR Selected Area (approximately 500 m, compared to approximately 5 km in the Pike model).

As such, only the Lock 5 – 6 section of the Pike model was used in this work, and the water level downstream of Lock 6 is relevant for the model calibration. The model may be slightly under sensitive at this location (Figure E5), 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. A slightly higher Mannings value was adopted for this section of the river, with a value of 0.03 s $m^{-1/3}$.

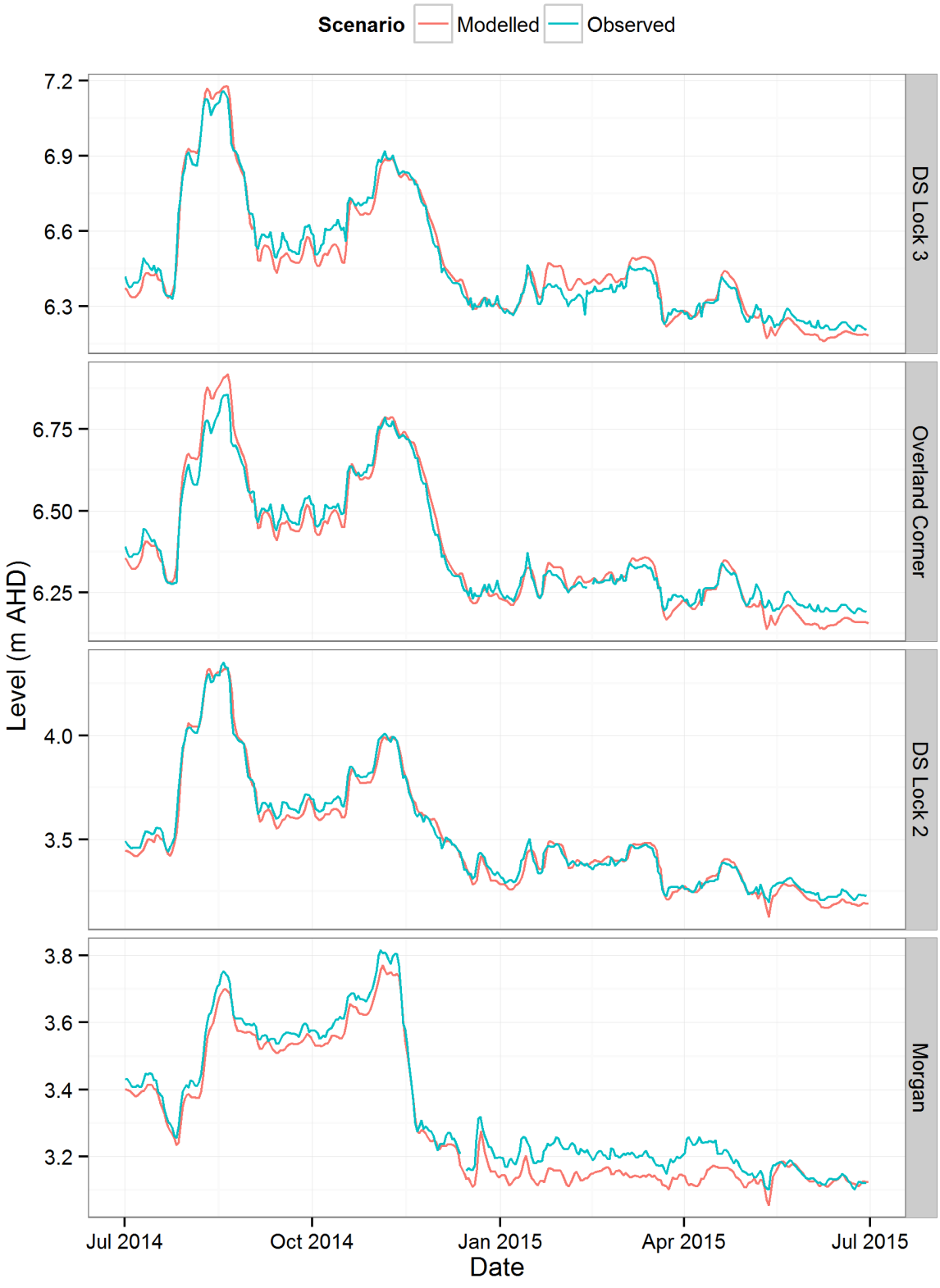

Figure E1. Water Levels used for calibration of the model from Lock 1 to Lock 3.

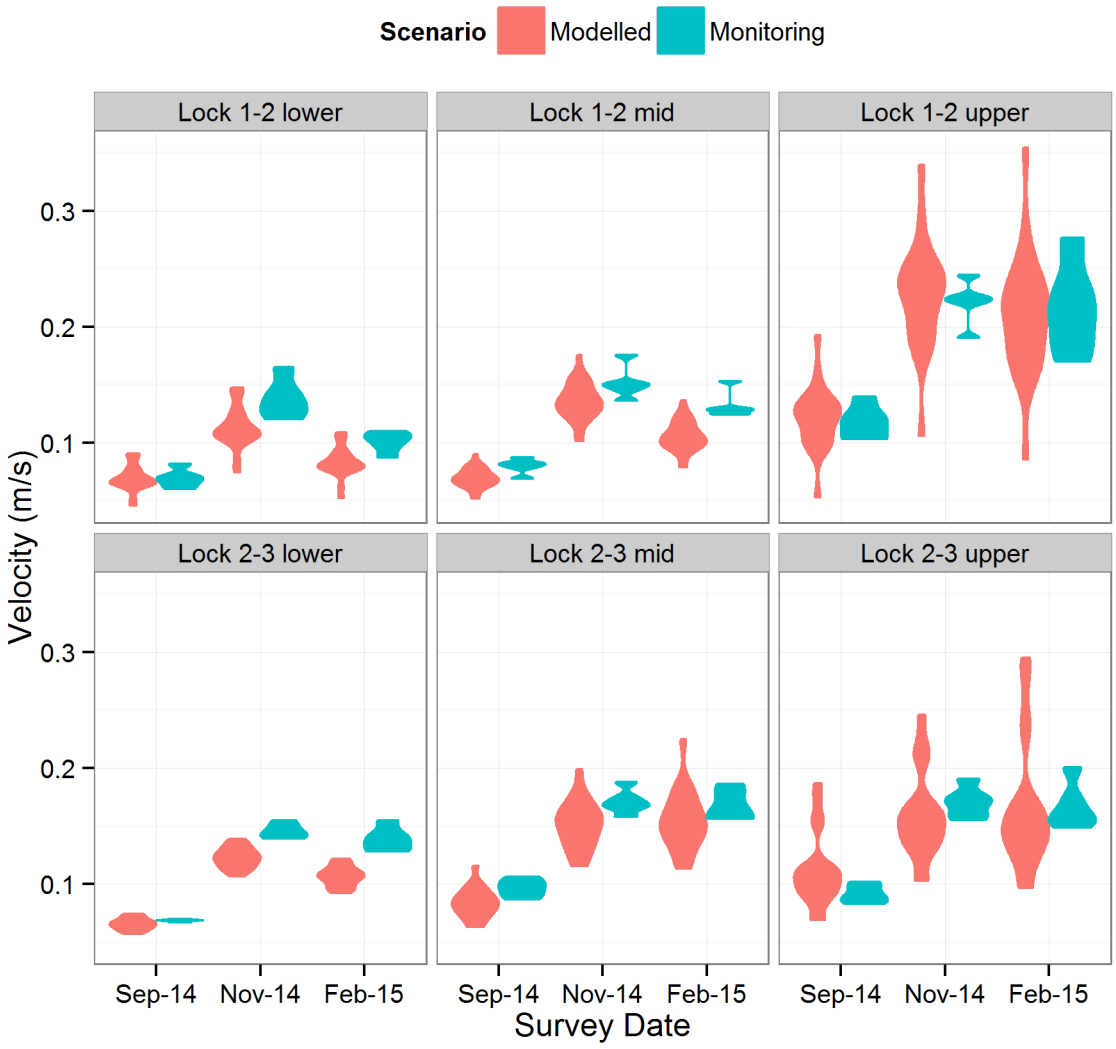

Figure E2. Modelled and measured velocity ranges between Locks 1 and 3.

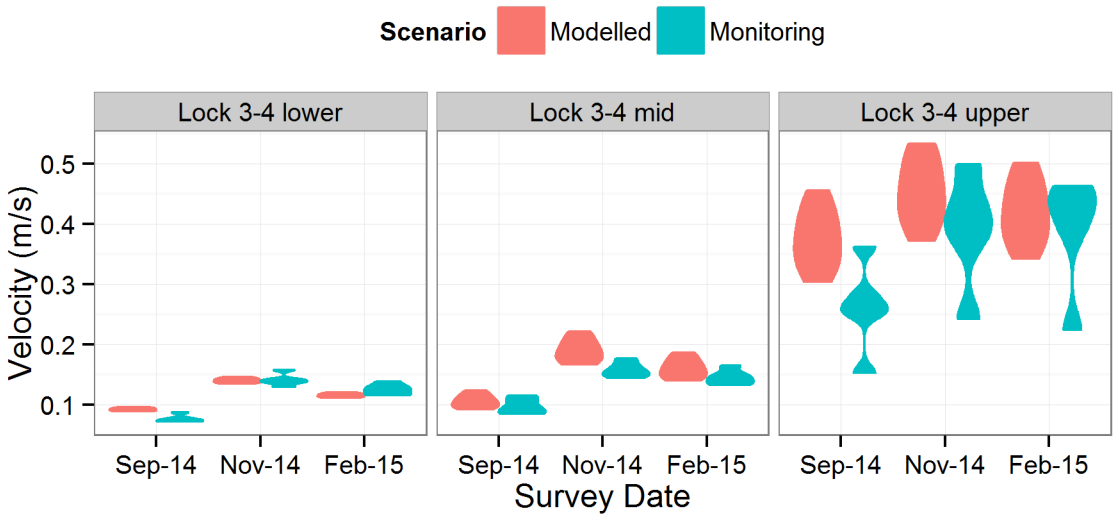

Figure E3. Modelled and measured velocity ranges between Locks 3 and 4.

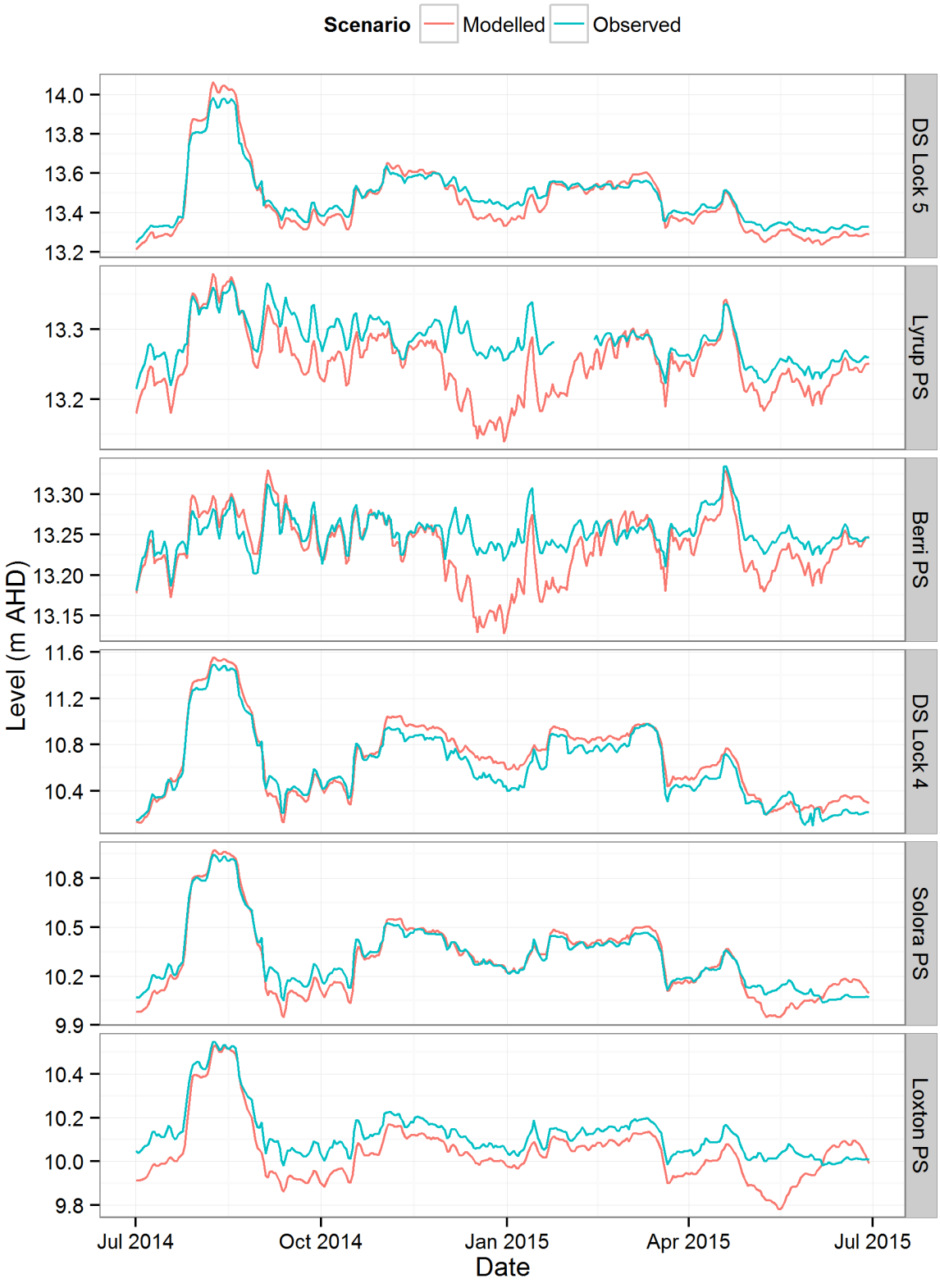

Figure E4. Water Levels used for calibration of the model from Lock 3 to Lock 5.

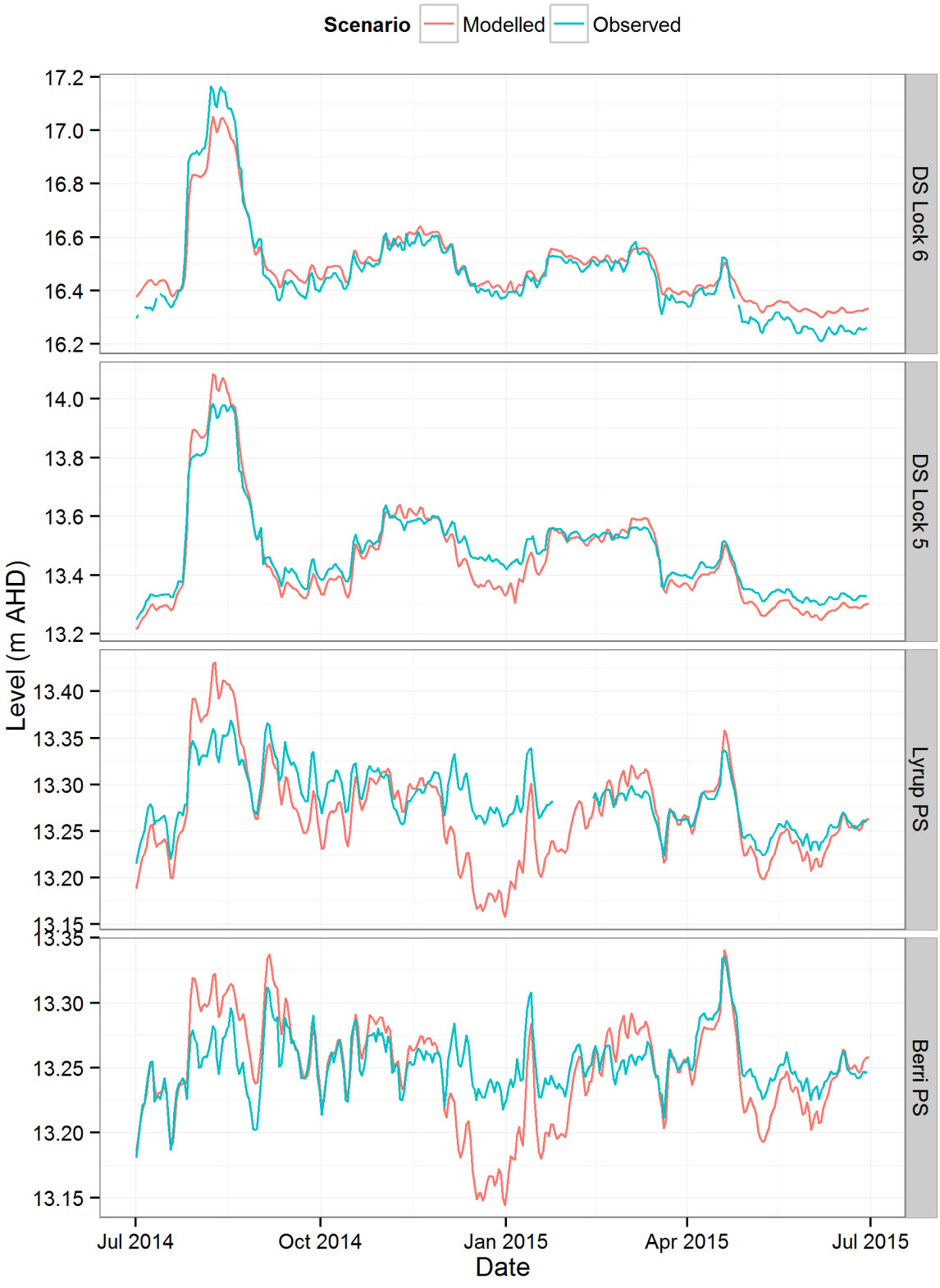

Figure E5. Water Levels used for calibration of the model from Lock 4 to Lock 6. Only outputs between Lock 5 and 6 are used from this model.

Environmental Water Scenarios

Once the models were configured and adequately calibrated, they can be used simulated the without environmental water case. 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 2014/15 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.

The only change to these model settings was the representation of diversions within SA. The environmental scenarios were adjusted to account for the purchase of entitlements within SA by the CEWH. Calculated diversions would be expected to be reduced in the 2014/15 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 111 GL of entitlements (not including the 8 GL that was used for environmental outcomes within SA, 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 111 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. This increase in diversions for the with environmental water scenarios

also reduces the magnitude of flow included in the upstream flow boundary condition the lower weir pool models.

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 as smaller. While not represented, the water levels at each lock are assumed to be the same across all scenarios. There weir pool raising events at Locks 1 and 2 in 2014 are represented in the modelling, however, environmental water was not considered to influence the undertaking of this event.

The difference in water level on each day due to all environmental water is presented in Figure E8, and due to Commonwealth environmental water alone in Figure E9. The shading is used to represent the time of year the difference occurred. It can be seen that environmental water resulted in increases in water level in the order of 0.2 m in the upper reaches of the weir pool. The increases in water level were much higher in the upper reaches of weir pool 3, due to the much shallower and narrower nature of the Murray River near where Katarapko Creek branches off from the Murray River.

Velocity

The results for the velocity in each weir pool can be seen in Figure E10. 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 10th, 50th (median) and 90th percentile velocities within the reach. This approach assumes a constant velocity between computation points, which may not be accurate, however 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 E10, with the range represented by the 10th and 90th percentiles represented by the shaded area.

For the period from October to November and again from January to March, when Commonwealth environmental water increased discharge from 5,200 – 6,700 ML day^{-1} to 9,000 – 10,000 ML day^{-1} (Figure 2), the median velocity in the weir pool increased from ~0.1 m s^{-1} to ~0.15 m s^{-1}, with some cross sections in the weir pool increasing into the range representing moderate-flowing habitat (Mallen-Cooper *et al.* 2011), seen as the blue shaded area between 0.18 and 0.3 m s^{-1}. Some fast-flowing sections can be seen in Weir Pool 3, which aligns with the velocities recorded in the upper reaches of this weir pool in 2014/15 (Figure E3), as well as the larger changes in water level observed in this location. However, these high velocities can be seen to be relative outliers in the weir pool, with the median velocity (solid line) much closer to the 10th percentile (lower bound of the shaded area).

The difference in the weir pool median velocity on each day due to all environmental water is presented in Figure E11 and due to Commonwealth environmental water alone in Figure E12. The shading is used to represent the time of year the difference occurred. The increase in velocity up to 0.05 – 0.075 m s^{-1} due to Commonwealth environmental water can be seen.

