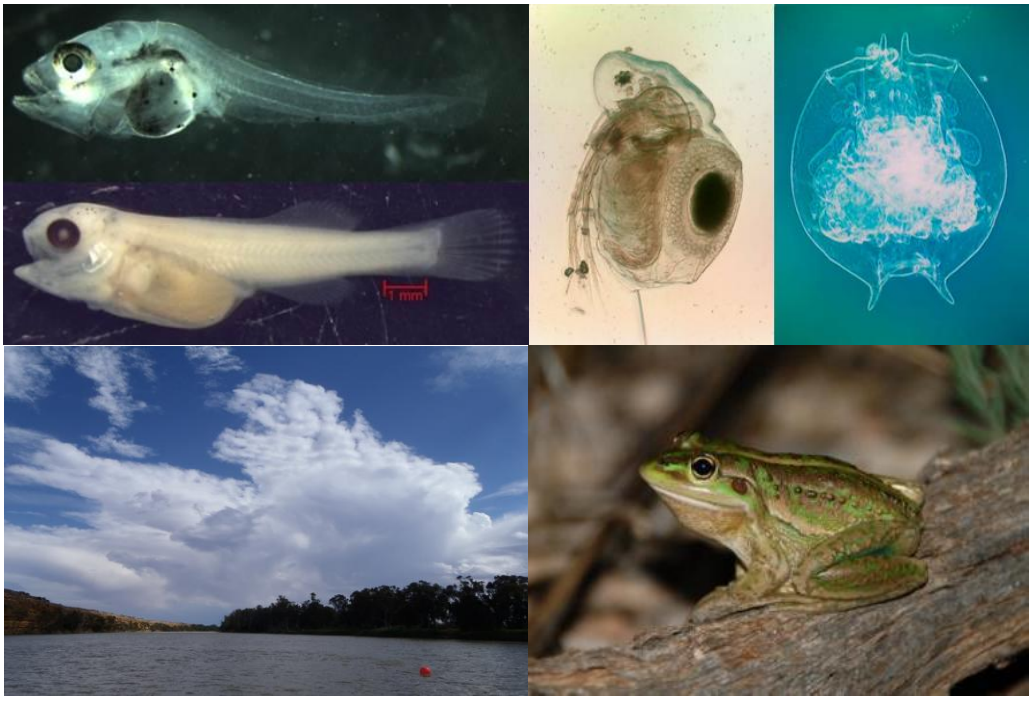
Report 2

Monitoring the ecological responses to Commonwealth environmental water delivered to the Lower Murray River in 2012-13

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



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DISCLAIMER

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EXECUTIVE SUMMARY

This project focuses on the intervention monitoring of the ecological responses to Commonwealth environmental water delivered to the Lower Murray River in 2012-13. The Lower Murray River encompasses a wide range of aquatic habitats that support diverse species of native flora and fauna. The complex ecosystems in the Lower Murray are strongly influenced by variation in riverine flow regime. During the prolonged drought that affected the Murray–Darling Basin (2001-2010), the ecological community in the Lower Murray River suffered severe stress. The 2010 flood and ensuing high flow years have led to some ecosystem improvement.

In 2012-13, ~786 GL of Commonwealth environmental water were delivered to the Lower Murray River, Lower Lakes and Coorong, in conjunction with other environmental flows (e.g. flows through the Murray–Darling Basin Authority The Living Murray Initiative). The flow releases to South Australia were coordinated through a series of watering events across the southern connected Basin to achieve multi-site environmental outcomes. Environmental watering, following the decline of unregulated flows, helped to maintain river flow at ~15,000 ML day^{-1} during November, created a flow pulse of ~19,000 ML day^{-1} in December in the Lower Murray River and supplemented freshwater flows to the Lower Lakes and barrage releases to the Coorong.

The current project investigated key ecological responses during 2012-13 in the main channel, wetlands, Lower Lakes and the Coorong, in line with expected ecological outcomes of Commonwealth environmental watering in the Lower Murray River. These included,

- larval fish and reproduction of flow-cued spawners
- larval fish food resources
- dissolved and particulate material transport
- floodplain wetland inundation and lateral connectivity
- fish lateral movement
- frog response
- Coorong modelling for *Ruppia tuberosa* and fish habitat

For each component, monitoring/modelling was conducted to address questions and test hypotheses based on our conceptual understanding of the life histories of relevant biota and ecological processes, and the responses that might be expected from the flow scenarios and environmental water delivery in 2012-13. This report provides a summary of the above studies and a synthesis of the ecological outcomes of the 2012-13 environmental watering in the Lower Murray River.

Key ecological outcomes

Monitoring in 2012-13 identified a number of ecological responses associated with the delivery of Commonwealth environmental water in the Lower Murray River. Key outcomes are summarised in Table 1.

Table 1. Summary of key ecological outcomes associated with environmental water releases to the Lower Murray River during 2012-13.

Objective of watering	Expected outcome	Indicator	Monitoring/modelling result
• support breeding of native fish • support recruitment of juvenile life stages	environmental watering will: • trigger spawning and lead to increased larval fish abundance in flow-cued spawners (i.e. golden perch and silver perch) • lead to fish recruitment success and enhance the recruitment of flow-cued spawning species (i.e. golden perch, silver perch • facilitate larval/post-larval fish dispersion	**Larval fish**	• presence of flow-cued spawning fish larvae was extended through to summer after delivery of environmental water • spatio-temporal changes in zooplankton assemblages associated with environmental watering • zooplankton from warmer regions highlighted the importance of longitudinal connectivity
• support ecosystem functions related to longitudinal connectivity • support key ecosystem functions related to connectivity between the river and the floodplain	environmental watering will: • better connect the channel with fringing riparian, wetland or floodplain areas enhance the food supply for larval fish • increase duration of flows and will enhance food supply for larval fish	**Food resources for larval fish**	• likely selectivity in feeding for larval fish • spatio-temporal changes in eukaryotic community associated with environmental water delivery may affect larval gut content
• support the transportation and	environmental watering will:	**Dissolved**	• the modelling outputs suggest that the flow regimes supported by Commonwealth

Objective of watering	Expected outcome	Indicator	Monitoring/modelling result
export of salt and nutrients through an open Murray Mouth	• increase the mobilisation of salts • increase the mobilisation of nutrients • increase suspended organic matter loads • increase the re-suspension of inorganic matter from the river bed and thus increase the transport of suspended inorganic matter from the basin and through the Lower Murray River	**and particulate material transport**	environmental watering increased the transport of dissolved and particulate matter through the Lower Murray River. • for some parameters, additional exports were only achieved because two sources of environmental water were provided, highlighting the importance of supplementary water provisions to the region.
• support key ecosystem functions, related to connectivity between the river and the floodplain	environmental watering will: • increase lateral connectivity of wetlands and low lying floodplain and floodplain inundation area.	**Inundation and lateral connectivity**	• the environmental watering during December, allowed for ~600 hectares increase in inundation area along the Lower Murray River. • the backwater curves showed 0.05-0.80 m increases in water level, depending on distance from the lock/weir and the section of the river. • the higher water levels potentially increased the inundation area of riparian vegetation in the main channel.
• support key ecosystem functions related to connectivity between the river	environmental watering will: • sustain flows and maintain the lateral movement of fish, particularly small bodied species. • extend high flow conditions and	**Lateral fish movement**	• there was high variability in fish assemblages, suggesting individual wetlands respond uniquely to environmental variables • delivery of environmental water did not appear to enhance reproduction and recruitment of carp

Objective of watering	Expected outcome	Indicator	Monitoring/modelling result
and the floodplain - support breeding of native fish - support recruitment of juvenile life stages	increase inundated aquatic habitat, potentially providing nursery ground for fish. - affect regional differences amongst fish assemblages		- no clear consistent pattern of movement throughout the wetlands was detected
- support key ecosystem functions, related to connectivity between the river and the floodplain	environmental watering will: - increase frog distribution, diversity and relative abundance in comparison to previous lower flow years - increase the extent of frog habitat within each of the South Australian River reaches.	**Frog response**	- frog calling and species richness was lower in summer than spring likely due to higher unregulated flows in spring - however, environmental watering in summer may have maintained habitat availability for frog breeding
- support ecosystem functions related to longitudinal connectivity	environmental watering will: - increase freshwater flow through the barrages and into the Coorong and improve salinity conditions and water levels in the Coorong thus enhancing *Ruppia tuberosa* and fish habitats	**Coorong (Modelling)**	- the *Ruppia tuberosa* and fish habitat models together with the hydrodynamic model (CHM v2.1) provide a useful tool for the evaluation and planning of environmental watering, including volume and timing of barrage outflows required to maintain viable populations of *Ruppia tuberosa* and extent of fish habitat for key species. - environmental water delivery in addition to an unregulated flow in 2012 provided a small benefit to *Ruppia tuberosa* populations, with up to 10%

Objective of watering	Expected outcome	Indicator	Monitoring/modelling result
			increase in the probability of propagule bank replenishment in the South Lagoon. • watering also benefited fish in the Coorong by improving habitat suitability up to 13% and increasing habitat extent up to 30 km (e.g. mulloway).

Key learning and management implications

Based on insights provided by the studies through the 2012-13 monitoring, and current knowledge of nutrient dynamics and flow related biology/ecology in the Lower Murray River, Lakes and Coorong, the following points should be considered with regard to the approach to environmental watering.

Lower Murray River

There was a strong presence of larval fish of large-bodied native species from late October to December. An environmental flow pulse with the appropriate timing and adequate magnitude and duration may enhance and extend the presence of some of these native fish larvae (e.g. golden perch and silver perch) and may lead to enhanced recruitment in the Lower Murray River. Maintaining a seasonal flow regime, potentially through the use of environmental water during spring and summer is important for some large-bodied native fish and in this study did not have an effect on spawning and recruitment of carp within the wetlands.

Whilst seasonal flow regimes are important in maintaining the ecological integrity of freshwater systems, within-channel flow management may present an opportunity to prolong and potentially enhance the lateral movements of native fish, with the direct benefits likely to be most pronounced in the wetlands upstream from Lock 1, whereby water levels are more influenced by flow discharge.

Nonetheless, due to the high spatial variability in the structure of fish assemblages attempting to access and exit wetlands and the physical, biological and hydrological differences among wetlands, the movement pattern will differ among wetlands in response to the regimes of flow delivery. This highlights the need for individual assessment of wetlands prior to the management interventions to support lateral fish movement.

In addition to flow regimes, managing flow integrity is critical; the source and continuity of the water delivered can play an important role in the outcomes achieved. For example, this study showed a strong influence of zooplankton species originated in the upper parts of the basin on the assemblages in the Lower Murray River and contributing to the local ecosystem.

A broad view of eukaryotic microorganisms present in the river, including zooplankton, was obtained through molecular analyses which identified a significant range of taxa, covering a broad range of trophic levels. It could potentially be used as a tool for more comprehensively assessing the mix of different trophic level pathways that underpin energy and material cycles in aquatic ecosystems that respond to flow and water quality.

The modelling approaches that describe aspects of water quality such as dissolved and particulate organic matter that were presented in this study could be used in planning for flow deliveries, by assessing the potential benefits/detriments of various watering actions in the hydrologically complex Lower Murray system. Continued refinement of the model will further improve its capacity to evaluate the influence of environmental water deliveries. The following specific points pertinent to material transport could be used to help guide future environmental watering:

- environmental watering 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 material.
- environmental flow deliveries during extended low flow periods are likely to have greater impacts on salt and nutrient concentrations 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.
- maximum exports of dissolved and particulate material from the Murray Mouth are likely to be achieved by delivering environmental water during periods of low oceanic water levels (summer). However, this may reduce water availability at other times, increasing the import of material from the Southern Ocean during those times. In contrast, delivery of environmental water to the region 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.

- net export of dissolved and particulate material can be achieved when discharges above threshold levels are provided. Whilst, these discharge thresholds are currently unknown and likely differ with seasonal changes in downstream water levels, supplementary water sources are important in providing adequate flows to export material from the system.

- flows during winter may result in limited assimilation of nutrients by biota (slower growth rates), whilst deliveries during summer could increase the risk of blackwater events and cyanobacterial blooms, depending on hydrological conditions. Flows during spring are likely to minimise these risks, but also maximise the benefits of nutrient inputs (e.g. stimulate productivity to support larval survival).

- multiple watering events in a given year could be used to meet different ecological objectives. For example one event in spring could be provided to increase nutrient assimilation, followed by a subsequent event to export material to downstream ecosystems.

Environmental watering that aims to increase frog breeding response and recruitment needs to provide water that will increase the inundation of vegetation during the months when frogs are reproductively active (spring/summer). This may be achieved through:

- an increase in water levels that leads to the inundation of riparian vegetation along the fringing edges of permanent water courses and wetlands,

- an increase in water levels that leads to the inundation of larger areas of temporary wetlands and floodplains, or

- localised pumping into temporary wetlands and floodplain areas.

- however, the extent of inundation of vegetation as a result of the above events will be influenced by how watering and flows have impacted on the vegetation in the years or months preceding the planned event.

Coorong

The volumes of environmental water currently available will have limited benefit on *Ruppia tuberosa* populations in the South Lagoon unless delivered in conjunction with an unregulated flow event. Even in this situation the unregulated flow will need to be of sufficient duration to provide barrage outflows during November and early December and environmental water used to manage flow recession to reduce the rate of water level decline in the South Lagoon to reduce the risk of stranding. For environmental flows alone to have a significant benefit much larger volumes of water will be required than are currently available.

Freshwater inflow is pivotal in maintaining estuarine fish habitat and populations in the Coorong. Environmental watering could be managed to maintain the connectivity and extend barrage outflows to improve the quality and extent of fish habitat in the Coorong.

Environmental watering during the summer months or in years with less barrage flows and higher salinity will provide a much larger effect of habitat improvement.

Flow delivery during late spring/summer is important as this period corresponds to the spawning and recruitment season of most estuarine fish species in the Coorong. Environmental flows could potentially help in maintaining a favourable salinity gradient, enhancing productivity and improving connectivity to facilitate fish recruitment.

Recommendations for future research and monitoring for the Lower Murray River and Coorong are provided in Section 7.

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 regime components provides critical knowledge to underpin environmental flow management to achieve the best ecological outcomes (Walker *et al.* 1995; Arthington *et al.* 2006).

The Lower Murray River represents a significant ecological asset to be targeted for environmental flows (DEWNR 2013). The complex system includes the main river channel, anabranches, floodplain/wetlands, billabongs, stream tributaries and the Lower Lakes, Coorong and Murray Mouth ecosystem, 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 recent decadal drought in the Murray–Darling Basin (MDB) (2001-2010), the ecosystem of the Lower Murray River 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). A natural flood in late 2010 and the following year's high flows with environmental water deliveries have led to some positive signs of ecological recovery (e.g. Gehrig *et al.* 2012; Nicol *et al.* 2013; Ye *et al.* 2013c).

Since 2011-12, significant volumes of Commonwealth environmental water have been delivered to the Lower Murray River, Lower Lakes and Coorong, in conjunction with other environmental flows (e.g. flows through the Murray-Darling Basin Authority The Living Murray Initiative), to facilitate ecosystem recovery post drought and restore ecological health (www.environment.gov.au/ewater/). The flow releases to South Australia were coordinated through a series of watering events across the southern connected Basin to achieve multi-site environmental outcomes (www.environment.gov.au/ewater/).

1.2 Hydrology

The 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 Lower Murray River is heavily modified by upstream diversions and extraction, and a series of 11 low-level (<3 m) weirs constructed in the 1930s-1940s, changing a connected flowing river to a series of weir pools (Walker 2006). Consequently, there has been a profound impact on riverine processes and the ecological community in the Lower Murray River (Walker 1985; Walker and Thoms 1993).

From 1996 to 2010 the MDB experienced a severe drought; inflows into the Murray River system were approximately 40% of the historical mean (MDBA 2011). The drought was broken in late 2010 by a significant overbank flow, reaching a peak of approximately 93,000 ML day^{-1} in February 2011 in the Lower Murray River. In the subsequent two years, flow remained high although largely confined within the channel (< 50,000 ML day^{-1})(Figure 1).

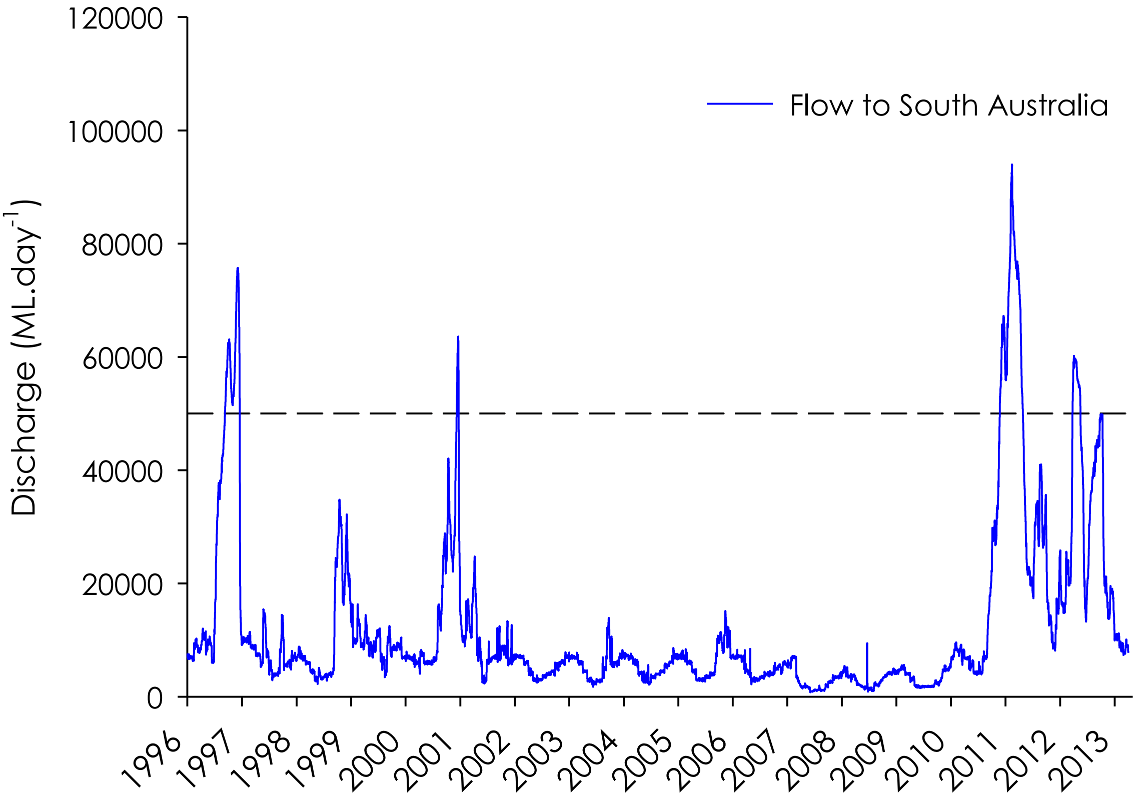


Figure 1. Daily flow (ML day^{-1}) in the Lower Murray River at the South Australian border from January 1996 to April 2013. Dotted line represented approximate bankfull flow in the main channel of the Lower Murray River.

In 2012-13 there was unregulated flow to South Australia throughout the winter, reaching ~50,000 ML day^{-1} in late September. This was followed by a steep drop in October to ~20,000 ML day^{-1} (Figure 1). Following the drop of unregulated flow there were a series of environmental water deliveries that maintained river flow at ~15,000 ML day^{-1} during November and created a flow pulse of ~19,000 ML day^{-1} in December (Figure 2). From January onwards flows remained around or below 10,000 ML day^{-1} even with continued environmental water delivery to the Lower Murray River.

Through the use of the Murray River Floodplain Inundation Model (RiM-FIM III), areas of inundation and backwater curves were estimated. The environmental watering during December, allowed for the inundation area to remain ~600 hectares larger in the Lower Murray River (the South Australian border to Wellington) than it would have been if no environmental water was allocated and the flow had receded to <10,000 ML day^{-1}. The backwater curves showed that water levels would have been

0.05-0.80 m lower, depending on distance from the lock/weir and the section of the river analysed, without the delivery of environmental water. The higher water levels potentially increased the inundation area of riparian vegetation in the main channel (for details refer to Ye *et al.* 2013b).

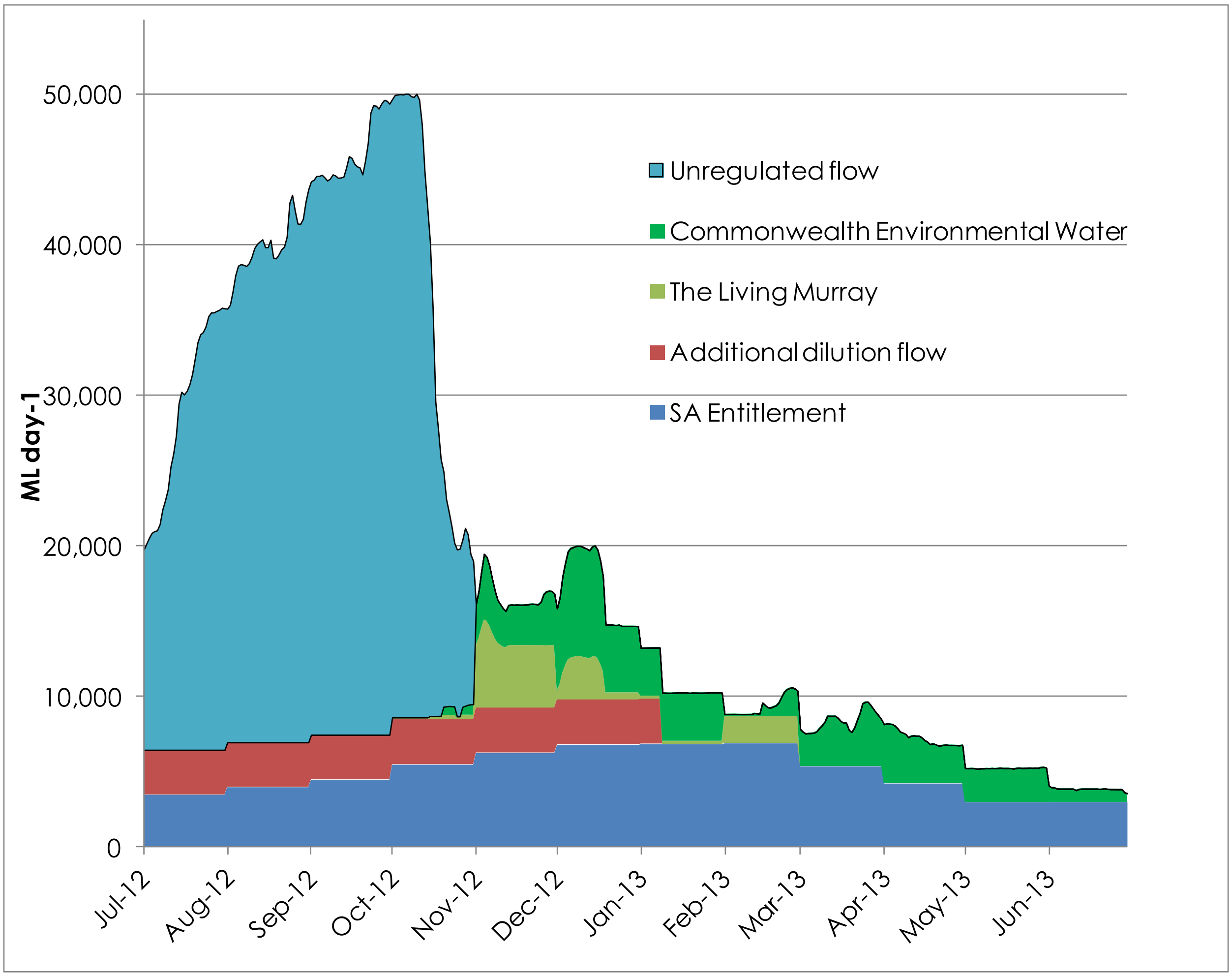


Figure 2. Flow to South Australia from July 2012 to June 2013.

1.3 Commonwealth environmental watering objectives, evaluation questions and monitoring tasks

Through the intervention monitoring of Commonwealth environmental water delivered to the Lower Murray River in 2012-13, structured, targeted investigations have been carried out to explore flow related ecological responses in the complex Lower Murray River system (i.e. the main channel and associated wetlands, and Coorong). 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 proposed complementary monitoring components (tasks) contribute toward a holistic understanding of ecosystem responses to flow management and ecological benefits (Figure 3).

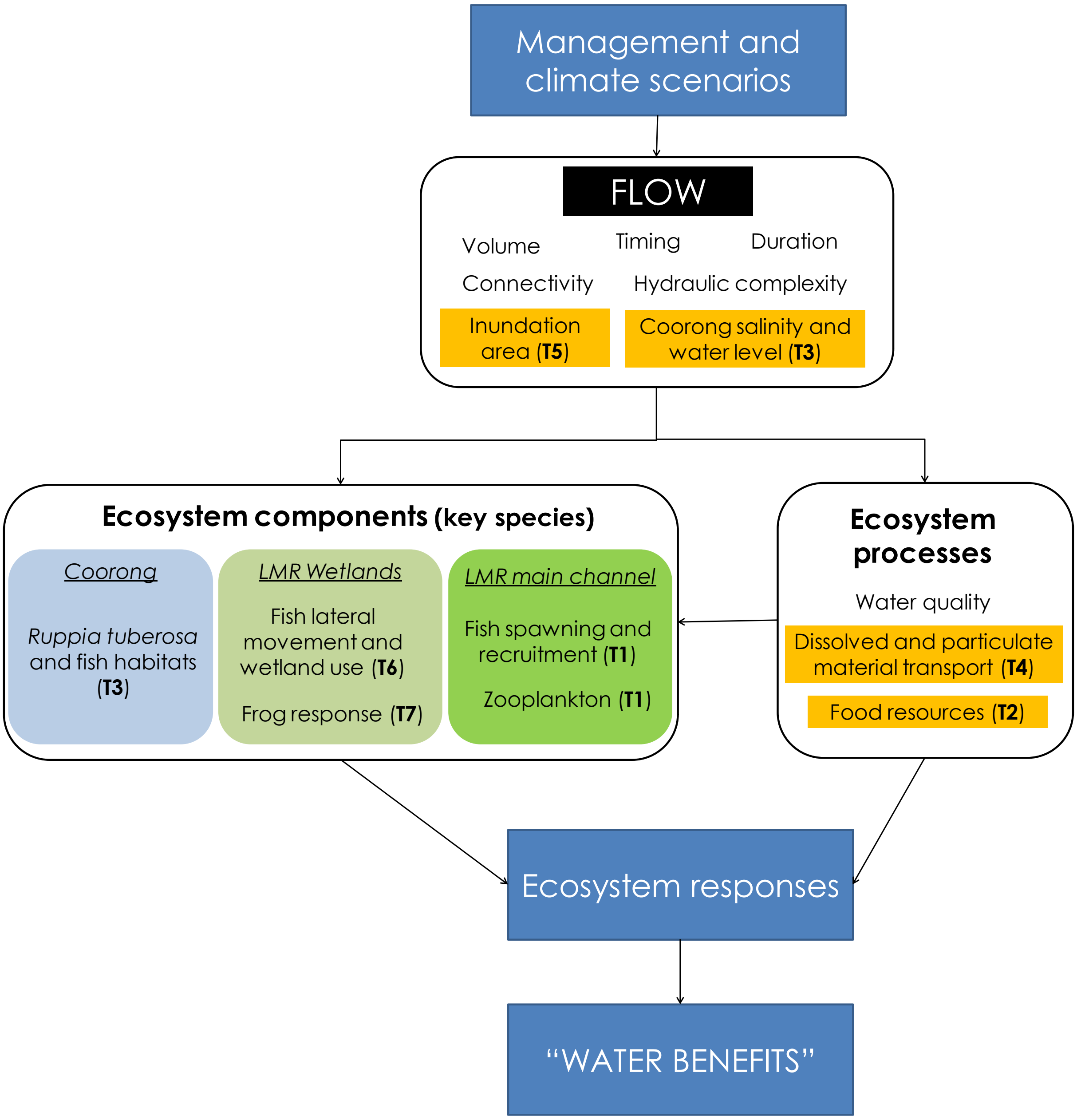


Figure 3. Conceptual diagram of how river ecosystems are affected by the key ecosystem driver (flow regime), subject to flow management and climate effects, and how complementary monitoring components (tasks) contribute toward a holistic understanding of ecosystem responses to flow management and ecological benefits in the Lower Murray River (LMR) (Note tasks are within ecosystem components and in highlighted orange boxes under flow and ecosystem processes).

Aligning with relevant Commonwealth environmental watering objectives for the Lower Murray River, we proposed to test a series of hypotheses based on our conceptual understanding of the life histories of relevant biota and ecological processes, and what responses might be expected from the flow scenarios with environmental water delivery in 2012-13. The work has been conducted through the following seven tasks with investigations into the main channel and wetlands of the Lower Murray River, and the Coorong, Lower Lakes and Murray Mouth (CLLMM) region.

Main channel

Larval fish

Commonwealth environmental watering objectives: Fauna

- provide a flow regime that supports breeding of native fish.
- provide a flow regime that supports recruitment of juvenile life stages.

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

- lead to different spawning responses in different fish species with varied level of flow dependence; it will trigger spawning and lead to increased larval fish abundance in flow-cued spawners (i.e. golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*)) in the Lower Murray River.
- enhance the food supply and quality for larval fish, leading to greater survival and growth rates.
- lead to fish recruitment success and enhance the recruitment of flow-cued spawning species (i.e. golden perch, silver perch) in the Lower Murray River.
- facilitate larval/post-larval fish dispersion; thus, will contribute to recruitment success in local fish populations (e.g. golden perch, silver perch and Murray cod (*Maccullochella peelii*)) in the Lower Murray River; natal origin of these fish will be influenced by the source of flow (e.g. may be the Darling River, mid or upper Murray River, as well as the Murrumbidgee and Goulburn rivers).

Food resources for larval fish

Commonwealth environmental watering objectives: Ecosystem function and connectivity

- provide a flow regime that supports ecosystem functions related to longitudinal connectivity
- provide a flow regime which supports key ecosystem functions related to connectivity between the river and the floodplain.
- provide a flow regime that supports fish breeding and recruitment.

Hypotheses: Increases in flow levels to better connect the channel with fringing riparian, wetland or floodplain areas enhances the food supply for larval fish.

Increases in the duration of flows connecting the channel with fringing riparian, wetland or floodplain areas enhances the food supply for larval fish.

At particular points in the hydrograph (fringing riparian connection, low level wetland connection, shedding floodplain connection), small increases in flow levels and/or duration significantly increase the food supply to larval fish.

Dissolved and particulate material transport

Commonwealth environmental watering objectives: Salt and nutrient

- provide a flow regime that supports the transportation and export of salt and nutrients through an open Murray Mouth.

Hypotheses: Salt transport: environmental watering will increase the mobilisation of salts from the Basin and increase the transport of salt passing from Lock 1 through the Lower Murray River and if there is sufficient flow, through the Lower Lakes and Murray Mouth.

Nutrient transport: environmental watering will increase the mobilisation of nutrients from the Basin and increase nutrient loads passing from Lock 1 through the Lower Murray River and if there is sufficient flow, through the Lower Lakes and Murray Mouth.

Suspended organic matter: environmental watering will increase suspended organic matter loads passing from Lock 1 through the Lower Murray River and if there is sufficient flow, through the Lower Lakes and Murray Mouth. In particular, increased floodplain inundation will result in increased loads of phytoplankton biomass and dead organic matter.

Suspended inorganic matter: environmental watering will increase the resuspension of inorganic matter from the river bed and thus increase the transport of suspended inorganic matter from Lock 1 through the Lower Murray River and if there is sufficient flow, through the Lower Lakes and Murray Mouth.

Wetlands

Inundation and lateral connectivity

Commonwealth environmental watering objectives: Ecosystem functions and connectivity

- provide a flow regime which supports key ecosystem functions, related to connectivity between the river and the floodplain.

Hypotheses: Lateral connectivity of wetlands and low lying floodplain is increased and floodplain inundation area is increased by environmental watering.

The evaluation was conducted through the use of the Murray River Floodplain Inundation Model (RiM-FIM III), with findings presented in Section 1.2 regarding increased areas of inundation and water levels associated with environmental water deliveries.

Lateral movements of fish

Commonwealth environmental watering objectives: Ecosystem function and connectivity/Fauna

- provide a flow regime which supports key ecosystem functions related to connectivity between the river and the floodplain.
- provide a flow regime that supports breeding of native fish.

- provide a flow regime that supports recruitment of juvenile life stages.

Hypotheses: Sustained flows will maintain the lateral movement of fish (from the River channel to the connected wetlands), particularly small bodied species.

Extended high flow conditions as a result of environmental watering will increase inundated aquatic habitat, potentially providing a nursery ground for fish.

The composition of fish assemblages attempting to access wetlands in the 'Murray Gorge' region will be different compared to those attempting to access wetlands in the 'Floodplain' region of the Lower Murray River.

Changes in environmental conditions (hydrology, i.e. water level, water temperature, salinity, flow direction, flow velocity) associated with different phases of environmental water delivery to South Australia will influence the abundance and diversity of native and non-native fish assemblages moving between the main channel and off-channel wetland habitats in the Lower Murray River.

Frog response

Commonwealth environmental watering objectives: Ecosystem functions and connectivity/fauna

- provide a flow regime which supports key ecosystem functions, related to connectivity between the river and the floodplain.

Hypotheses: Increased flow in the Lower Murray River (peak and duration) in spring/summer 2012-13 as a result of environmental watering will

- lead to an increase in frog distribution, diversity and relative abundance in comparison to previous lower flow years.
- increase the extent of frog habitat within each of the South Australian River reaches.

Coorong (modelling)

Ruppia tuberosa and fish habitat

Commonwealth environmental watering objectives: Ecosystem function and connectivity (Coorong)

- provide a flow regime that supports ecosystem functions related to longitudinal connectivity

Hypotheses: Increased freshwater flow through the barrages and into the Coorong due to environmental watering will reduce salinity and increase water levels in the Coorong, thus enhancing *Ruppia tuberosa* and fish habitats.

Evaluation questions

The following key evaluation questions will be addressed through the seven project tasks:

Has environmental water:

1. Contributed to the maintenance or improvement of the lateral connectivity between wetlands and the low lying floodplain in the Lower Murray River?
2. Supported the spawning and recruitment of native fish in the Lower Murray River?
3. Contributed to the provision of adequate larval fish food resources in the Lower Murray region?
4. Contributed to the transport of salt, nutrients and other dissolved and particulate matter through the Murray Mouth?
5. Improved the lateral movements of fish?
6. Supported the breeding of frogs in the wetlands of the Lower Murray River?
7. Provided benefits for *Ruppia tuberosa* and fish habitat in the Coorong?

8. How do these results inform future watering?

This report presents the findings and outcomes during the 2012-13 intervention monitoring for environmental watering in the Lower Murray River (South Australia).

2 BIOLOGY AND ECOLOGY BACKGROUND FOR TASKS

2.1 Main channel

Larval fish

In Australia, studies have been conducted to improve our understanding of the significance of flows and/or floodplains on key aspects of fish population dynamics including spawning and recruitment (e.g. Harris and Gehrke 1994; Humphries *et al.* 1999; King *et al.* 2003; Mallen-Cooper and Stuart 2003; Graham and Harris 2005; Arthington *et al.* 2006; King *et al.* 2007; Ye *et al.* 2008; Ebner *et al.* 2009; Cheshire *et al.* 2012). Indeed two analogous fish recruitment models have been developed based on observations from temperate Australian floodplain rivers, the flood recruitment model (FRM) (Harris and Gehrke 1994) and the low flow recruitment hypothesis (LFRH) (Humphries *et al.* 1999). The FRM, developed following the principles of the flood pulse concept (Junk *et al.* 1989), proposes two methods by which increased flows may enhance recruitment in river fish: flooding directly triggers spawning, and/or flooding indirectly enhances the survival of larvae and juveniles by providing suitable food and habitat on the inundated floodplain (Harris and Gehrke 1994). Despite widespread acceptance of the principles of the flood pulse concept and FRM, many Australian riverine fish have developed more plastic life histories with reproduction occurring during periods of low flows (e.g. Humphries *et al.* 1999; King *et al.* 2003; Zeug and Winemiller 2008; Cheshire 2010). The LFRH (Humphries *et al.* 1999) highlights that some species can successfully spawn and recruit during low within-channel flows. Specifically, larval fish studies during the recent extended drought period have demonstrated that the LFRH is applicable in the Lower Murray River for small to medium-bodied native fish species (Cheshire 2010). Whereas the 2010/11 high unregulated flows and extended flood period, allowed the investigation of the FRM in the Lower Murray River, indicating some positive responses with increased abundance and distribution of larval fish in several large-bodied native species (golden perch, silver perch, Murray cod and freshwater catfish) (Cheshire *et al.* 2012).

Larval fish mortality is commonly associated to either predation or starvation, and can be exceptionally high (i.e. 90-99%) (Kelso and Rutherford 1996; Trippel and

Chambers 1997). The shift from endogenous (depends on internal yolk sac) to exogenous (depends on external resources) feeding is when starvation usually occurs and is referred to as the *critical period* (May 1974). It has been suggested that a temporal overlap between fish spawning and zooplankton abundance is needed for larval fish growth and survival (Cushing 1990). To provide the required high densities of zooplankton, floodplain inundation has been suggested as a necessary event (Welcomme 1985; Junk *et al*. 1989; Harris and Gerke 1994). In the MDB there is relatively little information on zooplankton composition in the main channel (but see Shiel *et al.* 1982; Shiel 1985; King 2004). Zooplankton density and species composition will affect larval fish survival. The species composition of the zooplanktonic community will determine the actual proportion of that community that is available as a food resource. The size and biochemical constitution (e.g. protein and fatty acids) of these food items are of particular importance. Larval fish mouth gap determines the size of zooplankters that may be consumed. The abundance of certain fatty acids in larval fish diets has been shown to be essential to larval fish development and growth (Coutteau *et al.* 1997; Tocher 2010).

Our understanding of the role of flows and flooding in the life-history cycles of many of the MDB fish has improved in recent years (e.g. Humphries *et al.* 1999; King *et al.* 2003; Mallen-Cooper and Stuart 2003; Graham and Harris 2005; Ebner *et al.* 2009; King et *al.* 2009; Cheshire 2010; Cheshire *et al.* 2012). However, there are significant knowledge gaps on the specific environmental conditions required for successful fish spawning and recruitment and the complex mechanisms of how flow or other environmental factors affect fish (King *et al.* 2009). Such knowledge is critical for the restoration of ecologically important components of the flow regime in order to rehabilitate native fish populations through environmental flow management (Walker et *al.* 1995; Arthington *et al.* 2006).

Food resources for larval fish

The trophic linkages of food webs determine the pathways and efficiency of organic carbon (food energy) transfer through ecosystems. In a river ecosystem, sources of carbon originally fixed into organic material by photosynthesis can be detritus from the floodplains, or produced within the river by algae, attached biofilms and macrophytes. In some cases fish can directly utilise this material, but in many cases the organic material is processed through the microbial community into forms that are suitable for use by higher organisms.

The importance of micro-organisms (bacteria, protists, ciliates, fungi, algae and zooplankton) as a major source of food for the larger organisms in freshwater systems is well recognised (Schmid-Araya and Schmid 2000; Pernthaler and Posch 2009). Flagellated protists that are approximately 5 µm in size are major consumers of bacteria in freshwater habitats and they excrete surplus nutrients to the surrounding environment. Ciliates will graze on bacteria and algae and prey on other ciliates and flagellated protists. Zooplankton can feed on algae, protists, ciliates and the zoospores of fungi. Within this range of microorganisms, decomposers, detritivores, herbivores and carnivores all play a role in utilising, re-packaging and transferring the organic material between trophic levels. The characteristics of these communities and their inter-connections reflect the environmental conditions such as flow, turbidity and water quality, the result being that species are linked together in a highly complex network over a wide range of spatial scales. Microscopic eukaryotes are abundant in freshwater, but due to their size and a lack of taxonomic knowledge for these groups (Creer *et al.* 2010; Zinger *et al.* 2012) surveying these taxa for their response to changing environmental conditions has been restricted. With recently improved information, the analysis of micro-eukaryotic community structure is developing as a useful tool in sustainably managing aquatic environments and water resources (Bradford *et al.* 2013).

Native fish populations in the Murray River have declined over the last 100 years to about 10% of the pre-European level (MDBC 2013). Although the causes are still not well defined, water extraction, regulation of flow, invasive species, and climate change are all considered to be contributing factors. A lack of knowledge of the food web position of the larvae and juveniles of native fish in the Murray River makes it difficult to pin down the processes that may have contributed to their population decreases. A critical stage in the life cycle of fish is when larval fish switch from endogenous to exogenous feeding. This is a time when the availability of suitable prey can determine their survival and fish year-class strength. Zooplankton are often considered to be major food resource for fish larvae but recent studies have shown that ciliates can account for 60% of the total carbon biomass consumed, however they are fragile and easily digested so were often not identified in traditional studies

(Zingel *et al.* 2012). Food resources utilised by larvae can be identified by molecular amplification of gut content, which will detect the short fragments of prey items that are the products of digestion (Chow *et al.* 2011; Terahara *et al.* 2011; O'Rorke *et al.* 2012).

Dissolved and particulate material transport

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

- salinity, which is a measure of total dissolved salts and is a particularly important 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; Aldridge *et al.* 2012; Mosley *et al.* 2012).
- dissolved inorganic nutrients, which are readily assimilated by biota and are essential resources for growth and survival (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 material. It has a strong influence on light availability, which is important for structuring aquatic ecosystems

(Geddes 1984 a,b). It is influenced by flow through increased productivity as described previously, as well as the mobilisation of inorganic material 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 material (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; reduced nutrient concentrations through 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 Lower Murray River, Lower Lakes and Coorong (Brookes *et al.* 2009; Aldridge *et al.* 2011; Aldridge *et al.* 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 material. In doing so, these flows may provide ecological benefits through the provision of habitat and resources for biota.

2.2 Wetlands

Lateral movements of fish

Restoring lateral connectivity is important for maintaining native fish populations through increased survival, recruitment, feeding and reproduction opportunities (Junk *et al.* 1989), as off-channel habitats increase habitat availability and complexity. However, carp are also likely to benefit and therefore such a risk needs to be managed (Thwaites and Fredberg 2014).

The Murray River is a highly regulated river system, which supports a diverse range of fish species. Understanding the influence of different freshwater flow scenarios on the lateral movement of both native and invasive species between main-channel and wetland habitats would facilitate the development of well-informed, flow-related management intervention strategies to (*i*) enhance reproduction and recruitment success for native fish species; and (*ii*) inhibit key aspects of the population dynamics of invasive species, such as carp.

Knowledge on the lateral movements of freshwater fish between main-channel and wetland habitats in the Murray River is limited. In 2006, Conallin *et al.* (2010) examined the lateral movement of fish in the Lower Murray River under low-flow conditions. That study identified variations in the lateral movements of adult and juvenile fish, but no directional consistency was identified, which likely reflected an absence of hydrological cues, due to the lack of flow during the drought. Furthermore, Conallin *et al.* (2010) predicted that directional movements would become apparent under increased flow conditions. In contrast, under variable flow conditions in the mid-upper Murray River (north-eastern Victoria), lateral movements of small-bodied native fish were identified to strongly correlate with fluctuations in water level - as water levels rose, fish moved from the main river channel into off-channel habitats, before returning to the main river as water levels receded (Lyon *et al.* 2010).

Frog response

River regulation and recent drought has had a substantial impact on ecosystem processes and aquatic biota, including frog populations. A reduction in the extent, duration and frequency of wetlands flooded, and increased incidences of drought, negatively impact on all frog species found within the South Australian Murray River Corridor (Carey and Alexander 2003; Hazell 2003; Piha *et al.* 2007). Species reliant on seasonal flooding and variable flow regimes are at risk as a result of reduced flooding frequencies, which is forecasted to be exacerbated by climate change (Gonzalez *et al.* 2011). Less flooding is likely to result in a decline in vegetation health and reduced habitat extent for fauna. It may also lead to an increase in salinity due to less available freshwater and drier climate conditions (Gonzalez *et al.* 2011). Recruitment can be impacted in species reliant on flooding cues, or are unable to complete breeding cycles prior to drying events (Lane and Mahoney 2002; Wassens 2011). As a result, those species that have narrow habitat and physiological requirements, such as low salinity tolerances, limited dispersal ability, low reproductive capacity and recruitment rates, are at greater risk of decline due to reductions in flooding (Gonzalez *et al.* 2011).

Frog species within the South Australian Murray River corridor are found occupying waterbodies with a range of hydrological charactersitics from permanent and newly inundated ephemeral wetlands and creeks, to highly modified environments such as dams (Gonzalez *et al.* 2011). Most of these species have a preference for temporary waterbodies for breeding however they are also known to breed in permanent waterbodies (Lane and Mahoney 2002; Pyke *et al.* 2002; Lauck *et al*. 2005; Wassens *et al.* 2008; Gonzalez *et al.* 2011; Mason and Hillyard 2011; Wassens and Maher 2011).

The Southern Bell Frog (*Litoria raniformis*) is a species that is reliant on flooding of temporary wetlands for breeding and, as a result, is particularly at risk due to reduced flooding (Gonzalez *et al.* 2011). Widely found across the south-eastern region of the Murray-Darling Basin, in recent years it has undergone major declines in distribution (Wassens 2011). This has led to the species being listed as nationally threatened (vulnerable under the *Environment Protection and Biodiversity Conservation Act* 1999), threatened in South Australia (*National Parks and Wildlife Act* 1972), endangered in New South Wales (*Threatened Species Conservation Act* 1995), vulnerable in Tasmania (*Threatened Species Protection Act* 1995) and threatened in Victoria (*Flora and Fauna Gurantee Act* 1988) (Turner *et al.* 2011; Wassens 2011).

Although most species can utilise wetlands with a range of water regimes, they are highly dependant on inundated vegetation and/or physical habitat (Anstis 2002; Hazell 2003; Schultz 2006; Healey *et al.* 1997; Wassens 2011) and prefer to breed in waterbodies where abundant, complex and diverse emergent, submerged and fringing vegetation exists (Jansen and Healy 2003; Wassens *et al.* 2007; Gonzalez *et al.* 2011; Wassens 2011; Wassens and Maher 2011). Vegetation performs a number of important habitat functions for frogs and tadpoles such as; providing sites for calling and attachment of eggs, a food source through input of organic matter and a substrate for the growth of biofilms, and refuge and protection from predators (Anstis 2002; Lane *et al*. 2007; Wassens and Maher 2011).

Because changes to hydrological regimes, and the presence and level of structural complexity of aquatic and littoral vegetation directly affect frog species (Healy *et al.* 1997; Tarr and Babbitt 2002; McNally *et al.* 2009), it is especially important for environmental water managers to have a good understanding of the impact on frog populations of hydrological regimes. The delivery of environmental water, particularly where it leads to an increase in inundation of wetlands and vegetation habitats, may lead to improvements in frog populations across the Murray-Darling Basin.

2.3 Coorong (modelling)

The Coorong is a dynamic estuarine lagoon system located at the terminus of the Murray-Darling Basin in South Australia. It has been heavily impacted by river regulation and water extraction upstream since European settlement; subsequently the current average annual flow has declined by 61% at the Murray Mouth (from 12,333 GL $year^{-1}$ to 4,733 GL $year^{-1}$; CSIRO 2008). The Coorong has a strong north-south salinity gradient, generally ranging from brackish/marine in the Murray Mouth area to hypersaline in the North and South Lagoons (Geddes and Bulter 1984; Geddes 1987). Salinities are spatiotemporally variable and highly dependent on the freshwater inflows from the Murray River, with varied salinities supporting different ecological communities (Brookes *et al.* 2009). In addition, the southern end of the South Lagoon receives small volumes of fresh/brackish water from a network of drains (the Upper South East Drainage Scheme) through Salt Creek.

Freshwater inflow is a crucial driver affecting salinity and water level regimes in the Coorong; these physical-chemical parameters have a strong influence on the habitat and ecological communities including the iconic macrophyte, *Ruppia tuberosa* (Nicol 2005), and a range of fish species in the Coorong (Ye *et al.* 2012; Livore *et al.* 2013). The hydrodynamic model developed by CSIRO over the past years (Webster 2007; Webster 2013) and now being available as the *Coorong Hydrodynamic Model* (CHM) v2.1 (Joehnk and Webster 2014) allows the simulation of salinity and water levels along the 102 km of Coorong by 1 km resolution. This was combined with our conceptual understanding of the life-history of *Ruppia tuberosa* and the effects of salinity and water levels on key processes to evaluate the ecological benefit based on flow regimes and 2012-13 environmental watering in the Coorong. In addition, exploratory analysis on the extent of estuarine fish habitat was also undertaken based on the salinity tolerance thresholds of seven key species in the Coorong.

Ruppia tuberosa

Ruppia tuberosa is a submergent halophyte that was historically common in the South Lagoon of the Coorong (Geddes and Brock 1977; Brock 1979; Brock 1981; Paton 1982; Geddes and Butler 1984; Geddes 1987; Paton 1996). It is one of the most salt tolerant angiosperms with a maximum salinity tolerance of 230 g/L for adult plants (Brock 1982a); however, much lower salinities are required for life cycle completion. Kim et al. (2013) reported that salinities lower than 85 g/L for 15 days are required for germination from seeds and 125 g/L for sprouting from turions (a specialised drought resistant asexual propagule produced by aquatic plants). Furthermore, exposure to elevated salinity followed by lower salinity stimulated germination in seeds but reduced viability of turions by over 90% (Kim et al. 2013). Brock (1982b) also noted that at elevated salinities *Ruppia tuberosa* did not flower and was restricted to reproducing asexually; therefore, lower salinities are required for the production of seed and subsequent replenishing of the sediment propagule bank.

Water levels are also a critical factor for *Ruppia tuberosa* in the South Lagoon of the Coorong. *Ruppia tuberosa* is highly sensitive to desiccation but has high light requirements; therefore, there is a narrow band where the species can occur in the highly turbid South Lagoon (Nicol 2005). *Ruppia tuberosa* colonises areas between 0 and -0.5 m AHD in May to June in the South Lagoon; areas below -0.5 m AHD are below the euphotic zone and areas above 0 m AHD are prone to desiccation due to wind driven water levels fluctuations (seiching) (Nicol 2005). These water levels need to be maintained until at least mid November, preferably mid to late December to ensure the life cycle is completed and the seed bank replenished (Figure 4).

Figure 5 represents the optimal salinity regime for *Ruppia tuberosa* in the South Lagoon of the Coorong. Salinity needs to be lower than 125 g/L for turions to sprout and 85 g/L for seed germination for at least 15 days (Kim et al. 2013). Salinity needs to be maintained below 100 g/L for the duration of the growing season to ensure plants reproduce sexually. Whilst seed production is restricted at salinities above 100 g/L, turions may be produced but this requires further study. The maximum salinity thresholds for adult plants (230 g/L), turion sprouting (125 g/L), seed germination (85 g/L), sexual reproduction (100 g/L) and turion viability (130 g/L) are also represented.

The information in Figure 4 and Figure 5 are summarised in Figure 6 , a conceptual model that represents the life-history of *Ruppia tuberosa* in the South Lagoon of the Coorong. The life-history of *Ruppia tuberosa* is represented by five stages; the sediment propagule bank, seedlings, juveniles, asexual adults and sexual adults. The sediment propagule bank consists of seeds and turions; turions will sprout (in May to June) when inundated with water that has salinity lower than 125 g/L and seeds will germinate when the salinity is below 85 g/L (Kim et al. 2013). Seedlings will persist and become juveniles providing the water level is maintained above +0.2 m AHD and the salinity remains below 100 g/L and in turn juveniles will become asexual adults if the aforementioned conditions are maintained until October. If the salinity remains below 100 g/L and the water level above +0.2 m AHD until mid November (preferably mid to late December) the plants will reach sexual maturity and replenish the propagule bank. However, if the salinity exceeds 100 g/L the plants will not flower but turions may be produced that will partially replenish the propagule bank. If water levels fall below +0.2 m AHD before mid November plants will die and the propagule bank will not be replenished.

The ecological bottlenecks identified were seed germination and turion sprouting because during periods of extended barrage closure the salinity in May and June often did not fall below the thresholds for germination or sprouting (Figure 6). Furthermore, the high salinities experienced over summer during periods of extended barrage closure may have reduced turion viability in the propagule bank. The other bottle necks identified were plants not reaching sexual maturity and the propagule bank not being replenished (Figure 6). Barrage outflows often cease during late October to early November, which causes a sudden drop in water level in the South Lagoon, stranding plants that have not reached sexual maturity.

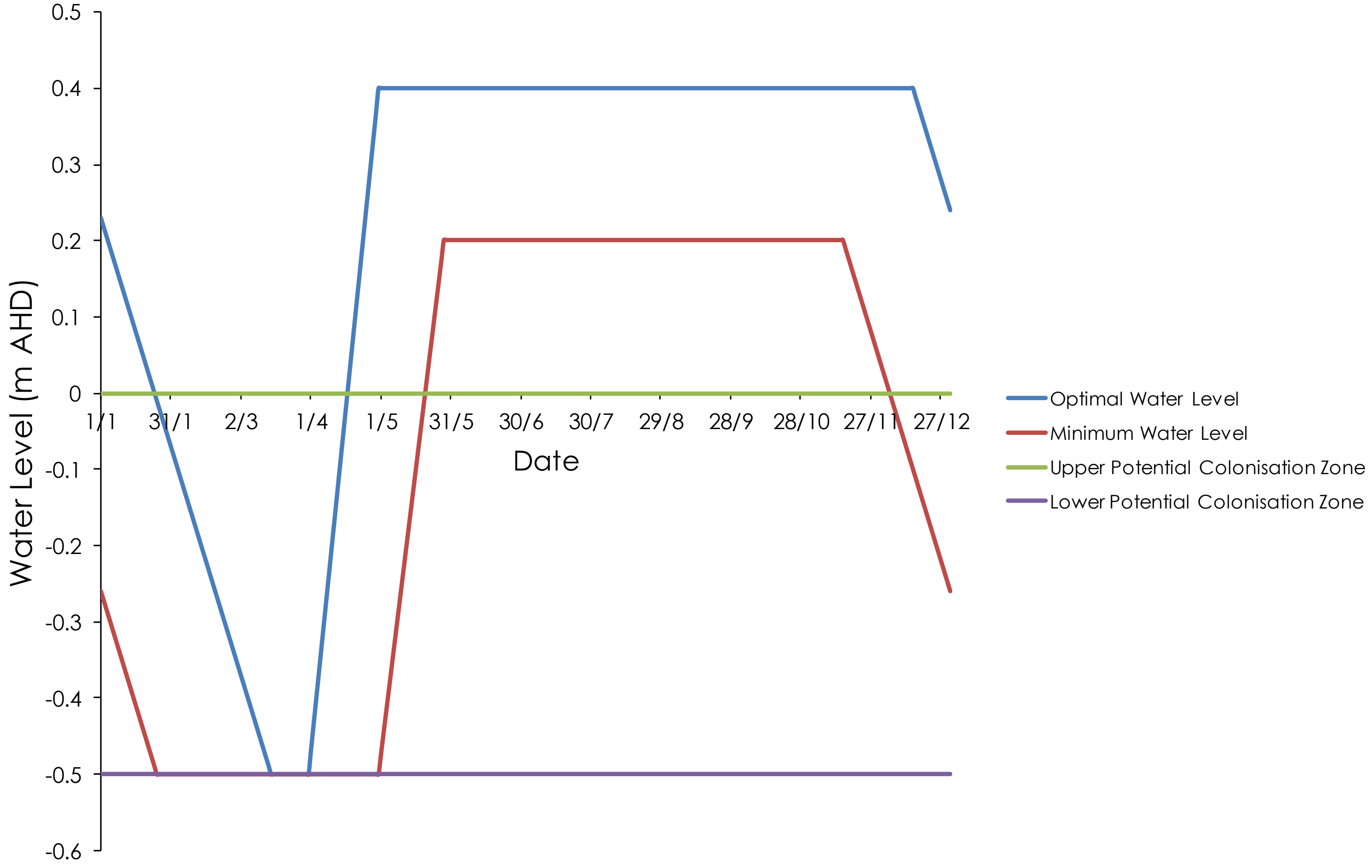


Figure 4. Optimal hydrograph for *Ruppia tuberosa* in the South Lagoon of the Coorong showing the minimum water levels throughout the year and the elevations where colonisation is likely to occur.

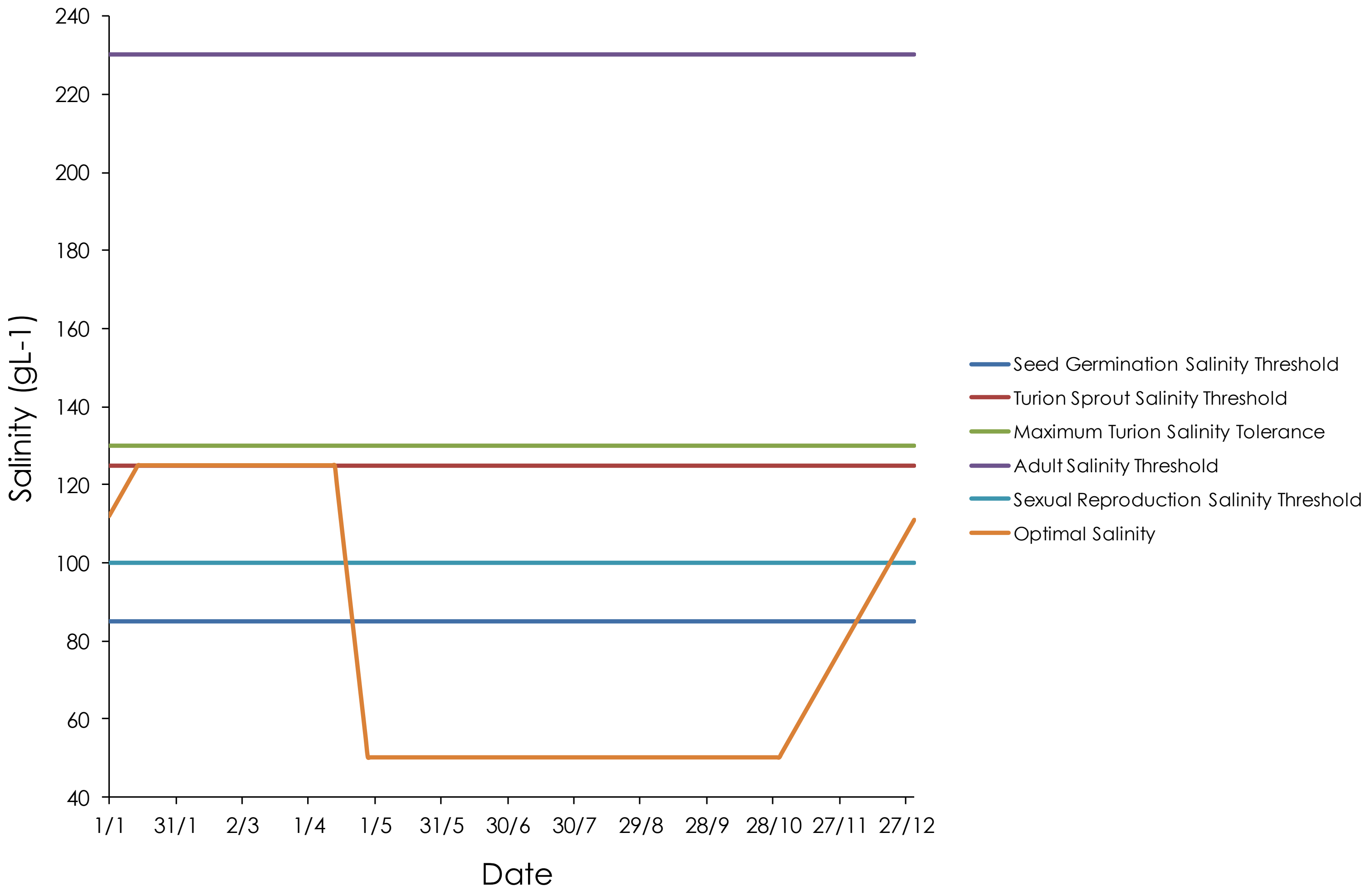


Figure 5. Optimal salinity regime *Ruppia tuberosa* in the South Lagoon of the Coorong showing the maximum salinity thresholds for adult plants, seed germination, turion sprouting, turion viability and sexual reproduction.

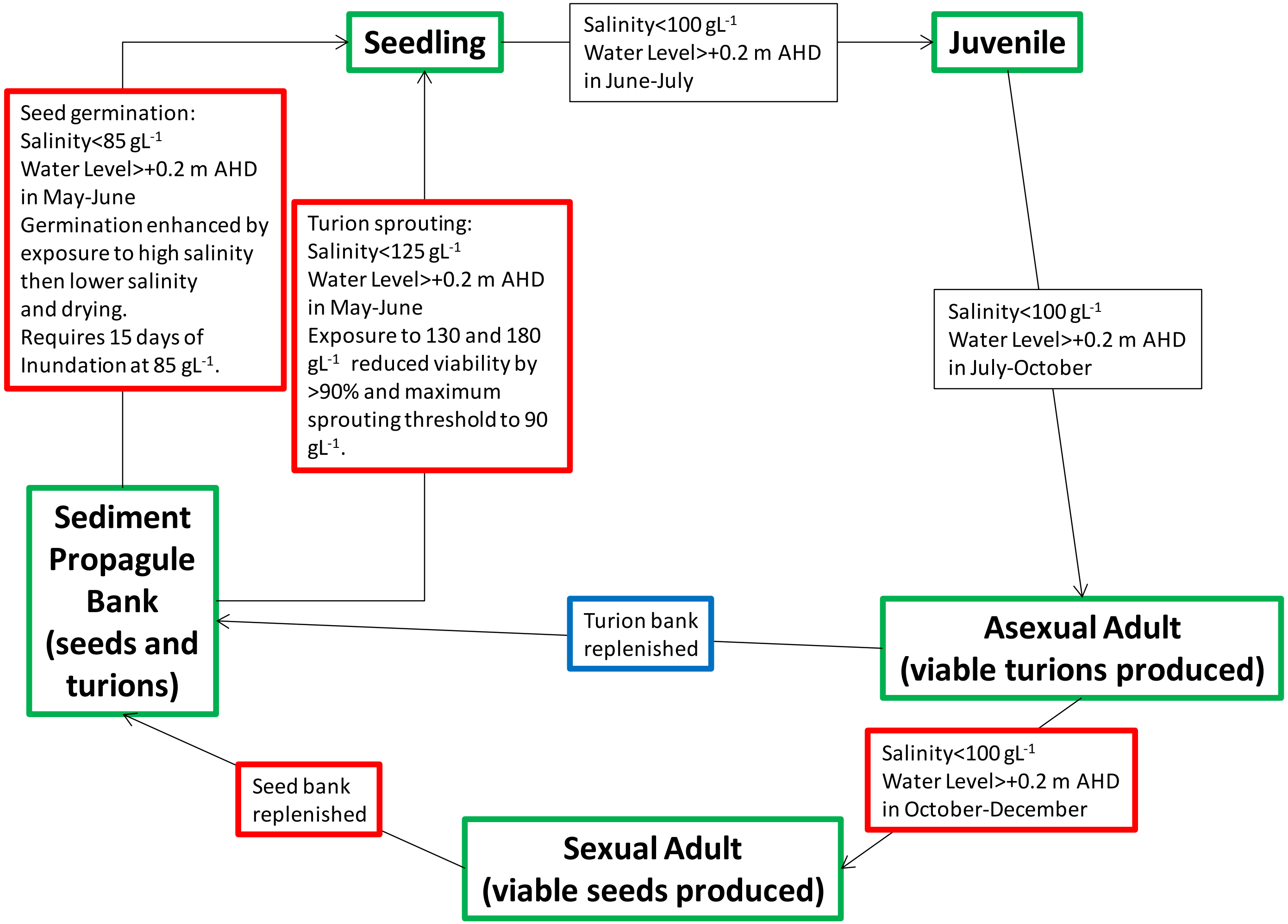


Figure 6. Conceptual model of the life-history of *Ruppia tuberosa* in the South Lagoon of the Coorong. Green boxes represent life-history stages, red boxes potential ecological "bottle necks" and the blue box requires more information.

Fish habitat

The Coorong supports a diverse range of fish species including freshwater, estuarine, marine and diadromous species. Although with different life-history strategies, many species are strongly associated with estuaries, using them as spawning, nursery and feeding grounds, refuge, or a migratory pathway (Whitfield 1999). Freshwater inflows and connectivity are crucial for maintaining the habitat, productivity and ecological integrity in estuaries. Over the past years, many studies in the Coorong have identified salinity as the key driver that influences fish assemblage structure (Noell *et al.* 2009; Zampatti *et al.* 2010) and the extent of estuarine fish habitat in the Coorong (Geddes 1987; Noell *et al.* 2009; Ye *et al.* 2011b). The contraction of effective fish habitat for a range of species due to freshwater flow reduction and increased salinities was well demonstrated during the millennium drought (2001-2010) (Noell *et al.* 2009; Ferguson *et al.* 2013; Ye *et al.* 2013a). The restoration of fish habitat was further shown in recent high flow years (2010-2013) following the substantial increase in barrage release and a broad salinity reduction throughout the Coorong (Ye *et al.* 2012; Livore *et al.* 2013).

A recent study investigated the tolerance thresholds of key Coorong fish species' juveniles to hyper-marine salinity and the relationship between thresholds and distribution of species and salinity levels in the field (McNeil *et al.* 2013). These species include important commercial and recreational fishery species, mulloway (*Argyrosomus japonicus*), yellow eye mullet (*Aldrichetta forsteri*), black bream (*Acanthopagrus butcheri*) and greenback flounder (*Rhombosolea tapirina*), and species with high ecological and conservation values, Tamar goby (*Afurcagobius tamarensis*), congolli (*Pseudaphritis urvillii*), and smallmouthed hardyhead (*Atherinosoma microstoma*).

Gradual acclimation tolerance trials were conducted in aquaria at two different test temperatures; 14 °C (representative cold 'winter' temperature) and 23 °C (representative warm 'summer' temperature). The lethal concentrations (i.e. tolerance thresholds) were compared to the distribution of fish across a natural salinity gradient in the field (Noell *et al.* 2009). The results suggest that threshold values, in particular 10% lethal concentration (LC_{10}) and 50% lethal concentration

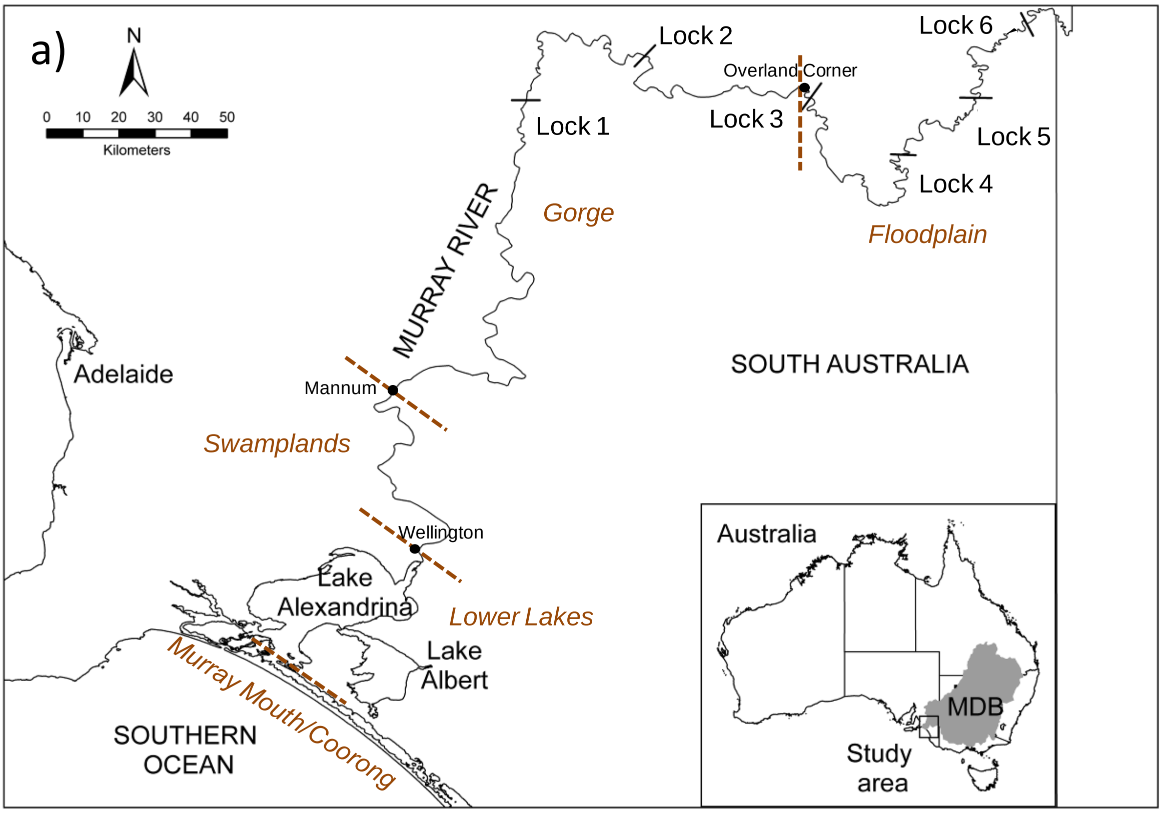
(LC_{50}), can approximate the maximum salinity extent of field distribution of species with moderate accuracy. As fish juveniles were generally reported to be more sensitive to salinity impacts than adults (Hart *et al*. 1991; Clunie *et al*. 2002), their salinities tolerance thresholds were used in the fish habitat modelling for the Coorong. LC_{10} was adopted as a more conservative threshold than LC_{50} to simulate suitable fish habitat in the field.

3 GENERAL METHODOLOGY

3.1 Study area

This broader study was conducted at various sites in the main channel and selected wetlands of the Lower Murray River and across the Coorong, Lower Lakes and Murray Mouth (CLLMM) region, South Australia (Figure 7). Sites in the Lower Murray River (the stretch of the Murray River between the South Australian border and the Lower Lakes) covered three distinct geomorphic regions:

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



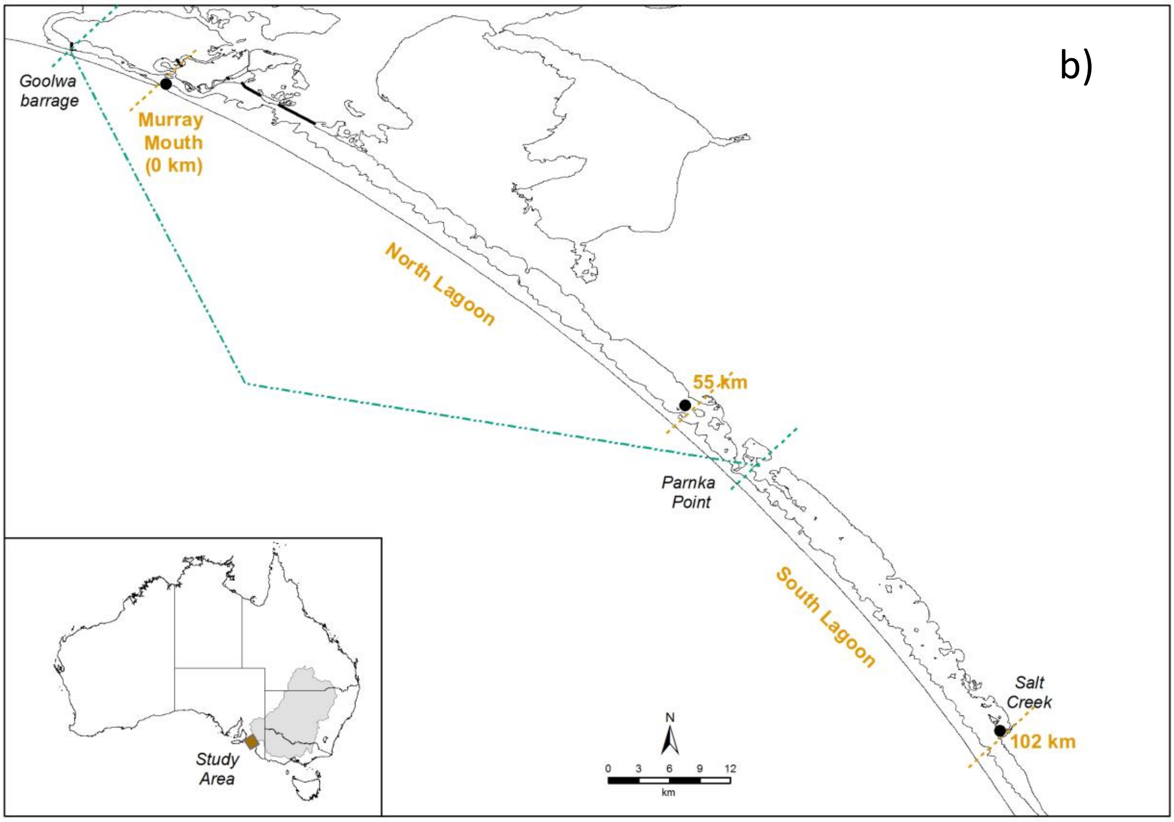


Figure 7. Map of a) the Lower Murray River, Lower Lakes and Murray Mouth showing geomorphic regions; b) the Coorong showing North and South lagoons and 0, 55 and 102 km markers used for Coorong modelling.

Sampling site information for each task is given below. For more detailed information (i.e. maps and GPS coordinates) please refer to Appendix I.

Main channel

Larval fish and food resources for larval fish: For these two tasks, three sites were sampled:

- LK-1 –in the main channel downstream of Lock 1 (Gorge region),
- LK-6 – in the main channel downstream of Lock 6 (Floodplain region) and
- LK-6A – in the main channel downstream of an anabranch system adjacent to Lock 6 (Floodplain region).

Dissolved and particulate material transport: The study area for this task was in the main channel from Lock 1 (Blanchetown) to the Murray Mouth, incorporating the Lower Murray River, Lower Lakes and Northern Coorong (Appendix I).

Wetlands

Lateral movements of fish: This study was conducted in four wetland sites in the Lower Murray River. Two of these sites were in the Floodplain region: Martins Bend and Overland Corner, and two in the Gorge region: Kroehns and North Purnong (Appendix I).

Frog response: This study included a total of 130 sites at 44 wetlands across three geomorphic regions: Floodplain, Gorge and Swamplands. Nine of these wetlands from two regions (Gorge and Floodplain) were used to assess the differences in frog species diversity and calling abundance between spring (no environmental water) and summer (with environmental water delivery). For detailed information on study sites refer to Hoffmann *et al*. (2014).

Coorong (modelling)

Ruppia tuberosa and fish habitat: The modelling was conducted for the areas from Murray Mouth, to the North and South lagoons of the Coorong (Figure 7b).

3.2 Task methods

Main channel

Larval fish

Larval fish assemblages in the Lower Murray River were sampled in order to detect changes in abundances during the reproductive season of some relevant freshwater native fish species. Samples were collected on a fortnightly basis from October 2012 to March 2013 (Table 2). Sampling was carried out by towing a Bongo net with a 500 µm mesh astern a 7 m boat, three times during the day and night (total of six tows site^{-1} trip^{-1}). Samples were then sorted and larval fish were identified and quantified in the lab. For more details please refer to Bucater *et al*. (2009).

Table 2. Larval fish sampling trips for the Lower Murray River between 2005-06 and 2012-13 (zooplankton sampling only occurred in 2012-13).

Trip Number	Indicative sampling week	Watering Years
1	2nd week in October	ALL
2	4th week in October	ALL
3	2nd week in November	ALL
4	4th week in November	ALL
5	2nd week in December	ALL
6	4th week in December	ALL
7	1st week in January	2012-13
8	3rd week in January	2005-06, 2010-11 and 2012-13
9	1st week in February	2011-12 and 2012-13
10	3rd week in February	2011-12 and 2012-13
11	1st week March	2011-12 and 2012-13
12	3rd week in March	2011-12

As part of this task, zooplankton assemblage was also sampled. Water samples using a Haney trap (volume = 4.5 litre) were collected to assess zooplankton abundance and community structure in the water column. Two Haney trap samples comprising a total of 9 litres were sieved through 32 µm mesh and the filtrate was preserved in 70% ethanol *in situ* to compose each sample. Three replicate samples were collected during the day and night of the same day; resulting in three day and three night samples for each site during each trip (for details see Ye *et al*. 2013b). Species were identified using keys in Shiel (1985) and references therein. The total count of zooplankters in 1 ml was multiplied by the number of millilitres in the original sample volume to provide an estimate of the density in the 4.5 litre trap volume. The accuracy of the method had previously been established to be ±2% by taking triplicate aliquots and calculating standard deviation and standard error.

For nutritional value of the zooplankton community, samples were collected by pumping subsurface water through three consecutive sieves of decreasing size (i.e. 1000 µm, 500 µm and 100 µm) for one hour with a direct line electric pump (~1100 L/hour) while larval plankton tows were carried out. One day and one night sample were collected at each site in all trips of 2012 except the first ($n = 9$). The filtrate obtained in 100 µm mesh sieves was washed and preserved on ice. As soon as practically possible, samples were frozen and returned to the lab where they were freeze-dried and biochemically analysed (for details see Ye *et al*. 2013b). Percentages of fatty acids that have been shown to be important in larval fish development were of particular interest (e.g. EPA, DHA, ARA, LIN and ALA).

Larval fish abundances will be expressed as number of individuals per cubic meter of water filtered. To model relationships between larval fish abundance as well as zooplankton abundance with one or more WQ predictor variables, as described by the Bray-Curtis resemblance matrix, we will use the DistLM (distance-based linear models) routine and the model-building criteria of forward R^2. Ordination of fitted values for the DistLM will be achieved through distance-based redundancy analysis (dbRDA), with vector overlays to show individual WQ parameters that were important in driving variation along dbRDA axes.

Food resources for larval fish

Water samples were collected for DNA analyses of the microorganism community composition from mid-river, the river edge and macrophyte beds. Water samples were also collected mid-river for phytoplankton enumeration. Zooplankton samples were collected from the open water using a Haney trap that captured larger microorganisms occurring in lower densities (see previous section). Zooplankton collections were sub-sampled for molecular analyses and microscopic enumeration (for details see Ye *et al*. 2013b). Larval fish were collected as described in the previous section.

DNA was extracted from both the water samples and the zooplankton samples and a fragment of the 18S rRNA gene that is highly conserved in eukaryotes was amplified using the Polymerase Chain Reaction (PCR) and sequenced by 454 pyrosequencing (Bradford *et al.* 2013). Larval fish were washed in distilled water and the alimentary tract rfemoved. DNA was extracted from each individual fish gut and treated in the same way as the water and zooplankton DNA samples except that peptide nucleic acid probes were added to block the amplification of fish tissue during the PCR step.

Unique DNA sequences representing Operational Taxonomic Units (OTUs) were identified from the water, zooplankton and fish gut samples (Bradford *et al*. 2013). Taxonomic identification of OTUs was made by matching the sequence reads to a curated reference sequence database (SILVA 18SrRNA database; Quast *et al*. 2012).

Non-metric, multivariate statistical analyses (PRIMER v6; Clarke and Gorley 2006), were used to test for significant differences in microeukaryote community composition between sites, trips, sampling locations, and diets of fish species. In general molecular sequence information was analysed as presence/absence data as the number of sequence reads is not considered a reliable measure of the numbers or biomass of organisms (Bradford *et al*. 2013).

Dissolved and particulate material transport

Water quality was monitored between October 2012 and April 2013 and supplemented with additional data sources (Appendix I). At each sampling site, measurements of water temperature, electrical conductivity, dissolved oxygen, pH and turbidity were taken at 0.5 m intervals through the water column. 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 (the sum of organic nitrogen and NH4), dissolved silica, total suspended solids, suspended organic matter and chlorophyll *a* using standard techniques. Total nitrogen was calculated as the sum of total Kjeldahl nitrogen and nitrate. Organic nitrogen was calculated as the difference between total Kjeldahl nitrogen and ammonium.

The physico-chemical information was used to validate a three-dimensional hydrodynamic-biogeochemical model, ELCOM-CAEDYM, which has been used extensively within the region (e.g. Aldridge *et al*. 2013). The model was initialised with data from a range of data sources, including Lock 1 inflows with the different combinations of environmental water use (provided by the MDBA). The flow data are treated as indicative only due to complexities around interstate water accounting. Assumptions such as these, result in uncertainty in the model outputs (refer to Aldridge *et al.* 2013 for more detail) and so outputs are not be treated as absolute values. 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 watering. For detailed information on the modelling approach, assumptions and validation, refer to Aldridge *et al.* (2013) and Hipsey and Busch (2012).

For this study, four simulations were run and compared for 1 October 2012 to 30 June 2013:

- with both Commonwealth environmental water and The Living Murray water;
- with Commonwealth environmental water, but without The Living Murray water;
- without Commonwealth environmental water, but with The Living Murray water;
- without both Commonwealth environmental water and The Living Murray water.

The influence of environmental watering on the concentrations of material was assessed through a comparison of modelled concentrations for the Lower Murray River (Wellington), Lower Lakes (Lake Alexandrina Middle) and Murray Mouth. The transport of material was assessed through modelled exports from the Lower Murray River (Wellington), Lower Lakes (Barrages) and Murray Mouth.

Wetlands

Lateral movements of fish

Field sampling

Fish assemblages moving through the river-wetland connection passage of four wetlands were sampled to describe the lateral movements of native and invasive fish before, during and after the delivery of the environmental water in 2012-13. Sampling was undertaken during six phases of flow delivery (Figure 8) to examine the influence of environmental water (EW) delivery and associated hydrological cues on fish movement to and from wetlands. The six flow phases were: (1) High – unregulated high flows; (2) Receding – regulated recession of flow; (3) Rising (EW) – increasing flow associated with the delivery of the environmental water; (4) High (EW) – stable and relatively high flow associated with the delivery of environmental water; (5) Receding (EW) – recession of flow associated with environmental water delivery; and (6) Low – low regulated flow (Figure 8). However, no sampling was done at Martins Bend and North Purnong during the High (EW) and Receding (EW) phases due to time constraints. As such, data for these two wetlands are not included in this report.

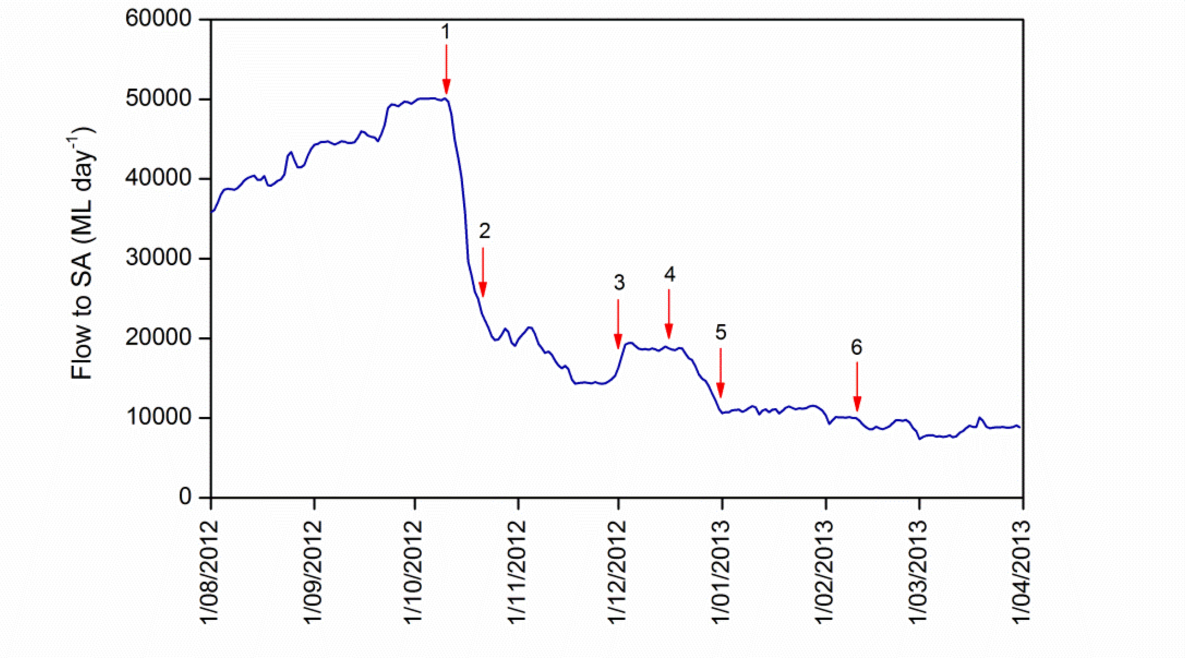


Figure 8. Hydrograph showing the Murray River flow (ML day^{-1}) across the border to South Australia for the period August 2012 – March 2013. Red, numbered (1-6) arrows indicate the six phases of flow during which sampling were undertaken.

On each sampling occasion, a network of fyke nets and drum nets were set in the river-wetland connection passage of each wetland, to catch fish moving bi-directionally between the main river channel and the wetland. All nets were set for 24 hours on three consecutive days (replicates). All fish captured were identified to species level, and counted. Up to 30 individuals of each species were randomly sub-sampled and measured for total length (TL mm) and released well outside of the net fleet in the direction they were originally moving. Each native species was classified to one of five functional groups based on their use of specific habitats and reproductive strategy (see Baumgartner *et al.* 2013). Each species was categorised as either a long-lived apex predator, flow-dependent specialist, foraging generalist, floodplain specialist, or diadromous. All invasive species were categorised into a single group.

On each sampling occasion, measurements of pH, dissolved oxygen, temperature and conductivity were recorded. Data for flow discharge, relative water level (meters AHD, level relative to Australian Height Datum) were obtained for both sites from the DEWNR Surface Water Archive (www.waterconnect.sa.gov.au). Estimates of the surface area of each wetland were obtained from the Murray River

Floodplain Inundation Model (described above) to estimate changes in wetland size (area of inundation) throughout the study period.

Data analysis

For data analyses, fish abundance data were standardised for catch-per-unit-effort (CPUE) to provide a relative abundance (no. of fish.hour^{-1}) prior to analyses. Fish assemblage structure was defined by the species composition and the relative abundance of each species within each replicate (day) over each flow phase for each wetland. Complex multivariate statistical analyses were undertaken using the PRIMER v6 package (Clarke and Warwick 2001) to: (1) investigate differences in assemblage structure moving 'In' and 'Out' of each wetland between and across flow phases; (2) determine the key species that drive the differences between assemblages; and (3) determine whether variation in fish assemblage structure was correlated to changes in hydrology. Detailed statistical analyses are described in Earl and Ye (In Prep).

The size distributions of key species moving into and out of wetlands were compared using a two-sample Kolmogorov-Smirnov test which was done using the IBM SPSS statistics package V.20. Fish length data were pooled across flow phases for each wetland to ensure sufficient sample sizes.