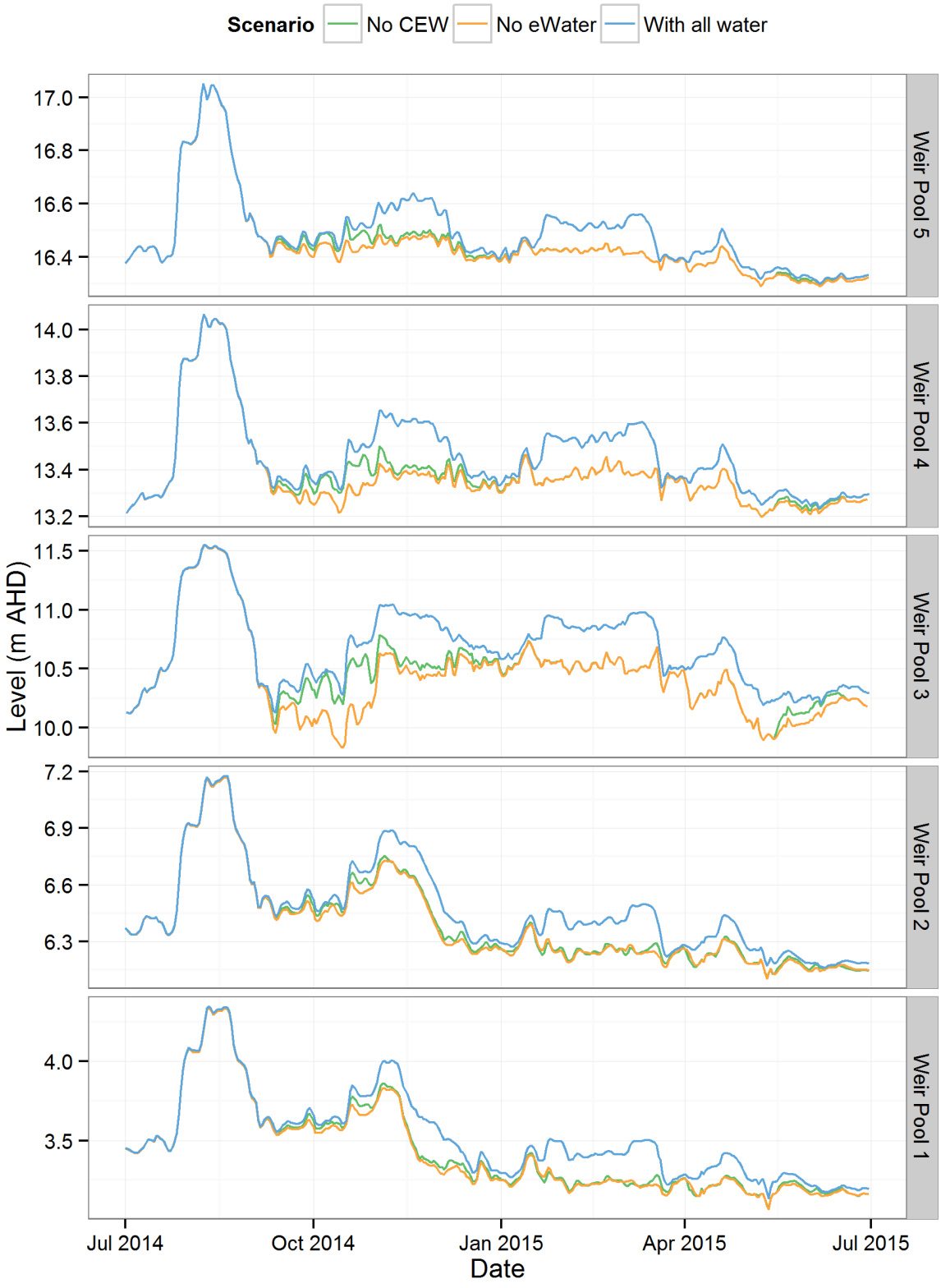

Figure E6. Modelled water level at the upstream end of each weir pool without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

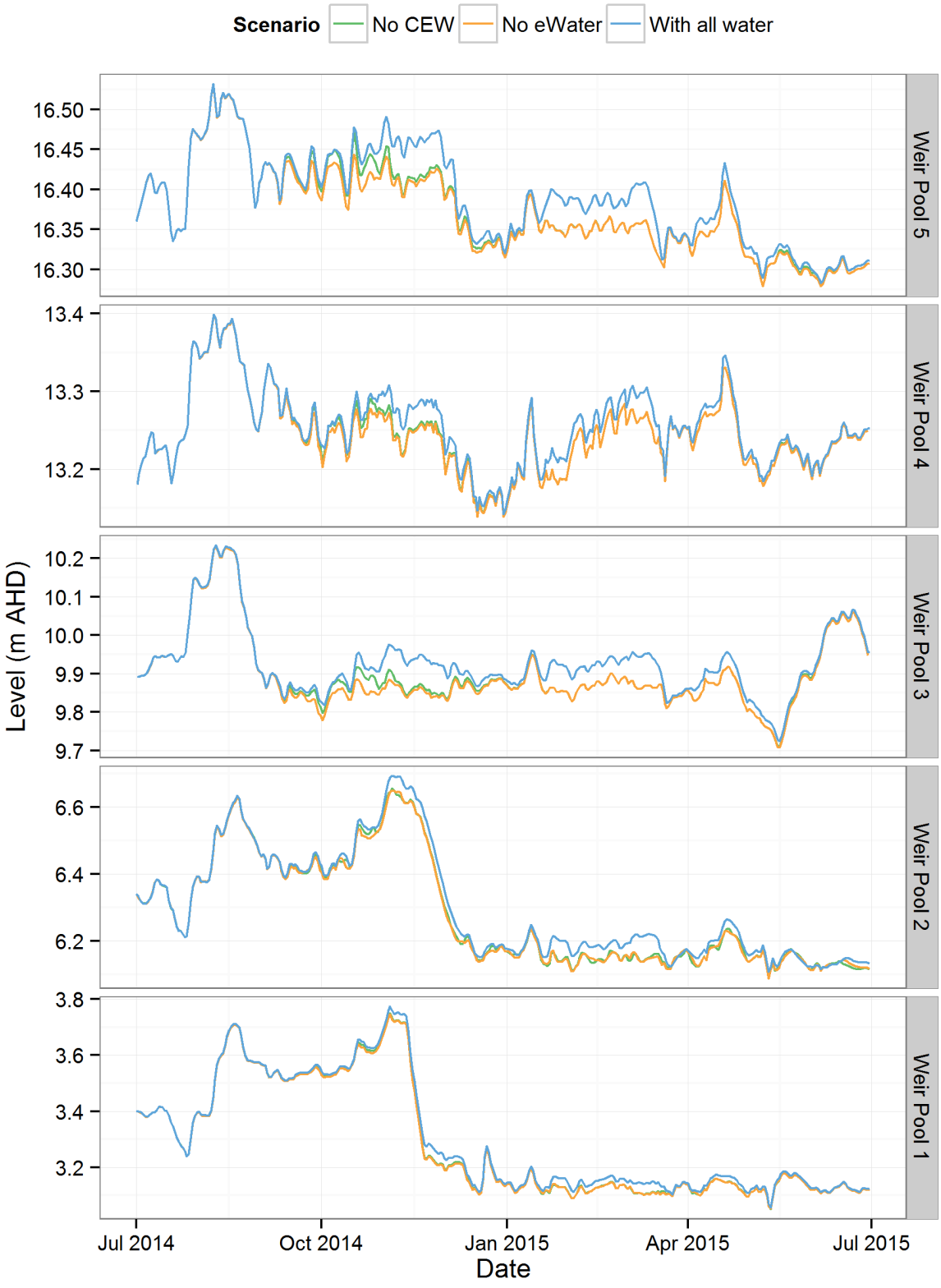

Figure E7. Modelled water level in the midpoint of each weir pool without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

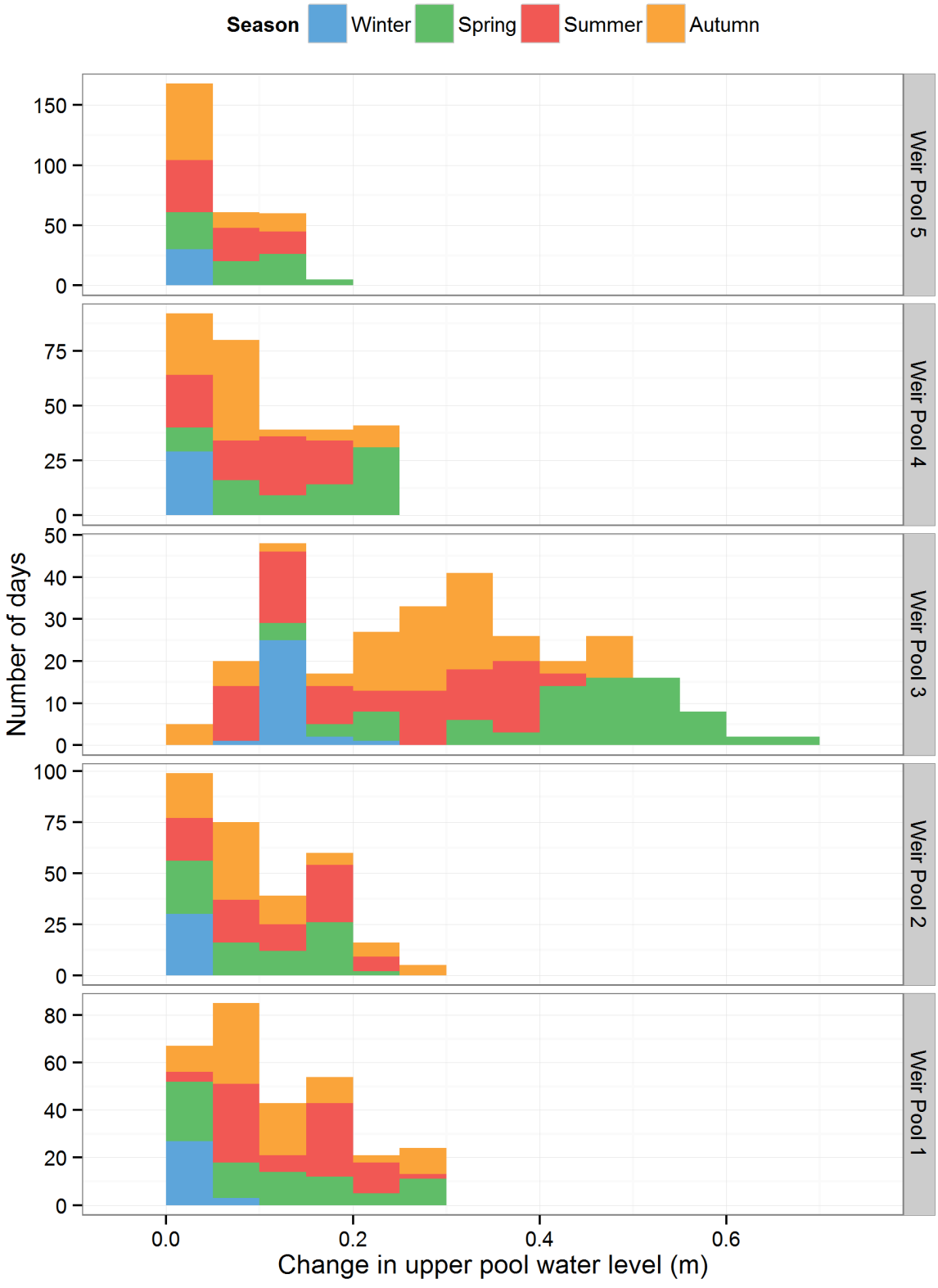

Figure E8. Change in upper pool water level each day due to all environmental water. Colours represent the timing of the change, shaded by season. The height of each bar represents the number of days over the year the change in water level due to environmental water was within that range.

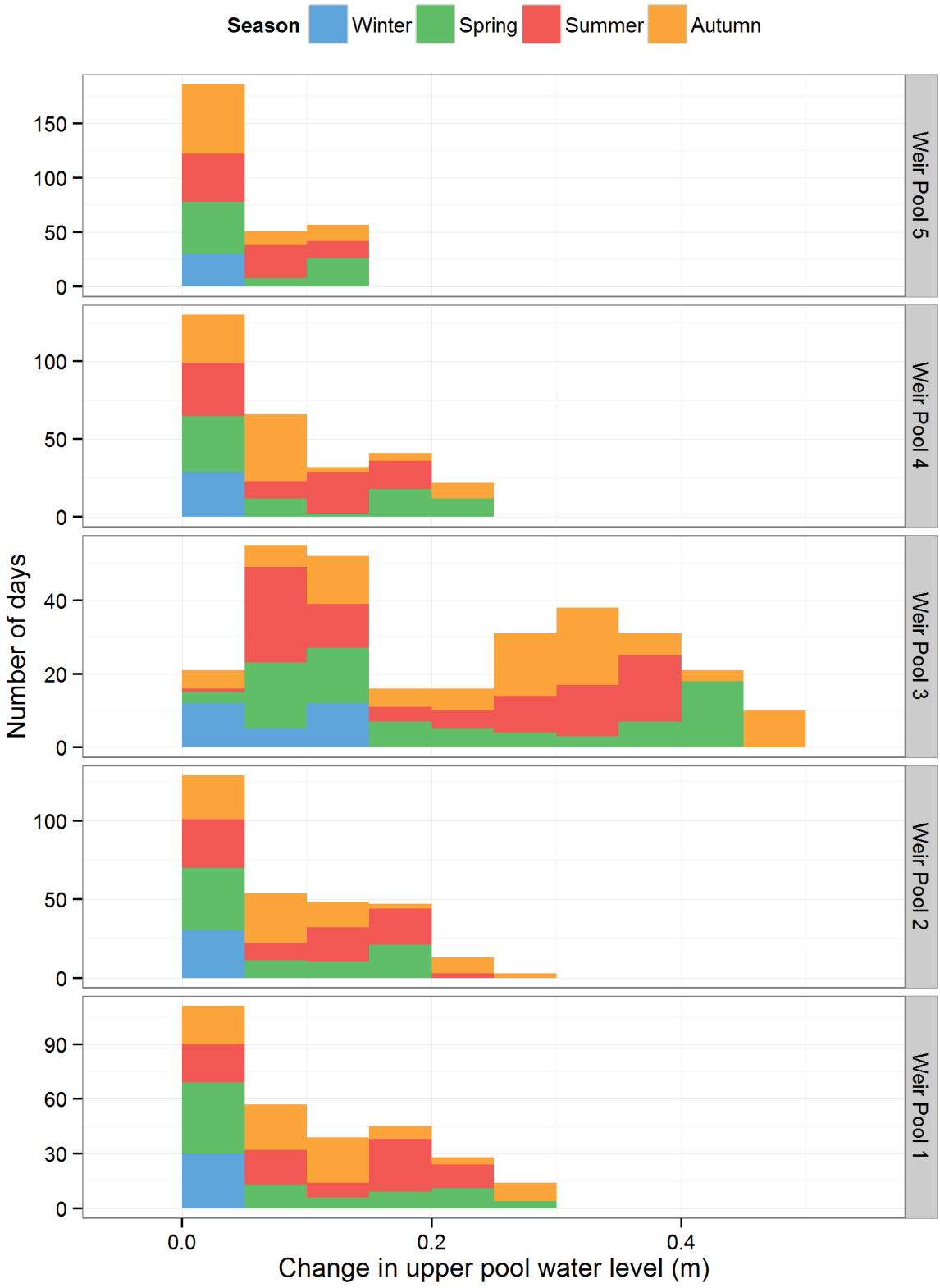

Figure E9. Change in upper pool water level each day due to Commonwealth environmental water. Colours represent the timing of the change, shaded by season. The height of each bar represents the number of days over the year the change in water level due to environmental water was within that range.

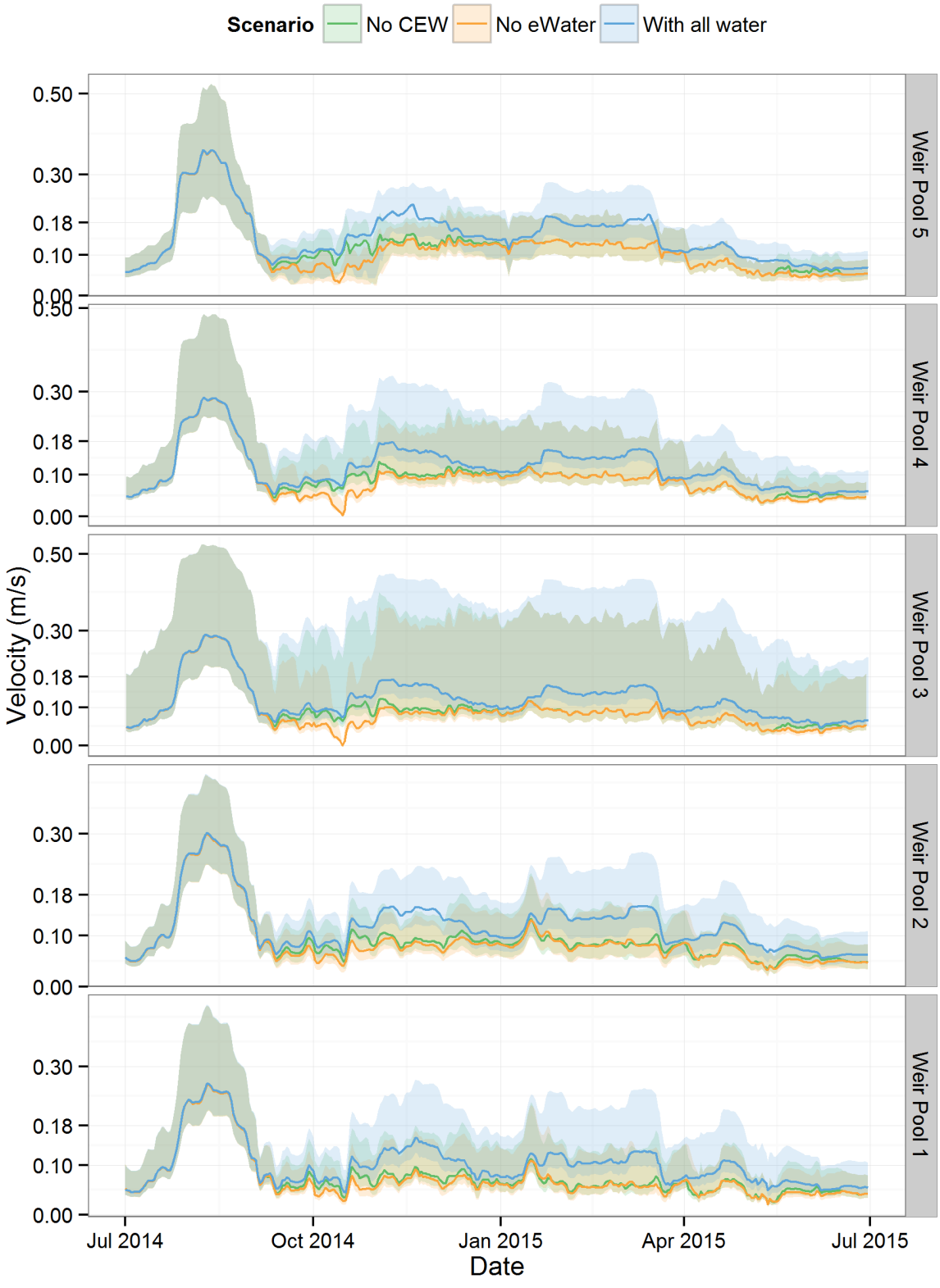

Figure E10. Median modelled velocity in each weir pool (line), with the 10th and 90th percentile 1D cross section velocities the shaded band. Scenarios presented are without environmental water (orange), without Commonwealth environmental water (green), and with all water (blue).

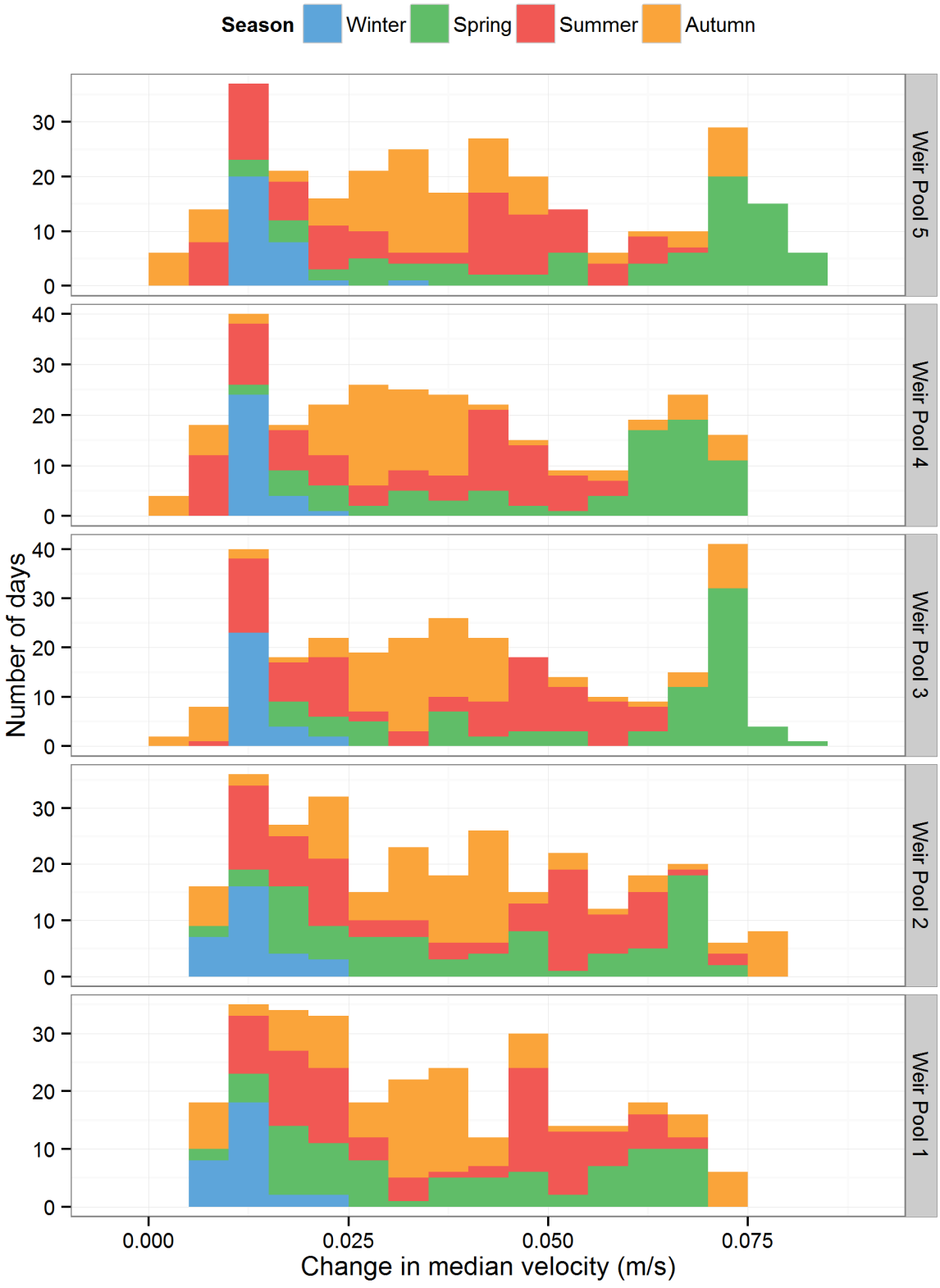

Figure E11. Change in weir pool median velocity each day due to all environmental water. Colours represent the timing of the change, shaded by season. The height of each bar represents the number of days over the year the change in velocity due to environmental water was within that range.