Carp reproduction and recruitment

To examine the influence of environmental water delivery on the spawning activity of common carp, all mature females (> 300 mm TL) captured in each region, during each flow phase, were retained for processing in the laboratory. The ovaries of each fish were removed and classified macroscopically to one of five stages of development based on their appearance, size and colour. The five stages were: (1) immature; (2) developing/resting; (3) developed; (4) ovulating (spawning imminent); and (5) spent/regressing (see Fowler *et al.* 1999 for detailed descriptions). All ovaries classified to ≥Stage 3, were subjected to microscopic analysis to provide a more accurate indication of recent or imminent spawning activity.

To further assess the influence of environmental water delivery on the spawning patterns for common carp, all juveniles <100 mm TL sampled were retained, dissected for the removal of their otoliths, and aged (in days) to estimate the date they were spawned. Estimated spawning dates for fish from each region were plotted in relation to the timing of flow delivery. Detailed descriptions of field sampling methods are described in Earl and Ye (In Prep).

Frog response

To evaluate the proposed hypotheses (Section 1.3), this project undertook the following tasks: 1) describe the frog community and distribution within the Lower Murray River during spring and summer 2012, 2) compare the diversity and abundance of frogs calling at wetlands before and during the environmental water provision and model environmental factors that may influence frog calling abundance and species richness and 3) compare frog breeding (through calling activity) in 2012 during the environmental watering with previous years to investigate frog breeding responses over a longer time scale.

Field sampling

To investigate the breeding response of frogs to environmental water delivery, frog call surveys were conducted between September and December 2012. Frog call surveys are a common method for detecting frog species and utilises the species-specific calls that male frogs use to attract mates to breeding sites during the first phase of a breeding response (Tyler 1994, Zimmerman 1994). This method has many advantages, such as allowing individuals to be detected without visual observation, which can be difficult due to the cryptic nature of many frogs (Zimmerman 1994). To allow comparison of the abundance and species richness of male frogs calling before and during the environmental watering, nine wetlands from two geomorphic regions (Floodplain and Gorge) were sampled repeatedly in September (no environmental water but higher flows occurred) and December 2012 (environmental water).

At each wetland the number of survey sites (replicates) varied depending on wetland size (larger wetlands requiring more sites) and accessibility at the time of surveying (due to flooding). To target areas inundated by the environmental water provided in summer, additional wetland sites were selected and assessed using the Rim-FIM (Overton *et al.* 2006) and on-site field assessment. Male frog calls were sampled following the method outlined in Your Wetland – Monitoring Manual (Tucker 2004). All wetland species of frogs within the Lower Murray River area call throughout the spring and summer months and frog calling is influenced largely by environmental and habitat variables, such as the peak of a flow event (Dostine *et al.* 2013; Wassens 2011). Habitat and environmental variables were also recorded during the surveys using a method adapted from the habitat assessment developed by Native Fish Australia (Hammer 2005).

Comparison of spring and summer 2012

To assess if there was an increased call response during the environmental water provision, the average species call richness (the number of species calling at a site) and frog call abundance (the estimated abundance of all male frogs calling at a frog site) and environmental variables at wetlands in spring (before e-water) and summer (during e-water) 2012 were compared using t-tests.

To investigate the relationship between environmental variables and the number of frogs and species recorded calling during frog surveys, generalised linear modelling was conducted with a quasi-Poisson distribution, appropriate for count data with over-dispersion (Crawley 2007). The dependent variables were frog call abundance (the estimated total number of calling males) and frog call richness (the total number of species calling) at each survey site considered against a number of environmental variables (Table 3). The explained deviance of the global model, which is the proportion of the model parameters that accounts for the variation of the dependent variable, was calculated by the following equation:

Explained variance = (null deviance - residual deviance)/null deviance.

Table 3. Explanatory variables used to explain frog call abundance at wetlands in 2012.

Variable	Description
Flows to SA	Recorded flow at the border entering SA on day of frog survey (ML day^{-1})
Electrical conductivity	Electrical conductivity of the surface water at frog survey site (µS/cm)
Air temperature	Air temperature recorded during survey (°C)
Humidity	Relative air humidity recorded during survey (%)
Emergent vegetation	Percent inundated emergent vegetation at frog survey site (%)
Submergent vegetation	Percent submerged vegetation at frog survey site (%)

Comparison to previous years

To compare male frog calling and abundance in summer 2012 (environmental water) with previous years, data from previous frog surveys conducted in the South Australian Murray-Darling Basin region in November and December were collated. To attempt to reduce variation due to some differences in sites sampled across different years, wetlands .were first allocated to one of three wetland types;

1. permanent (a wetland connected permanently at pool level),
2. managed (a wetland connected at pool level but managed through a flow-control structure to have wet and dry cycles) or
3. temporary (a wetland that is inundated when flows are above pool level).

Additionally, surveys conducted when wetlands were being actively managed (e.g. pumped, or closed and drawing down) were also excluded from analysis, as they were not connected and influenced by River flows and water level. Years where there were less than 12 sites sampled of that wetland type were also excluded in the analysis. Lastly, frog species were only included that spanned all geomorphic regions.

Mean rank frog call abundances were compared using Kruskal-Wallis test as data were non-parametric and sample sizes varied for each year. Post hoc pairwise comparisons were made between different years. All modelling and statistical analysis was conducted using R 3.0.1 (R Core Development Team 2013; package asbio). For more information on the survey methods please refer to Hoffmann *et al.* (2014).

Coorong (modelling)

Hydrodynamic model

The hydrodynamic model, *Coorong Hydrodynamic Model* (CHM) v2.1, developed by CSIRO (Webster 2007; Webster 2013; Joehnk and Webster 2014) was used to simulate water level and salinity along the Coorong for 102 km on a numerical grid reaching from the channel leading from the Murray Mouth to the North Lagoon down to the southern end of the South Lagoon (e.g. Joehnk and Webster 2014). The hydrodynamic model is based on daily barrage flow values from 1963 and updated regularly, currently until 31 August 2013 (data provided by MDBA). Salinities and water level along the North and South Lagoon were calculated on a daily time step with 1 km resolution driven with hourly and daily datasets describing tidal forcing, wind velocity, evaporation and precipitation. The modelling was run to simulate the whole period from 1963 to 2013.

Additionally, to evaluate the effect of environmental watering on ecosystems, three scenarios were calculated for the 2012-13 watering year:

1. reference, including all water sources;
2. with Commonwealth environmental water, without TLM water;
3. without both commonwealth environmental water and The Living Murray water.

The results of salinities and water level from scenario runs were subsequently used to simulate habitat characteristics for *Ruppia tuberosa* as well as fish species.

Ruppia tuberosa

Based on the effect of salinity and water level on the life-history of *Ruppia tuberosa*, an ecological response model that calculated the probability of replenishing the sediment propagule bank based on modeled hydrological conditions (i.e. output of the CHM) was developed. For the purpose of modeling, each life-history stage was treated as occurring over a discrete time period (in nature there would be considerable overlap of life-history stages) and a survival probability calculated for each time block that was in turn used to calculate the probability of replenishing the sediment propagule bank.

Model processes include seed germination, turion sprouting, seedling development to juvenile plants, juvenile development to asexual adult plants, and asexual adult development to sexual adult plants. Detailed modelling descriptions are provided in Joehnk *et al.* (2014). Each process is assigned a survival probability, which in the end results in a combined probability of sediment propagule bank replenishment.

Fish habitat

The basic fish model analysed salinity thresholds for juvenile fish of key species on an annual basis ("habitat suitability") and on a daily basis. The annual analysis provided probabilities of habitat suitability by calculating the annual exceedance probability for a certain salinity threshold. A simpler approach only detected the extent of a certain salinity threshold on a daily basis ("threshold analysis") along the Coorong starting at the Murray Mouth. The latter basically gives a simplified picture of the salinity contours along the Coorong over time.

Annual habitat suitability

The fish model looked for suitable habitats for juveniles of different fish species from Murray Mouth to the end of the South Lagoon of the Coorong, depending on ambient salinity levels for a period of one watering year. Each fish species was characterised by a certain salinity tolerance threshold, which varied between cold and warm seasons (Table 4). In our simplified expert model type system, the cold months included the period from mid April to mid October and warm months included the period from mid October to mid April. The model effectively calculated an annual exceedance probability for salinity. It assessed the daily salinity output of the hydrodynamic model for each 1 km grid along the 102 km Coorong, and counted the number of days where salinity was below the specific threshold of a fish species during the cold or warm period. The annual probability that a 1 km grid was a suitable habitat was then calculated. Details of parameter definition and model programming are presented in Joehnk *et al.* (2014).

Table 4. Period specific salinity thresholds for suitable habitats for seven fish species (adapted from McNeil *et al.* 2013).

Species name	Threshold cold months [g/L]	Threshold warm months [g/L]
Mulloway	60.3	51.1
Tamar goby	67.7	66.3
Black bream	78.6	81.8
Greenback flounder	81.1	72.9
Yelloweye mullet	83.8	68.3
Congolli	89.5	86.9
Smallmouthed hardyhead	99.5	97.1

Daily fish threshold analysis

To create an overlook over suitable habitats on a daily basis, the distance from the mouth (or the number of 1 km wide cells in the underlying hydrodynamic model) to which a certain threshold of salinity is not surpassed was also calculated. This was effectively a simplified contour plot depicting regions below and above a certain salinity threshold (see e.g. Figure 39). As above, salinity thresholds can vary between different periods (here warm and cold), and the same threshold values as given in Table 4 also apply here.

4 FINDINGS

4.1 Main channel

Larval fish

During the 2012-13 watering year, flow-cued spawning species larvae were present from October 2012 to January 2013 in the Gorge region (LK-1) and October 2012 to late December 2012 in the Floodplain region (LK-6 & LK-6A) (Figure 9 and Figure 15). Of particular interest is the fact that for a second consecutive year golden perch larvae have extended their presence into late summer, which was not observed in the drought (2005-2009). In 2011-12, golden perch larvae were collected until February whilst in 2012-13 they were collected until late January. All of the golden perch and silver perch larvae found late in January 2013, after the environmental flow pulse delivered in December 2012, were pre-flexion larvae which suggest they were spawned a few days before they were collected. This extended period of reproduction was not detected during the drought years (2005-2009), when pre-flexion golden perch and silver perch larvae were not collected at all. Whilst during the flood year 2010-11 pre-flexion larvae were only collected in November and December. The pattern of extended reproduction in 2012-13 was similar to that of 2011-12, albeit the abundance of golden perch larvae was lesser in 2012-13 (Figure 10).

The differences in flow conditions (e.g. timing, magnitude and duration) during 2011–12 and 2012-13 makes the interpretation of these results complex and no causal link can be directly and solely attributed to the presence of golden perch larvae late in the season. Delivery of environmental water may have promoted the extension of golden perch spawning to later in the season particularly in the Gorge geomorphic region where a large majority of the golden perch larvae collected late in the season were pre-flexion larvae suggesting they were spawned within the South Australian reaches of the Murray River. It is also possible that the releases of environmental water during summer may have enhanced larval drift from spawning populations upstream.

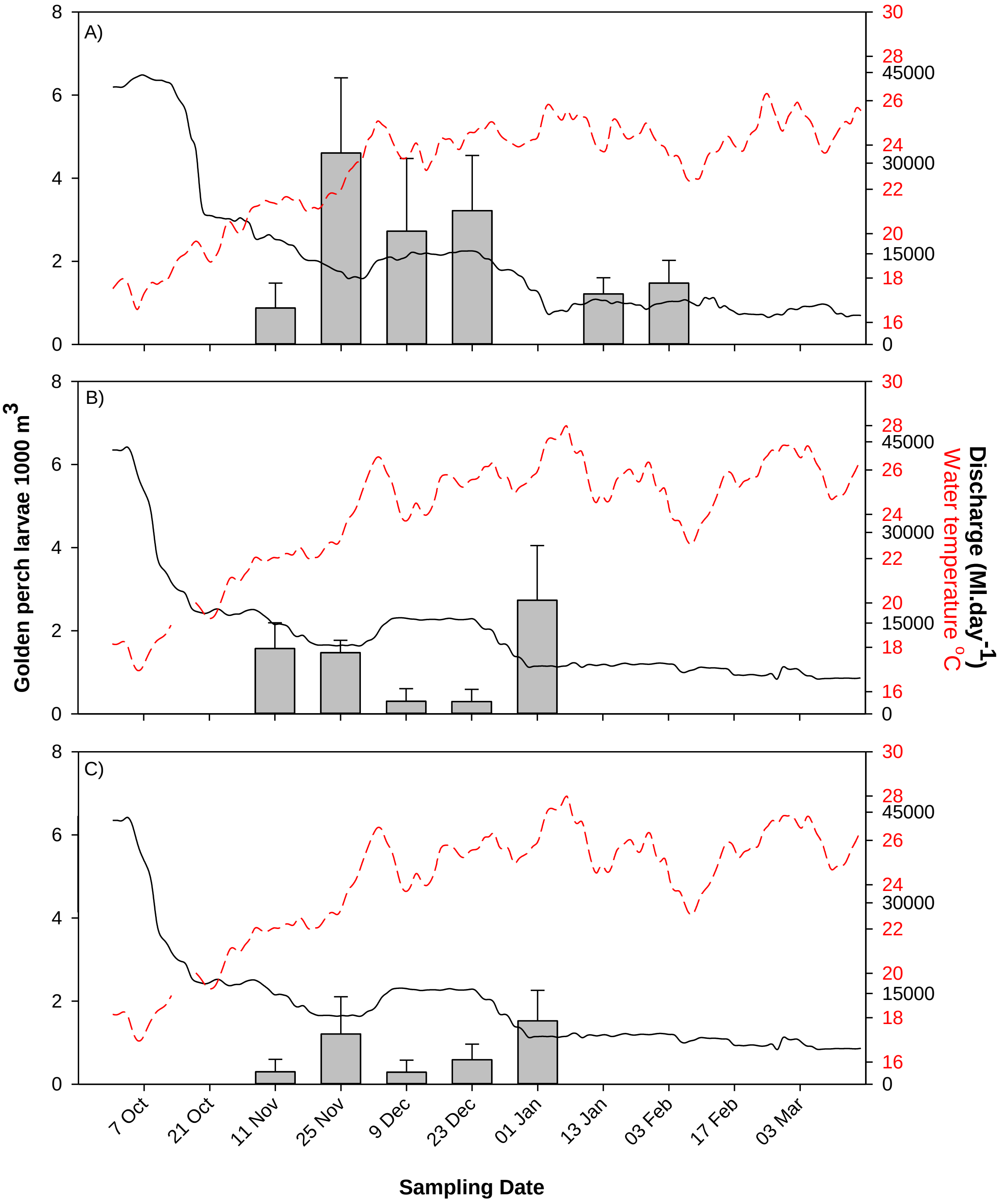


Figure 9. Mean (± S.E.) standardised catch of golden perch larvae between October 2012 and March 2013 at each site: A) Lock 1, B) Lock 6 and C) Lock 6A.

Generally the larval fish assemblages varied spatially and temporally. In the Floodplain region the larval fish assemblages at the two sampled sites were not different despite one of them being downstream of the exit of a major anabranch system, namely the Chowilla floodplain (Table 5). The site in the Gorge region was different from the two Floodplain sites (Appendix II) mainly due to higher abundances of bony herring, carp, carp gudgeon and golden perch larvae in the Gorge region (Table 5).

There was a seasonal change in larval fish assemblages in both geomorphic regions, which was related to an increase in temperature and a decrease in flow throughout the sampling period. This pattern is consistent and repeated throughout previous years sampling (Figure 11).

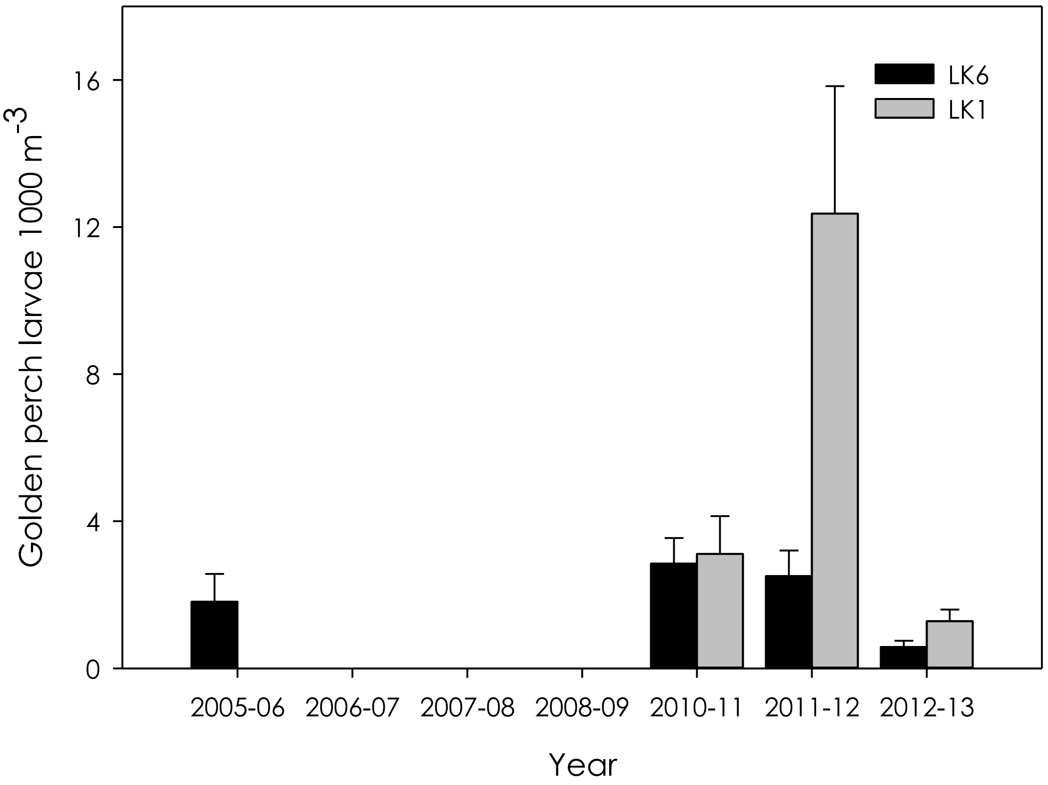


Figure 10. Mean (± S.E.) standardised (larvae 1000m^{-3}) catch of golden perch larvae throughout drought (2005-06 to 2008-09), flood (2010-11) and flow years (2011-12 and 2012-13). Note there was no sampling during 2009.

Table 5. Summary of estimated larval abundance (larvae 1000 m^{-3}) per site throughout the 2012-13 sampling period.

Site	LK-1	LK-6	LK-6A	Total
flat-headed gudegeon	842.5	1512.5	1092.5	3447.5
carp gudgeon	1024.6	544.8	516.3	2085.7
bony herring	780.1	110.6	82.5	973.3
carp	362.1	60.3	84.9	507.3
Australian smelt	192.6	5.1	34.1	231.8
golden perch	84.8	38.2	23.4	146.4
silver perch	37.5	3.4	5.1	46.1
Murray cod	10.6	7.1	7.2	24.9
freshwater catfish	8.9	10.9	3.6	23.5
Total	**3343.7**	**2293.1**	**1849.9**	

Previous work on larval fish assemblage by SARDI allowed a comparison between years of substantially different flow regimes, including the millennium drought and the 2010 floods. A graphical comparison using distance based linear models illustrates the differences in fish assemblages from 2005 (a low flow year with a small pulse), through the millennium drought 2006-2008 (no sampling occurred in 2009), during the flood year 2010 and the last two years characterised by some flow and environmental watering pulses (Figure 11). This comparison was only done for trips 1-6 (October to December inclusive), as these trips occurred in all sampled years. The environmental factors that were more closely related to the two main axes were flow and temperature. High flow was positively associated with all samples from 2010 and early trips in 2011 and 2012, whilst 2006, 2007 and 2008 showed a negative association.

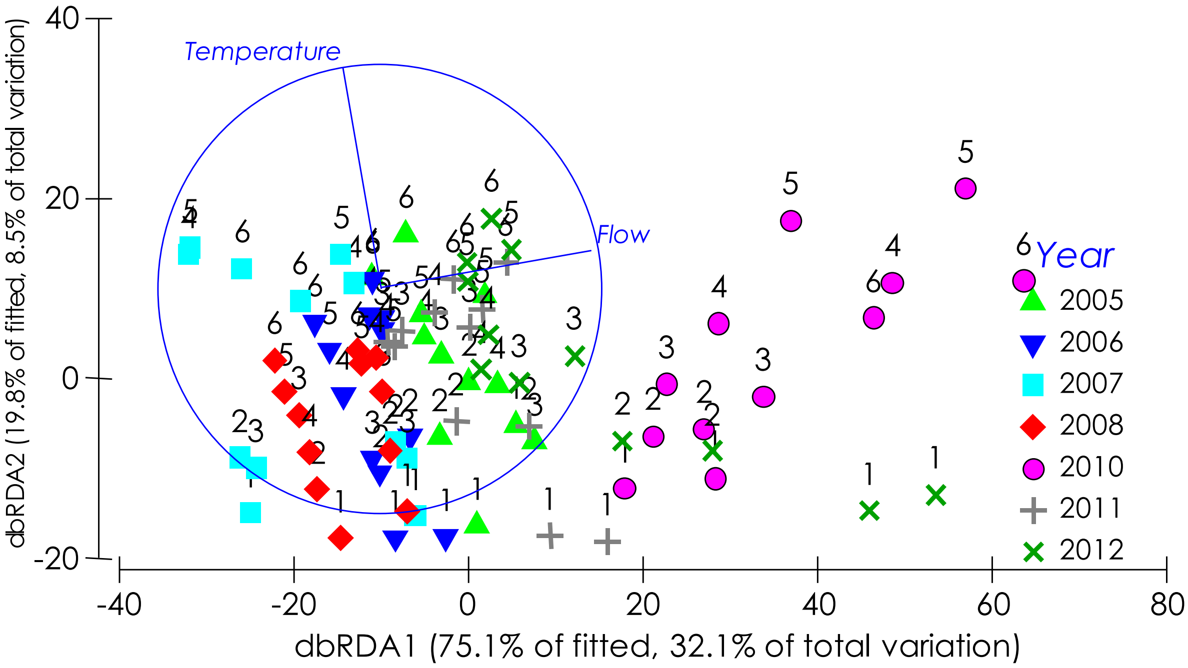


Figure 11. Distl M analysis of larval fish assemblages throughout the sampled years across sites LK-1 and LK-6. Vectors represent environmental variables that were significantly correlated to axes. Black numbers above symbols represent trip number.

Temperature explained the vertical distribution showing the seasonality exhibited in all years. What stands out from this comparison is that 2010 and 2006-08 are the extremes where assemblages are very dissimilar, whilst 2005, 2011 and 2012, particularly after trip 1 are very similar. When hydrographs for 2005, 2011 and 2012 are compared, it is clear that there was substantially more flow leading up to spring in 2011 and 2012 than in 2005, but the flow during spring were similar. This suggests that it is likely that larval fish assemblages during spring (the key reproductive season for many native fish) is driven by the flow occurring at the time regardless of previous flow history. This emphasises the need for delivery of environmental water during this critical reproductive period (spring and early summer) in order to obtain the most effective results for native fish reproduction in the Lower Murray River.

Zooplankton assemblage

The analysis of abundance and composition of zooplankters showed differences at spatial and temporal scales (Appendix III). Total abundances of zooplankters followed similar trends throughout the sampled period at all sites (Figure 12). However the species composition and abundances varied at both the temporal and spatial scale (Figure 13, Appendix III). The temporal variation can be attributed to an expected seasonal change as water temperature rose throughout spring to reach maximums in summer. This seemed to drive changes in species composition, with warm water species (e.g. *Filina pejleri, Hexarta intermedia* and *Synchaeta oblonga*) being more prevalent than in previous trips (Figure 12). At the spatial scale there was a clear separation in zooplankton assemblages between the two geomorphic regions, namely Gorge (LK-1) and Floodplain (LK-6 and LK-6A) regions. Pairwise comparison of zooplankton assemblages by sites showed significant differences between LK-1 and both LK-6 and LK-6A assemblages for every trip, whilst differences between LK-6 and LK-6A assemblages were only detected for trips 1, 5, 9 and 10 (Appendix IV). The differences between LK-6 and LK-6A during these trips were mainly driven by higher abundances of warm water species (e.g. *Synchaeta oblonga, Hexarthra intermedia, Filina pejleri, Brachionus diversicornis* and *Keratella tropica*) in LK-6A samples.

Principal coordinates analysis of the zooplankton assemblage at each site showed a distinct composition for trips 5 and 6 which occurred during the environmental water pulse delivered in December 2012 (Figure 13). SIMPER analysis (similarity analysis) indicated that the species that contributed to more than 50% of the similarity in zooplankton assemblages during these trips were *Bosmina meridionalis, Stenosemella lacustris* and *Difflugia gramen* which are all cosmopolitan species. Differences with the two previous trips (i.e. 3&4) at LK-1 were driven by decreases in *Trichocerca similis* and *T. pusilla* abundances, and an increase in the warm water species *Filina pejleri*. Whilst differences with the two previous trips at both LK-6 and LK-6A were driven mainly by the presence and increase of the warm water species *Hexarthra intermedia* and *Proalides tentaculatus*, respectively; and the decrease in abundance of *T. pusilla*.

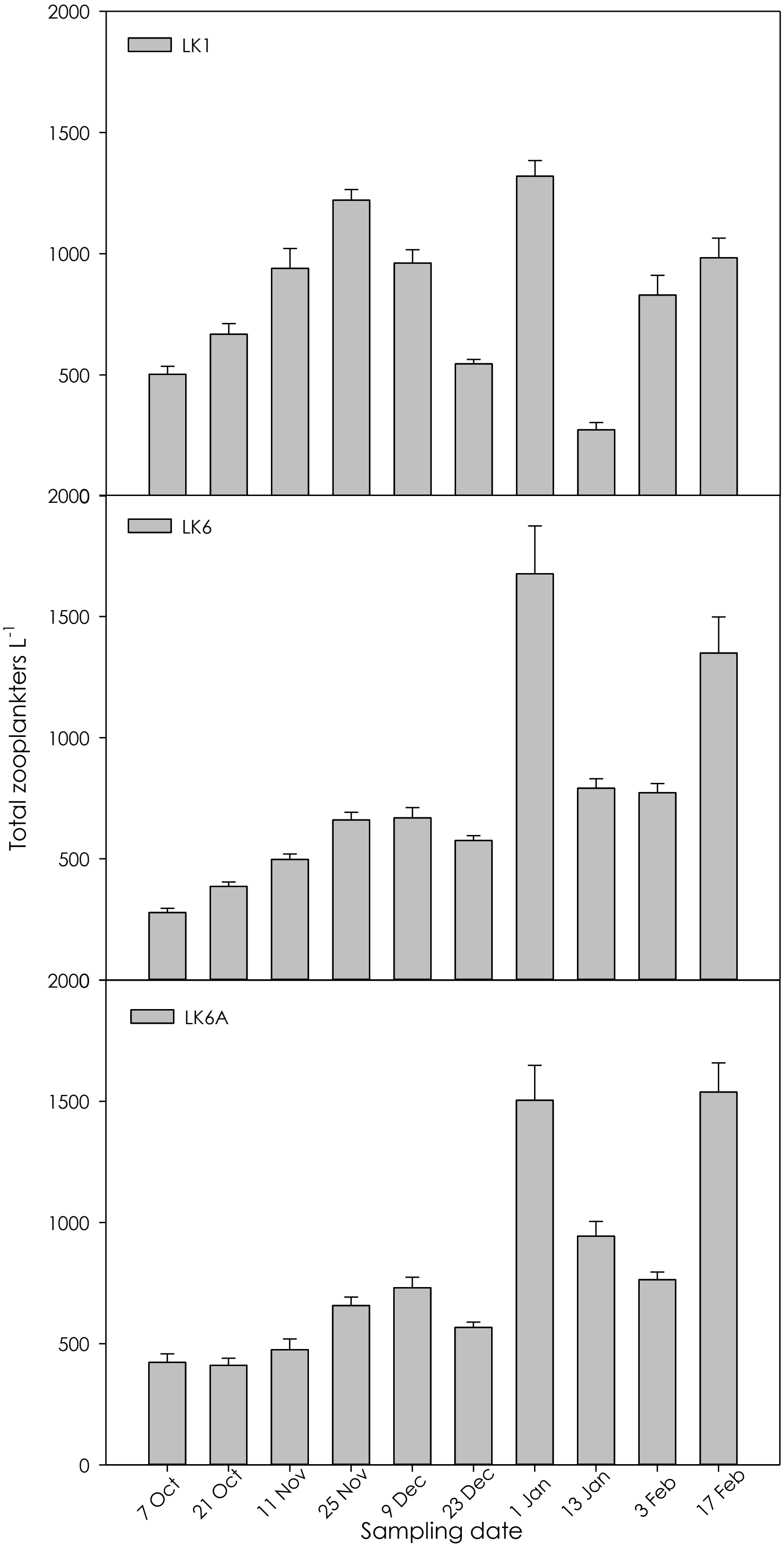


Figure 12. Total abundance of zooplankters (individuals L^{-1}) per trip at each sampling site for 2012-13.

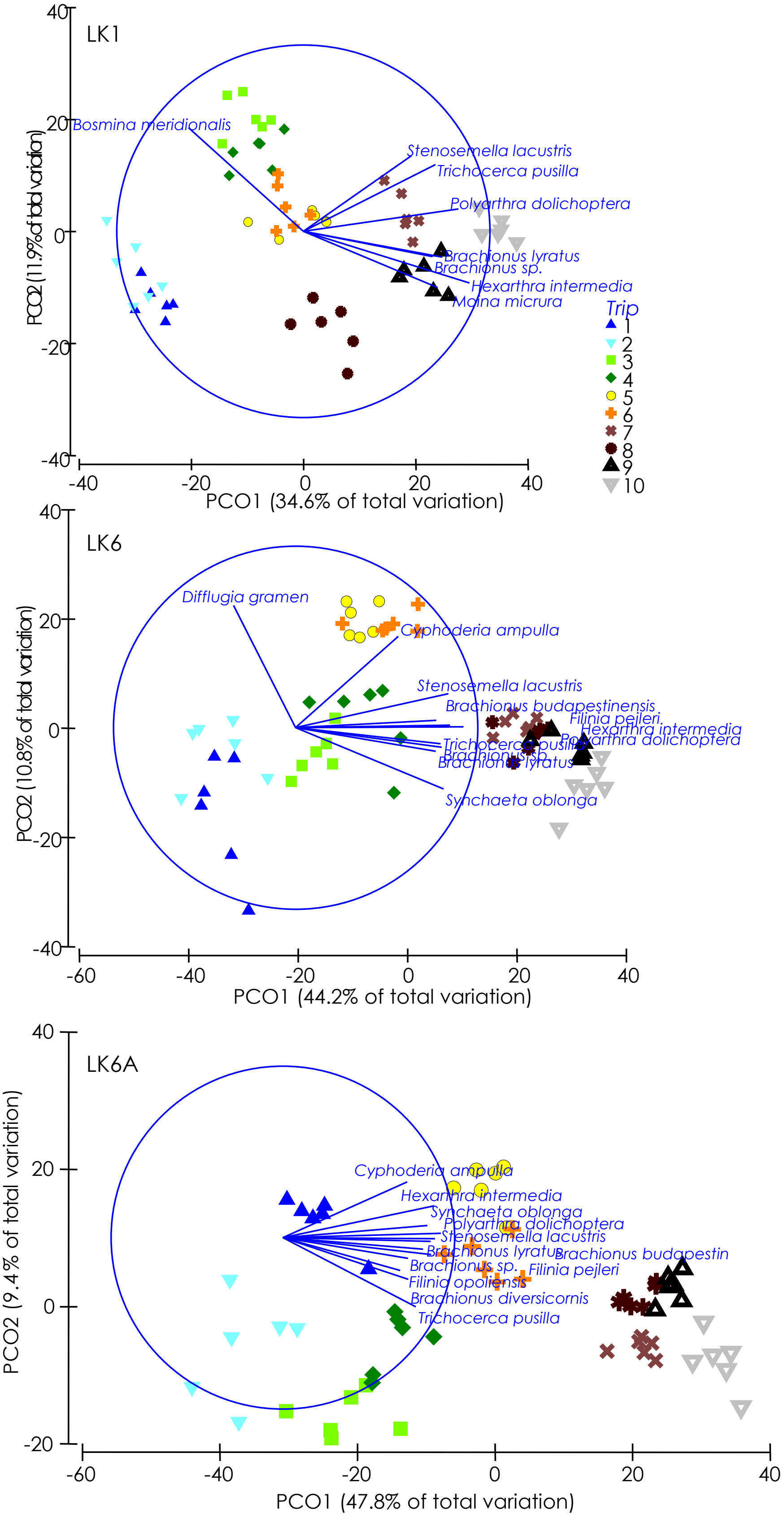


Figure 13. PC ordination of zooplankton assemblages at LK-1, LK-6 and LK-6A.

There is generally little information on the role of fatty acids in the development and growth of wild freshwater fish. Most of the information available comes from rearing experiments in aquaculture which focus on fast growth rates and low mortalities. However, most of the literature seems to agree that fatty acids play an important role in the development of the nervous system and that a lack of essential fatty acids leads to poor survival rates of early life stages (Coutteau *et al*. 1997; Tocher *et al*. 2008; Tocher 2010). Analysis of detected fatty acids extracted from the zooplankton assemblage from trips 2-10 showed a distinct temporal pattern. Principal coordinates analysis grouped samples in four distinct groups and the two axes explained 99.3% of the total variation (Figure 14). The group formed by samples from trips 4-6 were associated with palmitic acid (C16:1), myristic acid (C14:0) and eicosapentaenoic acid (EPA, C20:5). Whilst the group formed by samples from trips 7-9 which were associated with vectors for Linoleic acid (LA, C18:2) and α-Linoleic acid (ALA, C18:3). EPA, LA and ALA are fatty acids known to be important for the development of early life stages and juvenile freshwater fish (for review see Tocher 2010). It is worth noting that the trips (4-9) to which these fatty acids are related to coincide with the rise in abundance of the native fish larvae, particularly bony herring and golden perch (Figure 15). Diet analysis of native fish larvae would provide necessary information to determine what components of the zooplankton assemblage are selected (if any) by each species. Knowledge on the identity of the food source would provide the possibility of a targeted study to identify the nutritional needs of the native species.

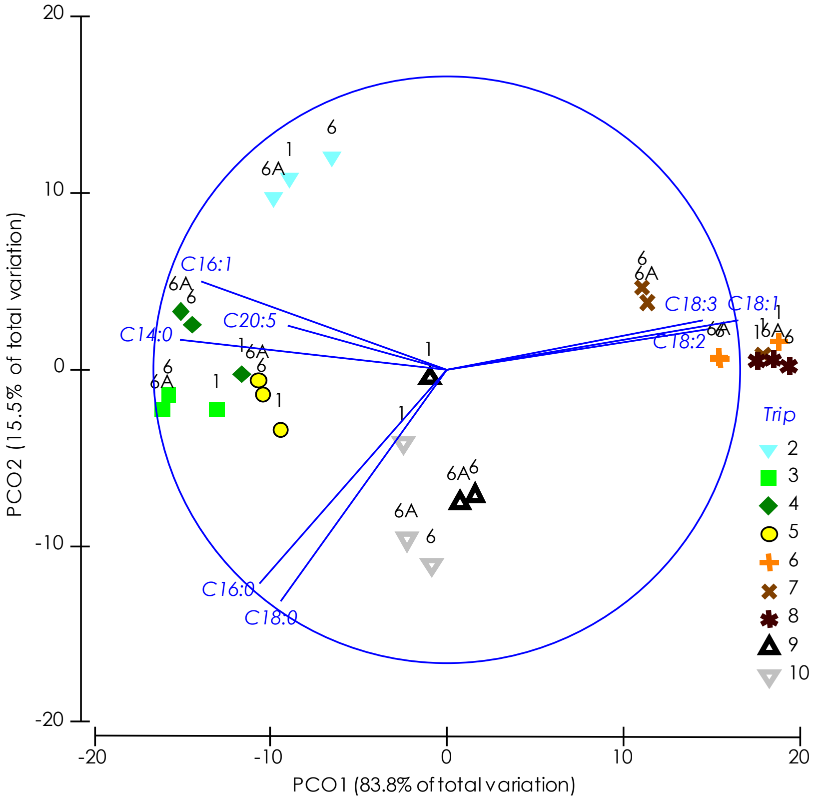


Figure 14. PC ordination of fatty acid relative abundance in zooplankton samples per trip. Black numbers above symbols indicate site.

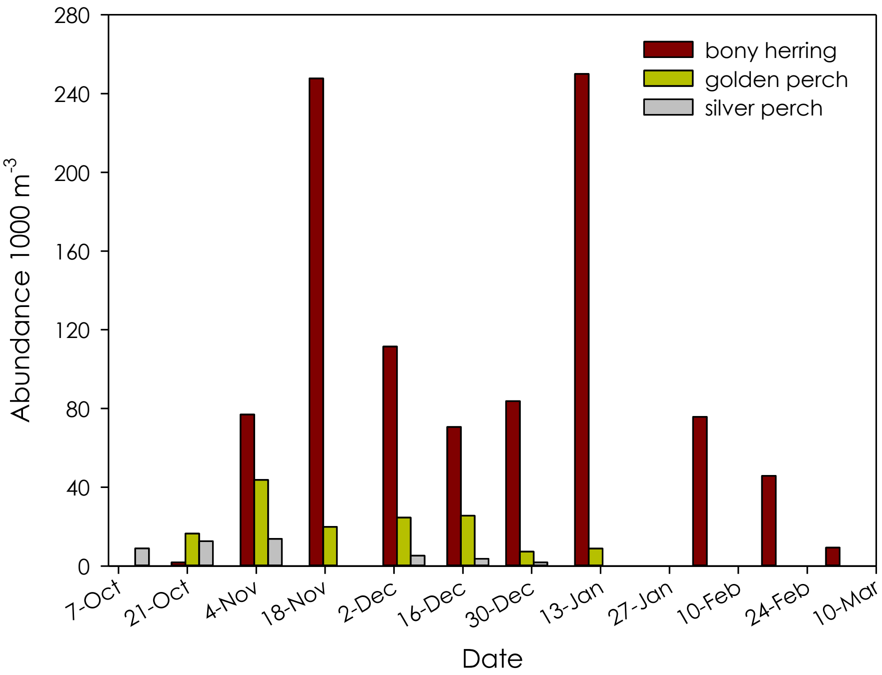


Figure 15. Abundance of larvae per trip (sites pooled) of three native species.

In this project a pilot study to establish diet of larval native fish (i.e. golden perch and Murray cod) through direct identification of gut content was conducted. Eight golden perch and six Murray cod larvae were dissected and gut content analysed through traditional taxonomic methods. Whilst the replication was very low, the results obtained are promising as golden perch collected at different sites and trips seemed to show preference for only a few species of zooplankton (Table 6). It was surprising to find that 7 of 8 golden perch had their stomach and gut filled with *Boeckella triarticulata*, which was a species that was only present in 10% of all zooplankton samples. Furthermore, when present it represented <2% of the total zooplankton abundance in any sample with an average abundance of 5.13 individuals L^{-1} (range: 3.11-9.78 individuals L^{-1}). Murray cod consumed three different species of zooplankton including *B. triarticulata*, however the small samples size does not allow for an interpretation of this data. Further research is needed to establish whether the trends observed are ubiquitous.

Table 6. Summary of gut content analysis of golden perch (n = 8; TL: mean = 9.46 mm, range: 6.6-11.5 mm) and Murray cod larvae (n = 6; TL: mean = 11.17 mm, range: 10.2-12.3 mm).

	Golden perch		**Murray cod**	
	Presence	Size range	Presence	Size range
Copepoda				
Calanoida				
Boeckella triarticulata	7/8	1100-1480 µm	2/6	880-1560 µm
eggs*	7/8		1/6	
Gladioferens sp.	1/8			
Cyclopoida				
Mesocyclops sp.			1/6	
Cladocera				
Bosmina meridionalis	2/8		1/6	

***likely to be *B. triarticulata* eggs, as they occurred concurrently with adult *B. triarticulata* and all adult individuals found in the gut content were bearing eggs.**

Food resources for larval fish

Molecular analyses of the water samples resulted in 1,213 – 1,252 unique sequences or Operational Taxonomic Unit (OTUs) per site from which 99 genera of microeukaryotes were identified, with a small amount of variation between sites (Table 7). Almost 29% of the total data set, had no close match to a reference sequence reflecting the large amount of undescribed and unsequenced diversity present in the river water. Sequences that could not be taxonomically matched were identified by their OTU number and grouped as unknown microeukaryotes.

Table 7. Summary of the DNA results obtained in the three different sites (LK-6, LK-6A, LK-1)

Sites	No. of samples	No. of sequence reads analysed	No. of operational taxonomic units identified	No. of genera identified
LK-6	40	243,229	1,252	89
LK-6A	40	226,943	1,265	94
LK-1	40	235,876	1,213	89

The number of different OTUs in each taxonomic grouping was used as a measure of diversity. At the level of Phyla, diversity was similar across sites, with the highest diversity seen in the Chlorophyta (green algae), Ciliophora (ciliates), Arthropoda (invertebrates), Rotifera (rotifers) and Bacillariophyta (diatoms). Based on the molecular analyses of water samples the diversity at LK-6 and LK-6A were statistically different from LK-1 ($t = 2.8497$ and 2.6307 respectively; P–value = 0.0001), but not from each other ($t = 1.1176$; P–value = 0.2123). Despite general similarities in overall diversity, the microeukaryotic community composition in the water changed sequentially over successive sampling trips producing a time series with significant differences at the 1% level between all trips except 9 and 10 (Figure 16).

Changes in community composition of phytoplankton and zooplankton were assessed from direct counts, as well as from analyses of subsets of the molecular OTUs identified as taxonomically belonging to these groups. Similar serial patterns of change were seen in the zooplankton and phytoplankton data over the sampling period in both the molecular and count data.

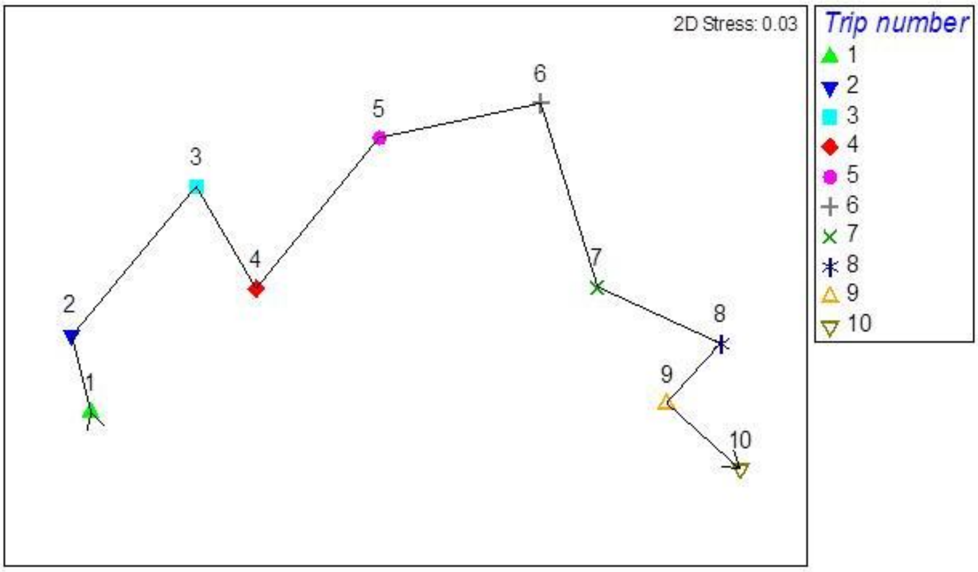


Figure 16. Multivariate ordination of water samples based on presence or absence of molecular operational taxonomic units summed for the three different sites and Bray-Curtis similarities between microbiotic sample data and a trajectory of the sampling trips.