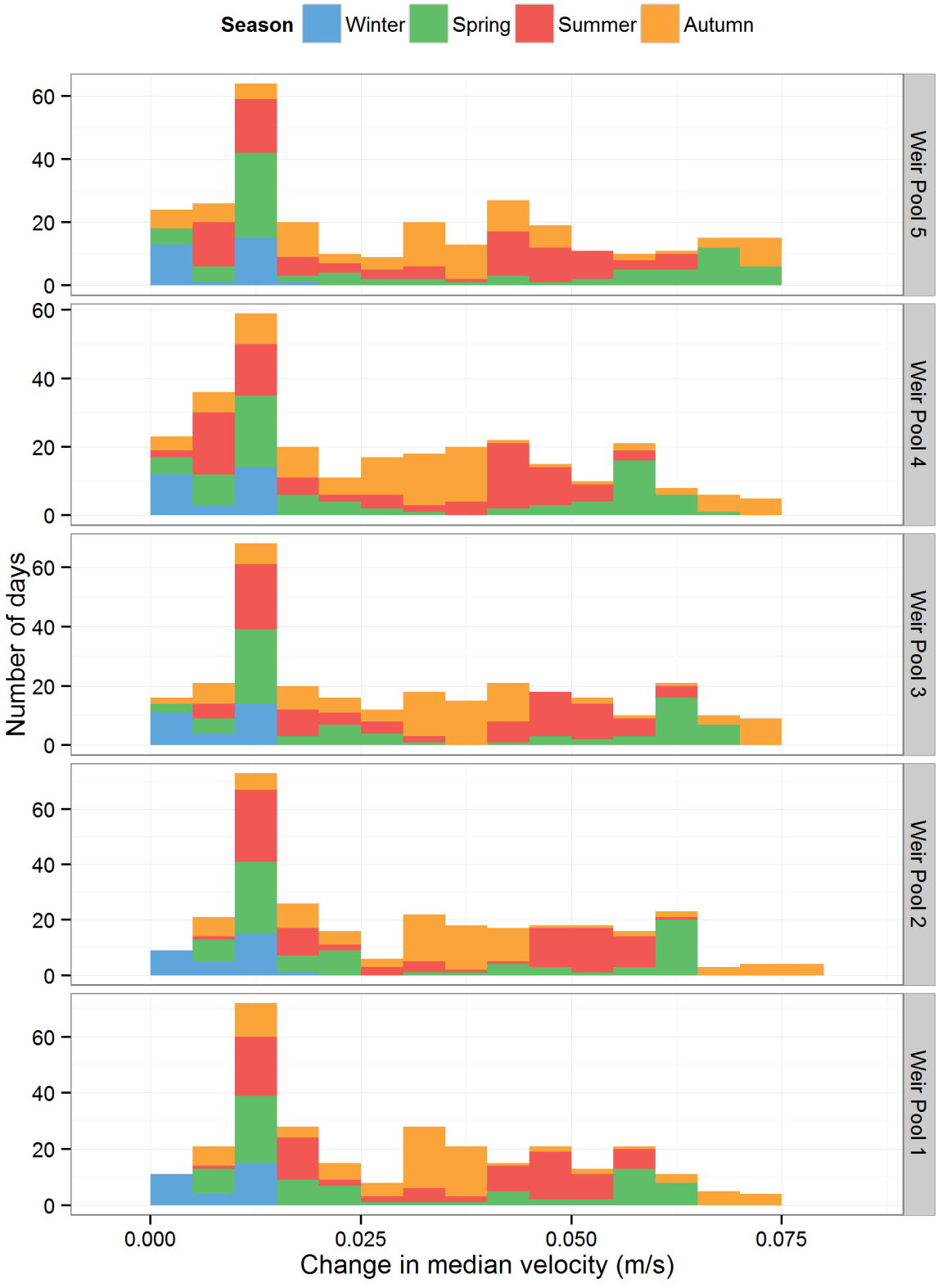

Figure E12. Change in weir pool median velocity each day due to Commonwealth environmental water. Colours represent the timing of the change, shaded by season. The height of each bar represents the number of days over the year the change in velocity due to environmental water was within that range.

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 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.
- Total suspended solids, which is a measure of the total amount of inorganic and organic particulate matter in the water column. It has a strong influence on light availability, which is important for structuring aquatic ecosystems (Geddes 1984a; 1984b). It is influenced by flow through increased productivity (as described previously), as well as the

mobilisation of inorganic matter from the floodplain and river channel (i.e. resuspension).

Altering the flow regime of riverine systems has had significant consequences for 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 sediment transport and increased sedimentation due to deposition; 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). 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 on 2014-2015, a hydrodynamic-biogeochemical model was set-up and 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 2014 and June 2015 (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, total suspended solids 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 dates	Data source
Murray River Channel	Morgan	Approximately weekly between 01/07/2014 and 30/06/15	SA Water
	Wellington	15/07/2014, 16/10/2014, 22/01/2015, 14/04/15	Murray Futures (DEWNR)
Lower Lakes	Lake Albert Middle		
	Lake Alexandrina Opening		
	Lake Alexandrina Middle		
Coorong	Point McLeay	14/07/2014, 16/09/2014, 15/10/2014, 13/11/2014, 10/12/2014, 21/01/2015, 23/04/2015	Murray Futures (DEWNR)
	Goolwa Barrage		
	Murray Mouth		
	Ewe Island		
	Mark Point		
	Parnka Point		

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. Furthermore, TUFLOW-FV-AED was used to assess the contribution of environmental water to dissolved and particulate matter during 2013/14. The model approach adopted within the AED model was conceptually similar to earlier studies (Hipsey and Busch 2012; Aldridge *et al*. 2013; Ye *et al*. 2015b) that adopted the CAEDYM model platform. 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.

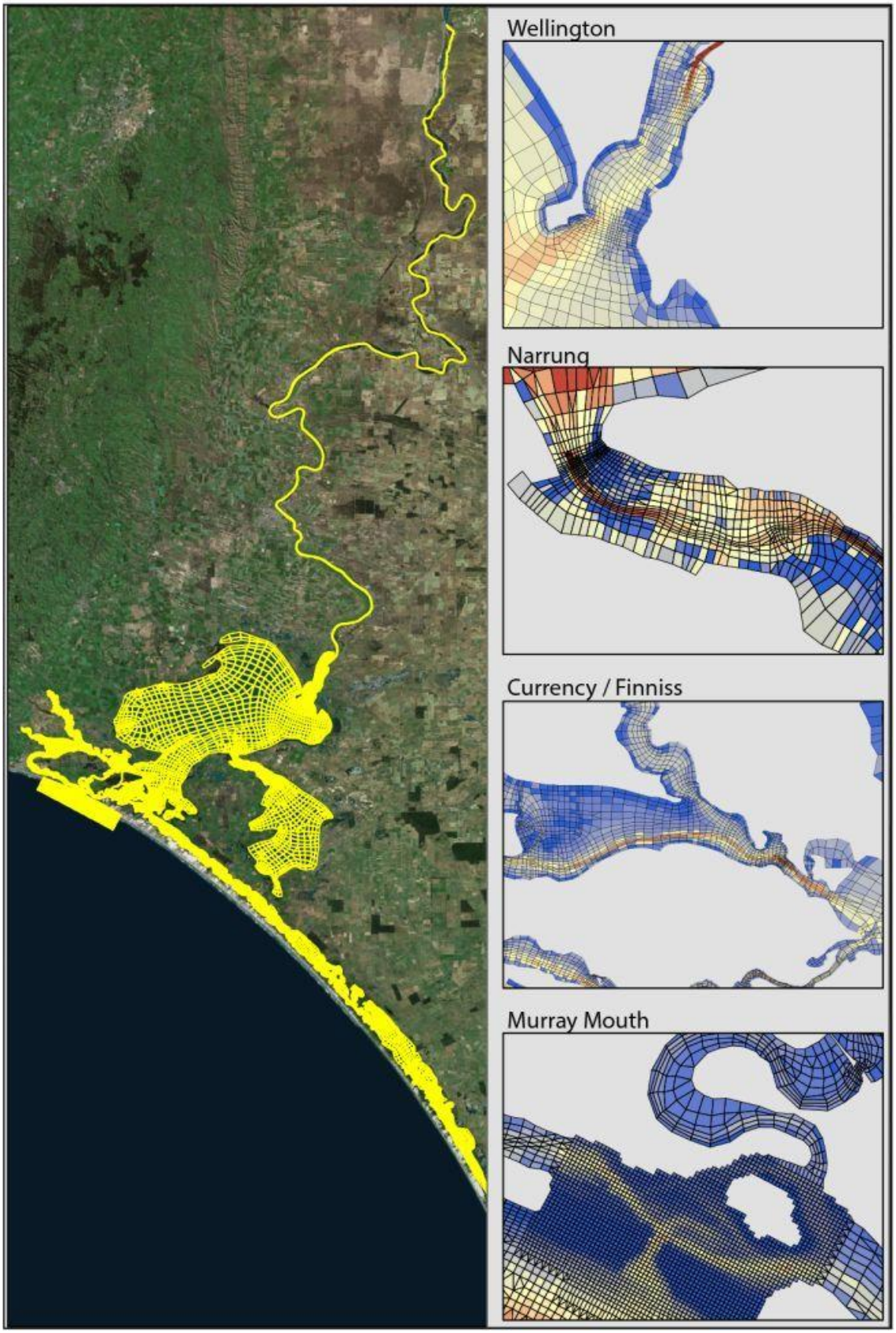

Figure F1. Overview of model domain applied in this study using TUFLOW-FV. Grid provided courtesy of Department of Environment, Water and Natural Resources.

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; and
- without any environmental water

These simulations were run for the period between July 2014 and June 2015.

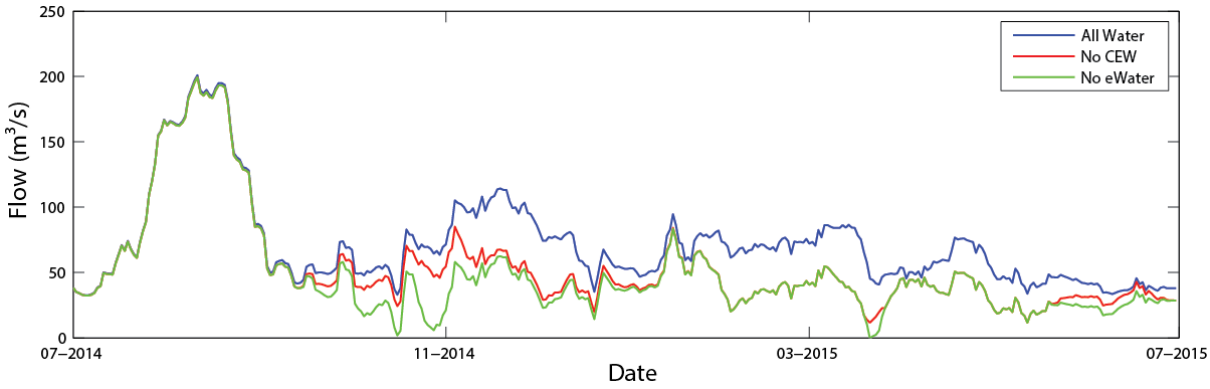

Figure F2. Overview of the three flow scenarios assessed by the model simulations. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater). Flows were applied to the model at the upstream Lock1 boundary.

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 during periods of high River Murray inflows is 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 members 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 Murray River Channel (Wellington), Lower Lakes (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, chlorophyll *a* and total suspended solids. Salinity is presented as practical salinity units (PSU), a measurement of the measured conductivity to standard 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 (Figure F3–Figure F10) than in Section 2.2 Matter Transport, including field collected data used for model validation.

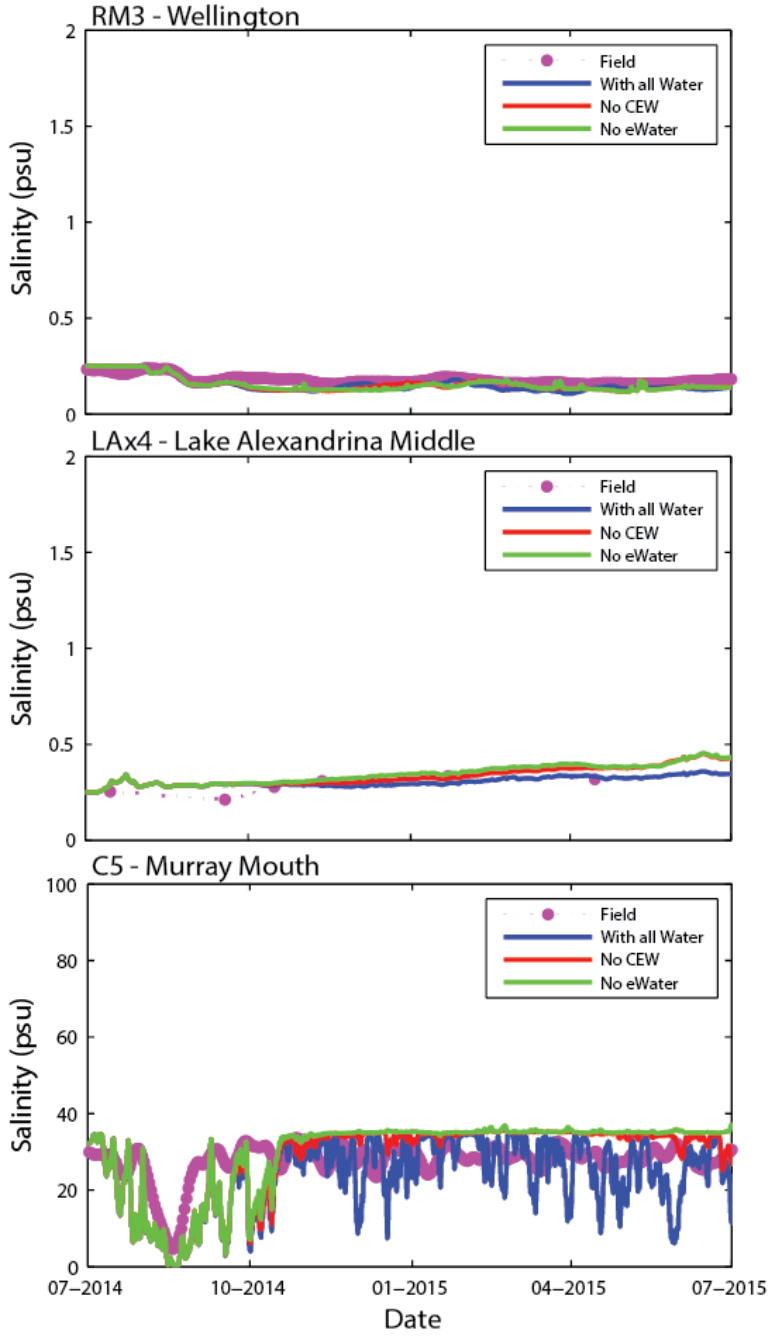

Figure F3. Observed and modelled practical salinity units (PSU) at selected sites. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

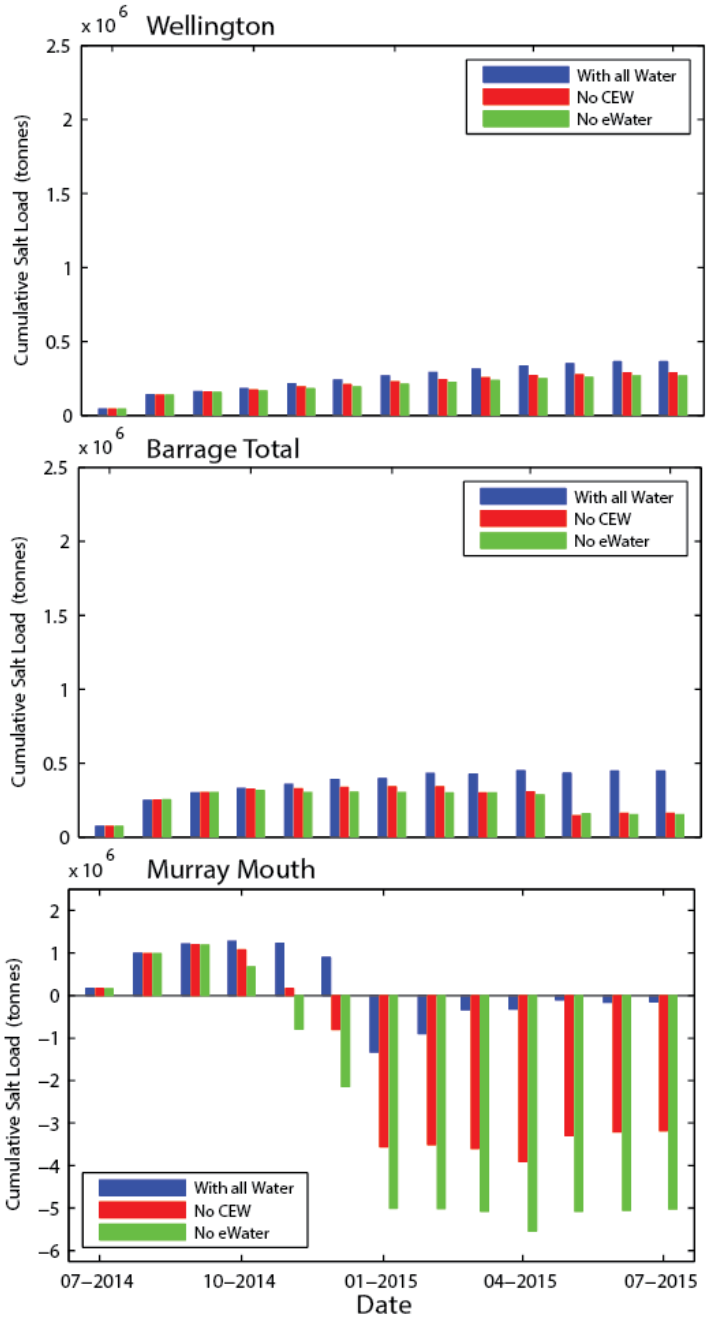

Figure F4. Modelled cumulative salt exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

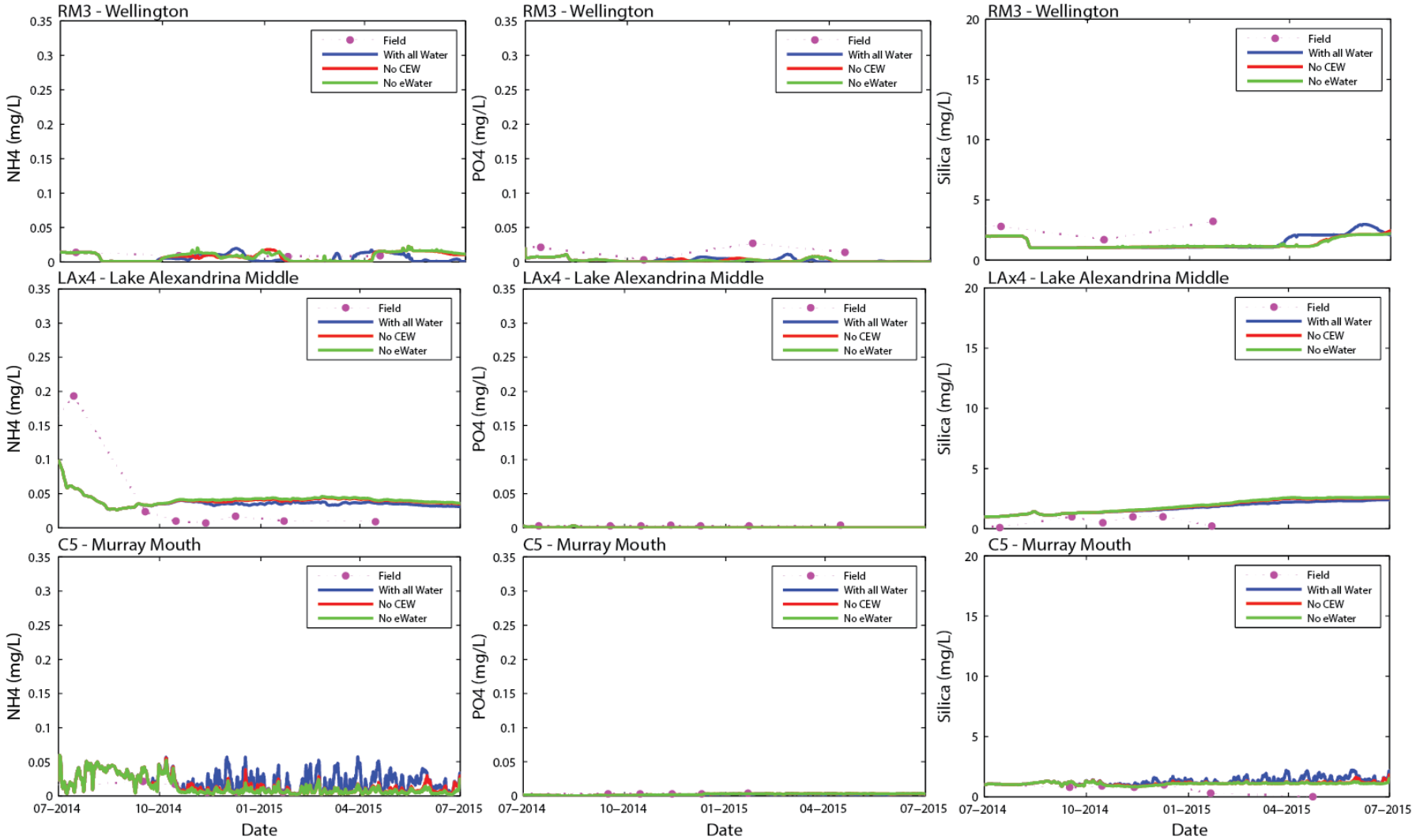

Figure F5. Observed and modelled ammonium (NH4), phosphate (PO4) and silica concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