Shifts in community composition were apparent in the zooplankton molecular data and the algae count data associated with the environmental water (Figure 17). The zooplankton community changed between trips 3 and 4, prior to the environmental water, with a further shift associated with its arrival (Figure 17A). Communities associated with the environmental water (trips 5 and 6) were different from those of trips 1-4 and trips 7-10. The phytoplankton counts indicated a major shift in community composition associated with the arrival of the environmental water between trips 4 and 5 (Figure 17B).

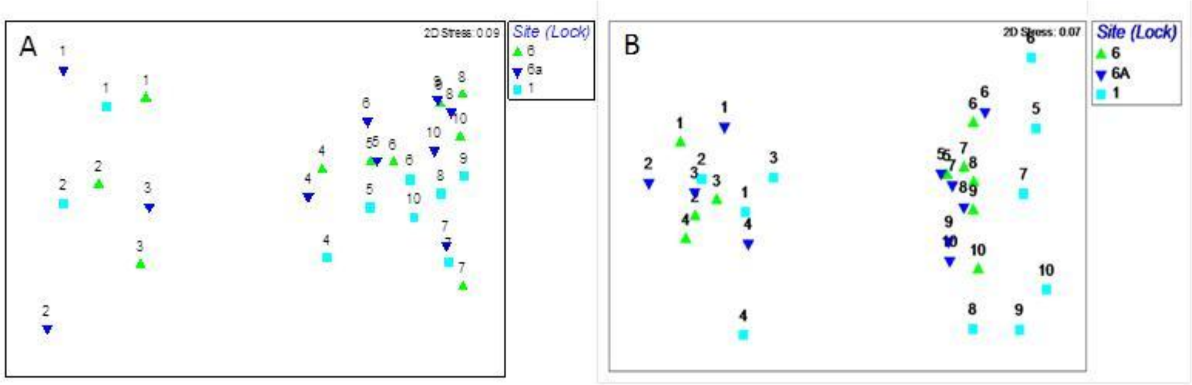


Figure 17. Multivariate ordination of zooplankton and algal samples by nMDS based on Bray-Curtis similarities between A) zooplankton samples (presence or absence of molecular operational taxonomic units), B) algal count samples (square root transformed species counts), Sampling trips 1-10 are marked.

Molecular taxa (OTUs) that could be identified to genus, and the algae and zooplankton count data were assessed against these changing biotic diveristy patterns. Taxa with a Spearman correlation ≥ 0.5 were considered to be associated with changes in community composition. These analyses identified a range of genera important before, during and after the environmental flow period. For example, different genera of green algae were important before (*Pyramimonas, Chlorhormidium, Crucigenia, Actinastrum, Tetracystis, Planctonema*) and after the environmental flow (*Polytoma, Golenkinia, Choricystis, Botryococcus, Parachlorella*). Similarly changes were observed in diatoms and fungi. A cladoceran (*Bosmina*), amoeba *(Difflugia grameni)*, ciliate *(Stenosemella lacustris)* and mollusc (*Elliptio)* were associated with the period before the environmental flows, while rotifers (*Collotheca, Proales*), ciliates (*Cyclidium, Dileptus*), heliozoans (*Sphearastrum, Choanocystis*), a protozoan (*Cyrtoiophosis*), a streptophyte (*Mesostigma*) and a cercozoan (*Thecofilosea*) were associated with trips during and following the environmental flow. Cyanobacteria (*Aphanocaspa, Cuspidothrix, Anabaenopsis*) and a dinoflagellate (*Peridiniopsis*) were also correlated with the post environmental flow trips. The diatoms *Actinocyclus* and *Cyclotella* were associated with the environmental water as were 25 other operational taxonomic units.

Larval fish gut content: Larvae of golden perch, silver perch, freshwater catfish and Murray cod were collected, but only golden perch and silver perch had samples extending through the period of the environmental flow (Table 8). Larvae of freshwater catfish and Murray cod were only collected during or prior to the environmental watering respectively, so that changes in gut content could not be assessed. The freshwater catfish and Murray cod larvae were large at between 10 to 15 mm while silver perch were small, <7 mm. Golden perch larvae were found in two size classes representing pre-flexion (<7 mm) and flexion stages (>10 mm). Numbers of larval fish with detectable gut content varied between species ranging from a total of 48 for golden perch and 6 for Murray cod, but all were in low numbers on each sampling occasion.

Table 8. Larval fish with diet detected by pyrosequencing. Number of OTUs is included as a measure of taxonomic diversity

Species	Common name	No. of larvae	Sites	Trips	No. of OTUs	Genera
Macquaria ambigua	Golden Perch	48	LK 6, 6a, 1	2, 3, 4, 5, 6, 7, 8	285	37
Bidyanus bidyanus	Silver Perch	19	LK 6, 6a, 1	1, 2, 3,5 ,6 ,7	178	25
Tandanus tandanus	Catfish	12	LK 6, 6a, 1	5, 6, 7	158	19
Maccullochella peelii	Murray cod	6	LK 6a, 1	3, 4, 5	69	12

In total 356 unique OTUs were identified from the larval fish gut DNA analyses. The number of OTUs detected for each species was related to the number of larvae analysed suggesting that larger sample numbers are required for a comprehensive analysis of gut content. Of the OTUs detected, 15 were found in multiple specimens of each fish species with considerable overlap between species. Insects (Coleoptera), copepods (Calanoida) and hydroids (Hydrozoa) were common items for multiple fish species. In contrast, sponges (Demospongiae) were found in the gut of pre-flexion golden perch and freshwater catfish, nematodes (Chromandorea) in the flexion stage of golden perch, and fungi (Ascomycota and Chytridiomycota) in all groups except the flexion stage of golden perch. Silver perch was the only species to have multiple beetle taxa in the gut content. Consequently larval fish species showed significant differences in gut content and they could be separated based on the taxa in their gut, including pre-flexion and flexion larvae of golden perch which had separate contents (Figure 18).

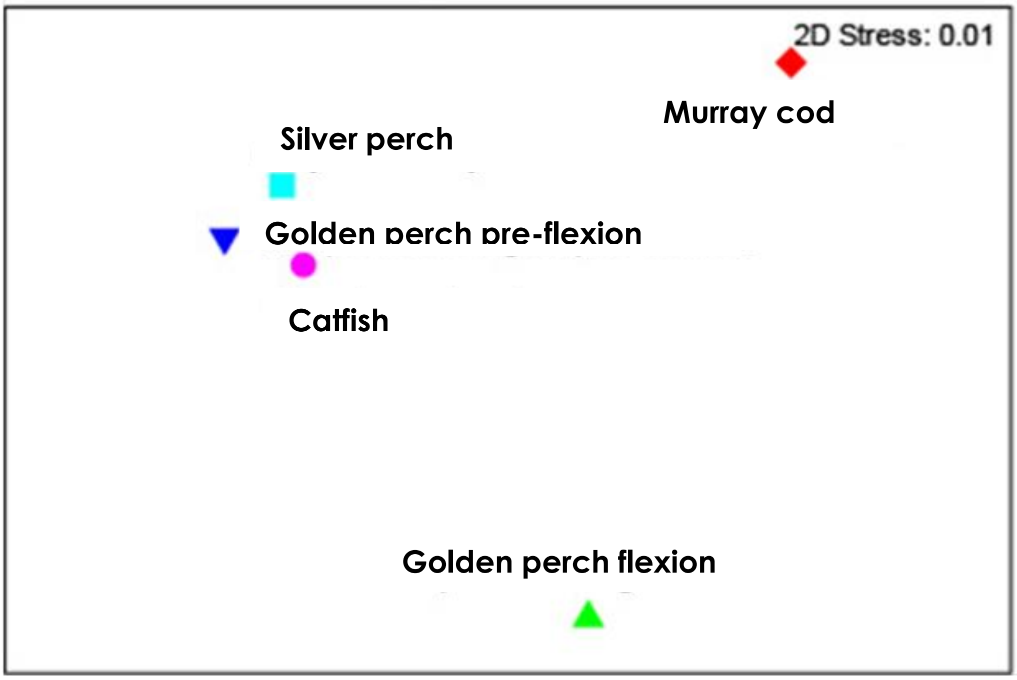


Figure 18. Multivariate ordination by nMDS of larval fish gut content summed for each species and then transformed to presence or absence of OTUs. Golden perch was divided into flexion and pre-flexion groups. Non-metric multidimension scaling ordination was carried out on the resemblance matrix of Bray-Curtis similarities between the larval fish groups.

Only for the pre-flexion larvae of golden perch, were there enough samples with detectable gut content to examine site and trip differences. Tests of differences between trips found that sampling trip 4 could be separated from trip 8, but there were no differences between sites.

Comparisons between community composition and larval fish gut content linked available food resources to larval fish. The cladoceran *Bosmina*, which was found in golden perch, catfish and silver perch, and the mollusc *Elliptio*, which was found in the gut of Murray cod, were both correlated with the trips prior to the environmental flow. OTUs that were correlated with the environmental water (>0.4) and found in the fish gut contents included a number of Copepoda, Fungi and Chlorophyta. The diatoms *Actinocyclus* and *Cyclotella* which were associated with the environmental water were not found in the gut of the larval fish. Taxa that were correlated with the sampling trips during and following the environmental flow were the rotifer *Colotheca*, which was found in the gut of silver perch; the chlorophyte *Choricystis* which was found in golden perch; the diatom *Thalassiosira* which was found in golden perch, catfish and silver perch; and the fungi *Catenophlyctis* and *Malassizia* which were found in all the larval fish species.

Dissolved and particulate material transport

The modelling suggests that in the Lower Murray River environmental watering altered the timing of salinity peaks, but had little effect on salinity levels (Figure 19). However, within the Lower Lakes and Coorong, salinity was reduced as a result of environmental water delivery, particularly Commonwealth environmental watering (Figure 19). Within the Lower Lakes this became most evident towards the end of the study period, with electrical conductivity up to 4,000 µS/cm lower with both Commonwealth environmental water and TLM water in comparison to without any environmental water. Furthermore, at the Murray Mouth electrical conductivity was up to 20,000 µS/cm lower with environmental water delivery at times (equivalent to a reduction of up to approximately 50%). This appeared to be a result of increased inflows of lower salinity into the Northern Coorong and a subsequent reduction in seawater incursions through the Murray Mouth.

Based on the modelling outputs environmental watering increased salt exports from the Lower Murray River and Lower Lakes and resulted in net salt export from the Murray Mouth rather than a net import (Figure 20). For the Lower Murray River exports increased steadily, with a total modelled export of approximately 400,000 tonnes to the Lower Lakes during the study period, of which environmental water use contributed approximately 40%. Environmental watering also increased exports from the Lower Lakes, although to a lesser extent due to the lower salinity concentrations within the Lower Lakes that resulted from environmental flows. The modelling suggests that at the Murray Mouth, the delivery of both sources of environmental water resulted in a net export of approximately 600,000 tonnes of salt over the study period, whilst for all other scenarios there was a net import of approximately 300,000 tonnes.

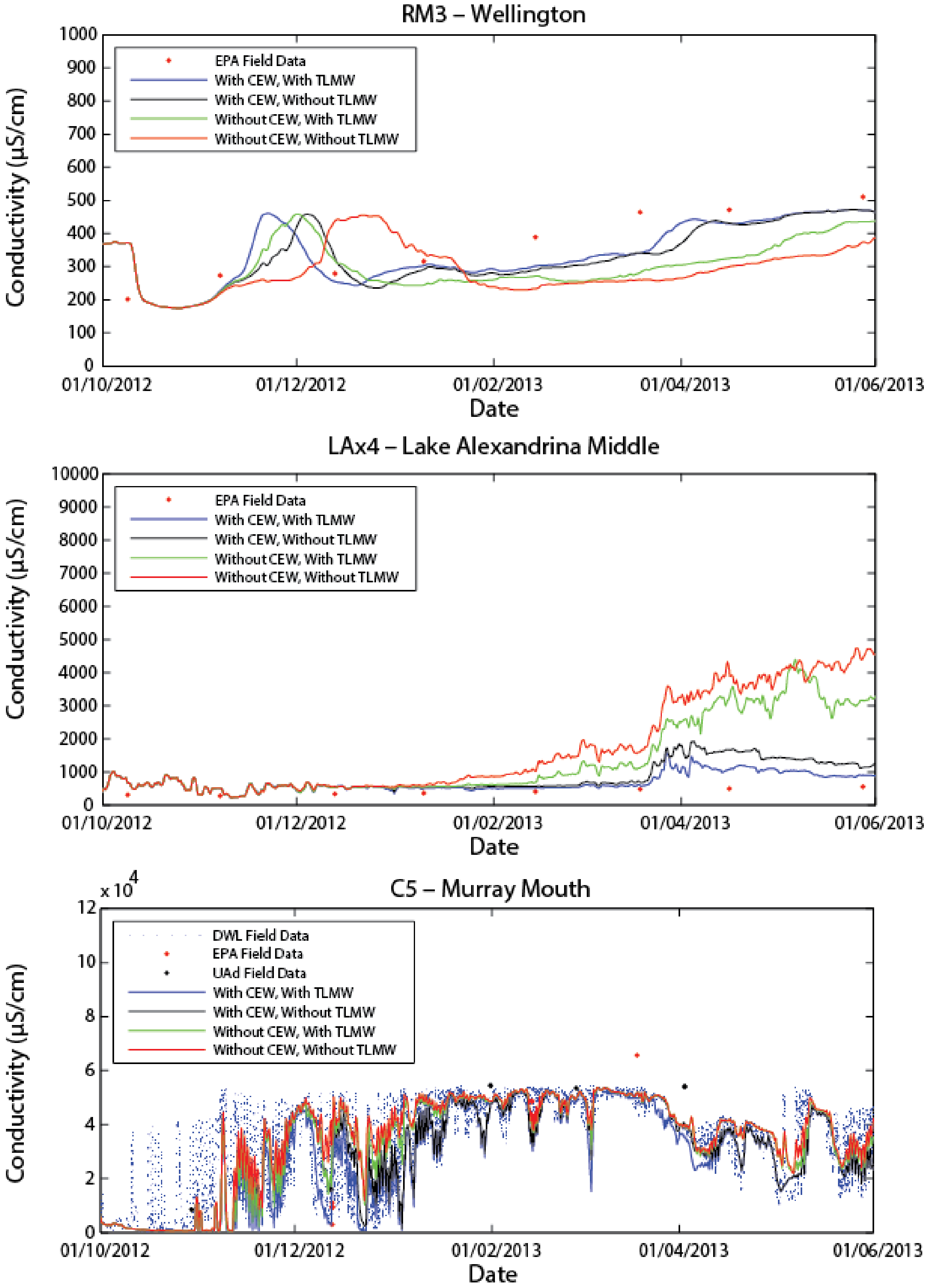


Figure 19. Observed and modelled electrical conductivity at selected sites. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

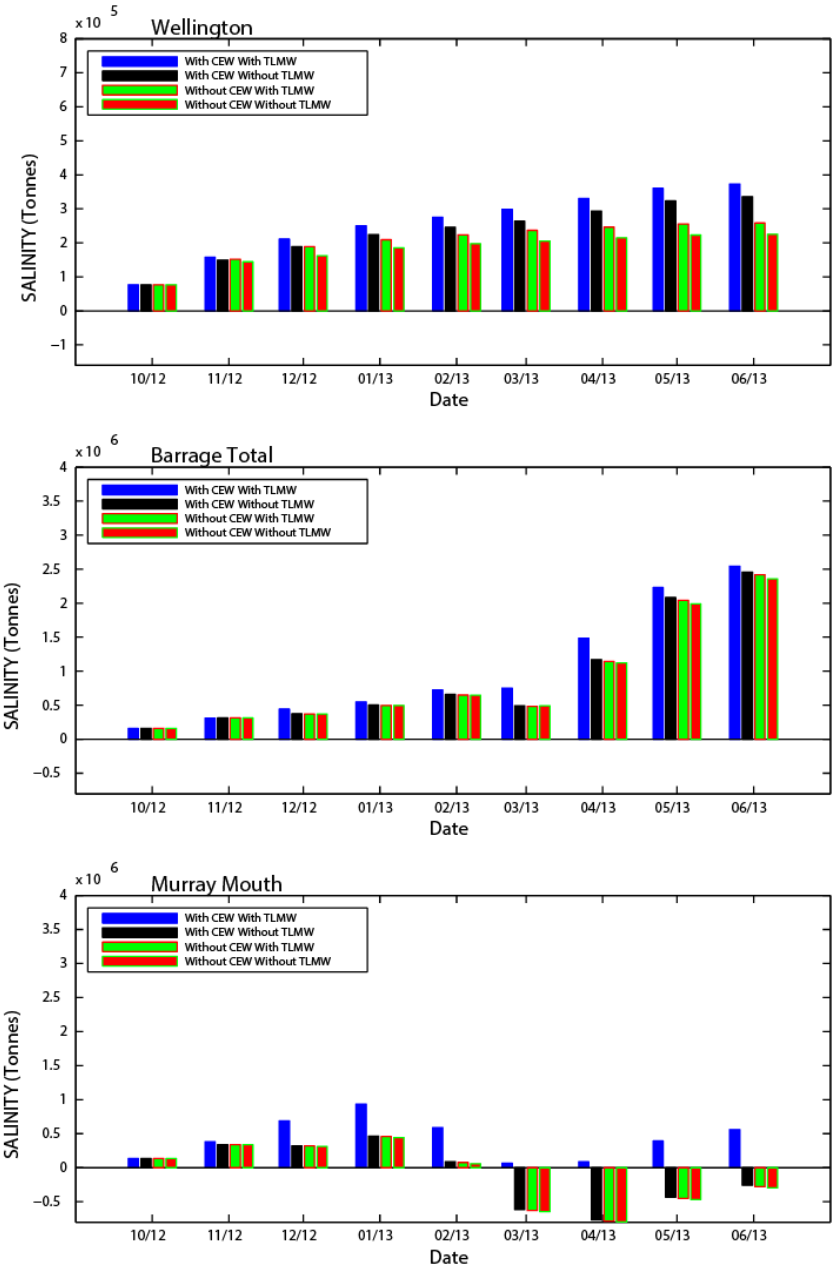


Figure 20. Modelled salt exports with and without environmental flows. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

There were only small differences in the modelled dissolved nutrient concentrations with and without environmental water, particularly given uncertainties associated with the modelled outputs (Figure 21and Figure 22). As a result, differences in modelled exports between the scenarios were largely a result of differences in discharge. There was an increase in the cumulative loads through the study period with greatest export from the Lower Murray River, followed by the Lower Lakes and

Murray Mouth, suggesting a retention or transformation of dissolved nutrients within the system (Figure 23). At all sites, environmental flow delivery increased the export of dissolved nutrients. At the Murray Mouth, exports were only apparent when both Commonwealth environmental water and TLM water were provided.

Within the Lower Murray River particulate organic nitrogen and phosphorus exports were also higher with environmental watering and increased proportionally with discharge (Figure 24). However, in both the Lower Lakes and Murray Mouth, additional exports were only apparent when both environmental water sources were provided. As for dissolved nutrients, the export of particulate nutrients decreased from the Lower Murray River to the Murray Mouth (Figure 24). Over the study period, the environmental watering accounted for approximately 40%, 10% and 20% of exports of particulate nutrients from the Murray River, Lower Lakes and Murray Mouth, respectively.

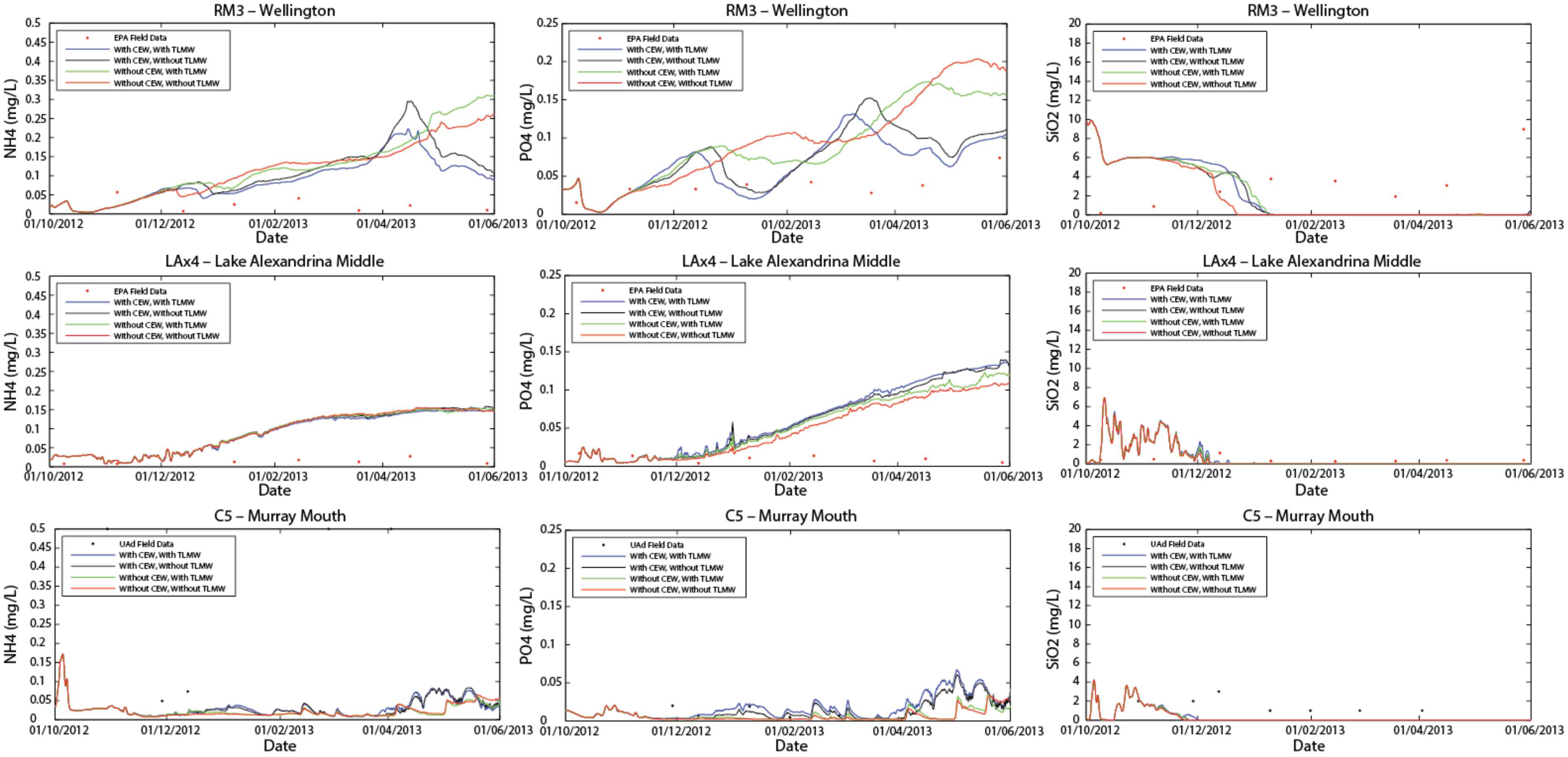


Figure 21. Observed and modelled ammonium (NH4), phosphate (PO4) and silica (SiO2) concentrations at selected sites. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

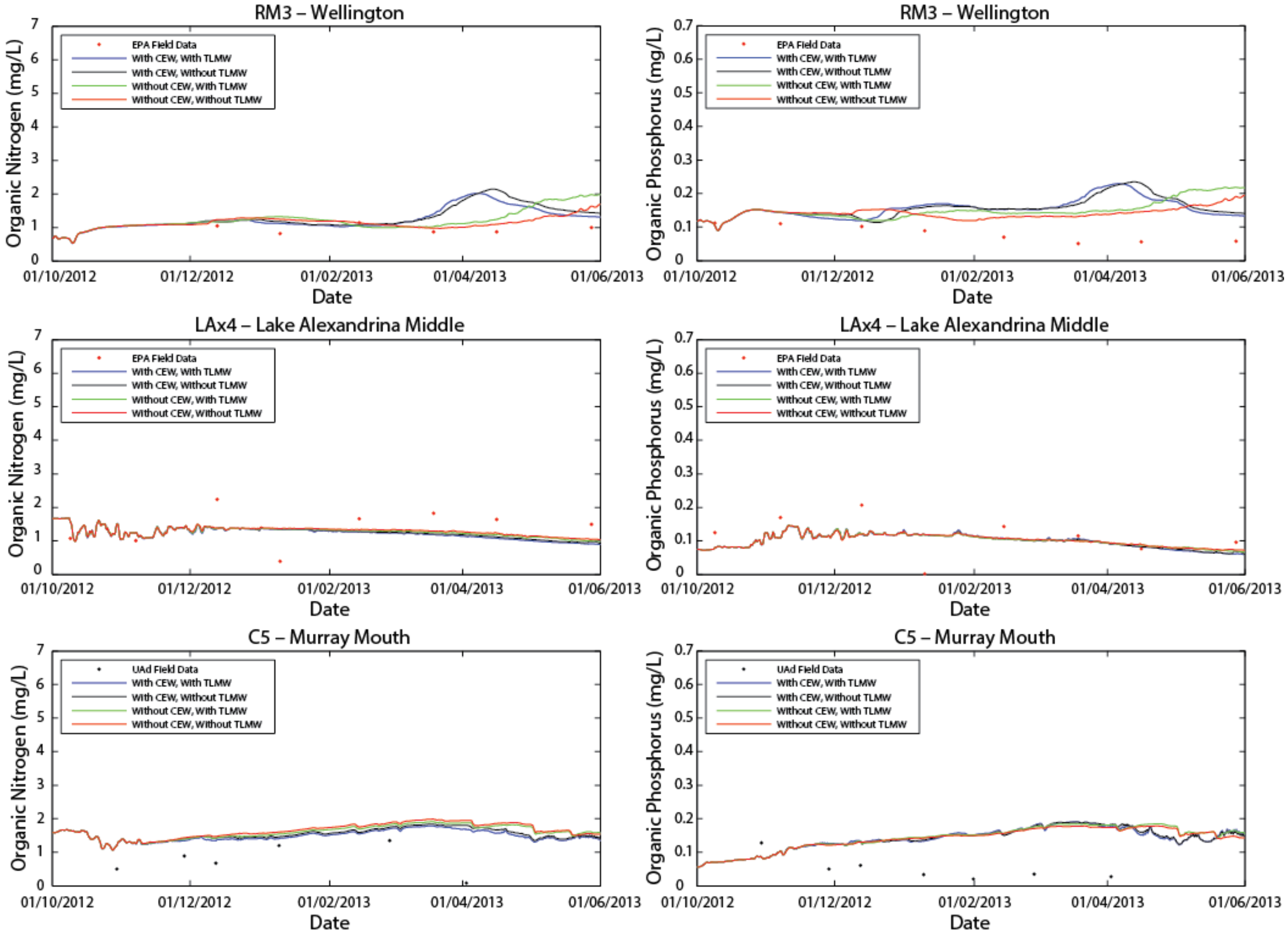


Figure 22. Observed and modelled particulate organic nitrogen and phosphorus concentrations at selected sites. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

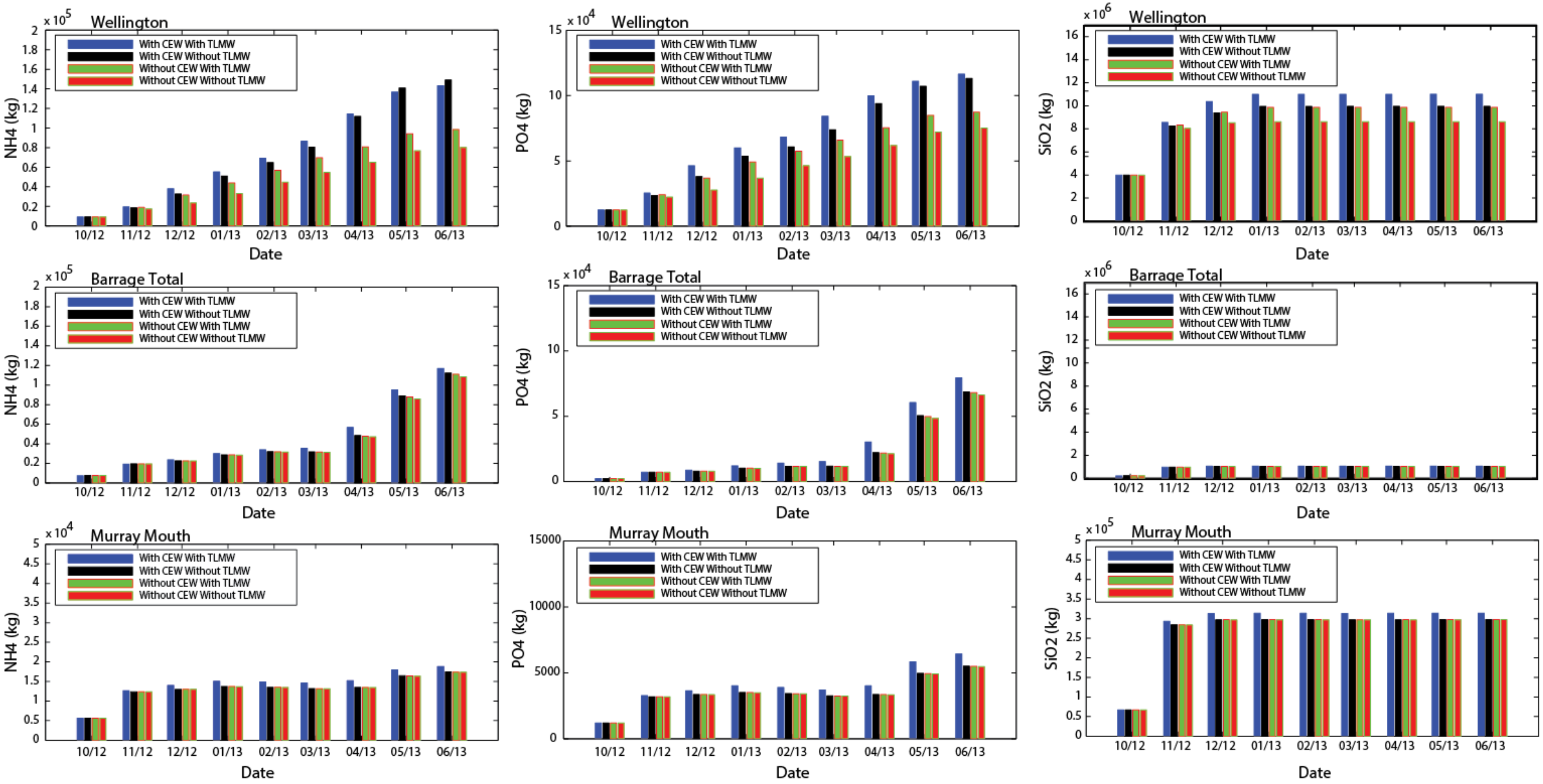


Figure 23. Modelled ammonium (NH4), phosphate (PO4) and silica (SiO2) exports with and without environmental flows. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

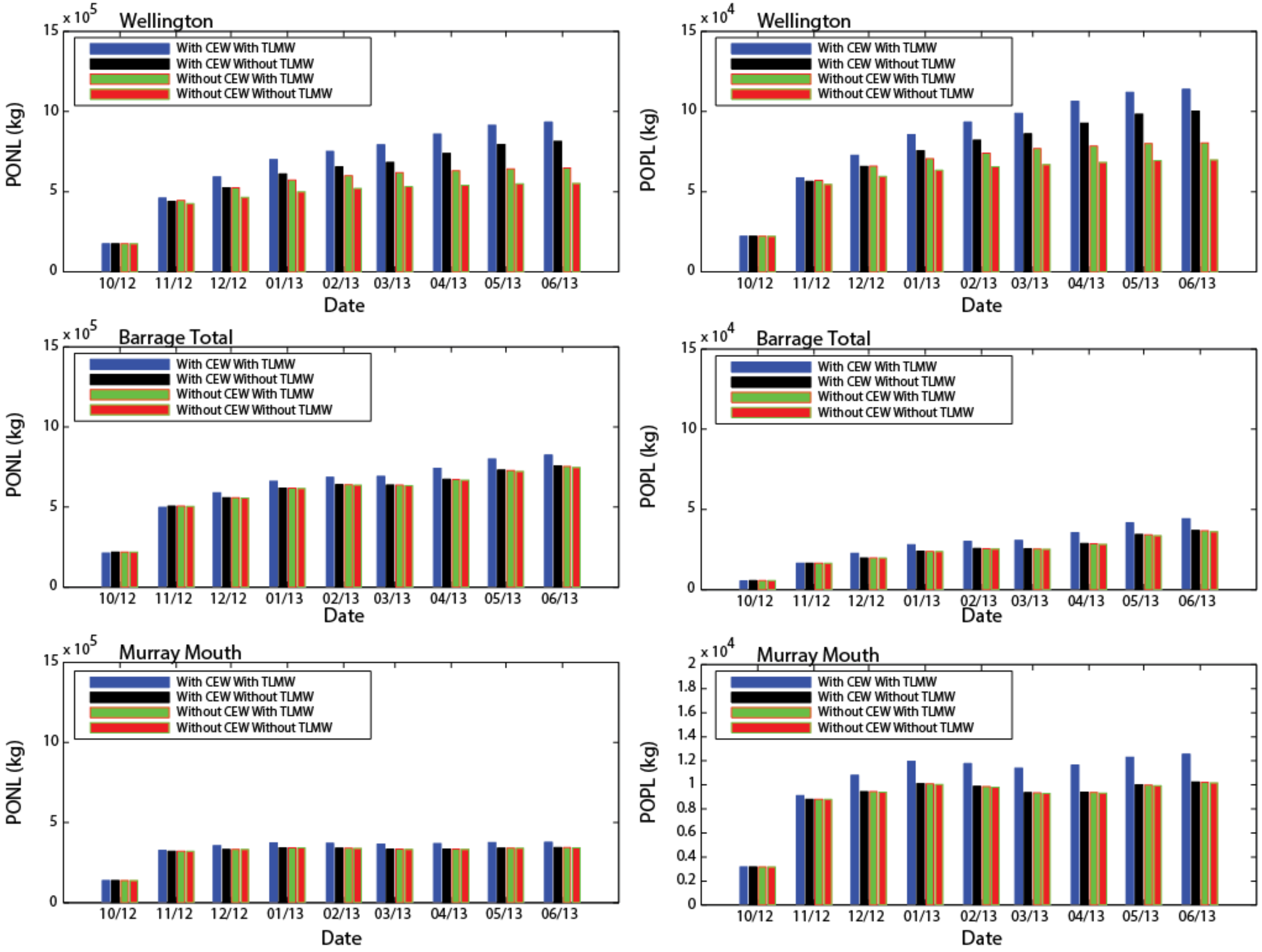


Figure 24. Modelled particulate organic phosphorus (POPL) and nitrogen (PONL) exports with and without environmental flows. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

Given uncertainty in the modelled outputs of chlorophyll *a* concentrations, there was no apparent influence of environmental watering on chlorophyll *a* concentrations (Figure 25). As a result, differences in modelled exports between the scenarios were largely a result of differences in discharge. The modelling suggests there was a steady increase in the exports of chlorophyll *a* from the Murray River and Lower Lakes, with environmental water contributing to approximately 40% of the total exports (Figure 26). Environmental watering also contributed significantly to export of chlorophyll *a* from the Murray Mouth, which appeared to be a result of the combined effect of the two water sources of environmental water. The export from the Murray Mouth was lower than that of the Lower Lakes, suggesting retention of chlorophyll *a* within the system. Exports of total suspended solids followed a similar pattern as chlorophyll *a*, with environmental watering increasing exports (Figure 26). Within the Lower Murray River, the magnitude of exports was proportional to the total discharge, although additional exports from the Lower Lakes and Murray Mouth only occurred when both sources of environmental water were provided.

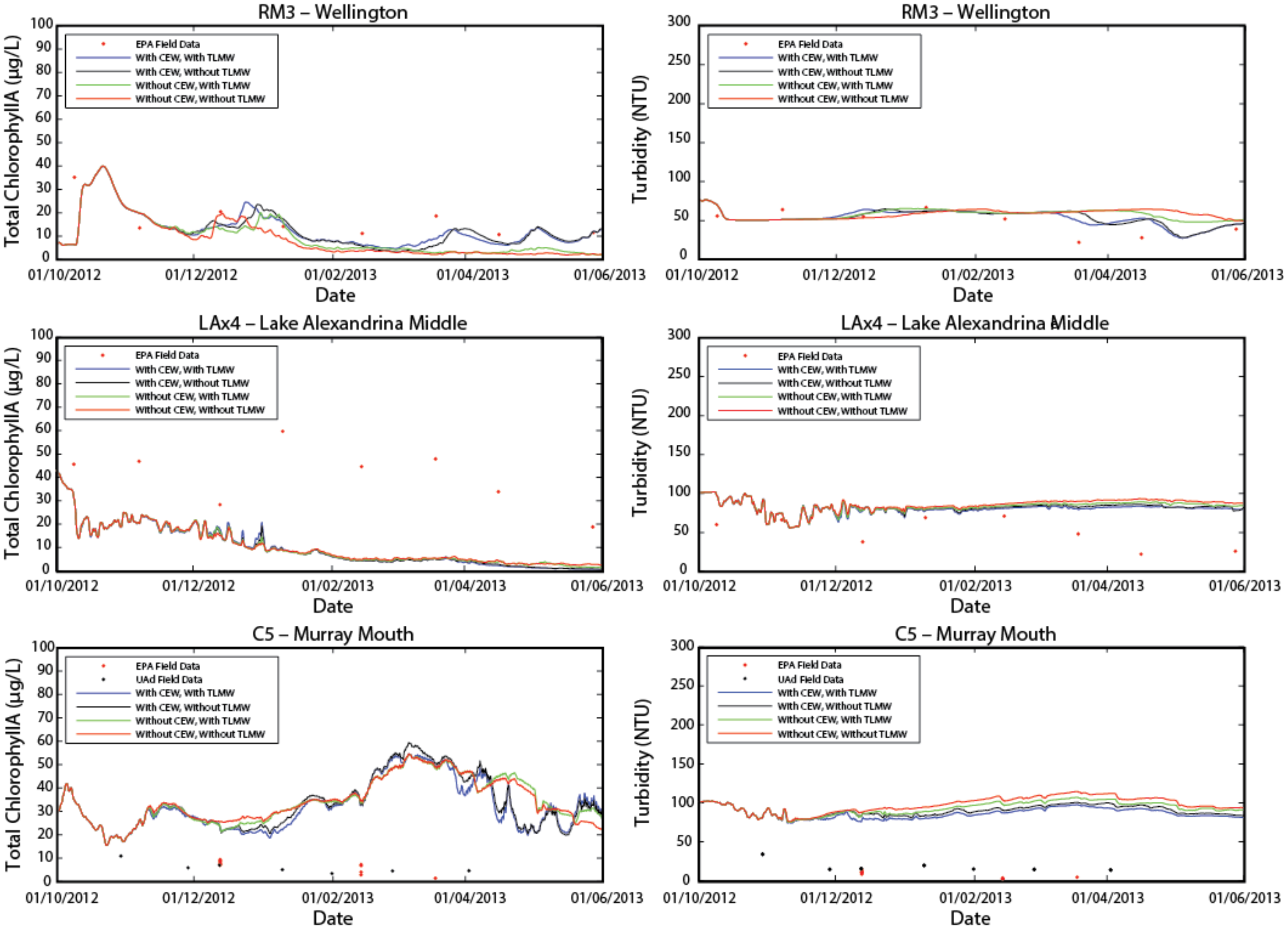


Figure 25. Observed and modelled (with and without environmental watering) chlorophyll *a* concentrations and turbidity with and without environmental flows. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

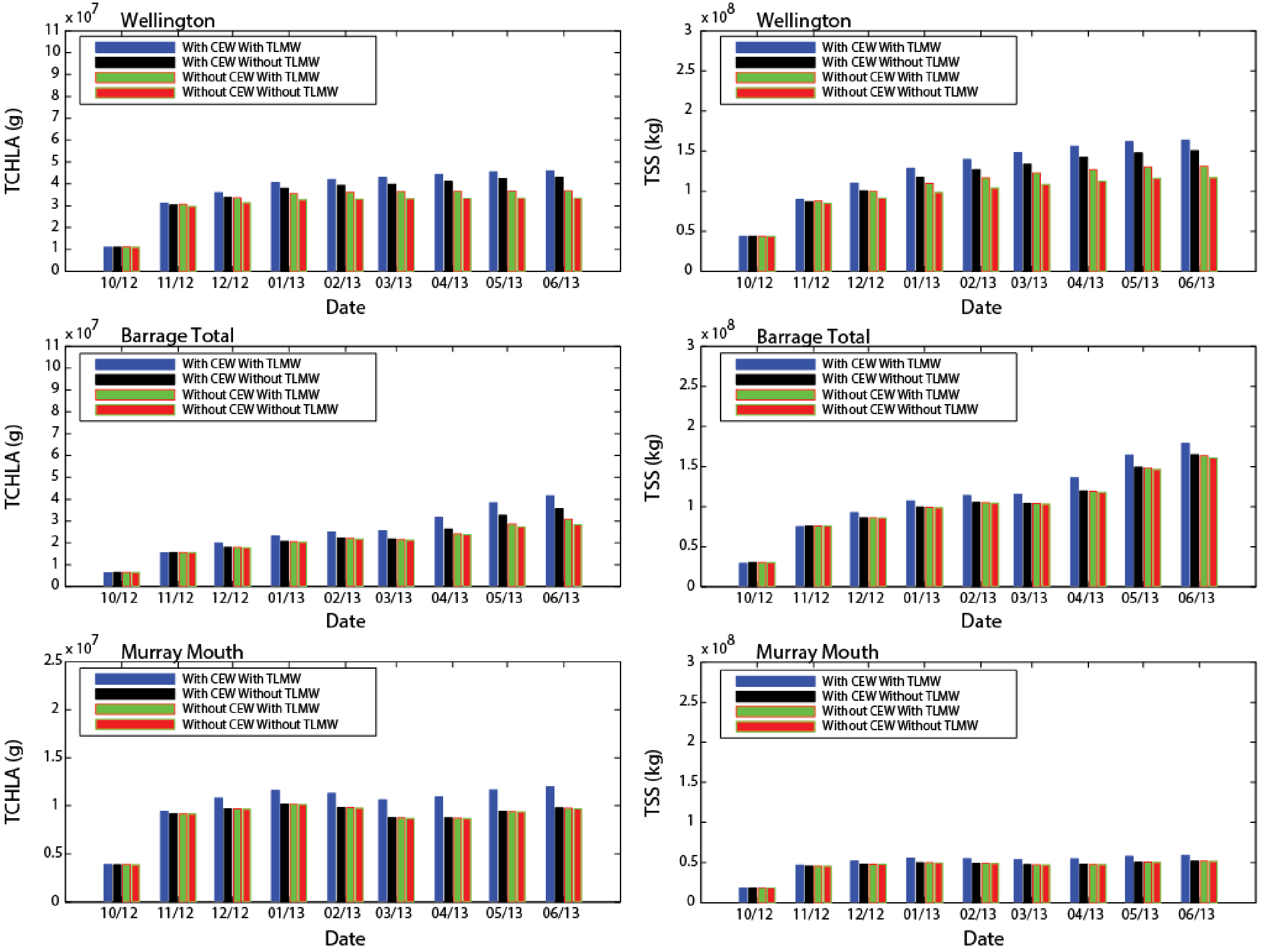


Figure 26. Modelled chlorophyll *a* (TCHLA) and total suspended solid (TSS) exports with and without environmental flows. Scenarios include with and without both commonwealth environmental water (CEW) and The Living Murray water (TLMW).

4.2 Wetlands

Lateral movements of fish

Catch summary, species richness and abundance

Overall, a total of 57,612 fish from 16 species (eleven native and five invasive) were sampled in the river-wetland connection passage across all four wetlands of the Lower Murray River in 2012-13. The foraging generalist carp gudgeon was the most abundant species and contributed to 36% of the total catch. Invasive common carp and Eastern gambusia were the second and third most abundant species sampled, respectively, and each accounted for ~16% of the total catch. Several other foraging generalist species including flat-headed gudgeon (14%), bony herring (10%) and unspecked hardyhead (5%) were also sampled in relatively high numbers. The remaining ten species, which included flow dependent specialists golden perch and silver perch, foraging generalists Murray rainbowfish and Australian smelt and the diadromous congolli, collectively accounted for only 3.6% of the total number of fish sampled.

Lateral movements of fish

Patterns of fish movement between the main river channel and wetlands in the Lower Murray River were complex and highly variable among flow phases at each wetland, and such differences were not consistent among wetlands. As such, fish movement patterns for each wetland were analysed and presented separately.

Overland Corner (Floodplain region)

For Overland Corner, a total of 12,152 fish from 14 species (nine native and five invasive) were observed moving between the wetland and main river channel (Table 9). During the unregulated High flow phase, abundances were low with only 50 and 103 fish sampled moving in and out of the wetland, respectively. During Receding flows, abundances increased substantially with a total of 4,923 fish sampled, of which 70% were invasive juvenile common carp and 20% were native foraging generalists including carp gudgeons (Table 9). During the delivery of the EW flow pulse, assemblages were dominated by native foraging generalists and numbers of flow dependent specialist golden perch were slightly higher.

Principal component analysis identified two broad groupings of fish assemblages by flow phase. One grouping consisted of assemblages sampled during the High flow phase and the other group comprised assemblages sampled during all other flow phases (Figure 27). However, groupings of assemblages according to the direction they were moving during each flow phase were less distinct. The total variation in fish assemblages was well captured by PCO1 and PCO2 (70.6%).

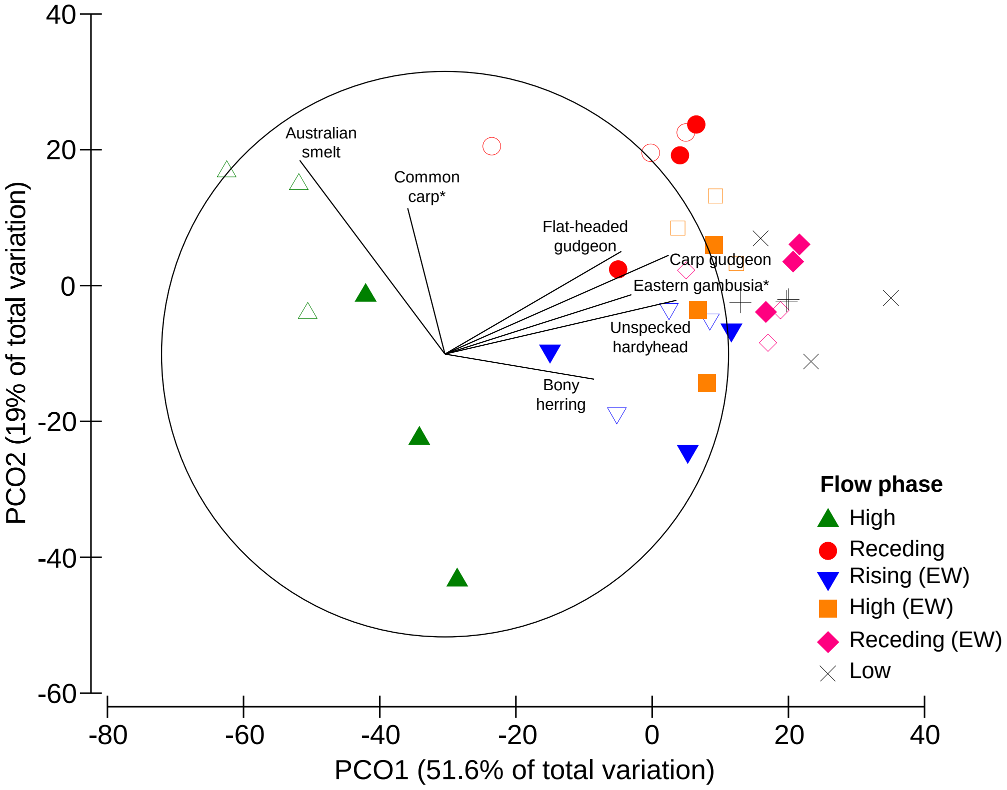


Figure 27. Principal component analysis of fish assemblages moving 'IN' (open symbols) and 'OUT' (closed symbols) of the wetland at Overland Corner during six flow phases in the Lower Murray River in 2012-13 (Low flow phase: X = IN; + = out). EW: environmental water event. (*): invasive species.

Table 9. Species and total number (standardised – 24 hour soak time) of fish captured using directional fyke nets and drum nets moving in and out of the wetland at Overland Corner over a 3-day period during each of the six phases of flow delivery. Functional groups: FD = flow dependent specialists, FG = foraging generalists, D = diadromous species, I = Invasive species (Abbreviation: *Craterocephalus stercus*. = *Craterocephalus stercusmuscarum fulvus*). EW: environmental water event.

Species		**Functional group**	**High**		**Receding**		**Rising EW**		**High EW**		**Receding EW**		**Low**	
Common name	**Scientific name**		**in**	**out**	**in**	**out**	**in**	**out**	**in**	**out**	**in**	**out**	**in**	**out**
Golden perch	*Macquaria ambigua*	FD	4	1	2		2	3	4	8	1	5		3
Silver perch	*Bidyanus bidyanus*	FD			1									
Unspecked hardyhead	*Craterocephalus stercus.*	FG			37	39	9	94	21	103	48	73	24	113
Carp gudgeon	*Hypseleotris* spp.	FG	4	2	770	237	72	270	188	453	1695	353	340	443
Murray rainbowfish	*Melanotaenia fluviatilis*	FG				1					3	5	2	13
Bony herring	*Nematalosa erebi*	FG	11		32	3	45	25	291	894	12	6	101	102
Flat-headed gudgeon	*Philypnodon grandiceps*	FG			41	25	7	23	101	372	61	45	9	15
Dwf flat-headed gudgeon	*Philynodon macrostomus*	FG				1			2	3	3	21		
Australian smelt	*Retropinna semoni*	FG	11	85	107	113	4	3	6	37		1	1	2
Common carp	*Cyprinus carpio*	I	18	15	1514	1949	70	45	137	75	56	12		6
Goldfish	*Carrasius auratus*	I	2		5		2	4	2	1				
Eastern gambusia	*Gambusia holbrooki*	I			5	16	4	4	10	9	52	21	3	14
Redfin perch	*Perca fluviatilis*	I			20	3		4	1	1				
Oriental weatherloach	*Misgurnus anguillicaudatus*	I										1		
	Total		50	103	2536	2387	214	475	764	1956	1931	543	481	711
	No. of species		6	4	11	10	9	10	12	11	9	11	7	9
	No. of native species		4	2	7	7	6	6	9	7	7	8	6	7

The native foraging generalist Australian smelt characterised fish assemblages during the high flow phase; whereas the invasive common carp were strongly correlated to assemblages sampled during the Receding flow phase (Figure 27; Table 9). The grouping of assemblages sampled during the delivery of environmental water and Low flow phase were characterised mostly by several foraging generalists (flat-headed gudgeon, carp gudgeon, unspecked hardyhead), the small-bodied invasive Eastern gambusia, and to a lesser extent the foraging generalist bony herring.

PERMANOVA was used to statistically compare the structure of assemblages moving in and out of the wetland and to determine if these directional movement patterns were consistent among flow phases. The interaction between flow phase and direction was not significant; however the effects of flow phase and direction on assemblage structure were significant (Appendix V). This suggests that the effects of flow phase on assemblage structure were the same for both directions, and effects of direction on assemblage structure were the same for all six flow phases.

For assemblages moving into the wetland, pairwise comparisons revealed significant difference in assemblage structure between the unregulated High flow phase and the Rising (EW) and Low flow phases. There were also significant differences between the Receding and Rising (EW) flow phases. For assemblages moving out of the wetland, pairwise tests revealed significant differences between the unregulated High flow phase and all other flow phases, and between the Receding (EW) and Low flow phases (see Earl and Ye (In Prep) for test results and for details on which species contributed most to the dissimilarity between flow phases).

Two of the five key species (common carp and bony herring) showed differences in size distributions relative to the direction of movement (Appendix VI). For both bony herring and common carp, individuals attempting to enter the wetland were significantly larger than those leaving the wetland. However, size distributions for each species comprised almost entirely small juveniles. No differences in size distribution related to the direction of movement were detected for golden perch, carp gudgeon or flat-headed gudgeon.

Kroehns (Gorge region)

For Kroehns, a total of 22,563 fish from 14 species (ten native; four invasive) were observed moving between the wetland and main river (Table 10). Prior to the delivery of the EW flow pulse, abundances were low and assemblages comprised mostly foraging generalists including carp gudgeon, unspecked hardyhead and Australian smelt. During the environmental flow pulse, abundances of the flow dependent specialist golden perch were relatively high; the foraging generalists flat-headed gudgeon and carp gudgeon were highly abundant; and the diadromous congolli were present in low numbers. Abundance of the invasive common carp remained relatively low throughout the six phases of flow. Invasive Eastern gambusia was most abundant during the Receding (EW) and Low flow phases (Table 10).

Principal component analysis identified four broad groupings of assemblages across the six phases of flow (Figure 28). Greatest separation occurred between High flows and all other flow phases. Receding flows were also relatively distinct from all other flow phases. Furthermore, Rising (EW) and High (EW) were relatively separate from Receding (EW) and Low flows. Groupings of assemblages according to the direction they were moving during each flow phase showed considerable overlap. The total variation in fish assemblages was well captured by PCO1 and PCO2 (74.7%).

The foraging generalist Australian smelt strongly characterised the assemblages sampled during the High flow phase (Figure 28). The foraging generalist flat-headed gudgeon was the best indicator of assemblages sampled during the Rising (EW) and High (EW) flow phases; whereas several foraging generalists' including carp gudgeon, dwarf flat-headed gudgeon, unspecked hardyhead and Murray rainbowfish were strongly correlated to assemblages sampled during the Receding (EW) and Low flows.

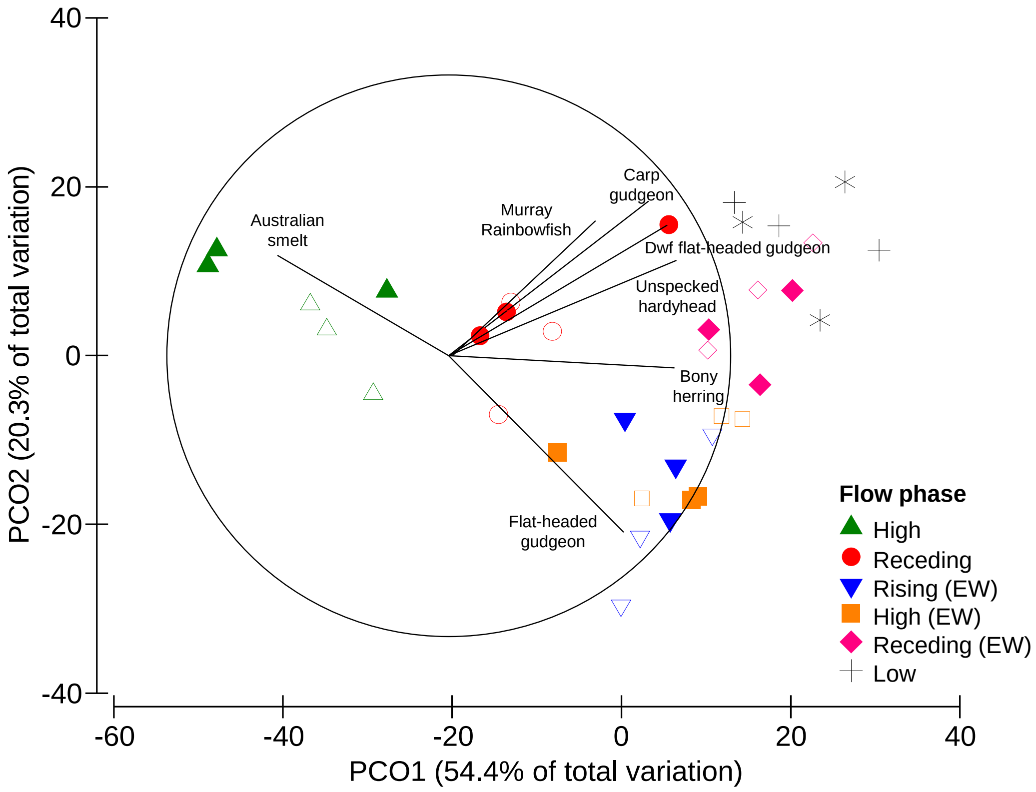


Figure 28. Principal component analysis of fish assemblages moving 'IN' (open symbols) and 'OUT' (closed symbols) of the wetland at Kroehns during six flow phases in the Lower Murray River in 2012-13. EW: environmental water event.

PERMANOVA was used to statistically compare the structure of assemblages moving in and out of the wetland and to determine if these directional movement patterns were consistent among flow phases. The interaction between flow phase and direction was not significant, nor was the effects of direction. However, there was a significant difference in assemblage structure between flow phases (Appendix V). As such, assemblages moving 'In' and 'Out' were pooled, as they were not significantly different, to assess how assemblages changed between flow phases.

Pairwise comparisons revealed significant differences in assemblage structure between all combinations of flow phases, except for between Rising (EW) and High (EW) (see Earl and Ye (In Prep) for test results and for details on which species contributed most to the dissimilarity between flow phases).

Table 10. Species and total number (standardised – 24 hour soak time) of fish captured using directional fyke nets and drum nets moving in and out of the wetland at Kroehns over a 3-day period during each of the six phases of flow delivery. Functional groups: FD = flow dependent specialists, FG = foraging generalists, D = diadromous species, I = Invasive species (Abbreviation: *Craterocephalus stercus* = *Craterocephalus stercusmuscarum fulvus*). EW = environmental water pulse

Species **Common name**	**Scientific name**	**Functional group**	**High**		**Receding**		**Rising EW**		**High EW**		**Receding EW**		**Low**	
			in	**out**	**in**	**out**	**in**	**out**	**in**	**out**	**in**	**out**	**in**	**out**
Golden perch	*Macquaria ambigua*	FD	4	9	2	9	35	5	7	2	17	24	2	
Silver perch	*Bidyanus bidyanus*	FD							1	1				
Unspecked hardyhead	*Craterocephalus stercus.*	FG	1		152	47	77	19	26	67	133	163	877	91
Carp gudgeon	*Hypseleotris spp.*	FG	20	38	360	195	219	195	134	393	1218	1302	2670	1965
Murray rainbowfish	*Melanotaenia fluviatilis*	FG			4				1			2	8	2
Bony herring	*Nematalosa erebi*	FG	1	2	1	13	45	43	36	71	171	182	287	56
Flat-headed gudgeon	*Philypnodon grandiceps*	FG		2	17	12	760	1320	796	2914	184	389	80	79
Dwf flat-headed gudgeon	*Philynodon macrostomus*	FG	2	2	5	7	1	1	3	3	107	124	7	18
Australian smelt	*Retropinna semoni*	FG	68	57	83	274	3	2		10	2	11	4	1
Congolli	*Pseudaphritis urvillii*	D							1	2	1	1		1
Common carp	*Cyprinus carpio*	I	94	158	91	95	217	67	73	154	272	290	47	29
Goldfish	*Carrasius auratus*	I	2	1	3	8			1			1	2	3
Eastern gambusia	*Gambusia holbrooki*	I			4		1	1		5	721	360	423	541
Redfin perch	*Perca fluviatilis*	I			65	8	15	7	3	15	10	12	1	
		Total	192	269	787	668	1374	1661	1082	3638	2835	2863	4409	2786
		No. of species	8	8	12	10	10	10	12	12	11	13	12	11
		No. of native species	6	6	8	7	7	7	9	9	8	9	8	8

As was the case for Overland Corner, of the key species detected moving into and out of Kroehns wetland, bony herring and common carp were the only two species which showed differences in size distributions relative to the direction of movement (Appendix VI). However, in contrast to Overland Corner, individuals of both species leaving the wetland were on average significantly larger than those attempting to enter the wetland. No differences in size distribution related to the direction of movement were detected for golden perch, carp gudgeon, unspecked hardyhead, Australian smelt or flat-headed gudgeon.

Linking environmental variables to lateral fish movement

Overland Corner (Floodplain region)

Flow discharge was the best predictor of fish assemblage structure moving between the river and wetland and explained 44.9% of the variation throughout the six flow phases (Appendix VII). Conductivity, pH, dissolved oxygen and water temperature were also identified as significant predictor variables and explained an additional 14.9%. As such, 59.9% of the variation in fish assemblage structure was explained by these five environmental predictor variables (Appendix VII). The horizontal distribution of samples from the High flow phase and all other flow phases was well explained by the strong negative correlation with flow discharge and the positive correlation with dissolved oxygen and to a lesser extent conductivity and temperature (Figure 29). The vertical distribution of samples from all flow phases was primarily driven by a relatively weak positive correlation with pH and an even weaker negative correlation with conductivity and temperature (Figure 29).

Kroehns (Gorge region)

For Kroehns wetland, relative water level was the best predictor of fish assemblage structure, which explained 48% of the variation throughout the six flow phases. Conductivity, pH, dissolved oxygen and temperature were also identified as significant factors, improving the proportion of the variation to 68.8% (Appendix VII). The horizontal distribution of samples from the High flow phase, to the Receding flow phase and again to the all other phases was best explained by the negative correlation with relative water level and the positive correlation with conductivity

(Figure 30). Seasonal temperature shifts were also positively correlated with the horizontal distribution of samples. While the vertical distribution of samples was less, separation of samples from Rising (EW)and High (EW) flow phases from all other flow phases was well explained by the negative correlation with dissolved oxygen and temperature and weak positive correlation with pH (Figure 30).

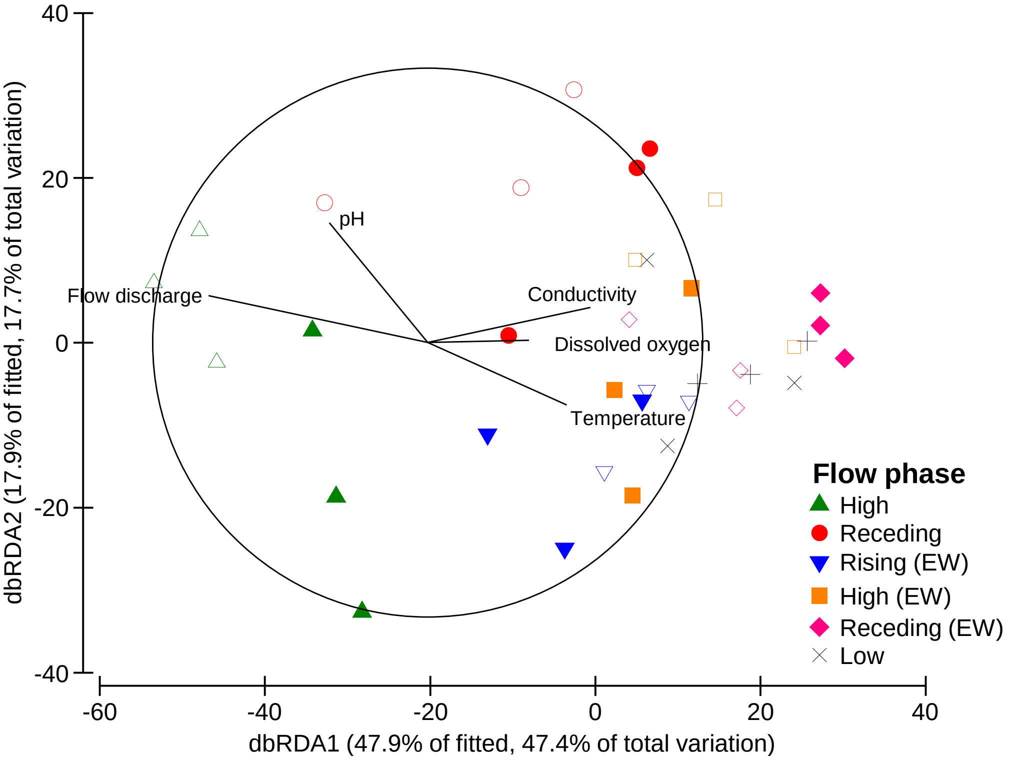


Figure 29. dbRDA ordination of the fitted model of fish assemblages moving 'IN' (open symbols) and 'OUT' (closed symbols) of the wetland at Overland Corner versus the environmental predictor variables during six flow phases in the Lower Murray River in 2012-13 (Low flow phase: X = IN; + = OUT). EW: environmental water event

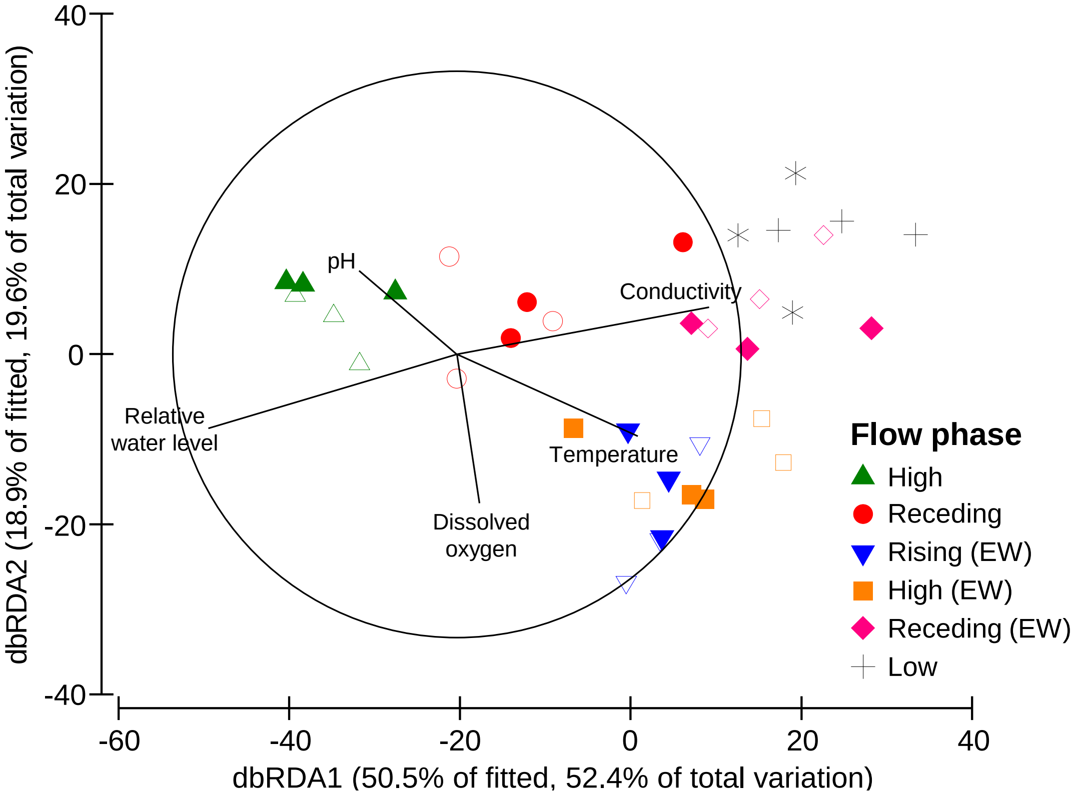


Figure 30. dbRDA ordination of the fitted model of fish assemblages moving 'IN' (open symbols) and 'OUT' (closed symbols) of the wetland at Kroehns versus the environmental predictor variables during six flow phases in the Lower Murray River in 2012-13 (Low flow phase: X = IN; + = OUT). EW: environmental water event.

Temporal variation in spawning and recruitment of common carp

Macroscopic staging of the ovaries of common carp was carried out to examine the reproductive development of individuals moving in and out wetlands. In the Floodplain and Gorge regions, ovaries of most females were classified as either Stage 3 (developed/ripe) or Stage 5 (spent/regressing). No spawning (Stage 4) fish were collected during the study period. The relatively high proportion of Stage 5 fish in both regions suggests that most females had spawned prior to the delivery of environmental water in December.

Microscopic analysis of the ovaries of common carp was done to provide a more accurate indication of when spawning occurred for common carp. No evidence of recent or imminent spawning, i.e. hydrated oocytes or post-ovulatory follicles, was detected in the ovaries of any fish. These results, along with those from macroscopic staging of ovaries, suggest that main spawning period for common carp in 2012 occurred prior to commencement of this study, i.e. before mid-October. Furthermore, these methods provide no evidence to suggest that the delivery of the CEW flow pulse enhanced reproductive development or spawning activity for this species in the Lower Murray River in 2012-13.

Recruitment and abundance of juveniles

Size frequency distributions for juvenile common carp sampled in the Floodplain and Gorge regions of the Lower Murray River in 2012-13 indicated that a major recruitment event occurred during unregulated flows conditions in 2012, i.e. mid to late October (Figure 31). While this dominant cohort of young fish (recruits) was evident in wetlands during all subsequent flow phases in 2012-13, no additional recruitment events were detected.

Spawning and hatch dates

Randomly selected juvenile common carp sampled from wetlands in the Floodplain and Gorge regions were aged to estimate their hatch date and thus provide insight into the timing of successful spawning for the species in 2012-13. In each region, new recruits were derived from spawning that occurred over a 4-month period from late August 2012 (Figure 32). However, approximately 55% and 62% of all recruits sampled in the Floodplain and Gorge regions, respectively, were derived from spawning that occurred during September, i.e. when unregulated high flows were passing through the Lower Murray River. Only a small proportion of recruits (<5%) were derived from spawning events whose timing coincided with the commencement of the delivery of the CEW flow pulse, i.e. early December.

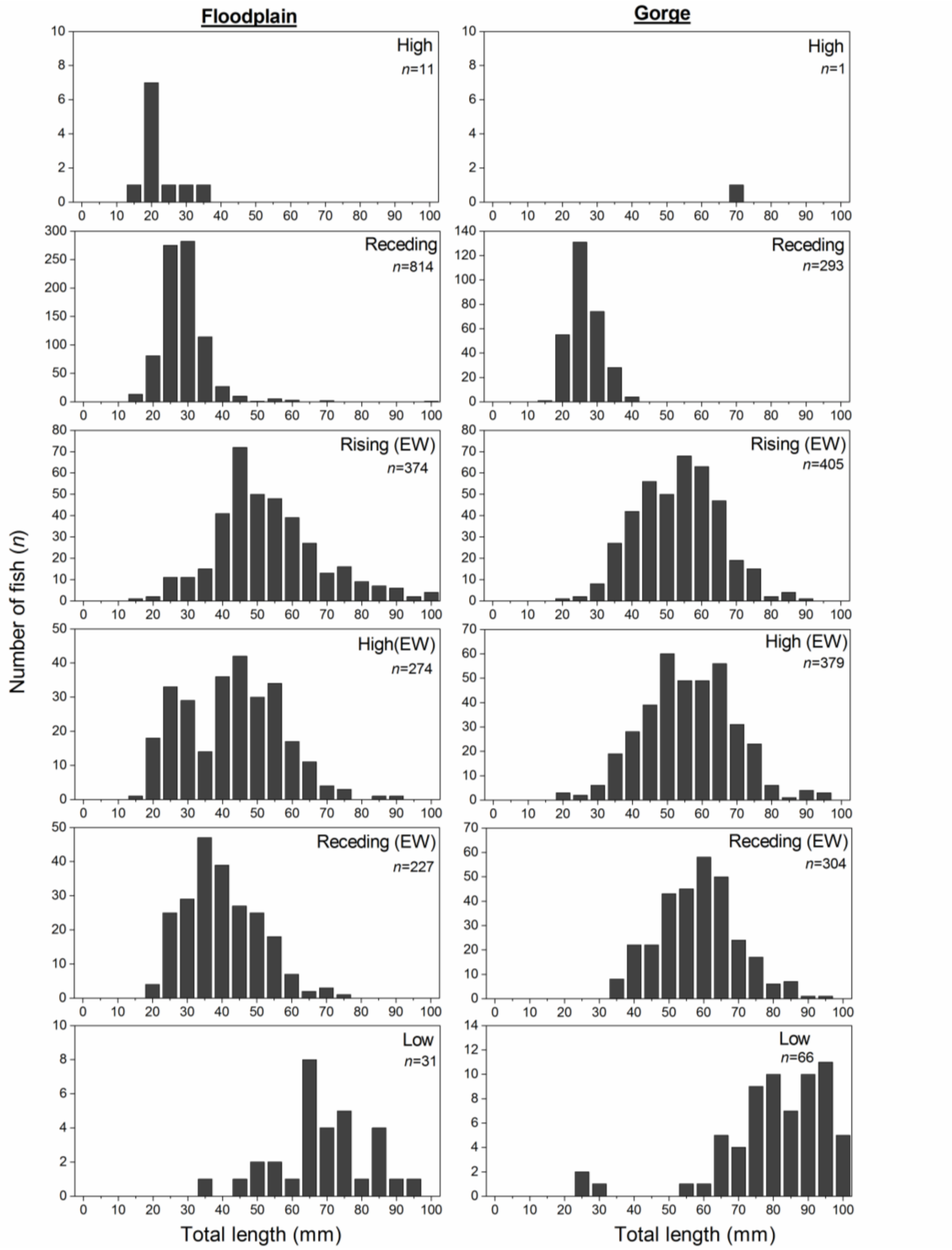


Figure 31. Length frequency distributions for juvenile common carp (≤100 mm TL) sampled in the river-wetland connection passage and within the wetlands for the Floodplain region (left) and Gorge region (right) for six phases of flow in 2012-13.

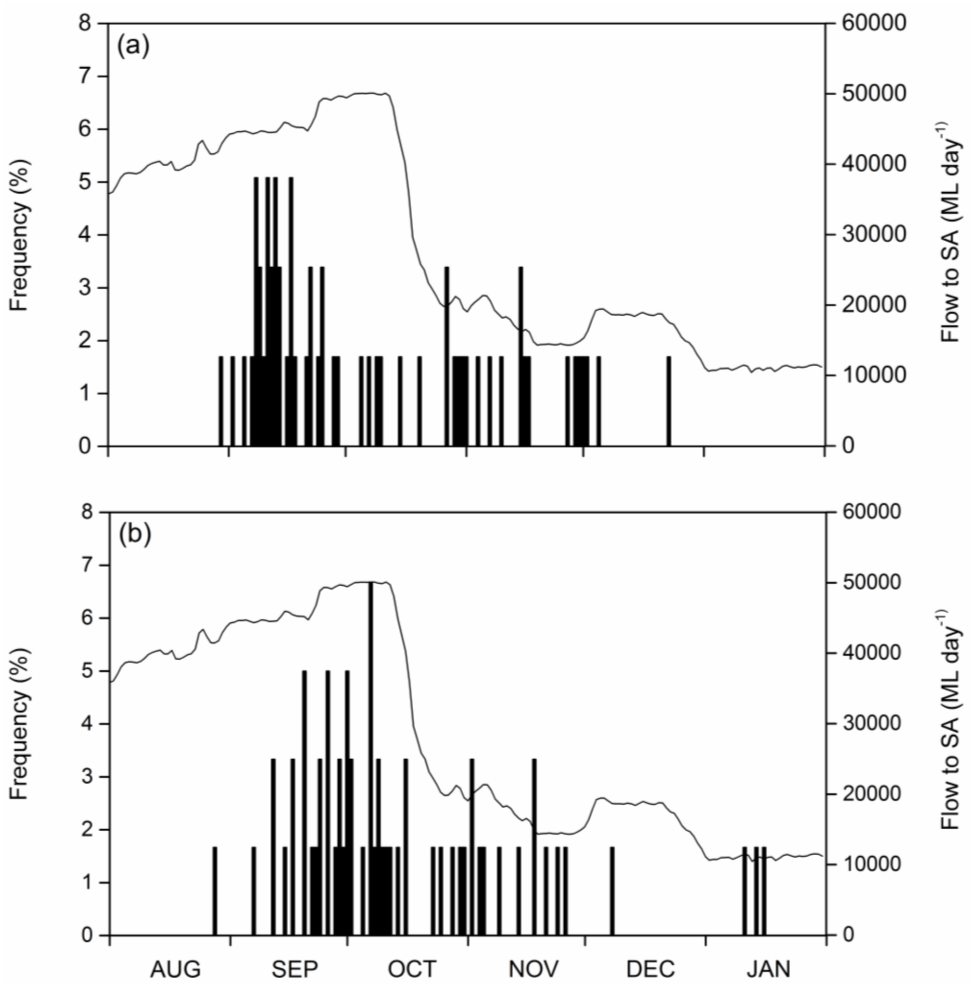


Figure 32. Estimated spawning date frequency (bars) for randomly-selected juvenile common carp sampled from (a) the Floodplain (n = 57) and (b) the Gorge (n = 62) region across all flow phases. Murray River flow (ML day^{-1}) over the South Australian border for the period from 1st August 2012 to 1 February 2013.

Frog response

Frog communities and distribution

A total of eight frog species were detected between September and December 2012 (Table 11). All eight frog species were detected in both spring, during unregulated flows (high flows), and summer during the environmental watering event. All eight species were recorded in the Gorge region and six species were recorded in the Floodplain and Swamplands regions. The distribution of two species, the Brown Tree Frog (*Litoria ewingii*) and Common Froglet (*Crinia signifera*), was limited to the Gorge and Swamplands geomorphic regions, whereas, the Eastern Sign-bearing froglet and Southern Bell Frog were only detected in the Gorge and Floodplain regions.

Table 11. Frog species detected at wetlands in the Lower Murray River regions in spring (n = 86) and summer (n = 112) 2012. X = species present in both spring and summer surveys. A brief description of frog species can be found in appendix XIV. Ea.=Eastern; So.=Southern.

Common Name	Scientific Name	Floodplain	Gorge	Swampland
Brown Tree Frog	*Litoria ewingii*		Spring	X
Common Froglet	*Crinia signifera*		X	X
Ea. Banjo Frog	*Limnodynastes dumerilii*	Spring	X	X
Ea. Sign-bearing froglet	*Crinia parinsignifera*	X	X	
Long-thumbed Frog	*Limnodynastes fletcheri*	X	X	X
Peron's Tree Frog	*Litoria peronii*	X	X	Summer
So. Bell Frog	*Litoria raniformis*	X	Summer	
Spotted Grass Frog	*Limnodynastes tasmaniensis*	X	X	X

Spring (high flows) and summer (environmental water) 2012

Comparing wetlands repeatedly sampled in spring (high flows) and summer (environmental water) revealed that average abundance of calling males at wetlands was significantly higher in spring than in summer ($p=0.005$) (Figure 33A). Average frog species call richness was also significantly higher in spring than in summer 2012 ($p<0.001$) (Figure 33B)(Appendix VIII).

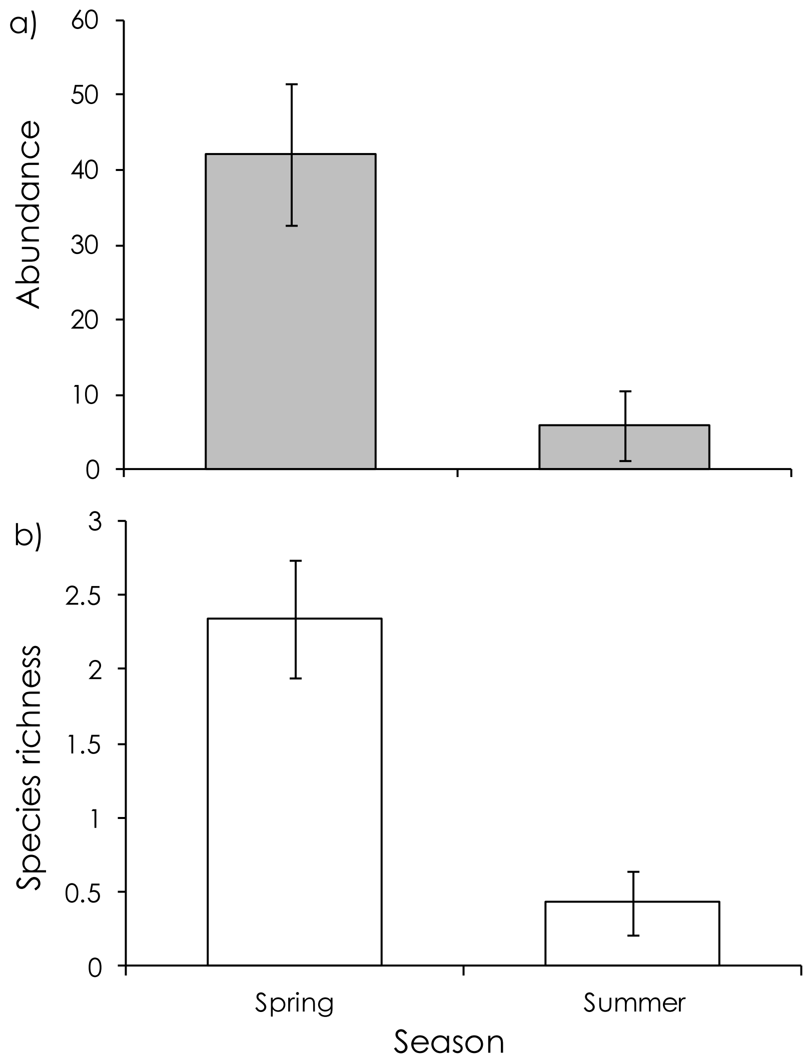


Figure 33. Mean (± Standard Error) (a) abundance and (b) species richness of calling males at wetlands (n = 9) in spring (high flows) and summer (environmental water) 2012.

Comparison of environmental variables at wetlands sampled during spring (high flows) and summer (environmental water) 2012 frog surveys revealed that flow to South Australia, and emergent vegetation decreased significantly from spring to summer and air temperature increased significantly over the sampling period (Hoffmann *et al*. 2014). Emergent vegetation was positively correlated with flows to South Australia (r^2=0.28, p=0.028, n=18). Electrical conductivity, humidity and submerged vegetation were not significantly different between spring and summer surveys.

Both frog call abundance and species call richness displayed a positive relationship with emergent vegetation (Figure 34 and Figure 35). Generalised linear modelling revealed that flows to South Australia and emergent vegetation were significant predictors for frog call abundance and species call richness at wetlands (Appendix IX and Appendix X)). Overall, the included environmental variables explained 62.4% and 46.7% of the variation in frog call abundance and species richness respectively.

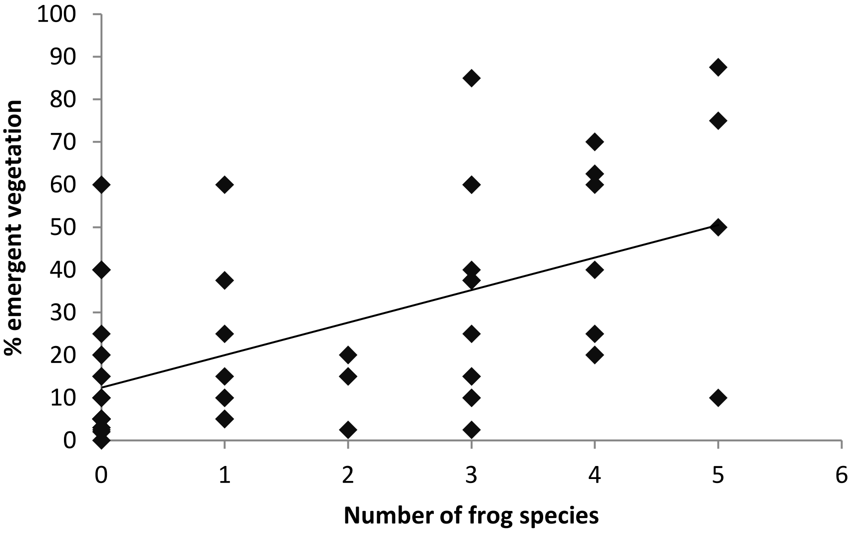


Figure 34. Relationship between species richness of calling males and emergent vegetation (%) at wetlands in spring and summer 2012.

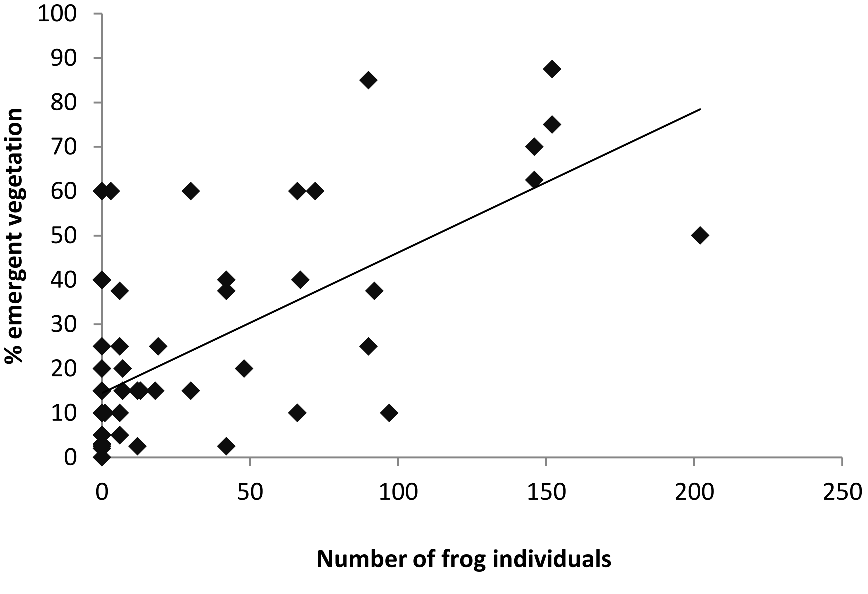


Figure 35. Relationship between the abundance of calling males and emergent vegetation (%) at wetlands in spring and summer 2012.

Comparison with previous summers

Between 2005 and 2009, flows at the South Australian border were at or below entitlement flows (low flows), with the exception of 2005, which had a relatively short period of above entitlement flows (Table 12). In 2010 to 2012, the Lower Murray River experienced higher unregulated flows, with a peak of 93,000 ML day^{-1} in March 2011. The environmental watering in 2012 increased flows to 19,557 ML day^{-1} in December.

Table 12. Maximum and minimum flows at the South Australian (SA) border (ML day^{-1}) between months of November to December for the years 2005 to 2012.

Year	Min flow at SA border between Nov/Dec (ML day^{-1})	Max flow at SA border between Nov/ Dec (ML day^{-1})	Description of flow conditions
2005	6,689	15,100	Increasing flow small pulse / weir manipulation
2006	4,015	6,257	Stable and low
2007	2,996	4,350	Stable and low
2008	3,845	4,935	Stable and low
2009	3,325	6,048	Stable and low
2010	28,465	67,218	Increasing
2011	8,158	25,119	Decreasing
2012	10,007	21, 218	Decreasing unregulated + increasing EW

Mean ranks of frog call abundance in managed wetlands (wetlands connected at pool level but managed through a flow-control structure to have wet and dry cycles) were statistically different between years (Kruskal-Wallis; $X^2 = 60.39$, df = 5, $p<0.001$). Frog call abundance in 2012 was significantly lower than 2005, 2006, 2009 and 2010, but not 2011 (Figure 36, Appendix XI). Frog call abundance in 2011 was significantly lower than 2010, which had the highest frog call abundance compared to all other years.