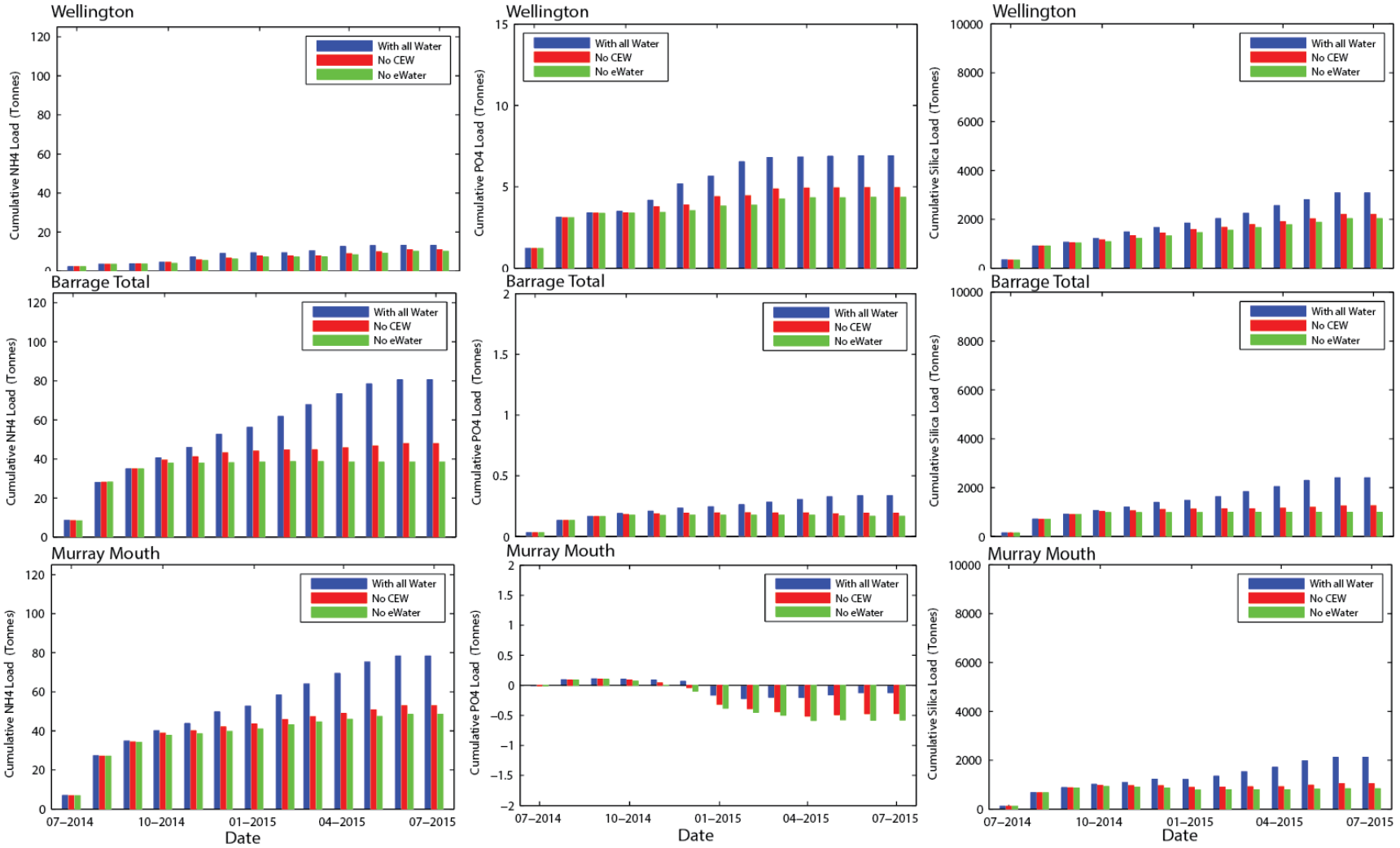

Figure F6. Modelled cumulative ammonium (NH4), phosphate (PO4) and silica exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

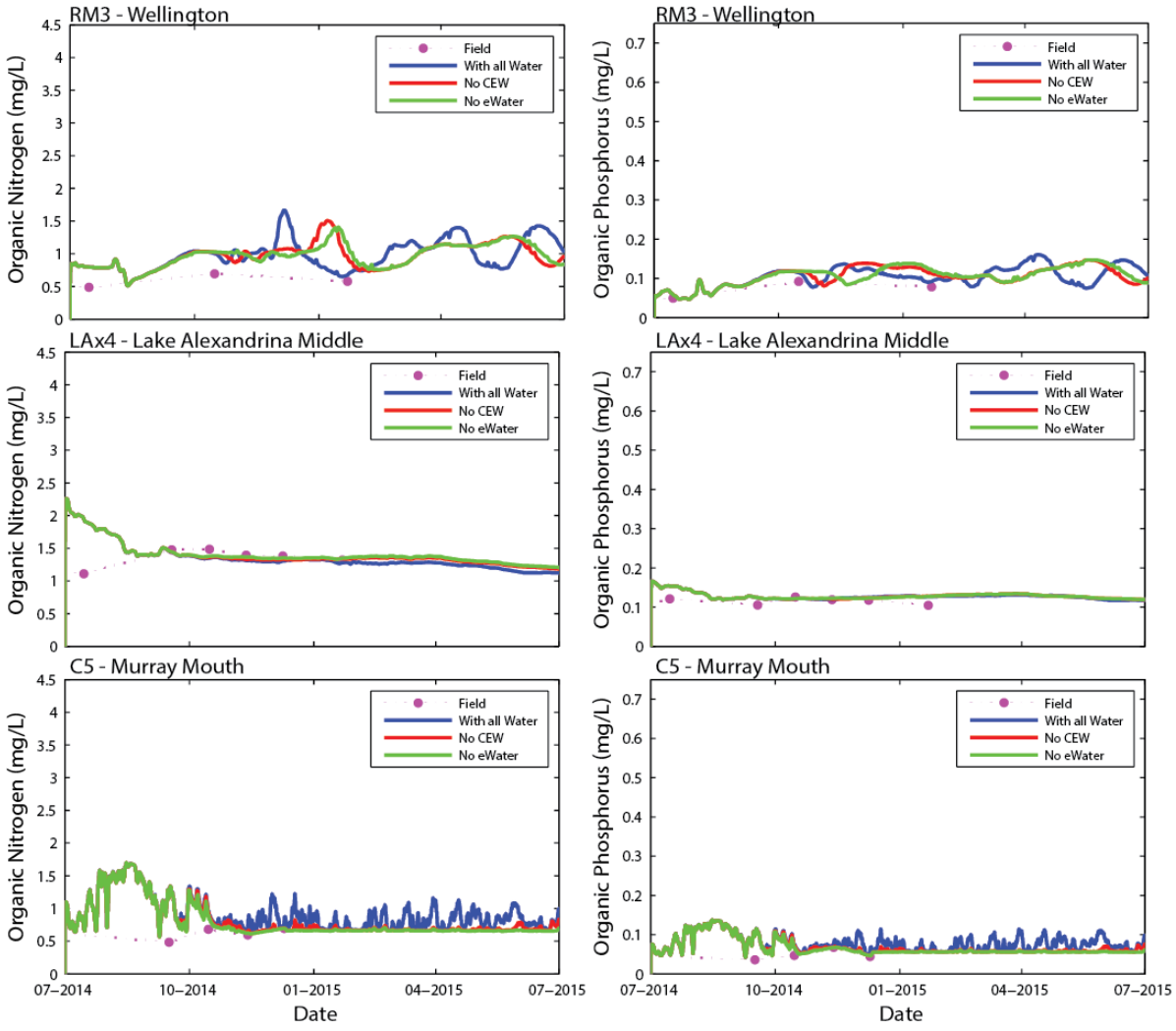

Figure F7. Observed and modelled particulate organic phosphorus concentrations at selected sites. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

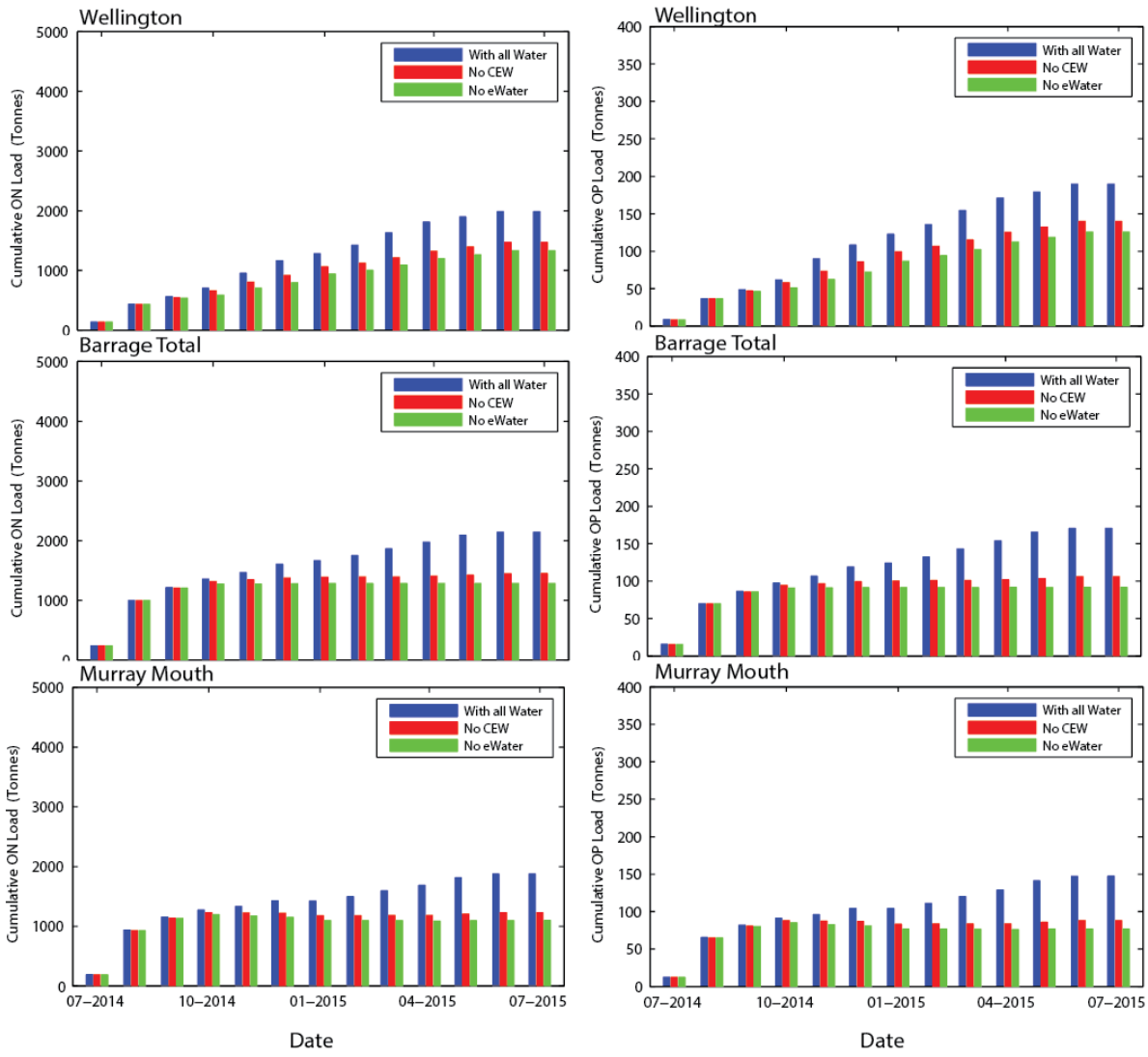

Figure F8. Modelled cumulative particulate organic nitrogen (ON) and phosphorus (OP) exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

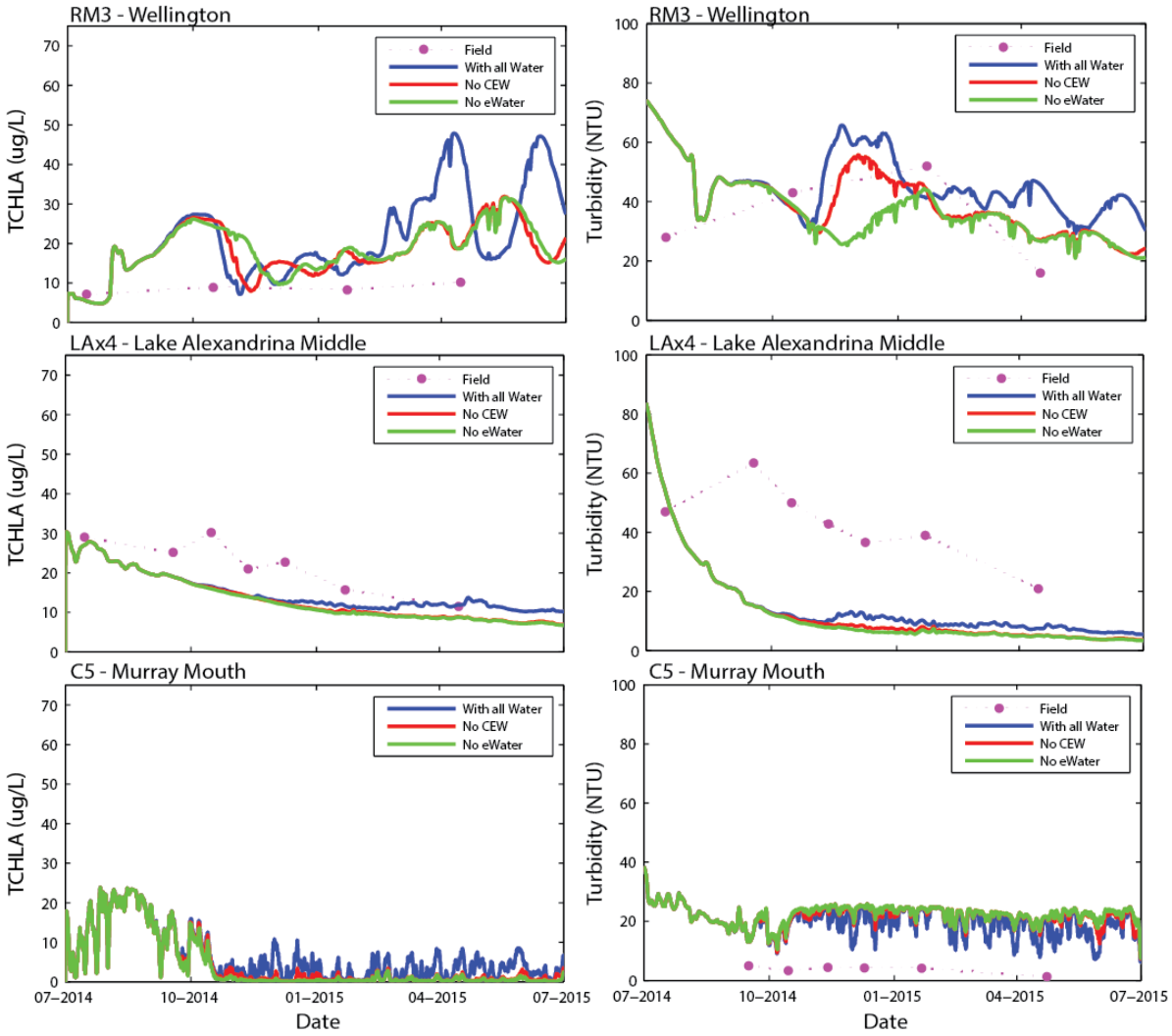

Figure F9. Observed and modelled (with and without environmental watering) chlorophyll a concentrations and turbidity with and without environmental flows. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

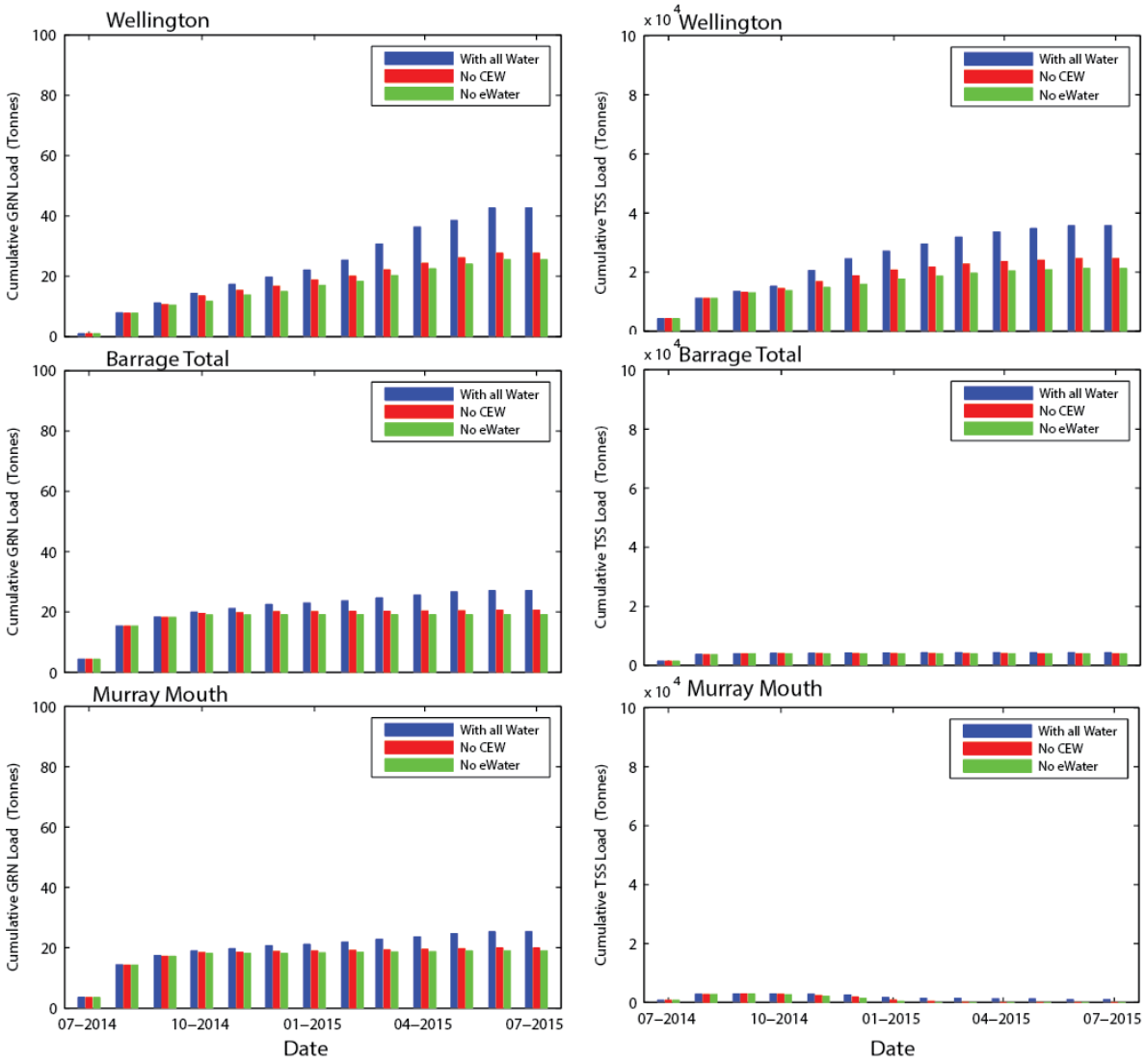

Figure F10. Modelled cumulative phytoplankton (as measured by carbon) and total suspended solid (TSS) exports (net) with and without environmental water delivery. Scenarios include with all water, without Commonwealth environmental water (No CEW) and without any environmental water (No eWater).

APPENDIX G: MICROINVERTEBRATES

Microinvertebrates

Background

Historically, the microinvertebrate assemblage of the LMR was derived from disparate upstream sources, primarily from the upper Murray River catchment, supplemented by periodical, albeit infrequent, flood contributions from the Darling system. There are no records of LMR microinvertebrate assemblages pre-impoundment, however a study of the extant LMR microinvertebrate community (Shiel *et al.* 1982) described the two major sources of plankton into the LMR, *viz.* a cool temperate lacustrine assemblage, including rotifers and microcrustaceans (cladocerans and copepods) in the westward-flowing heavily regulated Murray, and a rotifer-dominated warm-stenothermal plankton in the south-flowing unregulated Darling River. This mixed assemblage persists in the LMR to Lake Alexandrina.

More recently, LMR microinvertebrate densities in the context of a larval fish study were reported by Cheshire (2010), and most recently, the importance of floodplain wetland contributions to replenishment of the riverine microbiota was demonstrated by Furst *et al*. (2014) who monitored inflow and outflow of microinvertebrates from the Chowilla Floodplain during the 2010/11 millennium floods. The significance of the Chowilla Floodplain as an eggbank for microinvertebrate propagules had initially been demonstrated by hatching from various-aged Chowilla Floodplain sediments (Boulton and Lloyd 1992).

To assess the responses of microinvertebrates in the LMR Selected Area to delivery of Commonwealth environmental water in the LMR Selected Area during 2014/15, the following evaluation questions were addressed:

What did Commonwealth environmental water contribute:

- to microinvertebrate diversity?
- 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

Sampling sites and procedure

Microinvertebrate sampling was conducted approximately fortnightly between 3 November 2014 and 20 January at three sites within the floodplain and gorge geomorphic zones of the LMR Selected Area (Figure 5; Table G1), consistent 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.

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

Statistical analyses

Levene's test was used to test for equality of error variances prior to ANOVAs being conducted (SPSS, v19). Assemblage data were log transformed log(x+1) prior to multivariate analyses and all night-time samples were removed (except in the analysis which specifically compares day and night samples). Comparison of the microinvertebrate community between sites and sampling events were undertaken using multivariate analyses; namely Clustering with Simprov test, nMDS ordination, Two-factor PERMANOVA (Anderson and Ter Braak 2003), SIMPER and BIOENV. Bray-Curtis (Bray and Curtis 1957) similarities were used to construct the similarity matrices for all multivariate analyses. Physico-chemical variables and individual species with a Pearson Correlation Coefficient > 0.6 were overlain as vectors on the ordinations to see which water quality variables and species were influencing any observed patterns in the nMDS. All multivariate analyses were undertaken using the package PRIMER version 7 (Clarke and Gorley 2006). Physico-chemical data were normalised prior to inclusion in these analyses.