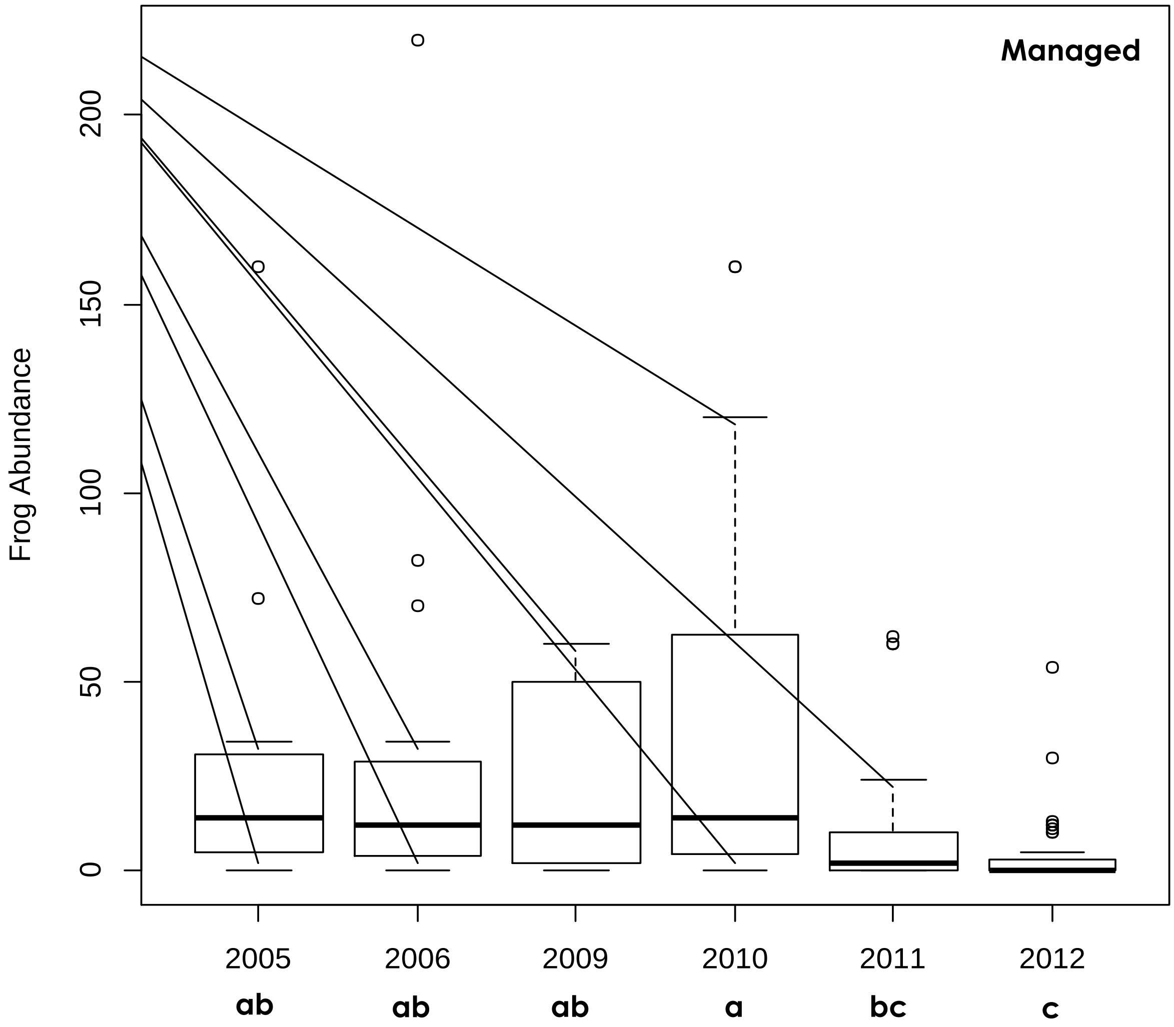


Figure 36. Boxplot of summer abundance of calling males at managed wetlands. Different letters below columns denote when frog abundances were significantly different from each other using Kruskal-Wallis post hoc tests ($p<0.05$). Note: Horizontal line=median, Ends of the box=1st and 3rd quartile, whiskers= 1.5 IQR, circles= outliers.

Mean rank frog call abundance at permanent wetlands (wetlands connected permanently at pool level) also varied significantly over time (Kruskal-Wallis; X^2= 27.93, df = 4, $p<0.001$), and frog calling abundance in 2007 and 2010 were significantly higher than 2011 and 2012 (Figure 37, Appendix XII). Summers of 2007, 2009 and 2010 did not differ significantly in mean rank frog call abundance.

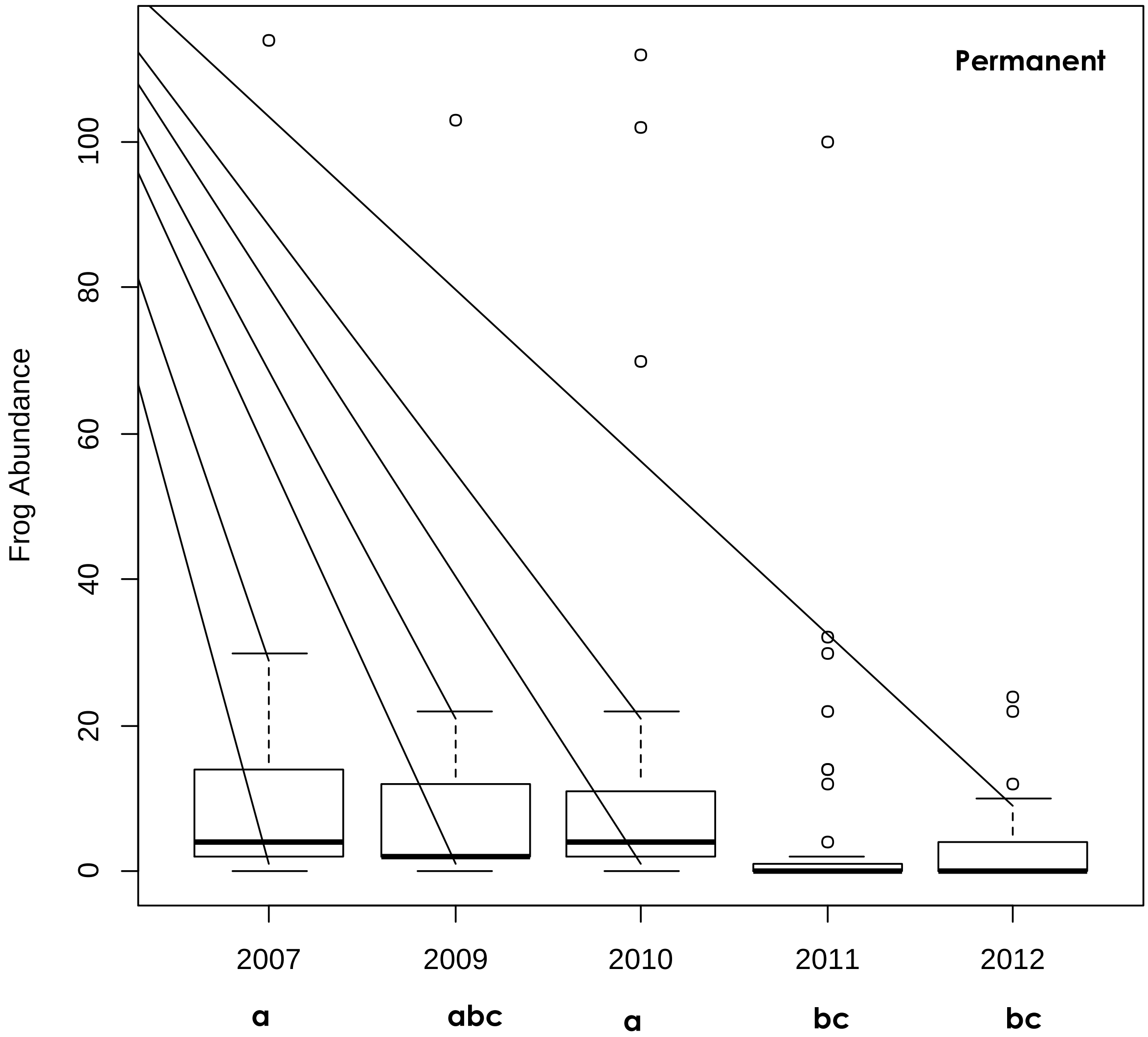


Figure 37. Boxplot of summer abundance of calling males at permanent wetlands. Different letters below columns denote when frog abundances were significantly different from each other using Kruskal-Wallis post hoc tests ($p<0.05$). Note: Horizontal line=median, Ends of the box=1st and 3rd quartile, whiskers= 1.5 IQR, circles= outliers.

As with managed wetlands and permanent wetlands, there was a significant difference in mean rank frog call abundance between years at temporary wetlands (wetlands that are inundated when flows are above pool level) (Kruskal-Wallis; X^2= 61.23, df = 2, p=<0.001). Mean rank frog abundance for 2010 was significantly higher than both 2011 and 2012 (Figure 38). Mean rank frog abundance in 2011 and 2012 were not significantly different.

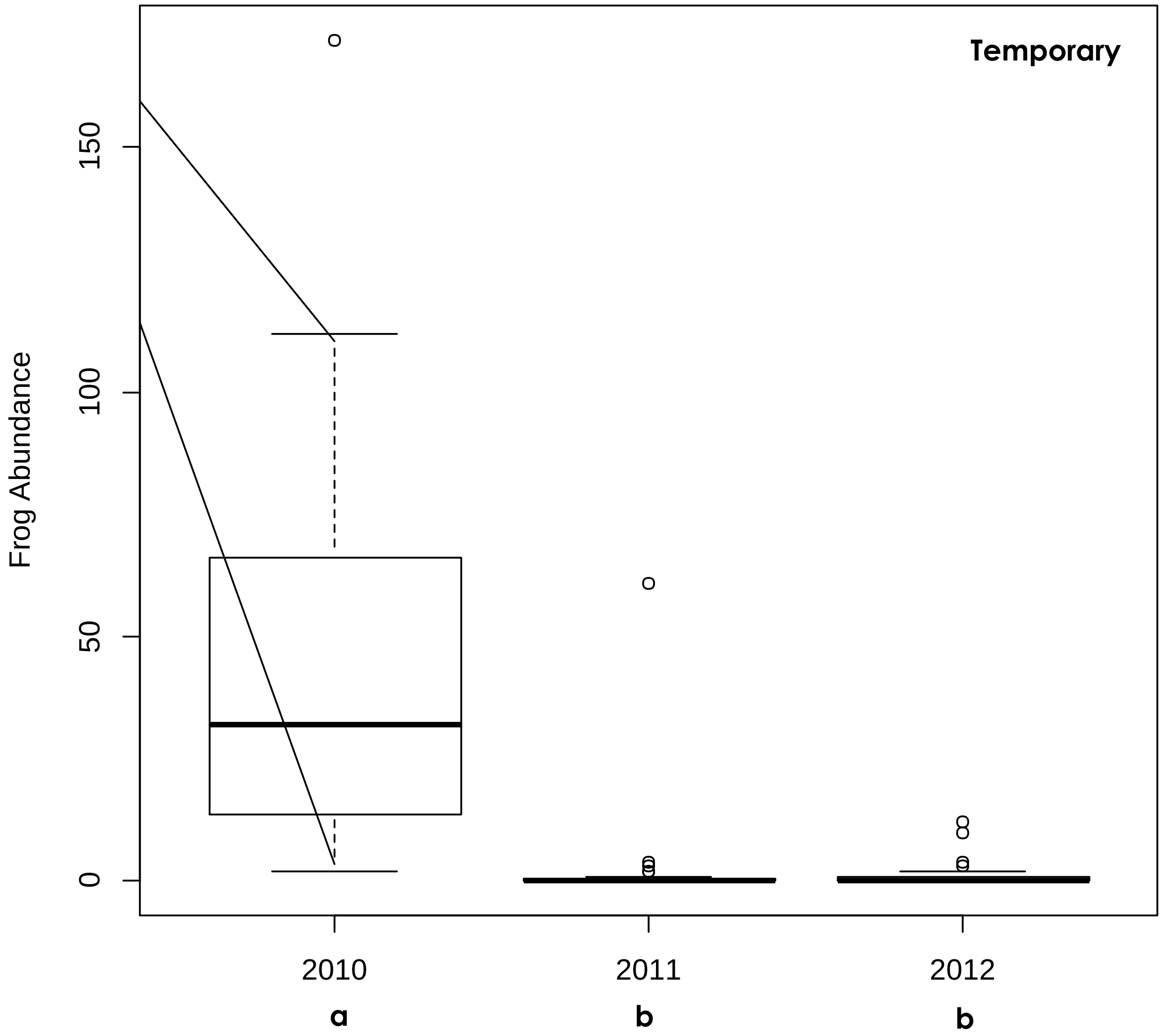


Figure 38. Boxplot of summer abundance of calling males at temporary wetlands. Different letters below columns denote when frog abundances were significantly different from each other using Kruskal-Wallis post hoc tests ($p<0.05$). Note: Horizontal line=median, Ends of the box=1st and 3rd quartile, whiskers= 1.5 IQR, circles= outliers.

Summer frog abundance of calling males at permanent and managed wetlands was not correlated with flows in the Lower Murray River across all years (*permanent;* rho=0.09, p=0.32, n=135; *managed;* rho=0.06 p=0.38, n=196). Frog call abundance at temporary wetlands was significantly correlated with flows to South Australia (*rho*=0.58, p> 0.001, n=103), however, these wetlands were only surveyed in 2010, 2011 and 2012, when higher flows inundated these areas.

4.3 Coorong (modelling)

Coorong hydrodynamic modelling

Model results for salinity and water level for the whole period 1963 – 2013 are shown in Figure 39. Since we mainly examined thresholds for habitat modelling, the results presented for a water level threshold of +0.2 m AHD and a salinity threshold of 85 g/L are exemplified. While water level will modify outcomes of habitat modelling, the main driver is the salinity tolerance of species. The threshold value used in Figure 39 is indicative for higher salinity tolerant juvenile fish, which could provide a preliminary understanding on habitat suitability.

Changes in water level and salinities during 2012-13 due to environmental watering are shown in Figure 40. Comparing to the reference scenario (with environmental watering from all sources), differences for Scenario 2 (with Commonwealth environmental watering only) were low but could become large for Scenario 3 if there were no environmental watering. The bigger changes in both water level and salinities were due to the larger contribution of Commonwealth environmental water in the summer months. Overall, the impact of missing environmental watering on salinities seems to be greater in the North Lagoon due to the then missing freshening effect of barrage inflows whereas the effect on water level seems to be more significant in the South Lagoon due to the decreased water inflow from the North Lagoon.

a)

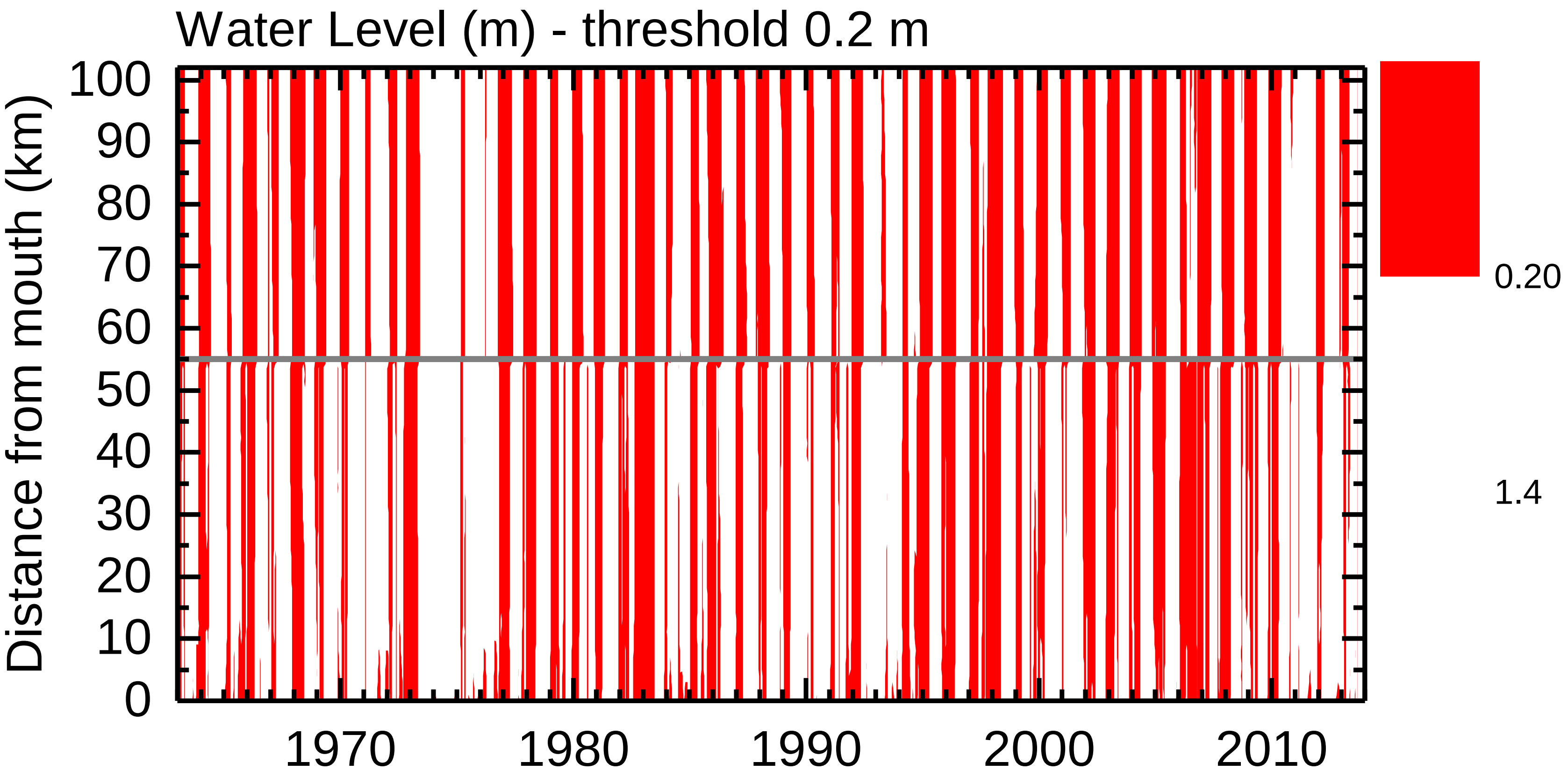


Figure 39. Water level (m AHD) and salinity (g/L) in the Coorong as modelled with CHM v2.1.0 for the full simulation period from 1963 to 2013 along the Coorong from mouth (0 km) to Salt Creek (102 km). Black horizontal line at 55 km delineates North and South Lagoon.
a) Water level visualised for a threshold of 0.2 m AHD, red areas indicate time periods when water level was ≤0.2 m AHD;

b)

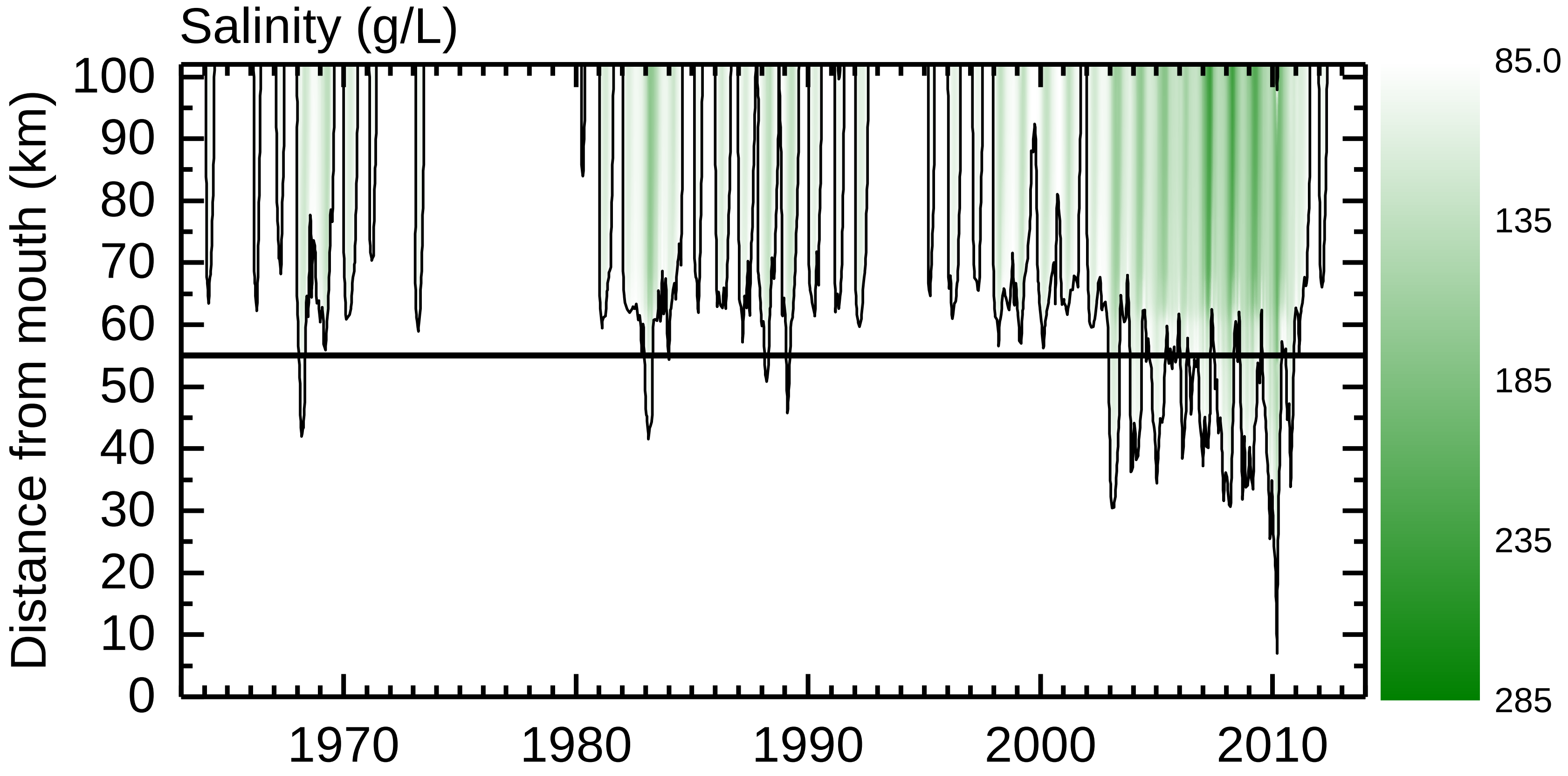


Figure 39 continued. Water level (m AHD) and salinity (g/L) in the Coorong as modelled with CHM v2.1.0 for the full simulation period from 1963 to 2013 along the Coorong from mouth (0 km) to Salt Creek (102 km). Black horizontal line at 55 km delineates North and South Lagoon. b) Salinities above 85 g/L (black contour line) to be compared with fish habitat simulations.

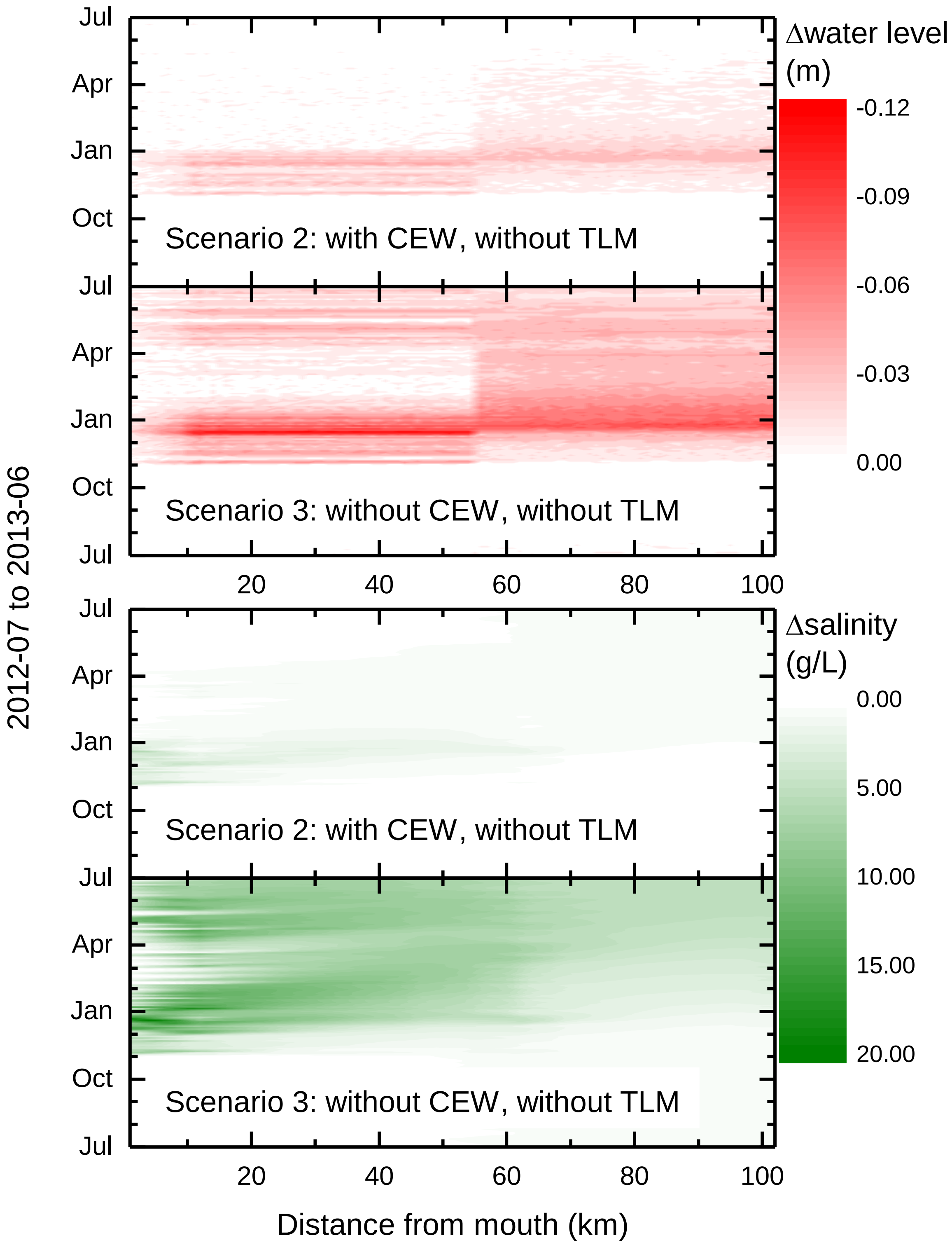


Figure 40. Changes in water level (upper graph) and salinity (lower graph) for scenarios 2 (with CEW, without TLM) and 3 (without CEW, without TLM) with respect to the reference (with CEW and TLM) for the period July 2012 to June 2013.

Ruppia tuberosa

Ruppia tuberosa simulation 1963 to 2013

The modeled probability of *Ruppia tuberosa* sediment propagule bank replenishment between 1963 and 2013 in the South Lagoon of the Coorong between Parnka Point (55 km from the Murray Mouth) and Salt Creek is shown in Figure 41. Over the 50 year model simulation period there has been a general decline in the probability of replenishment (Figure 41).

During the early to mid-1960s the probability of replenishment was generally high in the South Lagoon but this followed by a period of low probability in the late 1960s (Figure 41). During the 1970s the modeled probability of replenishment was high, except for two periods of approximately one year in 1972 and 1977 (Figure 41). During the 1980s the duration of the periods of hydrological conditions that resulted in low probability of replenishment increased and periods of high modeled probability of replenishment decreased (Figure 41). A further increase in the duration of unfavourable hydrological conditions was observed in the 1990s, with only three out of 10 years having hydrological conditions that would have resulted in a modeled probability greater than 25% (Figure 41). From 2001 to 2011 the modeled probability of replenishment was lower than 25% for the South Lagoon, except in 2001 in the northern 30 km of the lagoon (Figure 41). The 2010-11 flood resulted in favourable hydrological conditions for *Ruppia tuberosa* in the South Lagoon and; therefore, higher modeled probability of replenishment (Figure 41).

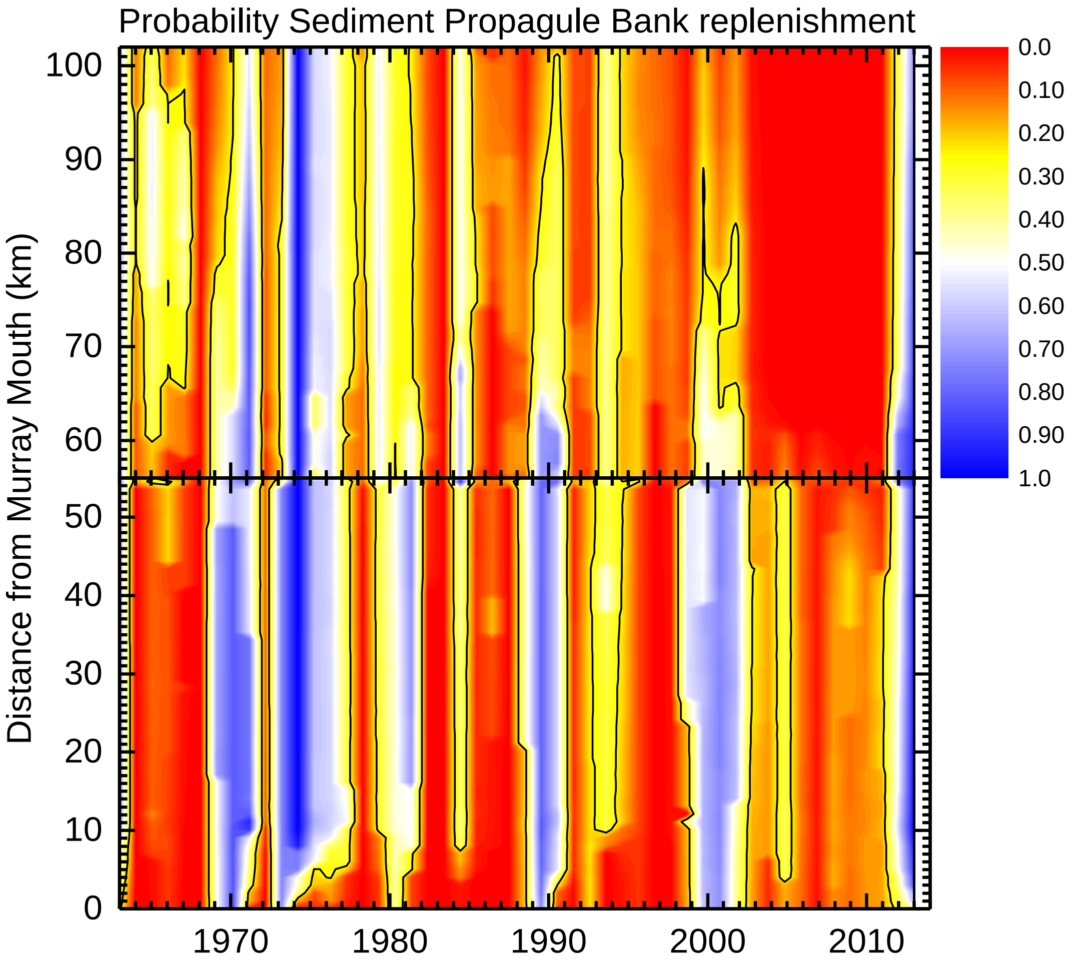


Figure 41. Model output showing the probability of *Ruppia tuberosa* sediment propagule bank replenishment from 1963 to 2013 in the North Lagoon (lower graph) and South Lagoon (upper graph) of the Coorong between Murray Mouth channel (0 km) and Salt Creek (102 km from the Murray Mouth). The contour line on the plot represents the 25% probability of sediment propagule bank replenishment.

Ruppia environmental watering evaluation for 2011 and 2012

In addition to the simulations of *Ruppia tuberosa* sediment propagule bank replenishment over a 50 year period the changes due to environmental watering and thus water level and salinity conditions were simulated for 2011 and 2012. The reference condition refers to actual barrage outflows (natural flow plus CEWO and TLM environmental water), scenario 2 refers to natural flows plus CEWO environmental water only and scenario 3 is modeled flow without environmental water. Since the *Ruppia tuberosa* response model is defined on calendar years sediment propagule bank replenishment probabilities was simulated for full calendar years, but information on environmental watering was only available from July 2011 onward. Thus the simulations in 2011 are biased towards relatively low effects on propagule bank replenishment. The sediment propagule bank replenishment probabilities together with its changes due to environmental watering are shown in Figure 42.

The probability of propagule bank replenishment in 2011 was generally low for the South Lagoon except in the most northern 10 km and there was little benefit (<2% increase in the chance of propagule bank replenishment) of environmental water (Figure 42a). In 2012 there was a high modelled probability of propagule bank replenishment throughout the South Lagoon (Figure 42a). The addition of Commonwealth Environmental water increased the chance of propagule bank replenishment up to 4% and with the addition TLM environmental water there was an increase of up to 10% in the northern 30 km of the South Lagoon (Figure 42b).

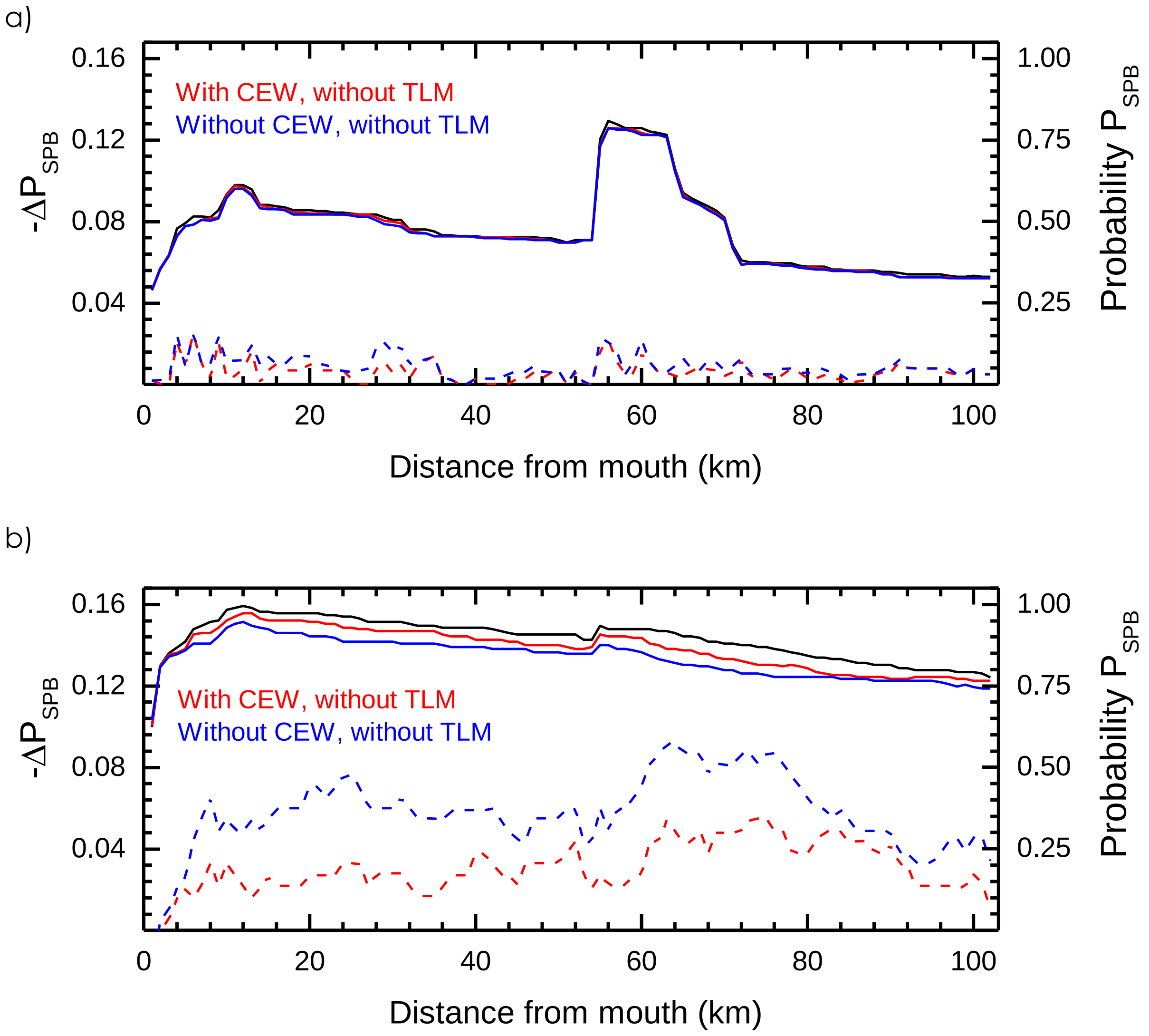


Figure 42. Sediment propagule bank replenishment probability for *Ruppia tuberosa* for a) 2011 and b) 2012. The black line represents the reference simulation including both, CEW and TLM water. Simulations with CEW and without TLM (red) and without CEW and TLM (blue) are given in terms of probability values for sediment propagule replenishment (right axis, solid lines) as well as changes with respect to the reference (dashed lines).

Fish habitat

<u>*Fish habitat simulation 1963 to 2013*</u>

In total seven fish species were included in the simulations using the salinity tolerance thresholds for warm and cold periods (Table 4). The results of the annual probability analysis in the reference scenario for the period from 1963 to 2013 are shown in Figure 43. The results for the simplified threshold analysis (see Appendix XV) Generally agrees with the annual exceedance probability analysis shown in Figure 43. Both daily and annual analyses show that during mid-1970s the entire Coorong provided suitable fish habitat in terms of salinities for all seven species whereas from early 2000 to 2010 almost all these species were excluded from the South Lagoon. For the species with least salinity tolerance (i.e. mulloway and Tamar goby), habitat suitability was less than 20% in the South Lagoon for most of the years from 1963 to 2013. In contrast, the most salt tolerant smallmouthed hardyhead habitat was suitable the South Lagoon throughout the years except during the millennium drought.

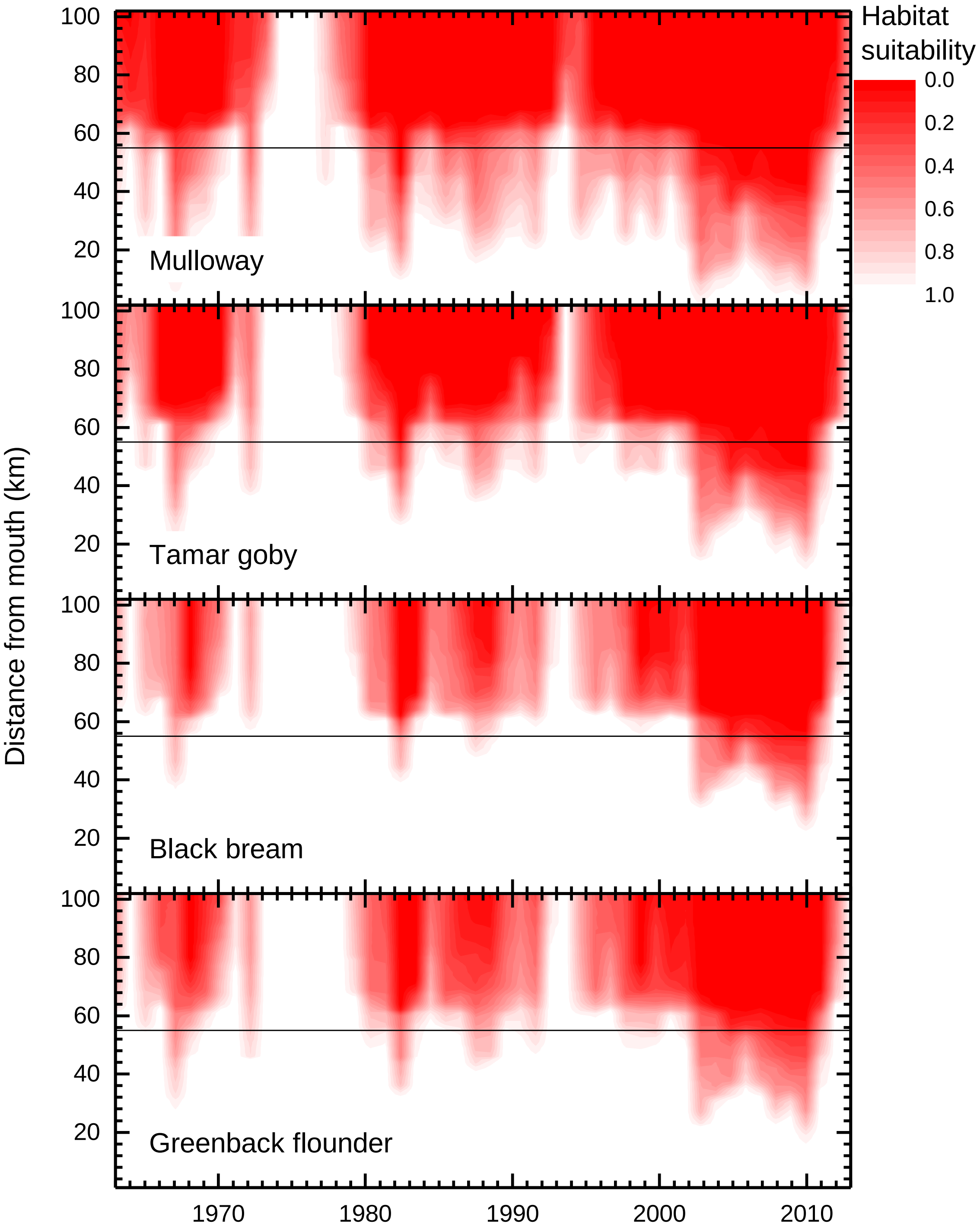


Figure 43. Habitat suitability for fish species along the Coorong from mouth (0 km) to Salt Creek (102 km) calculated for the whole simulation period 1963-2013 on an annual basis using salinity tolerance thresholds. The horizontal black line indicates the border between North (0-55 km) and South Lagoon (55-102 km).

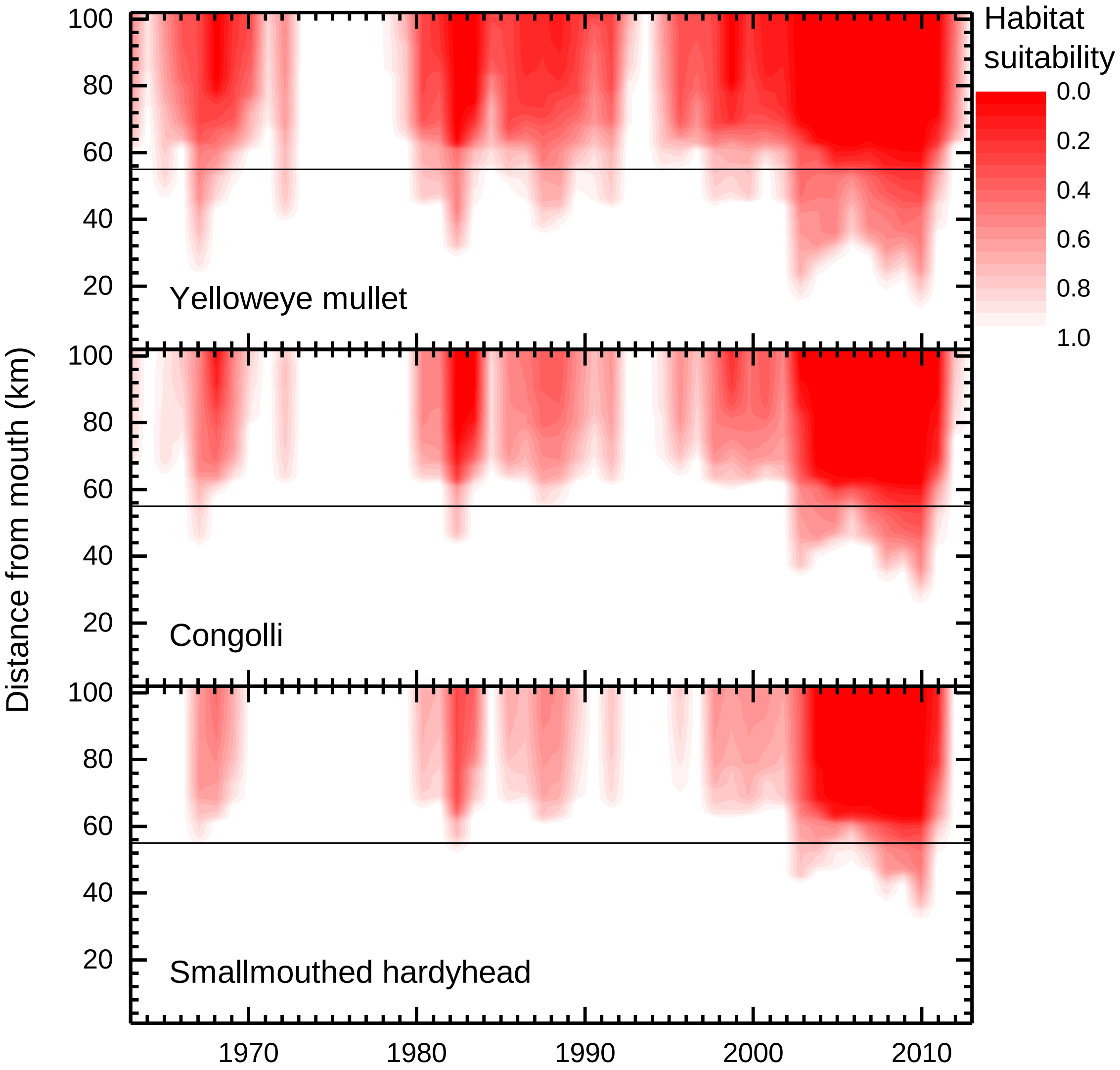


Figure 43 continued: Habitat suitability for fish species along the Coorong from mouth (0 km) to Salt Creek (102 km) calculated for the whole simulation period 1963-2013 on an annual basis using salinity tolerance thresholds. The horizontal black line indicates the border between North (0-55 km) and South (55-102 km) Lagoon.

Fish habitat environmental watering evaluation for 2012-13 (July to June)

Difference plots for changes due to environmental watering according to Scenarios 2 (with CEW, without TLM) and 3 (without CEW and TLM) are given in Figure 44 for the annual habitat suitability. However, only three out of the seven species, mulloway, Tamar goby and yelloweye mullet, showed changes due to changed salinity in the South Lagoon for 2012-13. The salinity thresholds of the other species were always below the simulated salinity levels along the Coorong, i.e. always classified as suitable habitat. Taking Mulloway as the most susceptible fish species in terms of salinity tolerance in our study, there would have been a reduction up to 3% (13%) in habitat suitability if less (or no) environmental water were delivered during 2012-13, i.e. for scenarios 2 (with Commonwealth environmental watering only) and 3 (with no environmental watering) respectively.

Threshold analysis was also used to analyse the extent, or the retreat, of fish habitat under barrage flow scenarios, i.e. differences between results for Scenarios 2 and 3 and the reference scenario. The retreat/extent of the three impacted fish species are shown in Figure 45. For mulloway, Scenarios 2 and 3 yielded a retreat in suitable fish habitat of 7 km and 30 km, respectively, for the cold months from April to June 2013.

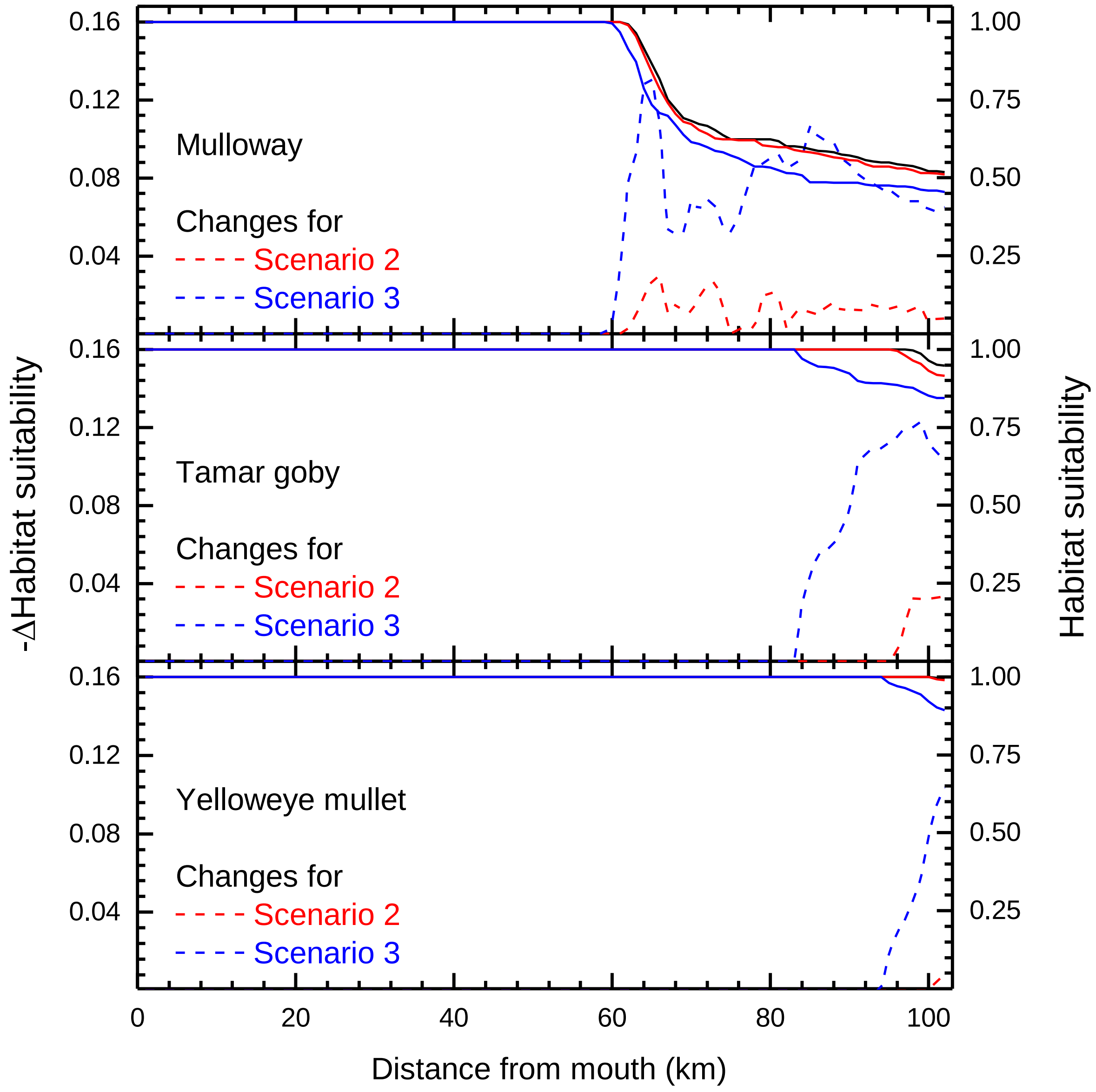


Figure 44. Changes in fish habitat suitability assuming less barrage flow in 2012-13 with respect to the reference simulation (including CEW and TLM water). The black line represents the reference simulation including both, CEW and TLM water. Simulations with CEW and without TLM (red) and without CEW and TLM (blue) are given in terms of probability values for fish habitat suitability (right axis, solid lines) as well as changes with respect to the reference (dashed lines). Species not shown did not experience changes this particular year. Sign of changes in habitat suitability chosen in a way that a positive value in the habitat change signifies a loss.

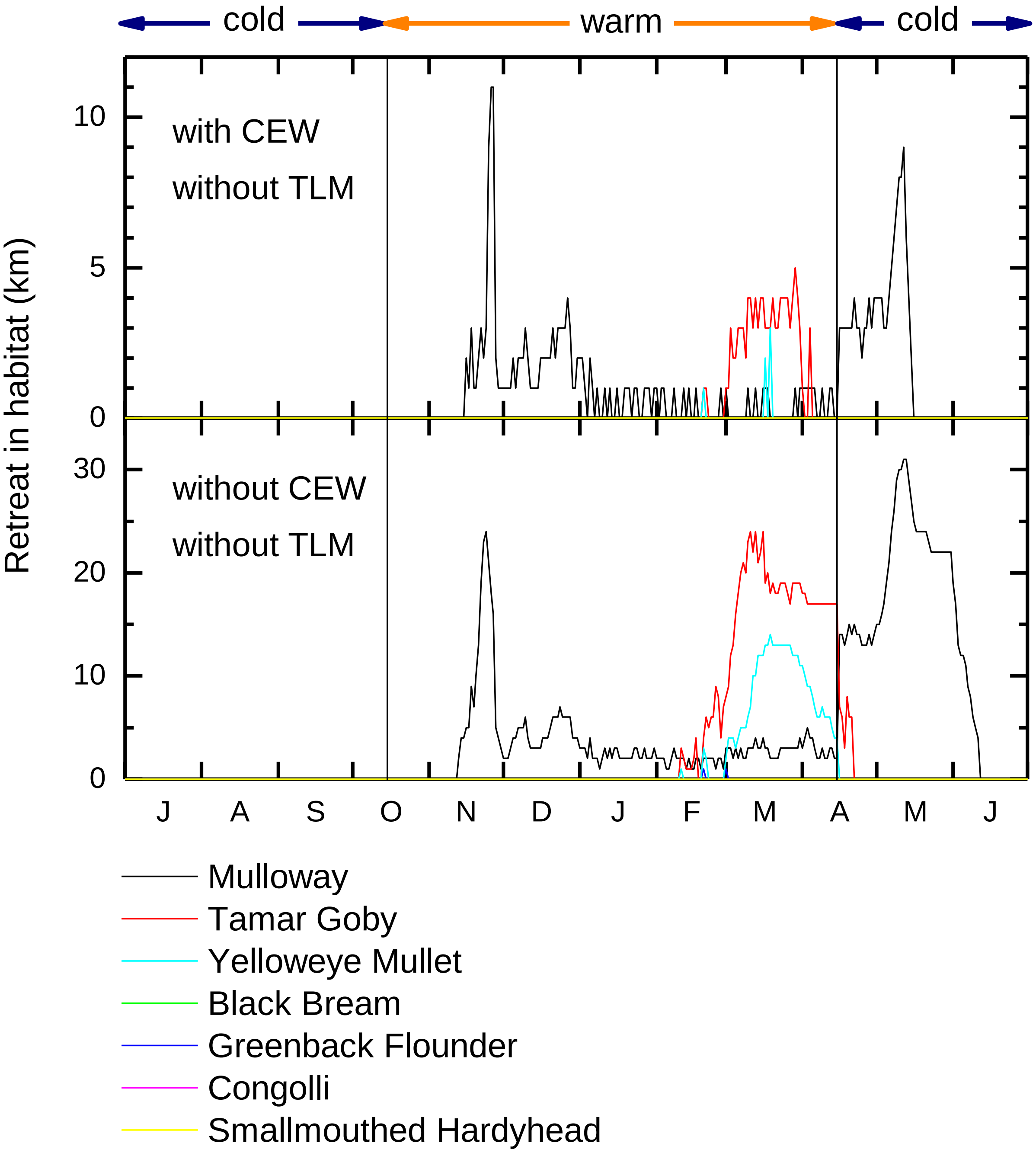


Figure 45. Differences in habitat reach with respect to the reference simulation (including CEW and TLM water). Upper graph shows the scenario result with CEW and without TLM water, while the lower graph shows the scenario results including neither CEW nor TLM water. Vertical black lines delineate cold from warm periods where different thresholds were used. Only three of the seven simulated species (mulloway, Tamar goby, and yelloweye mullet) showed changes in this specific year.

5 DISCUSSION AND EVALUATION

5.1 Main channel

Larval fish

The evaluation question this study aimed to answer was whether the flow regime supported by CEW promoted spawning and recruitment of native fish in the Lower Murray River. This question in part derives from the knowledge that some key large-bodied native species are flow-cued spawners, namely golden perch and silver perch, whilst recruitment success of other iconic species such as Murray cod improves when a lotic hydraulic environment is available.

The larval fish assemblage in 2012-13 was more closely related to those observed in 2005 when there was a very small within channel flow. The larval fish assemblage in the Lower Murray River was comprised of high abundances of small-bodied fish species such as carp gudgeon, flat-headed gudgeon and Australian smelt, which tend to numerically dominate the assemblages along with the generalist bony herring, which proved to be the most abundant species. These species are known to spawn annually and under a broad range of conditions. The larvae of small-bodied species were readily abundant throughout the whole of the Lower Murray River during the millennium drought (2006-2010), during which larvae of large-bodied native species were relatively scarce or not detected at all (Cheshire 2010, Cheshire *et al*. 2012). In order to appropriately address the evaluation question proposed above the focus must be centred on large-bodied natives, in particular those that are most dependent on flow for reproduction (e.g. golden perch).

Golden perch larvae were present from October 2012 to late January 2013 following the pattern observed in the previous reproductive season (i.e. 2011-12). During the millennium drought golden perch larvae were virtually absent from the water column throughout the entire reproductive season (e.g. October to February). After the return of flows in September 2010, golden perch larvae were again detected but only from October to December 2010. During the next two reproductive season (i.e. 2011-12 and 2012-13) winter flows were reasonably high and dropped in early spring (e.g. September), with environmental flow delivered later on in the summer (during January/February 2011-12 and during December 2012-13). In both these occasions, golden perch larvae were detected after the delivery of environmental water. Furthermore the majority of these larvae were very early stages (i.e. pre-flexion) suggesting they had been recently spawned (e.g. less than a week). Although this evidence is not enough to establish a causal link between environmental water delivery and golden perch spawning, it is an association that has been repeated in two consecutive years and that merits further research. The delivery of water may be stimulating resident golden perch to spawn and/or facilitating the drift of larvae that have been spawned further upstream to the Lower Murray River. Future research may look into the spatio-temporal origin of larvae that are present after an environmental watering event in order to elucidate this question. What can be established is that in two consecutive years the weeks following environmental watering showed an increased abundance of golden perch larvae, particularly in the Gorge region.

Zooplankton assemblage

The zooplankton assemblage is a key part of the riverine food web and as such, changes in its structure will have cascading effects on the higher and lower trophic levels of the web. In this part of the study the aim was to detect changes in zooplankton assemblages potentially related to environmental water deliveries and see if those changes could be related to larval fish diet and fatty acid composition of the assemblage.

The zooplankton assemblages showed significant spatio-temporal changes. Assemblages from each geomorphic region were distinct throughout the study suggesting that the processes that drive the assemblage composition in each region are independent. However there were certain similarities in some responses, the highest total abundance was reached in early January (i.e. trip 7) at all sites. This coincides with water temperature reaching summer peaks, as well as with the tail of the delivery of environmental water. Many of the species that were a trait of the assemblages observed during the delivery of environmental water were warm water species characteristic of the Darling system further north, suggesting that part of the water that was used for environmental purposes may have been originally sourced in that area highlighting the importance of longitudinal connectivity. There was also a significant change in fatty acid abundance within the zooplankton community which occurred during the delivery of environmental water between trips 5 and 6. Higher relative abundances of linoleic and α-linoleic acid were evident in the later trips, something that would potentially be beneficial for early life stages of freshwater fish (Tocher 2010).

The gut content analysis through direct observation revealed that both golden perch and Murray cod were not consuming the most abundant zooplankton; on the contrary they were consistently consuming scarce species. The prey items were however amongst the largest in the assemblage suggesting that golden perch and Murray cod larvae may have a maximum efficiency strategy where they consume prey that provide highest energy with the least effort or some other type of selectivity (Tonkin *et al*. 2006). These insights into native larval fish diets in the Lower Murray River provide information that is absent in the current literature. This line of research should be continued to gain better understanding of the processes involved in the early life stages of golden perch that may well determine recruitment success.

Food resources for larval fish

The key evaluation question for this study was whether supplying environmental water contributed to the provision of adequate larval fish food resources in the Lower Murray River. This was based on the hypothesis that increases in the level and duration of flows, even small changes that improved connections between the river channel and riparian, wetland or floodplain areas, would enhance the food supply for larval fish leading to greater survival, growths rates and recruitment.

Molecular and traditional techniques were used to enumerate and describe the phytoplankton and zooplankton communities. Molecular techniques provided information on a larger array of eukaroytic micro-organisms in addition to phytoplankton and zooplankton, including protists, ciliates, and fungi. There was overlap between genera identified from the molecular and visual datasets of algae and zooplankton, but occasionally the molecular data set identified additional genera. For the algae these included *Tetracystis, Planctonema, Polytoma, Golenkinia, Choricystis, Botryococcus, Parachlorella, Fistulifera, Synedra, Asterionella, Thalassiosira, Peridiniopsis, Mesostigma* and *Actinocyclus.* For the zooplankton these included *Proales, Cyclidium* (ciliate) and *Dileptus* (ciliate). These differences probably represent organisms that are in low numbers or difficult to see. Combining the molecular and traditional approaches provided the most useful information on community changes.

All data sets demonstrated sequential shifts in community composition over the sampling time series including a change in community composition in trips 5 and 6 potentially as a result of the environmental watering. Some species occurred before the environmental water arrived, some after it had arrived, while others appeared to be associated with the environmental water itself.

Genetic analyses of the gut contents of the larval fish demonstrated that only a small proportion of the microeukaryotes present in the water column were found in their gut contents. Of these there were a number of common items across species, but also additionally each fish species contained distinct food items. As a result, the gut contents of the different species of larval fish were significantly different from each other, and in the case of golden perch the pre-flexion and flexion stages had significantly different gut contents. Such detailed analyses of larval fish gut content have not been possible before and the findings suggest some selectivity of food items by the different larval fish species.

Some taxa commonly found in the gut of larval fish were consistently present in all trips. However, there were particular taxa that were correlated with the environmental water and found in the gut of larval fish. This indicates that some but not all of the microorganisms associated directly with the environmental watering were ingested by larval fish. Two planktonic microalgae diatom species that were correlated with the environmental watering, and did not occur at other sampling times, were not found in the gut of larval fish.

Taxa that were correlated with the sampling trips during and following the environmental flow and that occurred in the gut of larval fish included the rotifer *Colotheca,* green alga *Choricystis,* diatom *Thalassiosira,* and fungi *Catenophylyctis* and *Malassizia.* These results suggest that the environmental water may have altered conditions to favour these species for a period following the environmental watering.

These findings indicate that the larval fish are dissimilar in their feeding habits, with different species obtaining different food resources. In the case of the pre-flexion stage of golden perch where there were sufficient samples to analyse for trip differences there was some evidence that the gut content had changed after the environmental flow delivery.

Whether the gut content composition identified for the various larvae are the most suitable diets, or are different from those seen on other occasions, cannot be assessed due to a lack of data from a range of different conditions. However, it is expected that supplying suitable food resources will be critical to the successful growth and development of larval fish. The results obtained from this study show that the techniques to undertake such investigations are available. The demonstration of successful recruitment of fish associated with environmental flows requires linking changes in the fish population with relevant characteristics of the environmental flows. It is difficult in this case to reliably identify food resource links as the one-off environmental flow was short-lived and followed on from a period of high flows in the preceding months. In order to be able to interpret such events the responses to a range of watering events need to be monitored so that patterns in responses can be identified.

Dissolved and particulate material transport

The approach used for this study was valuable for evaluating changes in concentrations and transport of dissolved and particulate material associated with environmental water deliveries. Further refinement of the model will continue to improve the certainty of modelled outputs, particularly for the more sensitive parameters (dissolved nutrients and chlorophyll) within the Lower Lakes. The modelling outputs suggest that environmental flows in the Lower Murray can influence processes that are essential for providing habitat and resources for aquatic biota, further supporting the findings of Aldridge *et al*. (2013). In particular, during 2012-2013 environmental watering resulted in significant increases in the transport and export of salt, nutrients, chlorophyll (phytoplankton) and suspended solids through the Lower Murray River, Lower Lakes and Murray Mouth. The transport of this dissolved and particulate material is important for providing habitat for biota and resources that maintain the productivity of downstream ecosystems (Cook *et al*. 2010).

At the Murray Mouth, it appeared that the additional exports of dissolved and particulate material only resulted from the combined influence of Commonwealth environmental water and The Living Murray water, highlighting the importance of supplementary environmental water use to the system. This was particularly evident for salt exports, with net exports only observed when both environmental water sources were provided. This supports the view of Aldridge *et al*. (2013) that threshold discharges exist for the export of dissolved and particulate material, although the thresholds will vary temporally with changes in downstream water levels and differ between the Lower Murray River, Lower Lakes and Murray Mouth. The occurrence of these thresholds explains why environmental flows made such significant contributions to total exports.

It was evident that the increase in transport of dissolved and particulate material associated with environmental watering was largely a result of the increased discharge rather than increased concentrations, since concentrations were generally not influenced significantly. An exception to this was salinity within the Lower Lakes, with electrical conductivity lower with environmental water deliveries. This may be partly explained by how barrage operations are considered within the model (see Aldridge *et al*. (2013) for more detail). Nevertheless, reductions in salinity levels provide an important functional role by providing habitat for aquatic organisms. Salinities were also reduced considerably within the region of the Murray Mouth due to environmental watering. This is in agreement with previous studies (see Aldridge *et al*. 2012; Mosley *et al*. 2012; Aldridge *et al*. 2013) that have suggested that environmental water deliveries to the Lower Lakes and Coorong during periods of low-moderate flows are important for reducing salinity levels.

5.2 Wetlands

Lateral movements of fish

The importance of lateral connectivity between within-channel and wetland habitats for fish is well established (Junk *et al.* 1989; Stuart and Jones 2006), but knowledge of lateral fish movements and how they are influenced by flow is limited. The present study described the lateral movement of native and invasive fish into and out of wetlands in the Lower Murray River, before, during and after the delivery of the environmental flow pulse in 2012-13, and assessed the influence of flow-related hydraulic changes on fish movement. It was hypothesised that the delivery of the flow pulse would enhance the lateral movements of fish, which would ultimately benefit native fish populations through increased survival, recruitment, feeding and reproduction opportunities (Junk *et al.* 1989). However, there were concerns that invasive species, such as common carp, would also benefit. As such, this study also examined the reproductive development and recruitment of common carp to inform about the potential benefit of flow pulse delivery for this species.

Directional sampling in the river-wetland connection passage of four wetlands revealed a diverse (16 species) and abundant (~57,612 individuals) fish assemblage moving between wetlands and the main river channel of the Lower Murray River. Movements involved most fish species that were recorded in the most recent study on lateral fish movements in this region (Conallin *et al.* 2011), with the exception of common galaxias, small-mouthed hardyhead, pouched lamprey and Murray cod. However, the present study recorded two additional species, i.e. silver perch and oriental weatherloach. Overall, 11 of the 26 native species known to occur in the Murray-Darling Basin (Hammer and Walker 2004), were recorded in this study.

Patterns of lateral fish movement were complex and highly variable among flow phases at each wetland, and such differences were not consistent among wetlands. Such spatial variability is a common attribute of fish assemblages in freshwater systems (Jackson *et al.* 2001) and was not unexpected given the substantial variability in the physical, biological and hydrological attributes of wetlands in the Lower Murray River. Refer to Earl and Ye (In Prep) for further detailed discussion.

The lateral movement of most species at each wetland during each flow phase was generally bidirectional and demonstrated no clear, consistent pattern relative to changes in flow conditions. These results conflict those of numerous other studies (Hohausová *et al.* 2003; Stuart and Jones 2006; Castello 2008; Lyon *et al.* 2010) which detected substantial migrations in and out of off-channel habitats in response to seasonal changes in hydrological connectivity, whereby fish entered off-channel areas to exploit the relatively warm, food and shelter rich, and productive habitats, and moved out prior to disconnection.

In 2006, Conallin *et al.* (2011) detected substantial variations in the lateral movements of fish in the Lower Murray River, but similar to the present study, did not detect directional consistency of movements. Conallin *et al.* (2011) suggested that the lack of directionality likely reflected an absence of hydraulic cues, due to the low flow conditions during the drought, and predicted that directional movements would become apparent under increased flow conditions when key flow-related parameters such as water level, flow discharge and dissolved oxygen would likely be more influential. However, the results of the present study suggest that such hydraulic factors, associated with the decline in unregulated flows and subsequent delivery of a relatively small flow pulse of environmental water, did not influence the directionality of the lateral movements.

Despite the lack of directionality, there were shifts in the structure of laterally moving assemblages between flow phases. However, shifts were not consistent among wetlands, suggesting that flow delivery affected fish assemblages at each wetland in different ways. At Overland Corner, changes in the magnitude of flow discharge had a significant influence on the structure of fish assemblages moving to and from wetlands. This was not unexpected given the strong correlation between flow discharge and wetland size at Overland Corner, i.e. as discharge declined, the wetland contracted in size, reducing the amount of habitat available for fish (see

Section 1.2). During unregulated high flows, the number of fish moving laterally was relatively low. However, the decline in flow discharge during the Receding flow phase resulted in a significant increase in the number of fish, particularly common carp, moving in each direction. The delivery of environmental water appeared to enhance the lateral movements of native fish, particularly foraging generalist species, while the abundance of invasive species decreased and remained relatively low during this period. The benefit of the sustained flows associated with the delivery of environmental water to the lateral movement patterns of native fish was further highlighted by the subsequent decline in abundance during the Low flow phase, at a time when just 1% of wetland habitat remained inundated and available for these species.

Shifts in the structure of laterally moving assemblages between flow phases were also evident at Kroehns. However, unlike Overland Corner, changes did not appear to be directly related to variation in flow discharge. Rather, water level was the strongest environmental predictor of assemblage structure and there was no correlation between water level and flow discharge. Changes in water level below Lock 1 generally occur in response to local wind patterns which can have a surging effect on the large body of water between Lock 1 and the barrages separating the Lower Lakes (i.e. Lakes Alexandrina and Albert) from the Coorong. As such, the direct influence of increased flows associated with environmental water delivery on key hydraulic variables such as water level, wetland size and dissolved oxygen, was minimal compared to Overland Corner. Rather, hydraulic parameters showed a more distinctive seasonal shift, with a gradual increase in temperature and conductivity over the duration of the study. This seasonal shift corresponded to a gradual increase in the abundance of native fish moving into and out of the wetland at this site. As such, the direct influence of the environmental flow pulse (which supplemented flow discharge to ~15,000 ML day^{-1} at Lock 1), on the lateral movements of fish at Kroehns, and other perennially inundated wetland below Lock 1 remains unclear. Nonetheless, the delivery of a flow pulse of that magnitude which almost certainly improves water quality and enhances primary productivity in the main river channel can only be beneficial for the native fish populations that utilise habitats in that part of the system.

Common carp are the most dominant large-bodied fish in the Murray River (Davies *et al.* 2008) and commonly utilise wetland habitats for spawning and recruitment (Vilizzi and Walker 1999). In 2012, the timing of the delivery of environmental water to the Lower Murray River (e.g. early December) coincided with the middle of the spawning and recruitment period for common carp (Smith and Walker 2004). As such, there were concerns that the timing of environmental water delivery may enhance the reproductive development, spawning activity and the recruitment success of common carp. Analysis of these key demographic processes provided no evidence to suggest that this was the case. Whilst macroscopic analysis of common carp ovaries indicated that most females were fully developed during each of the six phases of flow, the proportion of spawning and or spent (i.e. recently spawned) females in the population remained relatively consistent throughout the duration of the study with no apparent response to changes in flow. Furthermore, ageing of small juvenile carp indicated that most fish spawned in 2012 were derived from spawning events that occurred from late August to November, with less than 5% derived from spawning that occurred in early December. Hence, there is no evidence, from the present study, to suggest that there was an increase in spawning activity and subsequent recruitment for common carp in response to the delivery of environmental water in 2012.

Frog response

Spring and summer 2012

The high flows that occurred in spring 2012, prior to the environmental watering event, and lack of control sites during the environmental watering, make it difficult to assess the direct influence of the environmental watering on frog populations. However, the environmental water delivery in 2012 provided habitat and breeding opportunity, as indicated by frog calls, for all eight wetland frog species found in the Lower Murray. Additionally, whilst tadpole surveys were not conducted at all wetlands, tadpoles were detected at many wetlands during spring and summer 2012. As tadpoles of all frog species detected in the study area require at least three months to metamorph (Anstis 2002), it is possible that tadpoles may have benefited from environmental watering in summer through extending the duration of inundation of habitat, however this was not quantified during this study.

Higher frog calling abundance and diversity was recorded in spring, during unregulated flows, than in summer, during the environmental water delivery. Modelling of the environmental variables determined that higher flows and emergent vegetation were significant predictors of frog calling richness and calling abundance in 2012. Therefore, calling in spring may have been associated with more inundated emergent vegetation. Most Murray-Darling Basin frog species prefer inundated vegetation to breed (Jansen and Healey 2003; Wassens and Maher, 2011) and many studies have found aquatic vegetation to be a habitat predictor for the presence and/or abundance of frogs (Healey *et al*. 1997; Hazell *et al.* 2001; Jansen and Healey 2003; Lemckert *et al.* 2006; Lane *et al.* 2007; Wassens and Maher 2011). For example, Wassens and Maher (2011) observed that frog breeding in the Lachlan River catchment occurred at sites with a higher percentage of aquatic vegetation than non-breeding sites. Vegetation is important for frog breeding as it performs a number of habitat functions for frogs and tadpoles such as; providing sites for calling and attachment of eggs, a food source through input of organic matter and a substrate for the growth of biofilms, and refuge and protection from predators (Anstis 2002; Lane *et al.* 2007; Wassens and Maher 2011).

Comparison with previous summers

Frog calling abundances varied significantly across all years for the three wetland types assessed. Summer 2012 (environmental water) had the lowest frog calling abundance and frog occupancy rates than all other years (across all wetland types), whereas 2010 had the highest frog calling abundances and occupancy rates. The summer of 2010 experienced the highest flow event (compared to other years surveyed) which inundated many dry wetlands (Hoffmann *et al*. 2014). Higher frog calling abundances and occupancy rates in 2010 were especially prominent in temporary wetlands and managed wetlands, which may reflect differences in the habitat available at each wetland type. Temporary and managed wetlands may have more fringing vegetation available to be inundated as a result of the higher flows, whereas permanent wetlands may have less available vegetation due to stable water levels constraining emergent vegetation to a narrow riparian band (van der Valk *et al.* 1994; Blanch *et al.* 2000). Whilst parameters of different wetland types were not considered in this study, work undertaken elsewhere in the Murray-Darling Basin showed that most frog species were found at higher rates in temporary sites that had higher percent cover of emergent and submerged vegetation compared with permanent sites (Wassens and Maher 2011). Nevertheless, permanent wetlands are important refuges in low flow periods (Wassens *et al.* 2008; Wassens 2010). During the high flows in summer 2010, frogs may have moved from permanent sites to newly inundated areas nearby with more preferred habitat, as some species have been observed to move up to 1 km away from the water's edge (Gonzalez *et al.* 2011).

The low calling abundances and occupancy rates detected in 2012 during the environmental watering may be in part due to the timing of the event as it followed two years of high flows. The prolonged high flows preceding the environmental watering may have led to a 'drowning and dying off' of vegetation around the riparian zone that occurred in the elevations just above pool level, resulting in sparser areas of emergent vegetation in summer 2012 (DEWNR unpublished data).

5.3 Coorong (modelling)

Ruppia tuberosa

For periods where there is information regarding the distribution and abundance of *Ruppia tuberosa* in the South Lagoon (the mid-1970s onwards) there is generally a good correlation between the model output and what was reported in the literature. During the mid to late 1970s when *Ruppia tuberosa* was abundant and widespread in the South Lagoon (Womersley 1975; Geddes and Brock 1977; Gilbertson and Foale 1977; Brock 1979; Brock 1981) the modeled probability of sediment propagule bank replenishment was high, except for two periods of approximately one year in 1972 and 1977 (Figure 41). These relatively short periods when there was a low probability of replenishment probably had little impact on the population dynamics of *Ruppia tuberosa* because this species has a persistent seed bank (i.e. not all of the seed in the sediment seed bank germinates at one time) (*sensu* Thompson and Grime 1979) and the unfavourable periods were followed by extended periods of favourable conditions when there was a high probability of propagule bank replenishment (Figure 41).

During the 1980s *Ruppia tuberosa* was still abundant and widespread in the South Lagoon of the Coorong (Paton 1982; Geddes and Butler 1984; Geddes 1987); however, the duration of the periods of hydrological conditions that resulted in low probability of propagule bank replenishment increased and periods of high probability decreased (Figure 41). The periods of high probability of propagule bank replenishment, whilst relatively short, appeared to be sufficient to maintain the *Ruppia tuberosa* population.

A further increase in the duration of unfavourable hydrological conditions was observed in the 1990s, with only three out of 10 years having hydrological conditions that would have resulted in a modeled probability greater than 25% of propagule bank replenishment (Figure 41). *Ruppia tuberosa* was widespread and abundant during the early 1990s (Leary 1993; Paton 1996; Nicol 2005), which corresponds to a period of high probability of propagule bank replenishment (Figure 41). However, by the late 1990s the distribution and abundance of *Ruppia tuberosa* was showing signs of decline particularly at the southern end of the South Lagoon (Freebairn 1998; Paton 2000; Nicol 2005; Whipp 2010), which corresponded with a period of modeled low probability of propagule bank replenishment (Figure 41).

From 2001 to 2011, the modeled probability of *Ruppia tuberosa* sediment propagule bank replenishment was lower than 25% for the South Lagoon, except in 2001 in the northern 30 km of the lagoon (Figure 41). This period corresponded with a sustained decline of *Ruppia tuberosa* distribution, abundance and propagule bank and by 2011 plants were absent from all but the most northerly section of the South Lagoon and the propagule bank was extremely depauperate (Paton 2001; Paton *et al.* 2001; Paton 2002; Paton 2003; Nicol 2005; Paton 2005a; Paton 2005b; Paton and Rogers 2008; Brookes *et al.* 2009; Whipp 2010).

The 2010-11 flood resulted in favourable hydrological conditions for *Ruppia tuberosa* in the South Lagoon; therefore, higher modeled probability of sediment propagule bank replenishment (Figure 41). Frahn *et al.* (2012) reported widespread but sparse occurrence of *Ruppia tuberosa* in the South Lagoon between Parnka Point and Salt Creek in December 2011. The abundance of *Ruppia tuberosa* when sampled in December 2011 was much lower than reported in 1980s, 1990s and early 2000s (Frahn *et al.* 2012). This was not surprising given the unprecedented period of poor hydrological conditions in the ten years prior to sampling, which needs to be taken into consideration when interoperating future model outputs (i.e. high modeled probability of sediment propagule bank replenishment may not result in widespread and abundant *Ruppia tuberosa* in the South Lagoon).

Model simulation for 2011 suggested that there was little benefit in providing environmental water; however, there was a benefit to *Ruppia tuberosa* populations by providing environmental water; in 2012 (Figure 42). It is worth noting that the water provided in 2012 was in addition to an unregulated flow and it is unlikely the volumes of environmental water available will provide any benefit to *Ruppia tuberosa* in isolation. The years when there was a high probability of propagule bank replenishment corresponded to years when there was an unregulated flow of sufficient duration that resulted in barrage outflows during late spring and early summer. Nevertheless, the volumes of environmental water available could be delivered during late spring and early summer to maximise benefits for *Ruppia tuberosa* by slowing the rate of water level decline during this period.

Fish habitat

Salinities in the Coorong are highly variable, and strongly driven by freshwater flows from the Murray River and tidal seawater exchange through the Murray Mouth (Geddes and Butler 1984; Joehnk *et al.* 2014). Typically, there is a strong north to south gradient with increasing salinities, which influence the distribution, abundance and assemblage structure of fish species (Ye *et al.* 2012; Livore *et al.* 2013). Fish habitat modelling using salinity tolerance threshold (LC_{10}) of key species provides a simplified preliminary assessment of the probability and extent of the suitable fish habitat in the Coorong, subject to barrage flow releases with or without environmental water deliveries.

For periods where there is information available regarding the distribution of the relevant species in the Coorong (the 1980s onwards) there is generally a good correlation between the model output and what was reported in the literature or through the Lakes and Coorong commercial fisheries data. For example, the modelled fish habitat reduction due to salinity increase particularly in the southern part of the Coorong during the drought periods well aligned with findings from fish and habitat studies in the Coorong during the 1982 drought (Geddes and Butler 1984) and the more recent millennium drought from 2006-2009 (Noell *et al*. 2009; Ye *et al.* 2011a). On the other hand, the range and habitat extension for a number of fish species following the restoration of Murray River inflows to the Coorong have also been illustrated in various field studies in 1983-84 (Geddes 1987) and post 2010-11 flood (Livore *et al.* 2013; Ye *et al.* 2013c).

Results of the daily threshold analysis are not significantly different from the annual exceedance probability analysis. However, habitat suitability in Figure 43 gives a more gradual picture. While the threshold analysis can give a good impression on the reach of a certain salinity threshold or fish habitat, it is not suitable to show intermediate regions of suitability. It always gives a binary (yes or no) output in contrast to the gradual habitat probability shown in Figure 43. The annual habitat probability is able to give a more detailed picture summarising changes in salinity and thus habitat suitability of an annual cycle. Like in the case of the *Ruppia* response model it could be extended in future to include likewise life cycle information and thus deliver an even finer picture of habitat suitability.

The evaluation of the effect of 2012-13 environmental watering on the annual habitat suitability suggested that only three out of the seven species, mulloway, Tamar goby and yelloweye mullet, were benefited by environmental watering (i.e. scenarios 2 and 3) due to changed salinity in the South Lagoon for 2012-13. For the other five species, the simulated salinity levels were below their thresholds throughout the Coorong during this year given barrage releases were moderately high (~4,500 GL.year^{-1}) in 2012-13 (Livore *et al.* 2013). If similar amounts of environmental water were delivered during the drought/low flow years, when most of the southern part of the Coorong became too saline for most of fish species, the effect on habitat change would have been larger thus more tangible benefit shown on fish habitat improvement. Despite this, the simulation modelling for 2012-13 demonstrated a significant impact on habitat suitability for three fish species with lower salinity tolerances by withholding/delivering environmental water in the South Lagoon, where habitat suitability is generally much smaller than in the North Lagoon. For example, mulloway, as the most susceptible fish species in terms of salinity tolerance in this study, there would have been up to 13% reduction in habitat suitability and 30 km habitat contraction if no environmental water were delivered to the Coorong. With Commonwealth environmental watering, the impact was alleviated to 3% suitability reduction and 7 km contraction.

6 CONCLUSIONS AND LEARNINGS

6.1 Main channel

Larval fish

Through the information that has been gathered since 2005 and with the more recent addition of environmental water delivery in summer it is clear that larval fish assemblages in the Lower Murray River are significantly affected and shaped by flow. If timing, magnitude and duration of environmental water deliveries are appropriate and adequate, a response in flow-cued spawning species is expected. The delivery of environmental water seems to have supported an extended presence of golden perch larvae in the Lower Murray River, by stimulating local spawning and/or facilitating larval drift from upstream sources. Furthermore the source of the environmental water may also play an important role in shaping not only the larval fish assemblage, but also the zooplankton assemblage that support fish larvae and many other functional groups such as some macroinvertebrates (e.g. freshwater shrimp and yabbies).

Golden perch larvae may have selective feeding habits. In order to support their needs during this critical life stage a better understanding of their diet in the early life histories is required. Building knowledge through further research will eventually lead to a better understanding of what conditions determine recruitment success of this species.

Food resources for larval fish

Despite consuming a broad range of microorganisms, different species of larval fish have different diets. This indicates that larval fish are selective in their use of resources. Large changes occur in the community composition of eukaryotic microorganisms in response to environmental changes, whether natural such as seasonal shifts, or perturbations such as environmental flows. This will influence the availability of food resources for larval fish, and a better understanding is needed of the changes in eukaryotic microorganism communities in response to these changes.

Supplying these needs will be critical to the successful growth and development of larval fish. The results obtained from this study show that the techniques to undertake such investigations are available, but the ability to interpret such events will be greatly enhanced by monitoring responses to a range of watering events that enable patterns to be identified.

Dissolved and particulate material transport

The modelling outputs from the study suggest that the flow regimes supported by Commonwealth environmental watering increased the transport of dissolved and particulate matter through the Lower Murray River, Lower Lakes and Murray Mouth. Whilst the influence of environmental flow on concentrations was only evident for salinity, environmental flow can significantly influence the transport and export of dissolved and particulate material. For some parameters, additional exports were only achieved because two sources of environmental water were provided, highlighting the importance of supplementary water provisions to the region. The increased transport of this material associated with environmental watering will play important functional roles for the studied ecosystems and the nearshore environment, providing habitat through reduced salinity levels and increasing productivity through the provision of resources.

6.2 Wetlands

Lateral movements of fish

A highly abundant and diverse fish assemblage was recorded moving between the main river channel and wetlands during this study period. The structure of fish assemblages moving laterally was highly variable among flow phases at each wetland, and such differences were not consistent among wetlands. Such variability reflected the spatial differences in the physical and hydraulic attributes among the two wetlands examined. Furthermore, movements of most species at each wetland during each flow phase were generally bidirectional and demonstrated no clear, consistent pattern relative to changes in flow conditions.

The variability in the structure of fish assemblages moving laterally could be explained in part by their hydraulic characteristics. However, the high variability among wetlands demonstrates that different wetlands respond in different ways to changes in the magnitude, timing, duration and frequency of flow delivery. Such variability highlights the need for individual assessment of wetlands prior to the management interventions to support lateral fish movement.

Furthermore, the timing of environmental water delivery in 2012-13, did not appear to enhance reproduction and the recruitment success of common carp. Evidence compiled in the present study indicated that >90% of the small juvenile common carp recorded moving were derived from spawning events that occurred prior to the delivery of the environmental flow pulse. Hence, there is no evidence to suggest that these key demographic processes for common carp benefited from the delivery of environmental water in December 2012.

Frog response

Overall, a breeding response was recorded for eight wetland frog species in summer 2012. Male frog calling response in summer 2012 (during the environmental watering) was lower than spring of 2012 and previous years. Analysis of data from previous years' studies indicates that there may have been less vegetation around the riparian zone following high flow events in 2010-2011. Thus, the extent of inundation of vegetation as a result of the 2012-13 environmental watering events may have been affected by the existing condition and availability of vegetation. The importance of inundated vegetation as breeding habitat for frog species in the Lower Murray River is well established. Hence, environmental flows that influence inundation of fringing vegetation would affect breeding habitat availability.