Results

Species richness

Using data specific to sites that were 5 km downstream of Lock 6 and Lock 1, for which collections were made during the day and at night, there was no significant

difference in microinvertebrate taxa richness between time of day samples (Two-way ANOVA; df = 1_{71}, p = 0.552; Table G2 and Figure G2). Nevertheless, the following univariate analysis used data specific to day-time collections only (i.e. night-time samples were removed prior to further analyses). Data from three sites within each Lock were used.

Table G2. Two-way ANOVA results comparing microinvertebrate taxa richness between locks (5 km below Lock 1 and 5 km below Lock 6) and time of day sampled (day vs night). Degrees of freedom, F and p-values are shown.

Source	df	F	p
Lock	1	0.32	0.576
Time of day	1	0.36	0.552
Lock*Time of day	1	2.49	0.119
Total	72		
Corrected total	71		

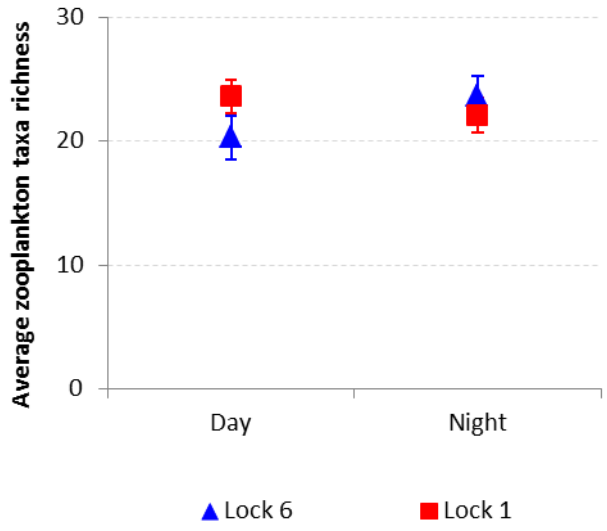

Figure G2. Average microinvertebrate taxa richness recorded from 5km below Lock 6 and 5 km below Lock 1 during different times of the day (day vs night-time collections).

TFor microinvertebrate taxa richness, there was a significant interaction between lock and sampling event (Two-way ANOVA; df = 5_{107}, p = 0.001; Table G3 and Figure G3), which indicates that the change in species richness of microinvertebrate communities through time was different between sites. Generally, microinvertebrate

richness increased steadily throughout the sampling period for Lock 6 and Lock 1 (Figure G3). Although species richness during mid-December 2014 was lowest at Lock 1, while richness at Lock 6 was high.

Table G3. Two-way ANOVA results comparing microinvertebrate taxa richness between locks and sampling events. Degrees of freedom, F and p-values are shown. Tukey's *post-hoc* results are presented in ascending order of mean microinvertebrate richness, with groups with no difference in means joined by a black line.

Source	*df*	*F*	*p*	Tukey's *post-hoc*					
Lock	1	0.33	0.568	**Lock 1**	**Lock 6**				
Sampling event	5	23.83	0.000	**19/20 Nov-14**	**3/4 Nov-14**	**1/2 Dec-14**	**14/15 Dec-14**	**7/8 Jan-15**	**19/20 Jan-15**
Lock*Sampling event	5	4.77	0.001						
Total	108								
Corrected total	107								

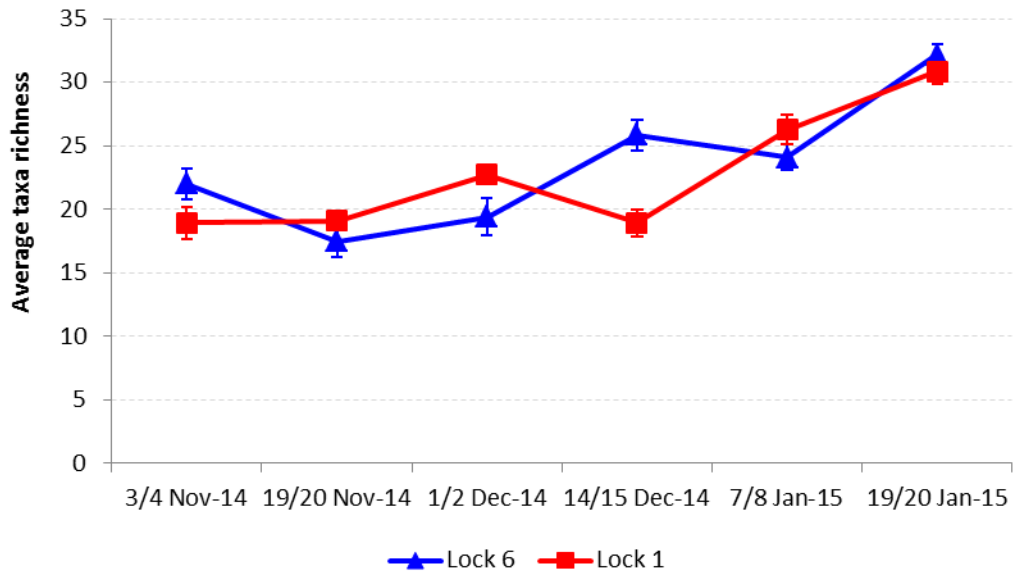

Figure G3. Average microinvertebrate richness (±se) from each Lock in each sampling event, showing change over time.

Microinvertebrate assemblages

Using all data collected, there was no significant difference in microinvertebrate assemblages between day and night-time collections (One-way ANOSIM; R = -0.01; p = 0.65; Figure G4). Nevertheless, the following multivariate analysis used data specific to day-time collections only (i.e. night-time samples were removed prior to further analyses). Data from three sites within each Lock were used.

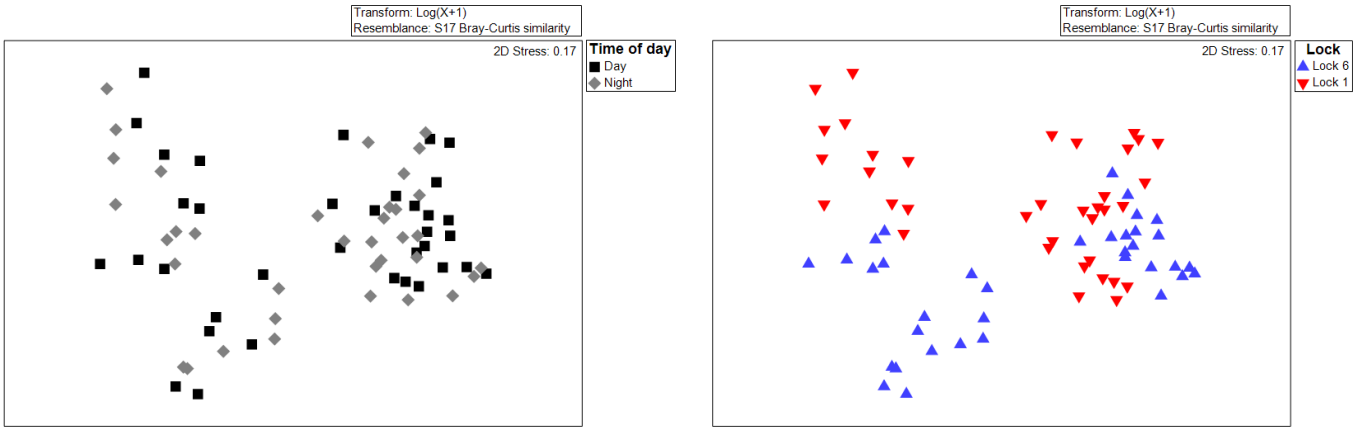

Figure G4. nMDS ordination of microinvertebrate assemblage data (log transformed) for below Locks 1 and 6 only using both day and night-time collection data. Samples are identified by time of day (left), and lock (right). nMDS was based on Bray-Curtis Similarities and 2D Stress was 0.17.

Using data collected during the day at Lock 6 and Lock 1, across all sampling events, patterns were evident in the non-metric MDS ordination of microinvertebrate assemblages (logx+1 transformed; Figure G5). Microinvertebrate assemblages appeared to separate well based on event, with individual events forming relatively tight groups and a temporal sequence apparent across the ordination, i.e. the early November sampling event was most similar to the mid-November event, but was most different to the last three events (mid-December, early January and mid-January; Figure G5). There was considerable overlap of samples from the mid-December and early January sampling events (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 more apparent in the earlier sampling events (early November and mid-November) than subsequent events, with microinvertebrate assemblages within these locks

appearing to become more similar over time, i.e. greater overlap of samples in the more recent events (Figure G5).

A significant interaction was detected between locks and sampling events (Two-factor PERMANOVA; df = 5_{96}, $p < 0.0001$; Table G4), suggesting inconsistent spatio-temporal variation among sampling events between two locks. Post-hoc pairwise tests conducted for each sampling event separately indicated significant differences in microinvertebrate assemblage between two locks for all events (see Table G5). Pairwise tests were conducted separately for each lock to examine differences over time (i.e. between sampling events) (see Tables G6 and G7).

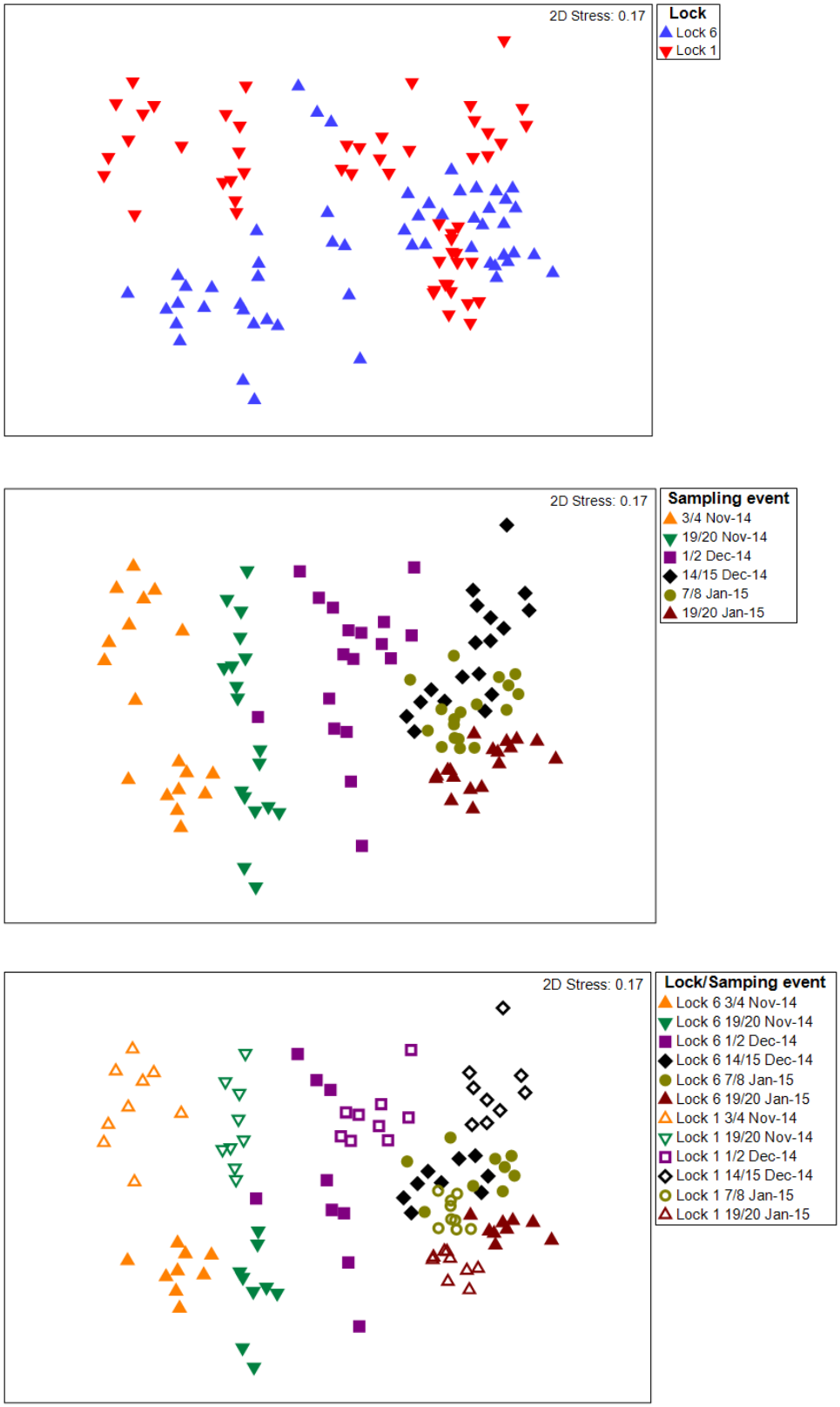

Figure G5. nMDS ordination of microinvertebrate assemblage data (log transformed), with samples identified by lock (top), sampling event (middle), and lock-sampling event (bottom). nMDS was based on Bray-Curtis Similarities and 2D Stress was 0.17.

Table G4. Two-factor PERMANOVA results comparing microinvertebrate assemblages between locks and sampling events. Degrees of freedom, pseudo-F and p-values are shown.

Source	*df*	*Pseudo-F*	*p*
Lock	1	14.20	<0.0001
Sampling event	5	24.56	<0.0001
Lock*Sampling event	5	7.30	<0.0001
Residual	96		
Total	107		

Table G5. Post-hoc pair-wise results of microinvertebrate log(x+1) abundance data betwen locks, within each sampling event, showing R-values (sample statistic)[1].and p-values.

Sampling event	Comparison between Lock 6 and Lock 1	
	R	p-value
3/4 Nov-14	0.969	0.001
19/20 Nov-14	0.889	0.0001
1/2 Dec-14	0.754	0.0002
14/15 Dec-14	0.725	0.00004
7/8 Jan-15	0.678	0.00004
19/20 Jan-15	0.963	0.00004

Lock 6

Within sites below Lock 6, all sampling events were significantly different from one another (Table G6 and Figure G6), discussed below. Generally separation between groups was high (i.e. R ≥ 0.70), with the exception of the mid-December and early January events, which were not as well separated although still significant (R = 0.57; see Table G6 and Figure G6).

Table G6. Within sites below Lock 6 pair-wise results of microinvertebrate log(x+1) abundance data amongst sampling events, showing R-values (sample statistic)[2]. * = groups significantly different.

[1] Sample statistic - R>0.75 = well separated groups, R>0.5 = groups overlapping but clearly different, and R>0.25 = groups barley separable.

[2] Sample statistic - R>0.75 = well separated groups, R>0.5 = groups overlapping but clearly different, and R>0.25 = groups barley separable.

	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan
3/4 Nov					
19/20 Nov	0.84*				
1/2 Dec	0.98*	0.78*			
14/15 Dec	1*	0.98*	0.82*		
7/8 Jan	1*	0.99*	0.97*	0.57*	
19/20 Jan	1*	1*	0.98*	0.94*	0.7*

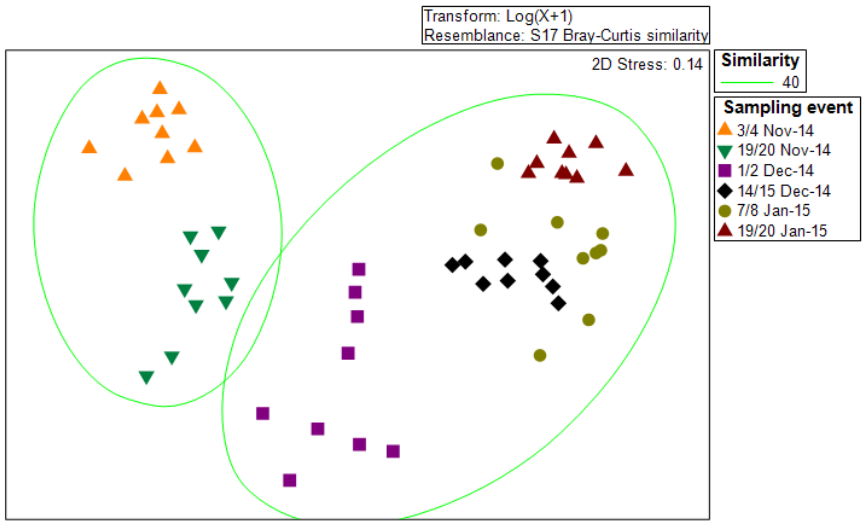

Figure G6. nMDS ordination of microinvertebrate assemblage data (log transformed) from Lock 6, with samples identified by sampling event. nMDS was based on Bray-Curtis Similarities and 2D Stress was 0.14. Samples are grouped within green circles at a Bray-Curtis similarity of 40% (SIMPROF).

SIMPER analysis was used to determine which species were driving the apparent differences between sampling events (all significant). Results are provided below in Table G7.

Table G7. Microinvertebrate taxa responsible for the dissimilarity between sampling events for 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 similarity (%) between sampling events is provided for each comparison.

Sampling event	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan	19/20 Jan
3/4 Nov						
19/20 Nov	50.13% **Conochilus sp. a [sm], Synchaeta sp., cladocera *Bosmina meridionalis*, and protists indet. glob. ciliate [sm], *Stenosemella* sp. and *Codonaria* sp.** Rotifers *Filinia pejleri*, *Trichocerca similis grandis* and *Trichocerca pusilla*					
1/2 Dec	39.83% **Protists indet. glob. ciliate [sm], *Stenosemella* sp. and *Codonaria* sp., the rotifers *Conochilus* sp. a [sm], *Synchaeta* sp., *Trichocerca similis* and indet. 2-toed rotifer [sm], and the cladocera *Bosmina meridionalis*** Rotifers *Polyarthra* sp. a [sm], *Synchaeta pectinata*, *Conochilus* sp. b [lg], *Filinia longiseta* and *Filinia terminalis*	47.63% **Protists *Codonaria* sp. and indet. glob. ciliate [sm], and rotifers *Trichocerca similis grandis*, *Trichocerca pusilla* and *Filinia pejleri*.** Rotifers *Polyarthra* sp. a [sm], *Conochilus* sp. a [sm] and *Filinia longiseta*				

Sampling event	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan	19/20 Jan
14/15 Dec	33.40% **Protists indet. glob. ciliate [sm], *Codonaria* sp., and *Stenosemella* sp., rotifers *Synchaeta* sp. and *Trichocerca similis*, and cladocera *Bosmina meridionalis*.** Rotifers *Polyarthra* sp. a [sm], *Keratella tropica*, *Hexarthra* sp. [?spp.] *Filinia terminalis*, *Brachionus angularis bidens* and *Brachionus diversicornis*, as well as calanoid and cyclopoid nauplii.	38.02% **Protists indet. glob. ciliate [sm] and *Codonaria* sp., as well as rotifer *Trichocerca similis grandis*.** Rotifers *Polyarthra* sp. a [sm], *Keratella tropica*, *Hexarthra* sp. [?spp.], *Filinia terminalis*, *Conochilus* sp. a [sm], *Brachionus diversicornis* and *Brachionus angularis bidens*, and calanoid and cyclopoid nauplii.	48.39% Rotifers *Keratella tropica*, *Hexarthra* sp. [?spp.], *Polyarthra* sp. a [sm], *Filinia terminalis*, *Brachionus diversicornis* and *Brachionus angularis bidens*, and calanoid and cyclopoid nauplii.			
7/8 Jan	27.46% **Protists indet. glob. ciliate [sm] and *Codonaria* sp., the rotifers *Synchaeta* sp., *Conochilus* sp. a [sm] and *Trichocerca similis*, and the cladocera *Bosmina meridionalis*.** *Polyarthra* sp. a [sm] *Keratella tropica*, *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Trichocerca* sp. b [tiny] and *Anuraeopsis coelata*, protist *Difflugia gramen*, as well as calanoid and cyclopoid nauplii and cyclopoid copepodites.	34.99% **Protists indet. glob. ciliate [sm] and *Codonaria* sp., and rotifers *Trichocerca similis grandis*, *Filinia pejleri* and *Synchaeta* sp.** Protist *Difflugia gramen*, and rotifers *Polyarthra* sp. a [sm], *Keratella tropica*, *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Anuraeopsis coelata* and *Trichocerca* sp. b [tiny], as well as	40.92% **Protists indet. glob. ciliate [sm] and *Codonaria* sp., and rotifers *Synchaeta pectinata*, *Synchaeta* sp., *Conochilus* sp. a [sm], and *Filinia longiseta*.** Protist *Difflugia gramen*, rotifers *Keratella tropica*, *Hexarthra* sp. [?spp.], *Filinia terminalis* *Trichocerca similis*	58.69% **Protist *Codonaria* sp. and rotifers *Conochilus* sp. a [sm] and *Brachionus diversicornis*.** Protists *Difflugia gramen* and *Stenosemella* sp. and rotifer *Trichocerca similis grandis*		

Sampling event	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan	19/20 Jan
		cyclopoid and calanoid nauplii.	*grandis*, *Polyarthra* sp. a [sm] and *Anuraeopsis coelata*, calanoid nauplii, and cyclopoid nauplii.			
19/20 Jan	27.27% **Protists indet. glob. ciliate [sm], *Codonaria* sp. and *Stenosemella* sp., and rotifers *Synchaeta* sp., *Conochilus* sp. a [sm] and *Trichocerca similis*.** Rotifers *Filinia terminalis*, *Polyarthra* sp. a [sm], *Anuraeopsis fissa*, *Keratella tropica*, *Hexarthra* sp. [?spp.], *Brachionus diversicornis*, *Anuraeopsis coelata*, *Conochilus* sp. b [lg], *Collotheca* cf. *tenuilobata* and *Brachionus falcatus*, and nauplii of cyclopoid copepods.	32.94% **Protists indet. glob. ciliate [sm] and *Codonaria* sp., and rotifers *Trichocerca similis grandis* and *Synchaeta* sp..** Protist *Difflugia gramen*, and rotifers *Filinia terminalis*, *Polyarthra* sp. a [sm], *Anuraeopsis fissa*, *Keratella tropica*, *Hexarthra* sp. [?spp.], *Brachionus diversicornis*, *Anuraeopsis coelata*, *Trichocerca* sp. b [tiny], *Conochilus* sp. b [lg], *Brachionus falcatus,* and *Collotheca* cf. *tenuilobata*], as well as calanoid and cyclopoid nauplii.	38.32% **Protist indet. glob. ciliate [sm] and the rotifers *Synchaeta pectinata*, *Synchaeta* sp., *Filinia longiseta*.** Protist *Difflugia gramen*, rotifers *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Anuraeopsis fissa*, *Brachionus diversicornis*, *Keratella tropica*, *Anuraeopsis coelata*, *Conochilus* sp. b [lg], *Brachionus falcatus* and *Collotheca* cf. *tenuilobata*, and cyclopoid nauplii.	56.15% ***Synchaeta* sp., *Synchaeta pectinata*, *Polyarthra* sp. a [sm] and calanoid nauplii.** *Anuraeopsis fissa*, *Anuraeopsis coelata*, *Conochilus* sp. b [lg], *Brachionus falcatus*, *Collotheca* cf. *tenuilobata*, *Filinia terminalis* and *Keratella lenzi*.	55.69% *Anuraeopsis fissa*, *Conochilus* sp. b [lg], *Filinia terminalis*, *Brachionus diversicornis*, *Collotheca* cf. *tenuilobata*, *Brachionus falcatus* and *Anuraeopsis coelata*.	

Pearson correlations between individual species and patterns within the sampling event ordination generally concurred with the SIMPER analysis described above, with the greater abundances of the protists indet. glob. ciliate [sm] and *Codonaria* sp., and rotifers *Synchaeta* sp. and *Trichocerca similis*, and lower abundances of the protist *Difflugia gramen*, rotifers *Filinia terminalis*, *Anuraeopsis coelata*, *Brachionus diversicornis*, *Polyarthra* sp. a [sm], *Brachionus falcatus*, *Hexarthra* sp. [?spp.], *Trichocerca* sp. b [tiny], *Brachionus angularis bidens*, *Keratella tropica* and *Polyarthra* sp. a [sm], as well as cyclopoid nauplii and calanoid nauplii recorded during the early November event driving the difference between this event and the more recent events in mid-December, early January and mid-January.

Patterns within the below Lock 6 microinvertebrate assemblage ordination were influenced by differences in physical parameters between sampling events, including turbidity (NTU), water temperature (°C) and observed flow (ML day^{-1}) (BIOENV; Rho = 0.77, p = 0.0001). Turbidity and flow were greater during the mid-November sampling event, while water temperature was higher during the mid-December and early January events (see Figure G7).

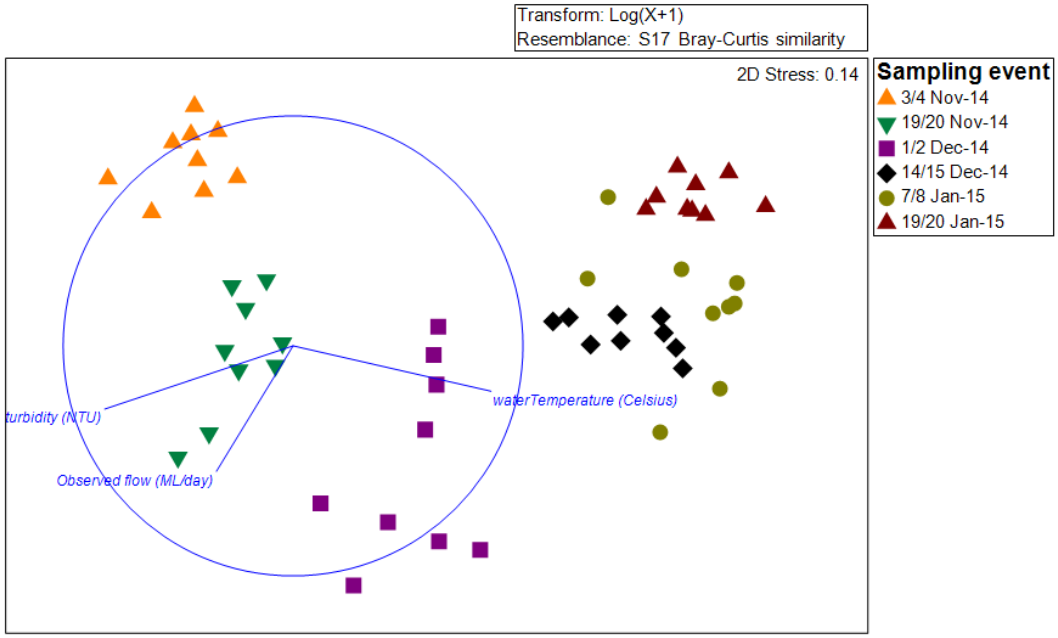

Figure G7. nMDS ordination of microinvertebrate assemblage data (log transformed) from Lock 6, with samples identified by sampling event. nMDS was based on Bray-Curtis Similarities and 2D Stress was 0.14. Samples are grouped within green c: Vectors of Pearson Correlations (correlation >0.6) with physical parameters overlain on the Lock 6 ordination. NB: water quality variables were normalised prior to analysis circles at a Bray-Curtis similarity of 40% (SIMPROF).

Lock 1

Similarly, within sites below Lock 1, all sampling events were significantly different from one another (Table G8 and Figure G8). In this case, all separations were high, with most sampling events having an R-value greater than 0.9 (Table G8).

Table G8. Within sites below Lock 1 pair-wise results of microinvertebrate log(x+1) abundance data amongst sampling events, showing R-values (sample statistic). * = groups significantly different.

	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan
3/4 Nov					
19/20 Nov	0.96*				
1/2 Dec	1*	0.98*			
14/15 Dec	1*	1*	0.88*		
7/8 Jan	1*	1*	0.99*	0.79*	
19/20 Jan	1*	1*	1*	0.98*	0.99*

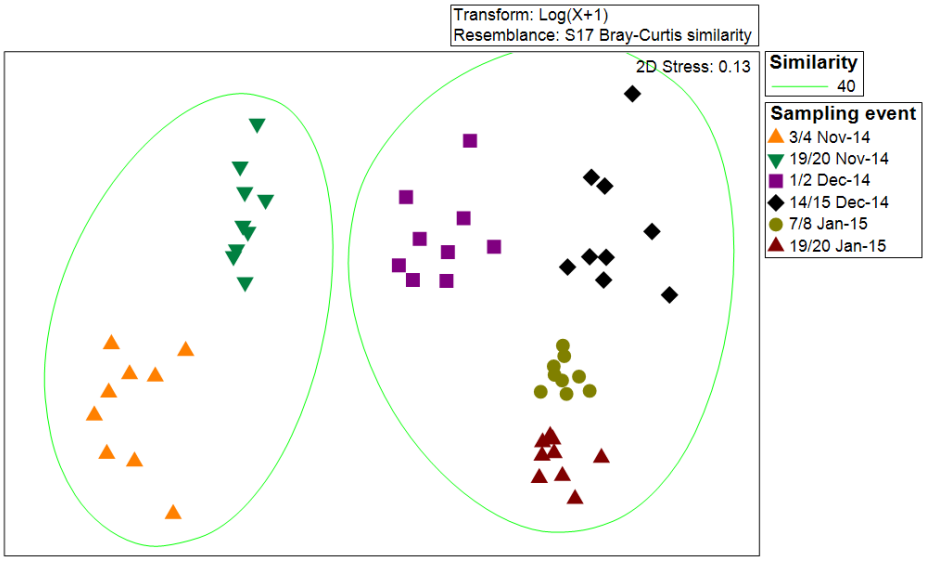

Figure G8. nMDS ordination of microinvertebrate assemblage data (log transformed) from Lock 1, with samples identified by sampling event. nMDS was based on Bray-Curtis Similarities and 2D Stress was 0.13. Samples are grouped within green circles at a Bray-Curtis similarity of 40% (SIMPROF).

Results from the SIMPER analysis comparing microinvertebrate assemblages within sites below Lock 1 between sampling events (all significant) is provided below in Table G9.

Table G9. Microinvertebrate taxa responsible for the dissimilarity between sampling events for 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 similarity (%) between sampling events is provided for each comparison.

Sampling event	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan	19/20 Jan
3/4 Nov						
19/20 Nov	44.13% ***Codonaria* sp., indet. glob. ciliate [lg], *Conochilus* sp. a [sm], *Proalides tentaculatus*, *Cyphoderia ampulla* and *Difflugia* cf. *fallax*.** *Trichocerca similis grandis*, *Synchaeta pectinata*, *Polyarthra* sp. a [sm] and *Trichocerca pusilla*.					
1/2 Dec	36.20% ***Synchaeta* sp., *Codonaria* sp., indet. glob. ciliate [lg], *Conochilus* sp. a [sm], *Difflugia* cf. *fallax* and *Stenosemella* sp..** *Polyarthra* sp. a [sm], *Trichocerca pusilla*, *Hexarthra* sp. [?spp.], *Keratella tropica*, flosculariid sp. [cf. *Sinanatherina*], *Conochilus* sp. b [lg], *Filinia pejleri* and *Trichocerca* sp. b [tiny].	46.39% ***Synchaeta* sp., *Trichocerca similis grandis* and *Synchaeta pectinata*.** *Polyarthra* sp. a [sm], *Hexarthra* sp. [?spp.], *Keratella tropica*, *Trichocerca pusilla* and flosculariid sp. [cf. *Sinanatherina*].				

Sampling event	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan	19/20 Jan
14/15 Dec	23.73% ***Codonaria* sp., *Synchaeta* sp., *Stenosemella* sp., indet. glob. ciliate [lg,] *Proalides tentaculatus* and *Cyphoderia ampulla*.** *Polyarthra* sp. a [sm], *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Keratella tropica*, *Trichocerca pusilla*, *Polyarthra* sp. b [lg], *Brachionus angularis bidens* and *Filinia pejleri*.	31.53% ***Synchaeta* sp., *Trichocerca similis grandis*, *Synchaeta pectinata*, *Stenosemella* sp. and *Codonaria* sp..** *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Polyarthra* sp. a [sm], *Keratella tropica* and *Polyarthra* sp. b [lg].	48.71% ***Stenosemella* sp. and *Trichocerca pusilla*.** *Filinia terminalis*, *Polyarthra* sp. b [lg], *Brachionus angularis bidens* and *Brachionus diversicornis*.	[redacted]		
7/8 Jan	28.77% ***Synchaeta* sp. and indet. glob. ciliate [lg].** *Polyarthra* sp. a [sm], *Keratella tropica*, *Filinia terminalis*, *Trichocerca pusilla*, *Hexarthra* sp. [?spp.], *Anuraeopsis coelata*, *Codonaria* sp., *Trichocerca similis grandis*, *Brachionus diversicornis*, *Polyarthra* sp. b [lg] and cyclopoid copepodites.	35.91% ***Synchaeta* sp., *Synchaeta pectinata* and *Trichocerca similis grandis*.** *Codonaria* sp., *Keratella tropica*, *Filinia terminalis*, *Hexarthra* sp. [?spp.], *Polyarthra* sp. a [sm], *Anuraeopsis coelata*, *Trichocerca pusilla*, *Brachionus diversicornis*, *Polyarthra* sp. b [lg] and cyclopoid copepodites.	48.22% ***Conochilus* sp. b [lg] and *Filinia pejleri*.** *Codonaria* sp., *Filinia terminalis*, *Anuraeopsis coelata*, *Keratella tropica*, *Brachionus diversicornis*, cyclopoid copepodites, *Trichocerca similis grandis, Polyarthra* sp. b [lg], *Hexarthra* sp. [?spp.] and *Brachionus budapestinensis*.	51.50% ***Brachionus angularis bidens*.** *Codonaria* sp., *Anuraeopsis coelata*, *Trichocerca pusilla*, *Trichocerca similis grandis*, *Keratella tropica*, *Stenosemella* sp., and cyclopoid copepodites.	[redacted]	

Sampling event	3/4 Nov	19/20 Nov	1/2 Dec	14/15 Dec	7/8 Jan	19/20 Jan
19/20 Jan	25.57% ***Synchaeta* sp., indet. glob. ciliate [lg] and *Bosmina meridionalis*.** *Trichocerca pusilla*, *Polyarthra* sp. a [sm], *Keratella lenzi*, *Hexarthra* sp. [?spp.], *Keratella tropica*, *Filinia terminalis*, *Trichocerca similis grandis*, *Filinia longiseta*, *Brachionus angularis bidens*, *Trichocerca* sp. b [tiny], *Collotheca* cf. *tenuilobata*, *Conochilus* sp. b [lg] *Synchaeta pectinata*, and cyclopoid copepodites.	34.96% ***Synchaeta* sp. and *Trichocerca similis grandis*.** *Keratella lenzi*, *Keratella tropica*, *Hexarthra* sp. [?spp.], *Trichocerca pusilla*, *Filinia terminalis*, *Codonaria* sp., *Filinia longiseta*, *Polyarthra* sp. a [sm], *Collotheca* cf. *tenuilobata*, *Trichocerca* sp. b [tiny], cyclopoid copepodite, Conochilus sp. b [lg], *Brachionus budapestinensis* and *Brachionus diversicornis*.	44.34% **The cladocera *Bosmina meridionalis*.** *Keratella lenzi*, *Codonaria* sp., *Filinia terminalis*, *Filinia longiseta*, *Trichocerca pusilla*, *Trichocerca similis grandis*, *Keratella tropica*, *Collotheca* cf. *tenuilobata*, *Hexarthra* sp. [?spp.], cyclopoid copepodites, *Brachionus angularis bidens*, *Brachionus budapestinensis*, *Synchaeta pectinata* and *Brachionus diversicornis.*	44.02% **Calanoid nauplii, *Bosmina meridionalis* and *Polyarthra* sp. b [lg].** *Keratella lenzi*, *Codonaria* sp., *Trichocerca pusilla*, *Trichocerca similis grandis*, *Filinia longiseta*, *Collotheca* cf. *tenuilobata*, *Keratella tropica*, *Conochilus* sp. b [lg], *Stenosemella* sp., *Synchaeta pectinata* and *Trichocerca* sp. b [tiny].	58.54% ***Anuraeopsis coelata*, *Polyarthra* sp. b [lg], calanoid nauplii and *Bosmina meridionalis*.** *Keratella lenzi*, *Filinia longiseta*, *Collotheca* cf. *tenuilobata*, *Synchaeta pectinata*, *Trichocerca pusilla*, *Conochilus* sp. b [lg], *Brachionus angularis bidens* and *Brachionus falcatus*.	[redacted]

Pearson correlations between individual species and patterns within the sites below Lock 1 sampling event ordination generally concurred with the SIMPER analysis results, with the greater abundances of *Synchaeta* sp. and *Stenosemella* sp. recorded during the early November event influencing the separation of this event from all others, greater abundances of *Polyarthra* sp. a [sm] recorded during the early December and mid-December events influencing the position of these events in the ordination, and greater abundances of *Hexarthra* sp. [?spp.], *Filinia terminalis*, *Trichocerca pusilla*, *Brachionus diversicornis*, *Keratella tropica*, cyclopoid copepodites, *Brachionus budapestinensis* and *Keratella lenzi* recorded during the two most recent events (early January and mid-January) influencing the separation of these events from all others.

Patterns within the sites below Lock 1 microinvertebrate assemblage ordination were influenced by differences in physical parameters between sampling events, including turbidity (NTU), water temperature (°C) and observed flow (ML day^{-1}) (BIOENV; Rho = 0.83, p = 0.0001). Turbidity and flow were greater during the early November and mid-November sampling events, while water temperature was higher during the early December and mid-December events at sites below Lock 1 (see Figure G9).

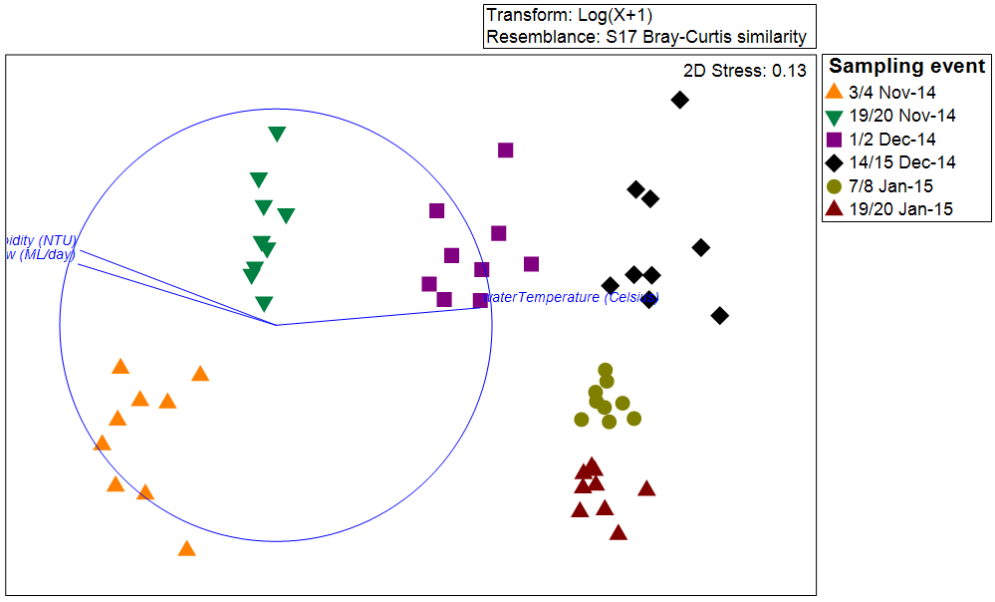

Figure G9. nMDS ordination of microinvertebrate assemblage data (log transformed) from Lock 1, with samples identified by sampling event. nMDS was based on Bray-Curtis Similarities and 2D Stress was 0.13. Samples are grouped within green circles at a Bray-Curtis similarity of 40% (SIMPROF). Vectors of Pearson Correlations (correlation >0.6) with physical parameters overlain on the Lock 1 ordination.

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 (*n* = 2), larvae of other large-bodied species were included in the gut-content analysis. Gut contents of golden perch (*n* = 2), Murray cod (*n* = 16) and freshwater catfish (*n* = 7) post-larvae, collected opportunistically through larval fish sampling as part of Category 3 Fish spawning and recruitment (Table G10), were analysed using traditional taxonomic methods. Most Murray cod (11/16) and freshwater catfish (4/7) guts were empty. Within species, there was no prey item that was present in more than one individual (Table G11), which may be a reflection of the different dates and locations that these individuals were collected from (Table G10). The cladoceran, *Bosmina meridionalis*, was abundant (11 individuals) in one golden perch larvae during mid-November 2014 (Table G11).

Low sample sizes of larvae and patchiness of samples at temporal and spatial scales (Table G10) did not allow for a quantitative comparison of fish diet to ambient microinvertebrate prey composition to determine feeding selectivity. In turn, the contribution of Commonwealth environmental water on the dietary composition of large-bodied fish larvae could not be evaluated.

Table G10. 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 downstream of Lock 1. Similarly, Lock 6, 6A and 6B sites are situated 5, 7 and 9 km downstream of Lock 6.

Species	Total length (mm)	Site	Date	Gut contents
Golden perch	11	Lock 6B	18/11/14	
Golden perch	14	Lock 6	1/12/14	
Murray cod	10	Lock 6	3/11/2014	
Murray cod	11	Lock 1	4/11/2014	
Murray cod	11	Lock 1	4/11/2014	Empty
Murray cod	12	Lock 1	4/11/2014	
Murray cod	10	Lock 6	18/11/2014	
Murray cod	11	Lock 6	18/11/2014	Empty
Murray cod	11	Lock 1B	4/11/2014	Empty
Murray cod	12	Lock 1	4/11/14	Empty
Murray cod	11	Lock 1	4/11/2014	Empty
Murray cod	11	Lock 1A	19/11/2014	Empty
Murray cod	11	Lock 1	4/11/2014	Empty
Murray cod	10	Lock 6	3/11/2014	Empty
Murray cod	10	Lock 1	4/11/2014	Empty
Murray cod	11	Lock 1	4/11/2014	Empty
Murray cod	11	Lock 1	4/11/2014	
Murray cod	11	Lock 1	4/11/2014	Empty
Freshwater catfish	13	Lock 1A	19/11/2014	
Freshwater catfish	13	Lock 6	18/11/2014	Empty
Freshwater catfish	15	Lock 6	18/11/2014	
Freshwater catfish	14	Lock 1A	19/11/2014	
Freshwater catfish	13	Lock 6	18/11/2014	Empty
Freshwater catfish	12	Lock 6	18/11/2014	Empty
Freshwater catfish	14	Lock 1	2/12/2014	Empty

Table G11. Summary of gut content analysis of post-larval golden perch (*n* = 2; total length (TL) = 11–14 mm), Murray cod (*n* = 5; TL = 10–12 mm) and freshwater catfish (*n* = 3; TL = 13–15 mm). %N represents the numerical proportion of a prey item towards the total within each species. indet. egg = egg that could not be classified to any taxonomic group.

Prey	Golden perch Presence	%N	Murray cod Presence	%N	Freshwater catfish Presence	%N
Copepoda						
Calanoida						
Boeckella triarticulata	1/2	15.0	1/5	14.3		
Calamoecia sp.					1/3	33.3
copepodites					1/3	33.3
eggs	1/2	15.0				
Cladocera						
Bosmina meridionalis	1/2	55.0			1/3	11.1
Alona quadrangularis			1/5	14.3		
Chydorus sphaericus	1/2	5.0				
Ceriodaphnia sp.			1/5	14.3		
Neothrix armata			1/5	14.3		
Rotifera						
Brachionus calyciflorus	1/2	5.0				
Keratella procurva			1/5	14.3		
unid. Rotifer	1/2	5.0				
Insecta						
Chironomidae			1/5	14.3	1/3	22.2
indet. egg			1/5	14.3		

*amorphous white flocculent material also present in some freshwater catfish guts

APPENDIX H: FISH SPAWNING AND RECRUITMENT

Background

Restoring flow regimes with environmental water delivery 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 corresponds with overbank flooding and increased discharge that remains in-channel (Mallen-Cooper and Stuart 2003; Zampatti and Leigh 2013a; 2013b). As such, throughout the MDB, golden perch is considered a candidate species to inform, and measure ecological response to, environmental water delivery.

Understanding the influence of hydrology on the population dynamics of golden 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 southern 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 golden perch 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.

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 2014/15, water samples were collected weekly–monthly from ten 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	15/09/14–16/02/15	12
Murray	Lock 6	16/09/14–03/03/15	13
Murray	Lock 9	17/09/14–10/02/15	11
Murray	Lock 11	17/09/14–23/02/15	11
Murray	Torrumbarry	15/09/14–16/02/15	9
Murray	Barmah	14/11/14–10/12/14	3
Darling	Weir 32	09/09/14–02/03/15	12
Edward–Wakool	Deniliquin	19/09/14–19/02/15	12
Murrumbidgee	Narrandera	19/09/14–09/02/15	10
Goulburn	Yambuna	01/10/14–09/12/14	9

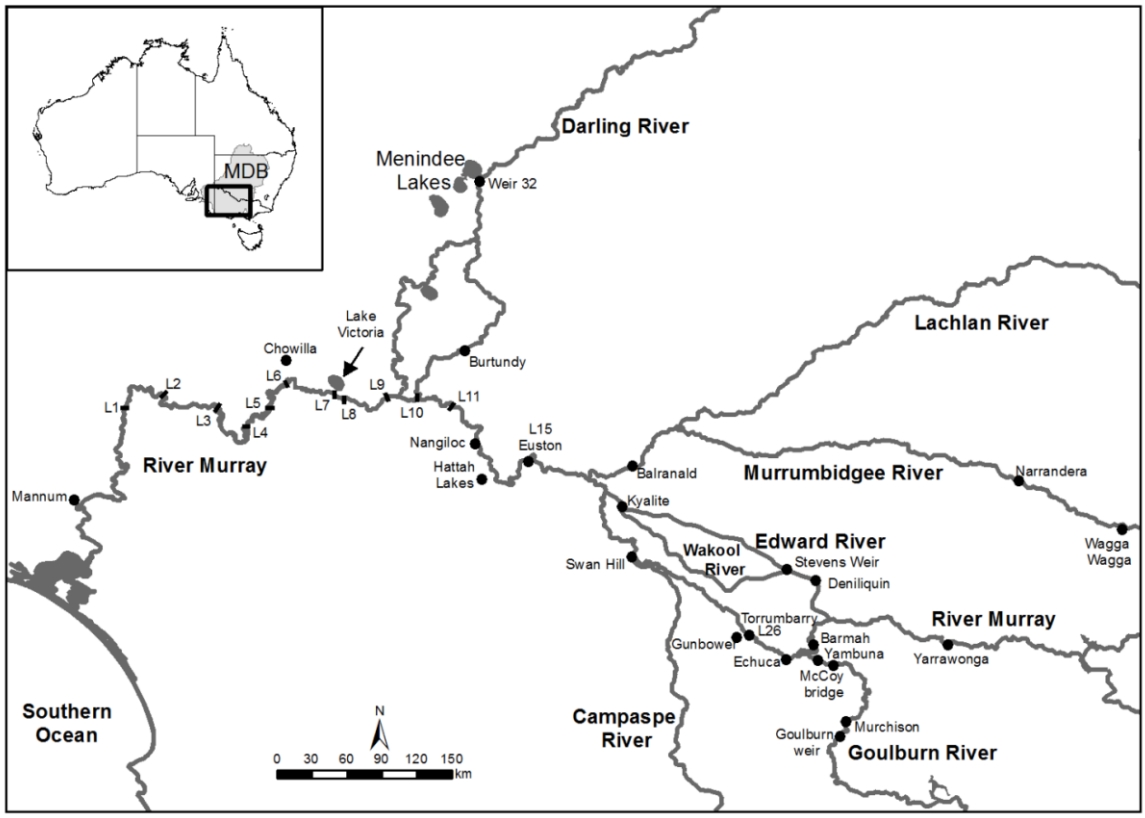

Figure H1. Map showing the location of the Murray–Darling Basin and the major rivers that comprise the southern Murray-Darling Basin, showing the numbered Locks and Weirs (up to Lock 26, Torrumbarry), the Darling, Lachlan, Murrumbidgee, Edward–Wakool, Campaspe and Goulburn rivers and Lake Victoria, an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

Sampling golden perch eggs and larvae

Larval fish sampling was conducted at three sites within the floodplain and gorge geomorphic zones of the LMR Selected Area, consistent with microinvertebrate sampling (Figure 5; Table G1).

Sampling young-of-year golden perch and population age-structure

Adult and juvenile golden perch were sampled by boat electrofishing at five and twelve sites in the floodplain and gorge zones, respectively, of the LMR Selected Area (Table H2).

Table H2. 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	Rilli Launch	S34.39307	E140.58388
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

Aliquots (20 ml) of each water sample were filtered through a 0.2 µm Acrodisc syringe-mounted filter into a clean polystyrene beaker and dried overnight in a HEPA-filtered fume cupboard. Previous analyses have shown that filtering after transfer to the laboratory, rather than after sample collection in the field, has no influence on measurement of $^{87}Sr/^{86}Sr$ (e.g. Palmer and Edmond 1989).

Strontium was extracted using a single pass over 0.15 ml (4 x 12 mm) beds of EICHROMTM Sr resin (50–100 µm). Following Pin *et al*. (1994), matrix elements were washed off the resin with 2M and 7M nitric acid, followed by elution of clean Sr in 0.05M nitric acid. The total blank, including syringe-filtering, is ≤0.1 ng, implying sample to blank ratios of ≥4000; no blank corrections were therefore deemed

necessary. Strontium isotope analyses were carried out on a "Nu Plasma" multi-collector inductively coupled plasma mass spectrophotometer (ICPMS) (Nu Instruments, Wrexham, UK) interfaced with an ARIDUS desolvating nebulizer, operated at an uptake rate of ~40 µL min^{-1}. Mass bias was corrected by normalizing to $^{88}Sr:^{86}Sr$ = 8.37521 and results reported relative to a value of 0.710230 for the SRM987 Sr isotope standard. Internal precisions (2SE) based on at least 30 ten-second integrations averaged ± 0.00002 and average reproducibility (2SD) was ± 0.00004.

Sampling golden perch eggs and larvae

Larval fish sampling was conducted approximately fortnightly between 3 November 2014 and 20 January 2014. Three day-time and three night-time plankton tows were undertaken on the same day at sites 5 km downstream of each lock, while one day-time plankton tow was undertaken at all other sites (Table G1). 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).

Figure H2. Retrieving a bongo net in the main channel of the Lower Murray River.

Sampling YOY golden perch and population age-structure

Adult and juvenile golden 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 March/April 2015 to maximise the chance of collecting YOY golden perch spawned in the spring–summer 2014/15 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 900 to 2880 seconds. All individuals were measured to the nearest mm (total length, TL) and a subsample of fish (*n* = 46–99) 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.

Figure H3. Electrofishing for young-of-year and adult golden perch (left), and daily increments on a young-of-year golden perch otolith (right).

Ageing

Larvae and YOY

To estimate the spawn date of larval and YOY golden perch, daily increment counts in otolith microstructure were examined in ten fish collected from the LMR Selected Area. Golden perch 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 (9, 5, and 3 µm). Sections were then polished using 0.3 µm alumina slurry to a thickness of 50–100 µm.

Sections were examined using a compound microscope (x 600) fitted with a digital camera and Optimas image analysis software (version 6.5, Media Cybernetics, Maryland, USA). 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, we investigated both length and age-frequency distributions. Fish retained for ageing (n = 145) 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.

87Sr/86Sr analysis

Larvae and YOY 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

Laser ablation – inductively coupled plasma mass spectrometry (LA-ICPMS) was used to measure $^{87}Sr/^{86}Sr$ in the otoliths of larval and juvenile golden perch. The experimental system consisted of a ''Nu Plasma'' multi-collector LA-ICPMS (Nu Instruments, Wrexham, UK), coupled to a HelEx laser ablation system (Laurin Technic, Canberra, Australia, and the Australian National University) constructed around a Compex 110 excimer laser (Lambda Physik, Gottingen, Germany) operating at 193 nm. Otolith mounts were placed in the sample cell and the primordium of each otolith was located visually with a 400× objective and a video imaging system. The intended ablation path on each sample was then digitally plotted using GeoStar v6.14 software (Resonetics, USA). Each otolith was ablated along a transect from the primordium to the dorsal margin at the widest radius using a 6 × 100 µm rectangular laser slit. The laser was operated at 90 mJ, pulsed at 10 Hz and scanned at 5 or 10 µm sec^{-1} (depending on the size of the otolith) across the sample. Ablation was performed under pure helium (He) to minimise the re-deposition of ablated material, and the sample was then rapidly entrained into the argon (Ar) carrier gas flow. A pre-ablation step using reduced energy (50 mJ) was conducted along each transect to remove any surface contaminants and a 20–30 sec background was measured prior to acquiring data for each sample. Corrections for krypton (Kr) and rubidium (Rb) interferences were made following closely the procedures of Woodhead *et al*. (2005) and mass bias was then corrected by reference to an $^{86}Sr/^{88}Sr$ ratio of 0.1194. Iolite Version 2.13 (Paton *et al*. 2011) that operates within IGOR Pro Version 6.2.2.2 (WaveMetrics, Inc., Oregon) was used to process data offline, with data corrected for potential Ca argide/dimer interferences.

A modern marine carbonate standard composed of mollusc shells ($^{87}Sr/^{86}Sr$ value of 0.70916 according to long-term laboratory measurements, identical to the accepted modern seawater value of 0.709160, MacArthur and Howarth (2004) was analysed after every 10 otolith samples to allow for calculation of external precision. Mean (±1 SD) values of $^{87}Sr/^{86}Sr$ values in the modern marine carbonate standard (n = 24) run throughout the analyses were 0.70918 ± 0.00017, with external precision

(expressed as ± 2 SE) calculated as ± 0.00006. Mean within-run precision, measured as ± 2 SE, was ± 0.00005.

Results

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

Water sample collection commenced in mid-September 2014 and extended, at the majority of sites, through until late February 2015. Overall, $^{87}Sr/^{86}Sr$ at most locations remained reasonably stable throughout the period of collection, with the highest ratios (>0.7190) measured in the Murray River at Barmah and the Edward River, and the lowest (<0.7080) in the Darling River (Figure H4). Water $^{87}Sr/^{86}Sr$ generally decreased longitudinally along the Murray River as tributaries with distinct and relatively temporally stable $^{87}Sr/^{86}Sr$ (e.g. Goulburn and Murrumbidgee rivers) contribute to discharge, although $^{87}Sr/^{86}Sr$ of the Murrumbidgee River showed overlap with $^{87}Sr/^{86}Sr$ at Lock 9 in the lower River Murray (below the Darling River junction) from late September to mid-October and at Lock 6 in the LMR from mid-November. Water $^{87}Sr/^{86}Sr$ was most variable at Lock 6 (0.7104–0.7148), particularly between February and March (Figure H4).

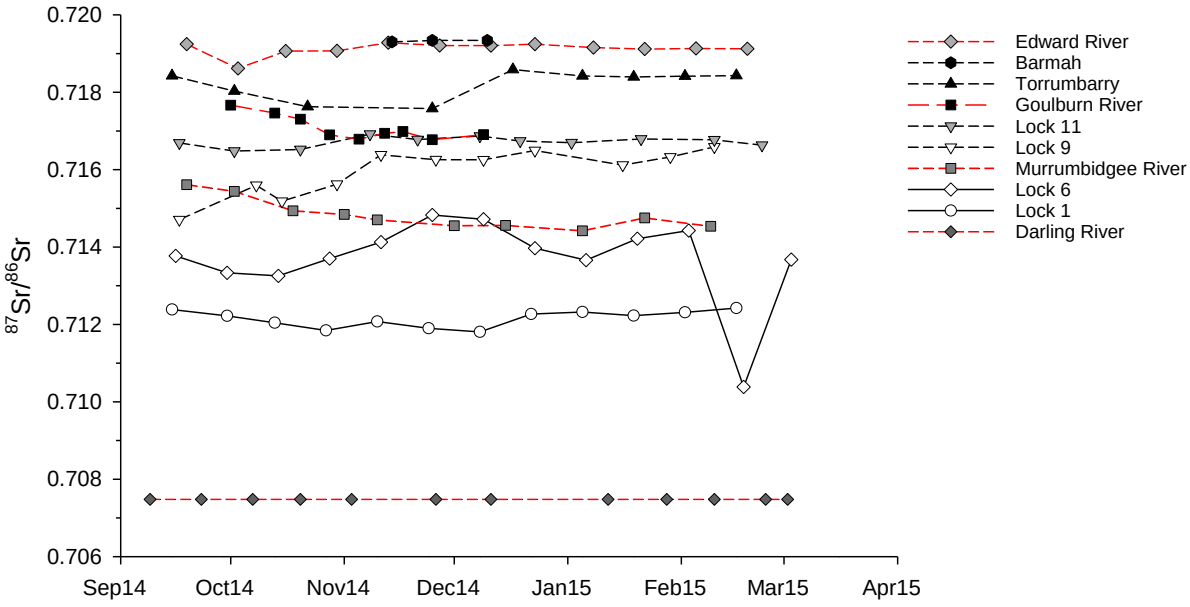

Figure H4. $^{87}Sr/^{86}Sr$ ratios in water samples collected from mid-September 2014 to early March 2015 in the Murray (Lock 1, 6, 9, 11 Torrumbarry and Barmah), Darling, Goulburn, Edward and Murrumbidgee rivers.

From October 2014 to March 2015, flow in the LMR (discharge at the South Australian border, QSA) ranged approximately 7,000–10,600 ML day^{-1} (Figure H5). In October and November 2014, flow ranged 9,000–10,000 ML day^{-1} before gradually decreasing to 7,000 ML day^{-1} in mid-January 2015. In mid- to late January 2015, flow increased sharply to 10,600 ML day^{-1}, where it remained 9,000–10,600 ML day^{-1} until mid-March 2015. QSA was comprised of flow from the upper Murray River, Lake Victoria, Murrumbidgee River and Victorian tributaries of the Murray River (Figure 4).

Flow in the mid-reaches of the Murray River at Euston increased from approximately 6,000 ML day^{-1} in early October 2014 to a maximum of approximately 13,700 ML day^{-1} in early November 2014 (Figure H5). Flow then decreased to 9,600 ML day^{-1} in late November 2014, before increasing to approximately 13,600 ML day^{-1} in early December 2014. Flow then decreased to approximately 6,500 ML day^{-1} in early January 2015 before rising again to peaks of 11,400 ML day^{-1} and 9,800 ML day^{-1} in late January 2015 and early March 2015, respectively. Flow in the Darling River at Burtundy was <250 ML day^{-1} from early September 2014 to April 2015 and absent through mid- to late March (Figure H5).

From early September 2014 to early March 2015, the contribution of Commonwealth environmental water to flow at the South Australian border ranged ~600–3,900 ML day^{-1}, with Commonwealth environmental water peaking at ~3,200–3,400 ML day^{-1} through 27 October to 29 November 2014 and at ~3,900 ML day^{-1} during early March 2015 (Figure 2). Environmental water from the MDBA's The Living Murray program was delivered from 8 September 2014 to 16 February 2015, peaking at ~2,400 ML day^{-1} through 30 September to 26 October 2014.

Throughout the sampling period, $^{87}Sr/^{86}Sr$ in water samples collected from Lock 9, 6 and 1 in the lower River Murray, below the Darling River junction, reflected water delivery from the mid-Murray River, and minimal input from the Darling River (Figure H5); During mid-February, however, $^{87}Sr/^{86}Sr$ at Lock 6 decreased sharply towards 0.710, most likely due to large volumes of water being delivered from Lake Victoria (Figure H5) which may have low $^{87}Sr/^{86}Sr$ due to storage of water from the Darling River in previous years.

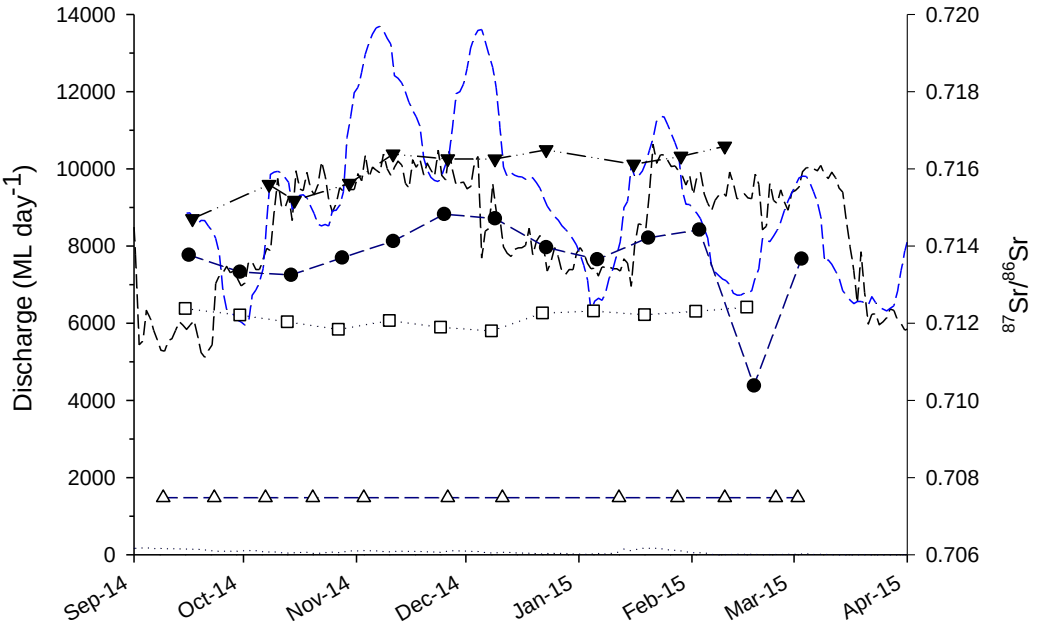

Figure H5. Mean daily discharge (ML day^{-1}) in the Murray River at the South Australian border (dashed black line) and Euston (dashed blue line), and Darling River at Burtundy (dotted black line). $^{87}Sr/^{86}Sr$ in water samples collected from mid-September 2014 to early March 2015 in the lower 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).

Golden perch

In 2014/15, low numbers of golden perch larvae were collected at sites downstream of Lock 1 (n = 5) and Lock 6 (n = 4), respectively. Larvae were collected on the first sampling trip in early November when water temperature was ~21°C. Relative abundances of golden perch larvae peaked at sites downstream of Lock 1 and Lock 6 in mid-November 2014 and early December 2014, respectively (Figure H6). All golden perch larvae collected at sites below Lock 1 were pre-flexion, whilst the majority of golden perch larvae collected at sites below Lock 6 were post-flexion. No golden perch larvae were collected after early December 2014.

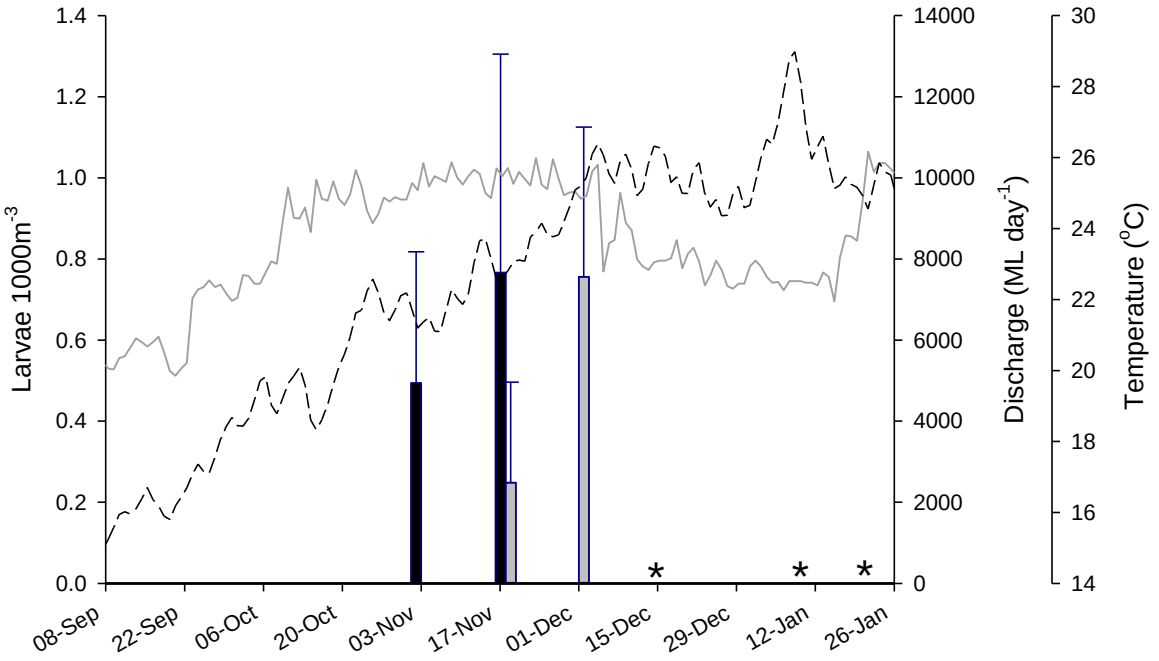

Figure H6. Mean (±S.E.) standardised abundance of golden perch larvae collected in the LMR Selected Area at sites downstream of Lock 1 (dark bars) and Lock 6 (light bars) in 2014/15, plotted against discharge (ML day^{-1}) in the Lower Murray River at the South Australian border (solid grey line) and water temperature (°C) (dashed black line). Sampling was undertaken fortnightly from 3 November 2014 to 20 January 2015. Sampling trips where golden perch larvae were not collected are represented by asterisks.

Spawn dates and otolith $^{87}Sr/^{86}Sr$ of larval and young-of-year golden perch

In 2014/15, we were able to determine daily ages and hence estimate spawn dates for nine larval and one YOY golden perch collected from the LMR Selected Area. Ages ranged 2–105 days for fish collected from 4 November 2014 to 2 April 2015 indicating a spawning period from 2 November to 18 December 2014 (Table H3; Figure H7).

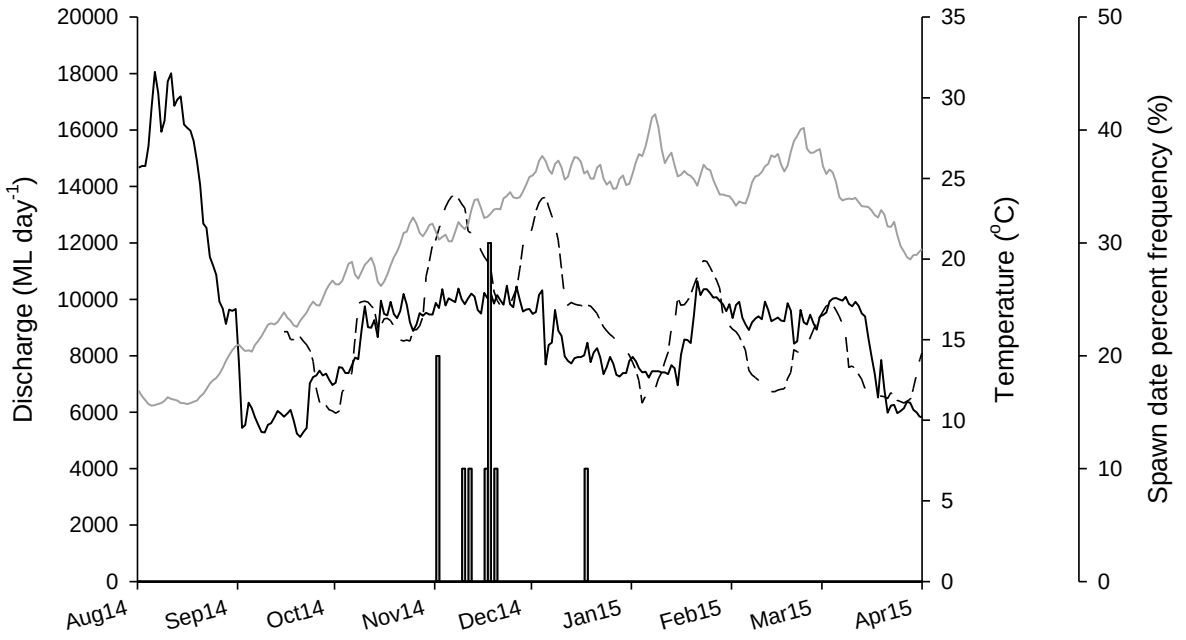

Figure H7. Back-calculated spawn dates for larval and young-of-year golden perch (grey bars; *n* = 10) captured from the LMR Selected Area during 2014/15, plotted against discharge (ML day^{-1}) in the Lower Murray River at the South Australian border (solid black line) and Euston (dashed black line) and water temperature (°C) (grey line).

Pre-flexion golden perch larvae collected in larval tows in November 2014 at sites downstream of Lock 1 ranged in length from 4–5 mm and were 2 days old (Table H3). At sites downstream of Lock 6, post-flexion larvae collected in larval tows from November to December 2014 ranged in length from 8–14 mm and age from 9–19 days (Table H3). One juvenile golden perch collected by electrofishing in April 2015 at Murray Bridge in the gorge geomorphic zone of the LMR Selected Area was 54 mm in length and 105 days old (Table H3).This juvenile golden perch was not collected during LTIM sampling but during sampling for an another fish ecology project in the LMR Selected Area.

Of the ten golden perch larvae/YOY for which we could determine daily age, two were analysed for $^{87}Sr/^{86}Sr$ (Table H3). The otoliths of the remainder of larval golden perch were too small for LA-ICPMS analysis. The YOY fish collected at Murray Bridge on 2 April 2015 was spawned on 18 December 2014 and had otolith core $^{87}Sr/^{86}Sr$ indicative of the LMR, downstream of Lock 1 (i.e. >0.7118 and <0.7124) (Table H3; Figure H8). The larval fish, collected below Lock 6 on 1 December 2014 was spawned on 12 November 2014 and exhibited otolith core $^{87}Sr/^{86}Sr$ indicative of the

lower River Murray, downstream of the Darling River junction, or Murrumbidgee River (~0.714–0.716) (Table H3; Figure H8).

Table H3. Capture location and date, length (mm), age (days), spawn date and otolith core $^{87}Sr/^{86}Sr$ values for larval and young-of-year golden perch collected from the floodplain and gorge geomorphic zones of the LMR Selected Area. Daily ages and spawn dates for four individuals (*) were estimated based on ages of golden perch with similar total lengths.

Zone	Capture location	Capture date	Length (mm)	Age (days)	Spawn date	$^{87}Sr/^{86}Sr$
Gorge	Murray Bridge	2/04/2015	54	105	18/12/2014	0.711883
Gorge	Lock 1	4/11/2014	5	2*	2/11/2014	-
Gorge	Lock 1	20/11/2014	5	2	18/11/2014	-
Gorge	Lock 1	4/11/2014	5	2	2/11/2014	-
Gorge	Lock 1	19/11/2014	5	2*	17/11/2014	-
Gorge	Lock 1	20/11/2014	5	2*	18/11/2014	-
Gorge	Lock 1	20/11/2014	4	2*	18/11/2014	-
Floodplain	Lock 6	1/12/2014	14	19	12/11/2014	0.714924
Floodplain	Lock 6	19/11/2014	8	9	10/11/2014	-
Floodplain	Lock 6	12/11/2014	8	11	20/11/2014	-

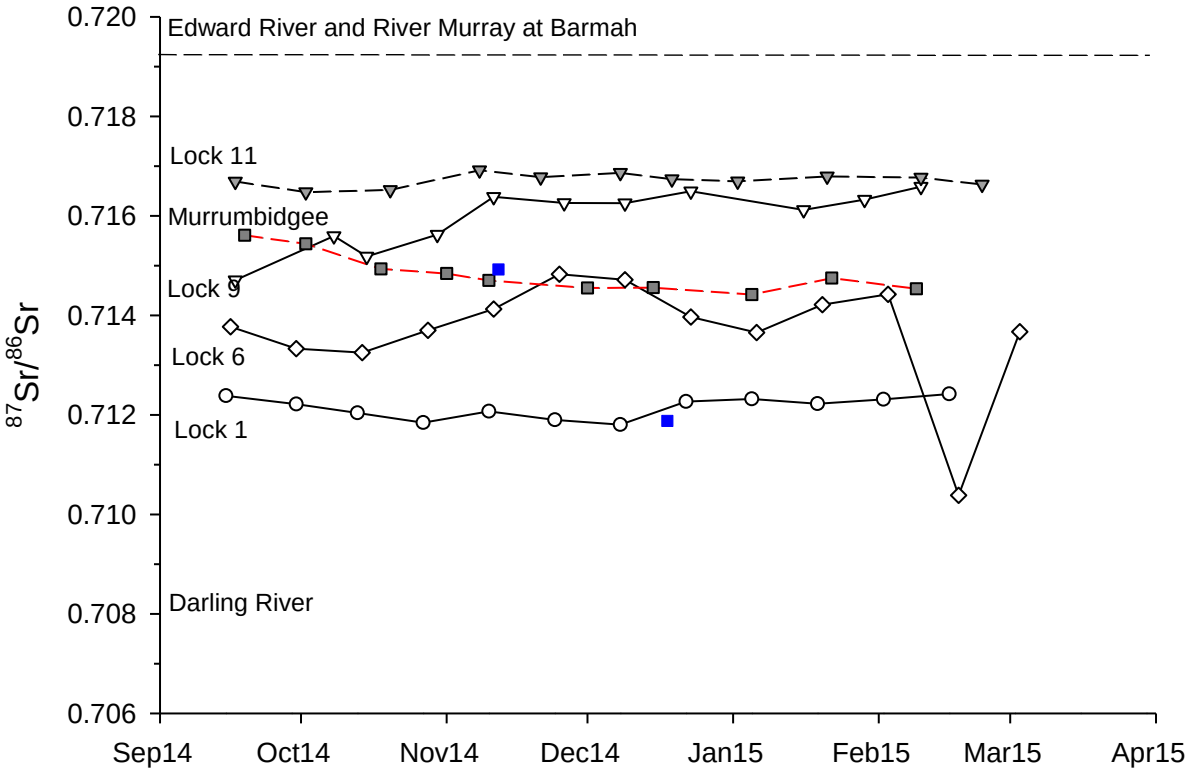

Figure H8. $^{87}Sr/^{86}Sr$ in water samples collected from late September 2014 to early March 2015 at sites in the southern MDB. $^{87}Sr/^{86}Sr$ in the Darling River and Edward River/Murray River at Barmah are presented as dashed straight lines as these were temporally stable and represent the maximum and minimum $^{87}Sr/^{86}Sr$ measured in water samples in the southern MDB in 2014/15. Closed blue squares represent spawn date and otolith core $^{87}Sr/^{86}Sr$ of larval/YOY golden perch (*n* = 2) collected in the LMR Selected Area from December 2014 to April 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. A transect of otolith $^{87}Sr/^{86}Sr$ for the YOY golden perch captured at Murray Bridge (downstream of Lock 1) on 2 April 2015 (spawned on 18 December 2014) indicates this fish was spawned in the LMR Selected Area, likely below Lock 1 (Figure H9a). A transect of $^{87}Sr/^{86}Sr$ for the larval golden perch captured downstream of Lock 6 on 1 December 2014 (spawned on 12 November 2014) shows on $^{87}Sr/^{86}Sr$ early in the fishes life history reflective of the lower River Murray between Lock 9 and Lock 6 or the Murrumbidgee River. This fish, however, retains otolith $^{87}Sr/^{86}Sr$ reflective of the lower River Murray (between Lock 9 and lock 6) throughout its life, thus was most likely spawned in the lower River Murray upstream of Lock 6. If this fish was spawned in the Murrumbidgee River we would have expected otolith $^{87}Sr/^{86}Sr$ to be elevated, at some stage, to values similar to those in water at Lock 11 as the fish

would have transitioned from the Murrumbidgee River to the mid-Murray River and eventually lower River Murray.

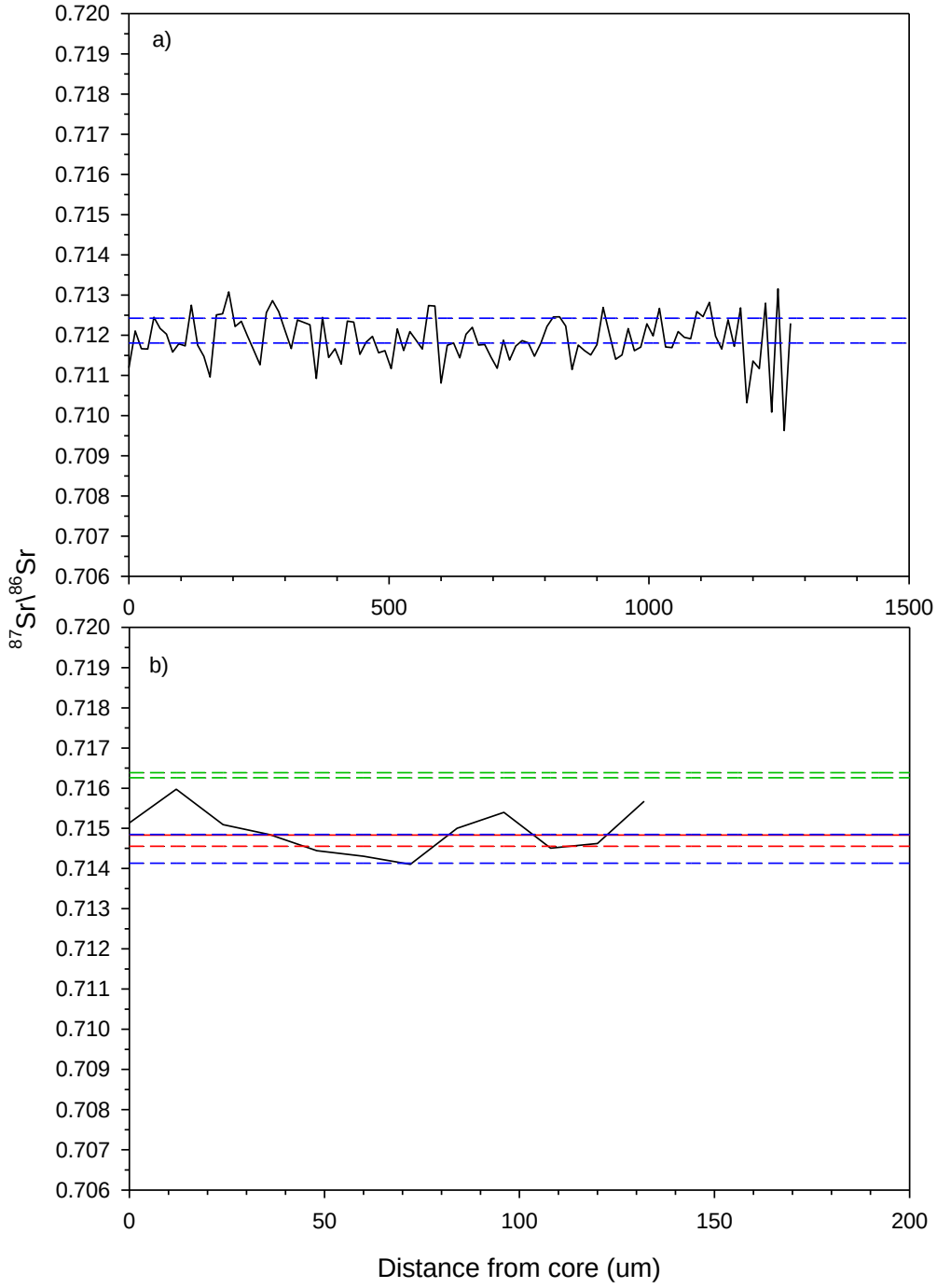

Figure H9. Individual life history profiles based on otolith Sr isotope transects (core to edge) for two golden perch aged (a) 105 and (b) 19 days collected at Murray Bridge (downstream of Lock 1, gorge) and downstream of Lock 6 (floodplain), respectively, in the Lower Murray River Selected Area. Dashed lines denote minimum and maximum $^{87}Sr/^{86}Sr$ ratios in the (a) River Murray at Lock 1 (blue), (b) River Murray at Lock 6 (blue) and Lock 9 (green), and Murrumbidgee River at Yambuna (red).

Golden perch length and age structure

In 2015, golden perch sampled in the gorge and floodplain geomorphic zones of the LMR Selected Area ranged in age from 2+ to 18+ years, with dominant cohorts of age 5+ and 4+fish, spawned in 2009/10 and 2010/11, respectively. Age 5+ fish comprised 61 and 23% of the sampled population in the floodplain and gorge geomorphic zones, respectively, whilst age 4+ fish comprised 24 and 33% of the population in the floodplain and gorge zones, respectively (Figure H10). In the gorge geomorphic zone, age 14+ and 18+ fish spawned in 2000/01 and 1996–97 comprised 12 and 14% of the sampled population, respectively (Figure H10). No Age 0+ or 1+ fish, spawned in 2014/15 and 2013/14, respectively, were collected during electrofishing in the LMR Selected Area in 2015 (Figure H10).

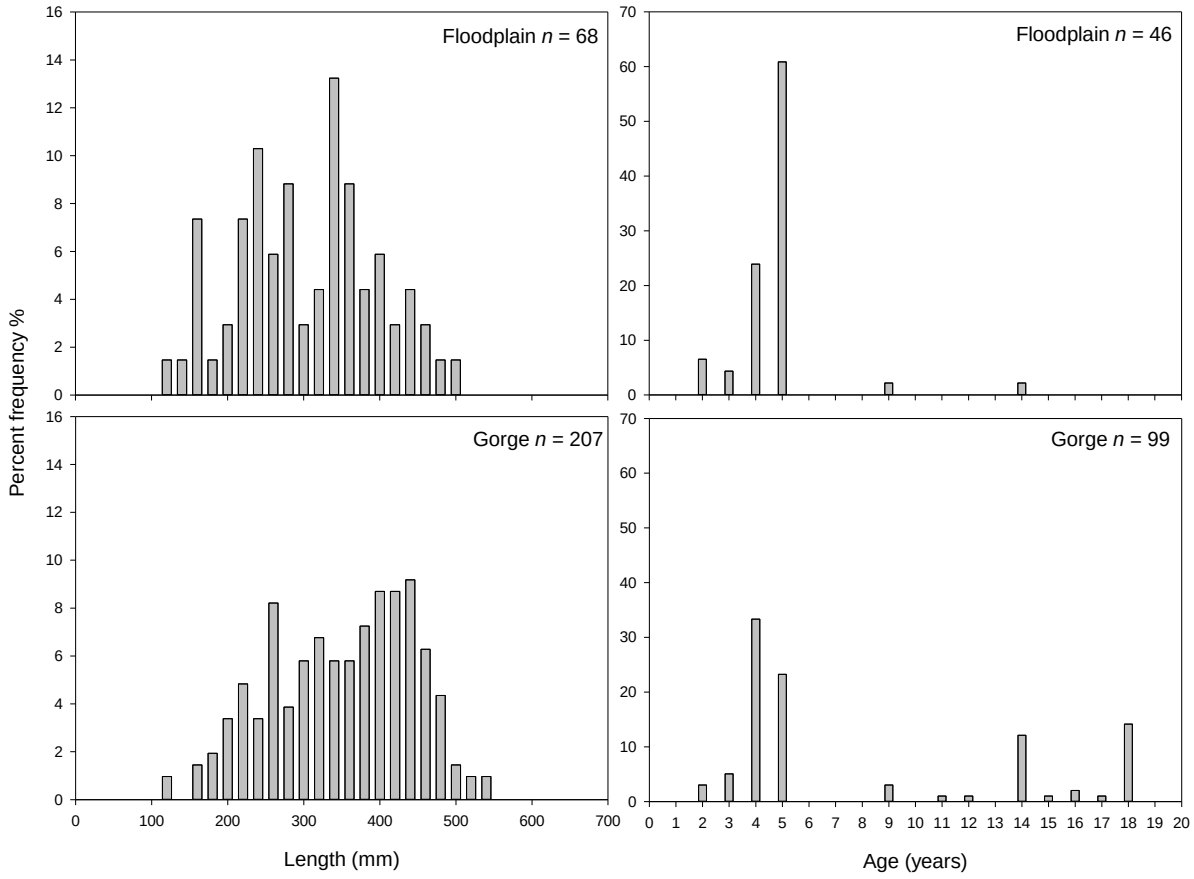

Figure H10. Length (left column) and age (right column) frequency distribution of golden perch collected by boat electrofishing from the floodplain (top) and gorge (bottom) geomorphic zones of the LMR Selected Area in March/April 2015.

Discussion and evaluation

In 2014/15, flow in the LMR Selected Area was maintained at a reasonably stable 9,000–10,000 ML day^{-1} in October and November before gradually decreasing to 7,000 ML day^{-1} in mid-January 2015. Through this period, Commonwealth environmental water compromised a maximum of ~3,400 ML day^{-1} from 27 October to 29 November 2014 Sampling for golden perch eggs and larvae from early November 2014 to end January 2015 revealed low numbers of golden perch larvae (*n* = 9) in the LMR selected area from early November to early December 2014. The age of these larvae (2–19 days) and/or otolith $^{87}Sr/^{86}Sr$ indicate these fish were spawned from 2–20 November in the LMR Selected Area or upstream in the lower River Murray. An individual YOY golden perch (total length = 54 mm) was collected at Murray Bridge in the LMR selected area as part of alternative electrofishing sampling in early April 2015. Otolith microstructure and $^{87}Sr/^{86}Sr$ indicated that this fish was spawned on 18 December downstream of Lock 1. Consequently there was a low level of golden perch spawning in the LMR selected area in conjunction with the delivery of Commonwealth environmental water in November and early December 2014.

In 2015, golden perch populations in the floodplain and gorge geomorphic zones of the LMR Selected Area were dominated by age 5+ and 4+ fish, representing 85% and 61% of the sampled populations, respectively. In the floodplain geomorphic zone, the remainder of the population comprised predominantly young fish (i.e. age 2+, 7%, and 3+, 4%). In the gorge geomorphic zone, however, the remainder of the population was comprised of generally older fish (i.e. age 18+, 14%, and 14+,12 %). No age 0+ or 1+ golden perch were collected.

Overall, these data demonstrate episodic recruitment of golden perch during the period of the Millennium drought (2001–2009), but more consistent recruitment from 2010 to 2013. Consecutive year-classes (i.e. age 2+–5+) from 2010–2013 were spawned in association with in-channel and overbank increases in flow in the lower River Murray and the Darling River. The addition of these year classes improved the resilience and hence health of golden 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 subsequently promote golden perch recruitment (to at least YOY) in the LMR Selected Area.

In 2015, the absence age 0+ and 1+ golden perch in the LMR population indicates negligible recruitment from spawning in 2013 and 2014. Indeed, golden perch recruitment main remain poor in the southern MDB in 2015/16 as El Nino conditions worsen leading to rain-fall deficiencies and forecast low streamflows (BOM 2015).

Conclusions

These findings support current conceptual models of the flow-related ecology of golden perch in the lower River Murray, with spawning and recruitment being associated with spring–summer in-channel flow variability in the lower River Murray (nominally greater than 14,000 ML day^{-1}) 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). The absence of these hydrological characteristics in 2014/15 led to limited spawning and negligible recruitment of golden perch to age 0+ in the LMR selected area. Hence, in relation to golden perch, 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 2014, flow between the mid and lower River Murray was fragmented and homogenised (through the operation of Lake Victoria) to aid in the operation of the Chowilla Regulator to artificially inundate floodplain habitats. In association, golden perch spawning in the LMR was limited and recruitment to age 0+ was negligible. At the same time, spawning and recruitment of the invasive species, common carp, was promoted by engineered artificial floodplain inundation (SARDI unpublished data). 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 through the delivery of Commonwealth environmental water will require consideration of maintaining the longitudinal integrity of flows and the potentially discordant outcomes of alternative flow management scenarios, including engineered artificial floodplain inundation.

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 (SA Section of the Murray River).
LTIM	Long-Term Intervention Monitoring
M&E	Monitoring and Evaluation
MDB	Murray–Darling Basin
SARDI	South Australian Research and Development Institute
TL	Total length
TLM	The Living Murray
VEWH	Victorian Environmental Water Holder
YOY	Young-of-year