6.3 Coorong (modeling)

The developed simple *Ruppia* and fish habitat models together with the operational hydrodynamic model (CHM v2.1) are capable of answering the question of impact on its macrophyte and fish habitats of additional or withdrawal of water to the Coorong. The combined modelling system could be easily used not only to hindcast the effect of environmental watering but also allows for future scenario/impact modelling of the effect of timing and quantity of environmental water on the ecosystem of the Coorong.

Ruppia tuberosa

The correlation between *Ruppia tuberosa* distribution and abundance reported in the literature and modeled probability of sediment propagule bank replenishment shows there is potential for the model to be used as a management tool. The model could be used to determine volume and timing of barrage outflows required to maintain viable populations of *Ruppia tuberosa*. Furthermore, there is potential to use the model to investigate scenarios that may result in "false starts" (i.e. favourable conditions for seed germination and turion sprouting followed by unfavourable conditions for life cycle completion).

Fish habitat

The preliminary fish habitat modelling based on salinity tolerance provides a simple tool to evaluate the habitat suitability and potential distributional extent of key fish species in the Coorong. The model output largely complied with available field data. The model could be used as a first cut to determine volume of barrage outflows to maintain the extent of suitable habitat for key fish species along the Coorong during the cold and warm periods. The 2012-13 environmental watering provided benefits for fish in the Coorong by improving habitat suitability up to 13% and increasing habitat extent up to 30 km. Nevertheless, the current analysis only provides a preliminary understanding of the potential effects of environmental watering on fish populations in the Coorong. Future fish response and habitat modelling could include life-history information of different species/guilds, which will provide a more comprehensive tool to evaluate and simulate environmental flow effect on their population dynamics in the Coorong.

7 RECOMMENDATIONS

7.1 Main channel

Larval fish

Environmental water management

Seasonal flow regimes are necessary to achieve ecosystem response and a first and strong presence of native large-bodied larval fish has been consistently present from late October to December due to the seasonal flows. A pulse of water with the appropriate timing and adequate magnitude and duration may enhance and extend the presence of these native fish larvae which may lead to enhanced recruitment in the Lower Murray River. Along with timing, magnitude and duration, the source and continuity of the water delivered can play an important role in the outcomes achieved. This study has shown that zooplankton assemblages were influenced by species that had originated in other parts of the basin and through the continuity managed their way down the river and contributed to local ecosystem.

Future monitoring/ research

In order to effectively manage river flows to generate the best possible outcomes for the riverine ecosystem it is imperative that a broad understanding of the processes that drive the ecosystem is obtained. Amongst those processes is native fish reproduction understood as "*the natural process among organisms by which new individuals are generated and the species perpetuated*". Future research should focus on the identification and characterisation of the environmental and biological conditions needed and how environmental watering and flow management could help to achieve these conditions, to facilitate reproduction success and restore sustainable and resilient native fish populations. This knowledge will inform the integrated management of flows at different scales with certain predictability of expected outcomes.

Food resources for larval fish

Environmental water management

The molecular analyses identified a significant range of eukaryotic microorganisms present in the river, and provided a basis for describing the changes in community composition in response to environmental influences including environmental flows. The molecular identification of phytoplankton and zooplankton provided similar patterns of responses to that obtained from traditional microscopic enumeration. Unlike the traditional techniques, the molecular methods reported on a broad range of microeukaryotes including protists, ciliates, fungi, algae, and zooplankton which is more difficult to achieve using the traditional methods. These findings demonstrate that the molecular techniques are sufficiently mature to be applied reliably in monitoring programs.

There are benefits in monitoring microorganisms to assess the influences of environmental conditions, including short term events such as environmental flows, on the ecology of river systems. One is that communities of eukaryotic microorganisms respond quickly to change, so providing patterns in community composition that change at a similar temporal and spatial scale as the perturbations. This helps to directly link community changes to the perturbations so that relationships between community composition and environmental conditions can be more readily identified.

In addition microeukaryotes cover a broad range of trophic levels including autotrophs, herbivores, carnivores, detritivores, and saprophytes. Current river health assessments based on narrow groups of indicator organisms are difficult to generalise across the biota and rarely identify environmental conditions that can be managed to improve conditions across trophic levels. The response patterns of microeukaryote communities provide a tool for assessing how the mix of autotrophic and heterotrophic pathways that underpin energy and material cycles in aquatic ecosystems respond to flow and water quality. Such assessments more comprehensively capture diversity across a range of trophic levels, better describing the extent to which groups performing different ecosystem services are influenced.

The molecular tools enable better understanding of the spatial and temporal changes found in the microeukaryotic assemblages providing a comprehensive view of the state of a river ecosystem from the perspective of the major influencers of water quality and food web connections.

Future monitoring/ research

There is a need to monitor responses in the communities of microeukaryotes to flow changes by sampling before, during, and after an environmental watering event. Preferably measurements would also need to be made at intervals throughout the natural flow regime to describe changes associated with seasonal shifts and to meteorological, water quality and flow events. The molecular techniques that have been devised are fit for purpose, but could be further improved by sequencing multiple genes instead of just one, and by adjusting the methodology as technological improvements are made. Linking changes in microeukaryotic communities with environmental conditions requires that suitable measurements are being made to appropriately characterise the environment.

Dissolved and particulate material transport

Environmental water management

Based on insights provided by this study and previous studies, including Aldridge *et al*. (2013), the following points could be used to help guide future environmental water use:

- environmental watering 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 material;
- environmental flow deliveries during extended low flow periods are likely to have greater impacts on salt and nutrient concentrations 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;

- maximum exports of dissolved and particulate material from the Murray Mouth are likely to be achieved by delivering environmental water during periods of low oceanic water levels (summer). However, this may reduce water availability at other times, increasing the import of material from the Southern Ocean during those times. In contrast, delivery of environmental water to the Lower Murray River 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;
- net export of dissolved and particulate material can be achieved when discharges above threshold levels are provided. Whilst, these discharge thresholds are currently unknown and likely differ with seasonal changes in downstream water levels, supplementary water sources are important in providing adequate flows to export material from the system;
- flows during winter may result in limited assimilation of nutrients by biota (slower growth rates), whilst deliveries during summer could increase the risk of blackwater events and cyanobacterial blooms, depending on hydrological conditions. Flows during spring are likely to minimise these risks, but also maximise the benefits of nutrient inputs (e.g. stimulate productivity to support larval survival);
- multiple watering events in a given year could be used to meet different ecological objectives. For example one event in spring could be provided to increase nutrient assimilation, followed by a subsequent event to export material to downstream ecosystems.

Future monitoring/research

Continued refinement of the model used in this study will further improve its capacity to evaluate the influence of environmental water deliveries on dissolved and particulate material concentrations and transport in the Lower Murray. In the future, such modelling approaches could also be used in planning for flow deliveries, by assessing the potential benefits/disbenefits of various watering actions. Without such assessments it is difficult to reach general recommendations about optimal use of environmental water for the transport of dissolved and particulate material as the Lower Murray is a hydrologically complex system. For planning, the relationship between season and discharge thresholds could be investigated for the purposes of setting flow targets for the delivery of environmental water. Continued refinement of the model used in this study will further improve its capacity to assess the response of dissolved and particulate material in the Lower Murray to environmental water. This includes the continued collection of appropriate water quality data.

7.2 Wetlands

Lateral movement of fish

Environmental water management

Whilst seasonal flow regimes are important in maintaining the ecological integrity of freshwater systems, this study suggests that within-channel flow management may present an opportunity to prolong and potentially enhance the lateral movements of native fish of the Lower Murray River. The direct influence of environmental flows on lateral fish movement appears to be highly variable among wetlands due to the high variation in the physical, biological and hydrological attributes among wetlands. However, the influence of the environmental flow pulse is likely to be most pronounced in the wetlands upstream from Lock 1, whereby water levels, which ultimately affect lateral connectivity, are directly correlated with flow discharge. For example, at Overland Corner, maintained within-channel flows of >15,000 ML day^{-1} facilitated the lateral fish movements. As flow decreased to <10,000 ML day^{-1}, i.e. during the Low flow phase, the size and diversity of laterally moving fish assemblages decreased. We speculate that if monitoring continued into autumn of 2013, lateral movements would cease completely due to further contraction in wetland size and increasing salinity within the remaining wetland habitat. As such, maintenance of moderate within-channel flow is essential to facilitate access for fish to these sheltered and food-rich habitats.

Nonetheless, the high spatial variability in the structure of fish assemblages attempting to access and exit wetlands demonstrates that different wetlands will respond in different ways to changes in the magnitude, timing, duration and frequency of flow delivery. This highlights the need for individual assessment of wetlands prior to the management interventions to support lateral fish movement.

Future monitoring/research

Observations of the lateral movement patterns of native and invasive fish in each region and how they were influenced by flow and non-flow related hydrological stimuli, provide an invaluable comparative database for future research and monitoring. The results of the present study suggest that the direct benefits of the environmental flow pulse to the lateral movement of native fish populations are likely to be most apparent in the wetlands, upstream from Lock 1. As such, future monitoring of the direct influence of changes in flow delivery on lateral fish movement should focus on wetlands in this region. Moreover, as sampling for each flow phase during the present study occurred over a 3-day period, it is not unreasonable to assume that large-scale migrations of key species in response to changes in flow may have been missed. As such, future assessments should place a greater emphasis on understanding the temporal changes in fish assemblage structure inside the wetland, rather than focusing so strongly on the movement patterns occurring in the river/wetland connection passage. Nonetheless, the high spatial variability in the structure of fish assemblages moving in and out of wetlands demonstrates the need to understand the effect of flow on lateral fish movement at other key wetlands in the Lower Murray River.

Frog response

Environmental water management

Inundation of riparian and floodplain vegetation, through an increase in water levels, is important in providing frog breeding opportunities during spring and summer. This may be achieved through:

- the delivery of environmental water that increases within river channel flows,

- localised pumping into temporary wetlands and floodplain areas,
- weir pool raising events, and/or
- unregulated flows.

In addition to the importance of inundated habitat for frog breeding, the management of environmental water needs to consider the duration of the event and the development times of tadpoles.

Future monitoring/research

Further studies of frog response as a result of environmental watering should include more targeted monitoring of recruitment, such as tadpole presence and metamorphosis to provide futher insight into the effect of envrionmental watering on breeding success. Additionally, there are other factors that may influence breeding response and recruitment success such as predation by fish and hydroperiod. These require further investigation.

7.3 Coorong (modelling)

Ruppia tuberosa

Environmental water management

The volumes of environmental water available will have little (if any) benefit on *Ruppia tuberosa* populations in the South Lagoon unless delivered in conjunction with an unregulated flow event. Even in this situation the unregulated flow will need to be of sufficient duration to provide barrage outflows during November and early December and environmental water used to manage flow recession to reduce the rate of water level decline in the South Lagoon to reduce the risk of stranding.

Future monitoring/research

The current R*uppia* response model together with the operational hydrodynamic model (CHM v2.1) provides a useful tool for evaluation and scenario modelling to assess the effect of environmental watering on *Ruppia*. Additional information regarding the salinity thresholds for flowering and seed production will improve the ecological response model and the environmental triggers for flowering in *Ruppia tuberosa* will inform environmental water management. Continued monitoring of the propagule bank and extant population of *Ruppia tuberosa* in the Coorong will improve model calibration.

Research into the effects of sub-lethal salinity of submergent plant species that were historically present in the Coorong (e.g. *Ruppia megacarpa, Lamprothamnium macropogon, Lepilaena cylindrocarpa*) could result in similar models being developed for these species to evaluate the feasibility of reintroduction. Furthermore, information regarding the salinity and water level preferences of fringing species (samphire and salt marsh species) could be used to develop ecological response models to assist in the management of fringing habitats.

Fish habitat

Environmental water management

Freshwater inflow is pivotal in maintaining estuarine fish habitat and populations in the Coorong. Environmental water delivery could provide an incremental benefit by reducing salinity levels along the north to south gradient in the Coorong, often leading to an extension of habitat into the South Lagoon particularly for species with less salinity tolerances. Environmental watering during the summer months or in years with less barrage flows and higher salinity will provide a much larger effect of habitat change. Flow delivery during late spring/summer is important as this period corresponds to the spawning and recruitment season of most estuarine fish species in the Coorong. Environmental flows could potentially help in maintaining favourable salinity gradient, enhancing productivity and improving connectivity to facilitate fish recruitment.

Future monitoring/research

The preliminary fish habitat model together with the operational hydrodynamic model (CHM v2.1) provides a useful tool for broad evaluation and scenario modelling to assess the effect of environmental watering on fish habitat suitability in the Coorong. In order for more comprehensive analysis and evaluation of the effect of environmental watering on fish habitat and populations, more complex fish models are required. These could be extended from the existing fish habitat model by including the information of life-history and flow/salinity effects on key life stages and processes of different species/guilds. Therefore it's important to identify such information and fill knowledge gaps. Further investigation and data gathering could be achieved through ecological monitoring of the effects of flow, including environmental water delivery, on fish assemblages and habitat in the Coorong. Such knowledge will not only inform the future development of fish-flow response models but also contribute to the system understanding of the flow related ecology, which will underpin environmental water management to maintain and improve ecosystem health and resilience in the Coorong.

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9 APPENDICES

Appendix I. Site information for sampling tasks: GPS coordinates of sampling sites.

Larval fish and food resources for fish

Site	Region	South	East
LK-1	Gorge	-34.3847	139.6225
LK-6	Floodplain	-34.0171	140.8738
LK-6A	Floodplain	-34.0142	140.8581

Dissolved and particulate material transport

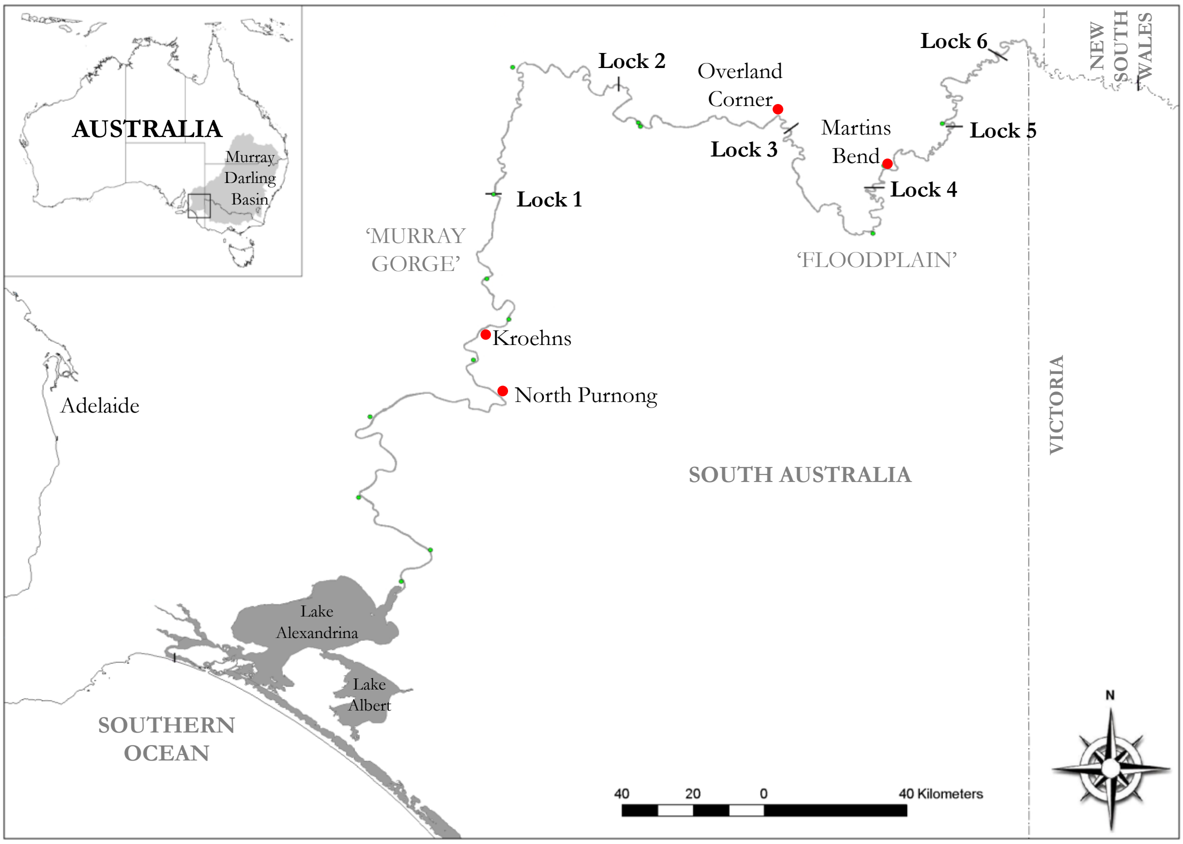
Sampling sites

Water body	Site name	Site ID	X	Y	Zone
Lower Murray River	Blanchetown (Lock 1)	RM-1	373768.9	6195837	UTM 54S
	Wellington	RM-3	353152.5	6086654	UTM 54S
Lower Lakes	Lake Albert Middle	LAb2	346724.2	6056213	UTM 54S
	Lake Alexandrina Opening	LAx1	348468.4	6080369	UTM 54S
	Lake Alexandrina Middle	LAx4	331761.4	6077836	UTM 54S
	Point McLeay	LAx5	324379.4	6068672	UTM 54S
Coorong	Goolwa Barrage	C - 1	302100.7	6066302	UTM 54S
	Murray Mouth	C - 5	308001.3	6063098	UTM 54S
	Ewe Island	C - 9	315228.9	6062110	UTM 54S
	Mark Point	C - 11	325761.5	6054914	UTM 54S
	Parnka Point	C - 12	355250.6	6025735	UTM 54S

Additional sites and sources

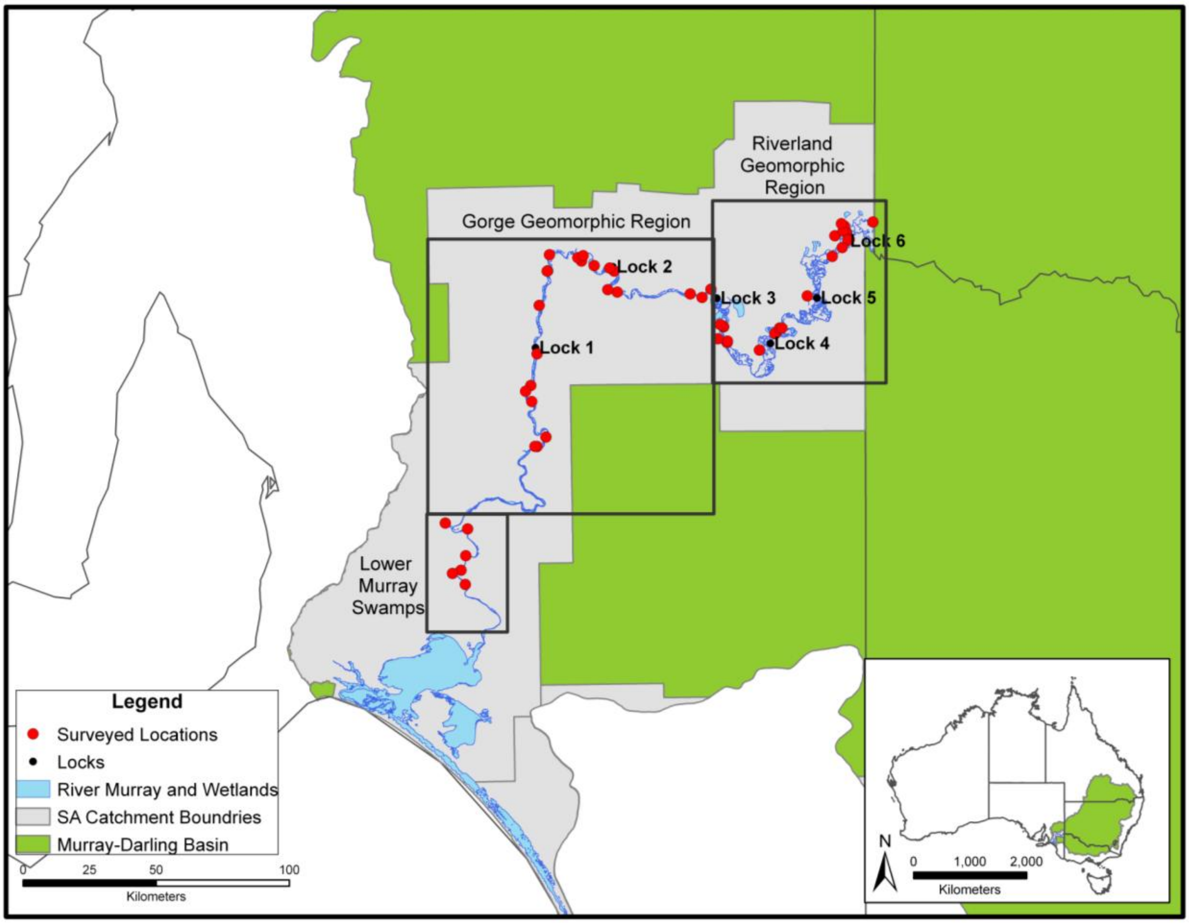
Data source	Sites	Sampling dates
CEWO	Blanchetown	30/10/2012, 29/11/2012, 13/12/2012, 10/01/2013, 01/02/2013, 28/02/2013, 03/04/2013
CEWO	Coorong	29/10/2012, 28/11/2012, 12/12/2012, 09/01/2013, 31/01/2013, 27/02/2013, 02/04/2013
SA Water	Blanchetown and Wellington	28/06/2012, 26/07/2012, 23/08/2012, 20/09/2012, 18/10/2012, 14/11/2012, 12/12/2012, 10/01/2013, 7/02/2013, 7/03/2013, 4/04/2013, 30/04/2013, 29/05/2013, 26/06/2013
Murray Futures (DEWNR/EPA)	Lower Lakes	18/07/2012, 14/08/2012, 10/09/2012, 09/10/2012, 07/11/2012. 13/12/2012, 10/01/2013, 14/02/2013 19/03/2013 16/04/2013 28/05/201312/06/2013
Murray Futures (DEWNR/EPA)	Coorong	12/09/2012 13/12/2012, 13/02/2013, 18/03/2013

Lateral movements of fish



Site	Region	South	East
Overland Corner	Floodplain	34.093	140.203
Martins Bend	Floodplain	34.176	140.371
Kroehns	Gorge	34.424	139.345
North Purnong	Gorge	34.502	139.354

Frog response



Geomorphic Region	Wetland	Easting	Northing
Gorge	Akuna Wetland	430910	6218342
	Brenda Park Lagoon	377297	6227012
	Devon Downs - Connecting creek	373327	6161037
	Devon Downs North	376675	6164600
	Devon Downs South	372570	6161089
	Hart Lagoon	403506	6219042
	Markaranka	394824	6228970
	Molo Flat	390001	6230698
	Morgan Conservation Park	377984	6233094
	Morgan's Lagoon	370998	6183989
	Murbpook Lagoon	374135	6214060
	Nigra Creek	402327	6226919
	Nikalapko	388640	6231958
	Noonawirra	369033	6181809
	Overland Corner	438691	6220152
	Ramco Lagoon	399896	6219959

	Reedy Creek	338875	6132277
	Schillers Lagoon	400609	6228062
	Scotts Creek	377173	6226939
	Sugar Shack	371356	6177928
	Sweeney's Lagoon	373335	6195845
	Taylor's Flat	390681	6232711
	Wigley Reach	435317	6217012
Floodplain (Riverland)	Beldora Wetland	444765	6200066
	Blackfellows Creek	443449	6205963
	Bookmark Creek	474900	6217601
	Bunyip Waterhole	489666	6241367
	Car Park Lagoons	456883	6197255
	Causeway Lagoon	462908	6203457
	Chowilla (Campsite 15)	488842	6243825
	Chowilla Creek/Bridge	489496	6241430
	Coppermine	485256	6240211
	Germein Swamp	443295	6206287
	Lake Littra	499558	6245360
	Little Duck Lagoon	462699	6203506
	Lock 6 Depression	490632	6238888
	Martins Bend	464500	6205531
	Monoman Creek/Bridge	488320	6242001
	Murtho Park/Wiela	484237	6232444
	Mussel Lagoons	442181	6206828
	Pilby Lagoon	490168	6238610
	Settlers Bend	465325	6205538
	Spectacle Lakes	444884	6200664
	Weila	487999	6235748
	Werta Wert	487706	6244737
	Yatco Lagoon	441244	6201482
Swamplands (Lower Murray Swamps)	Jury Swamp	346550	6120022
	Riverglades	344782	6114560
	Rocky Gully	341562	6113366
	Swanport	346398	6109197
	Wall Flat	347273	6130103

Appendix II. Pairwise tests of larval fish assemblages throughout 2012-13 sampling season for sites LK-1, LK-6 and LK-6A. Figures in bold indicate significant differences.

Sites	t	P(perm)	perms
LK-1 vs LK-6	2.4182	**0.001**	998
LK-1 vs LK-6A	2.5652	**0.001**	999
LK-6 vs LK-6A	1.5045	0.061	999

Appendix III. PERMANOVA results of zooplankton assemblage compared across 10 trips, 3 sites and 2 diel (day v. night). Bold indicates significant P values.

Source	*df*	MS	Pseudo-F	P(perm)
Trip	9	13086	6.7225	**0.001**
Site	2	5800	2.9799	**0.013**
Diel	1	766	1.4462	0.149
Trip x Site	18	1946	5.1850	**0.001**
Trip x Diel	9	628	1.4349	0.072
Site x Diel	2	204	0.4668	0.934
Trip x Site x Diel	18	437	1.1658	0.146
Res	120	375		

Appendix IV. Pair-wise comparison of zooplankton assemblages between sites across all trips. Bold indicates significant P values.

Trip	**1**		**2**		**3**		**4**		**5**	
Sites	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
LK-1, LK-6	2.52	**0.005**	2.58	**0.002**	2.48	**0.003**	2.54	**0.002**	2.38	**0.002**
LK-1, LK-6A	2.59	**0.009**	2.51	**0.001**	2.62	**0.003**	2.77	**0.003**	2.40	**0.006**
LK-6, LK-6A	1.40	**0.004**	1.27	0.075	0.98	0.481	1.17	0.143	1.33	**0.026**
Trip	**6**		**7**		**8**		**9**		**10**	
Sites	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
LK-1, LK-6	2.46	**0.002**	2.68	**0.001**	3.75	**0.004**	4.17	**0.007**	2.65	**0.004**
LK-1, LK-6A	2.73	**0.001**	3.11	**0.003**	4.11	**0.001**	3.73	**0.002**	2.85	**0.004**
LK-6, LK-6A	1.22	0.081	0.97	0.516	1.28	0.084	1.70	**0.003**	1.53	**0.011**

Appendix V. Results of the two-factor PERMANOVAs for a) Overland Corner and b) Kroehns, comparing fish assemblages (4th root transformed) between flow phases and direction of fish movement (e.g. in or out of wetlands from the main channel) in the Lower Murray River in 2012-13. Bold p values are significant.

a) Overland Corner – Floodplain region

Source	df	MS	Pseudo-F	P(perm)
Flow phase	5	4939	14.175	**0.001**
Direction	1	1405.6	4.034	**0.006**
Flow phase x Direction	5	597.08	1.714	0.059
Residuals	24	348.42		

b) Kroehns – Gorge region

Source	df	MS	Pseudo-F	P(perm)
Flow phase	5	4022.3	17.76	**0.002**
Direction	1	75.56	0.334	0.834
Flow phase x Direction	5	433.23	1.913	0.539
Residuals	24	226.5		

Appendix VI. Comparison of the size distributions (TL, mm) for key fish species detected moving into and out of wetlands at a) Overland Corner and b) Kroehns (BOTTOM) in 2012-13. Shown for each direction, are sample sizes (n), mean (±SD) total fish length and the Kolmogrov-Smirnov Z statistic. Where significant differences were detected the direction larger fish were moving is also shown. Significant p values highlighted in bold.

c) Overland Corner – Floodplain region

	IN		OUT				***Directional movement of larger fish***
Species	**n**	**Mean ±SD**	**n**	**Mean ±SD**	**KS**	***p***	
Golden Perch	13	174±104	19	130±56	0.71	0.697	-
Common carp	298	62±69	235	56±58	1.39	**0.041**	IN
Carp gudgeon	422	32±7	414	33±7	1.12	0.164	-
Bony herring	206	132±90	218	112±67	1.41	**0.038**	IN
Australian smelt	86	53±16	174	53±15	1.12	0.162	-
Flat-headed gudgeon	183	38±11	195	36±10	1.06	0.209	-

d) Kroehns – Gorge region

	IN		OUT				***Directional movement of larger fish***
Species	**n**	**Mean ±SD**	**n**	**Mean ±SD**	**KS**	***p***	
Golden Perch	63	104±29	46	122±48	1.25	0.088	-
Common carp	454	101±90	479	121±81	2.83	**<0.001**	OUT
Carp gudgeon	470	35±15	473	35±7	0.85	0.459	-
Bony herring	226	162±90	262	202±73	2.9	**<0.001**	OUT
Unspecked hardyhead	289	39±11	287	40±10	0.91	0.381	-
Flat-headed gudgeon	329	37±10	352	37±11	0.54	0.930	-
Australian smelt	150	61±10	166	61±11	0.5	0.964	-

Appendix VII. DistLM sequential results indicating which environmental variables significantly contributed most to the relationship with the multivariate assemblage data clouds (data for fish assemblages moving in and out of wetlands) for each wetland in 2012-13.

	Variable	Pseudo-F	P-value	Cumulative variation explained
Overland Corner	Flow discharge	27.707	**0.001**	0.459
	Conductivity	2.616	**0.042**	0.489
	pH	2.768	**0.023**	0.53
	Dissolved oxygen	2.689	**0.027**	0.568
	Temperature	2.311	**0.042**	0.599
	Wetland surface area	2.532	0.052	0.631
	Relative water level	0.739	0.567	0.64
Kroehns	Relative water level	31.562	**0.001**	0.481
	pH	4.447	**0.003**	0.543
	Dissolved oxygen	5.399	**0.001**	0.609
	Conductivity	4.256	**0.001**	0.656
	Temperature	3.065	**0.021**	0.688
	Flow discharge	1.49	0.22	0.703
	Wetland surface area	0.399	0.789	0.707

Appendix VIII. Results of t-tests comparing frog species richness and frog calling abundance in spring and summer.

	Spring		Summer			
	Mean	Std. Dev	Mean	Std. Dev	t-value	P
Frog species richness	2.348148	1.188412	0.42963	0.63365	4.273546	0.001
Frog calling abundance	42.09259	28.68386	5.883333	13.97757	3.404379	0.005

Appendix IX. Influence of environmental variables on frog species call richness at wetlands in spring and summer 2012.

Variable	Estimate	Std. Error	t-value	P	
(Intercept)	-1.49E+00	1.40E+00	-1.064	0.292	
Flow to SA	7.01E-05	1.72E-05	4.087	<0.001	***
Electrical conductivity	5.86E-05	8.34E-05	0.703	0.485	
Air temperature	-3.36E-02	3.56E-02	-0.944	0.349	
Humidity	-2.12E-02	1.29E-02	-1.643	0.106	
Emergent vegetation	1.50E-02	5.52E-03	2.724	0.008	**
Submerged vegetation	1.49E-01	2.07E-01	0.723	0.472	

Appendix X. Influence of environmental variables on frog call abundance at wetlands in spring and summer 2012.

Variable	Estimate	Std. Error	t-value	P	
(Intercept)	-7.91E-01	1.68E+00	-0.47	0.640	
Flow to SA	1.00E-04	2.70E-05	3.716	<0.001	***
Electrical conductivity	2.31E-05	1.65E-04	0.14	0.889	
Air temperature	-3.78E-02	4.35E-02	-0.87	0.388	
Humidity	-1.23E-02	1.29E-02	-0.955	0.343	
Emergent vegetation	2.74E-02	6.14E-03	4.467	<0.001	***
Submerged vegetation	1.68E-01	2.83E-01	0.594	0.555	

Appendix XI.Post-hoc comparisons of average mean rank abundance of calling male frogs across years at managed wetlands. Significant p values highlighted in bold.

		95% Confidence Interval		
Years	Difference	Lower	Upper	P-value
2005-2006	8.81579	-47.9991	65.63066	1
2005-2009	17.85425	-41.3525	77.06104	1
2006-2009	9.03846	-53.2929	71.36984	1
2005-2010	-1.61278	-45.285	42.05939	1
2006-2010	-10.4286	-58.2513	37.39418	1
2009-2010	-19.467	-70.1082	31.17415	1
2005-2011	46.85865	-0.01525	93.73254	0.050154
2006-2011	38.04286	-12.7205	88.80622	0.417439
2009-2011	29.0044	-24.4225	82.43129	1
2010-2011	48.47143	13.02784	83.91501	**0.000895**
2005-2012	71.13475	27.65375	114.6158	**2.40E-05**
2006-2012	62.31897	14.67073	109.9672	**0.001854**
2009-2012	53.2805	2.80409	103.7569	**0.029199**
2010-2012	72.74754	41.9306	103.5645	**0**
2011-2012	24.27611	-10.9317	59.48387	0.644784

Appendix XII. Post-hoc comparisons of average mean rank abundance of calling male frogs across years at permanent wetlands. Significant p values highlighted in bold.

Years	Difference	95% Confidence Interval		P-value
		Lower	Upper	
2007-2009	8.58929	-27.3094	44.48798	1
2007-2010	6.33333	-24.4819	37.14859	1
2009-2010	-2.25595	-38.1546	33.64274	1
2007-2011	40.8109	13.1167	68.5051	**0.000353**
2009-2011	32.22161	-1.03654	65.47976	0.065371
2010-2011	34.47756	6.78337	62.17176	**0.004748**
2007-2012	36.00735	7.54798	64.46673	**0.00383**
2009-2012	27.41807	-6.47989	61.31603	0.231806
2010-2012	29.67402	1.21464	58.13339	**0.034243**
2011-2012	-4.80354	-29.85	20.2429	1

Appendix XIII. Post-hoc comparisons of average mean rank abundance of calling male frogs across years at temporary wetlands. Significant p values highlighted in bold.

Years	Difference	95% Confidence Interval		P-value
		Lower	Upper	
2010-2011	52.34524	34.52197	70.1685	**0**
2010-2012	49.91667	33.04432	66.78902	**0**
2011-2012	-2.42857	-17.4436	12.58641	1

Appendix XIV. Photographs of frog species recorded in wetlands

Crinia signifera. Common Name: Common Froglet/ Common Eastern Froglet.

Crinia parinsignifera. Common Name: Murray Valley Froglet/ Eastern Sign-bearing Froglet.

Limnodynastes dumerili. Common Name: Eastern Banjo Frog/ Eastern Pobblebonk.

Limnodynastes fletcheri. Common Name: Long-thumbed Frog/ Barking Marsh Frog.

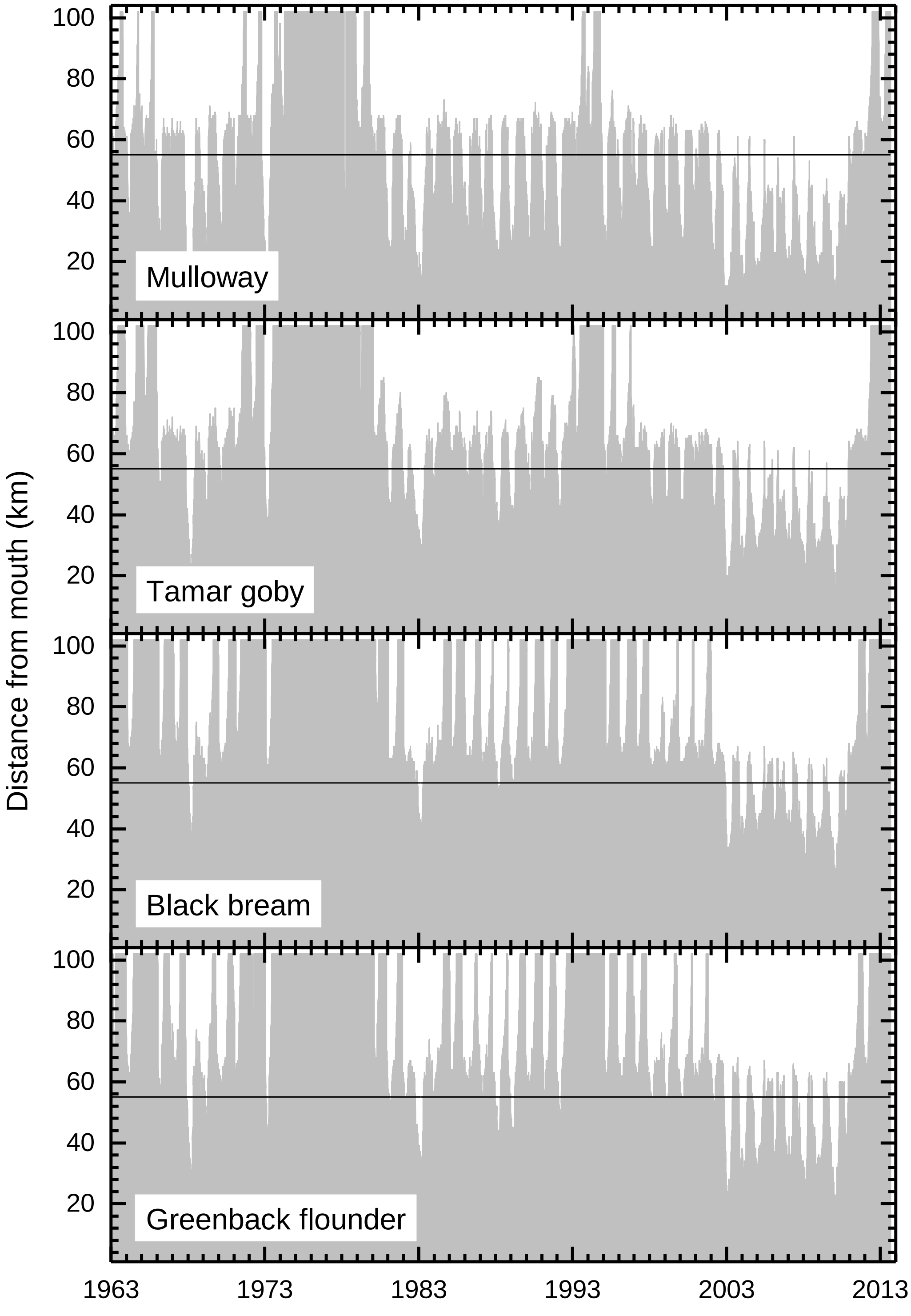
Limnodynastes tasmaniensis. Common Name: Spotted Grass Frog/ Spotted Marsh Frog.

Litoria ewingii. Common Name: Brown Tree Frog.

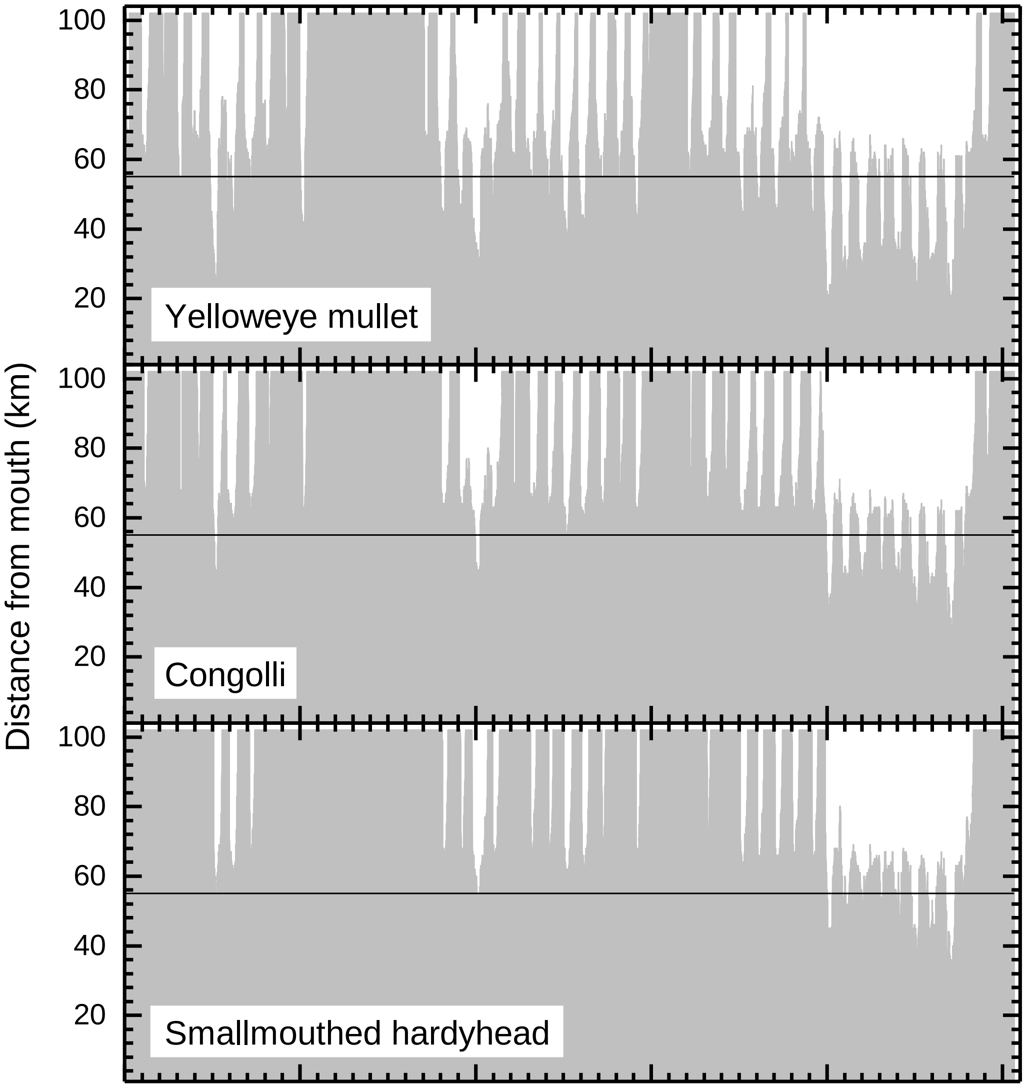
Litoria peronii. Common Name: Peron’s Tree Frog.

Litoria raniformis. Common Name: Southern Bell Frog/ Growling Grass Frog.

Appendix XV



Habitat suitability for fish along the Coorong from mouth (0 km) to Salt Creek (102 km) calculated for the whole simulation period 1963-2013 on a daily basis using salinity tolerance thresholds. The horizontal black line indicates the border between North (0-55 km) and South (55-102 km) Lagoon.



continued: Habitat suitability for fish species along the Coorong from mouth (0 km) to Salt Creek (102 km) calculated for the whole simulation period 1963-2013 on a daily basis using salinity tolerance thresholds. The horizontal black line indicates the border between North (0-55 km) and South (55-102 km) Lagoon.

Acronyms

AHD	Australian Height Datum
CEW	Commonwealth Environmental Water
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEWNR	Department of Environment, Water and Natural Resources
EPA (org.)	Environment Protection Authority
EW	Environmental water
FRM	Flood recruitment model
LK-1	Lock 1
LK-6	Lock 6
LK-6A	Lock 6 anabranch
LMR	Lower Murray River
LFRH	Low-flow recruitment model
MDB	Murray-Darling Basin
NRMB	Natural Resources Management Board
Rim-FIM	River Murray Flood Inundation Model
TLM	The Living Murray
TLMW	The Living Murray Water
SAMDB	South Australian Murray-Darling Basin
SARDI	South Australian Research and Development Institute
TL	Total length

Genetics abbreviations

ALA	a-Linoleic acid
ARA	Arachidonic acid
EPA (acid)	Eicosapentaenoic acid
DHA	*Docosahexaenoic* acid
LA	Linoleic acid
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction