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Monitoring the ecosystem responses to Commonwealth environmental water delivered to the Edward-Wakool river system, 2012-13

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AUTHORSHIP

This project was undertaken as a collaboration among Charles Sturt University, NSW DPI (Fisheries), Murray Catchment Management Authority, Monash University and NSW Office of Environment and Heritage. All authors contributed to project design, implementation and reporting. There was on-going collaboration among team members during the project and internal and external review of the report. The table below lists the lead authors for each section of the report.

Section	Section Title	Lead authors
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9.4	Objective 4 - Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations	Watts, Wooden and Kopf
9.5	Objective 5 - Support breeding and recruitment of native fish	McCasker, Kopf, Wooden, Baumgartner
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EXECUTIVE SUMMARY

This report documents the monitoring and evaluation of Commonwealth environmental watering in the Edward-Wakool system in 2012-13. It provides details of the environmental objectives of the watering actions, study design, indicators, methodology, and an assessment of ecosystem responses to environmental watering with respect to the objectives set by the Commonwealth Environmental Water Office. Results and conclusions from the monitoring and evaluation underpin recommendations for future environmental watering in this system.

Edward-Wakool system

The Edward-Wakool system is a major anabranch and floodplain of the Murray River. It is a complex network of interconnected streams, ephemeral creeks, flood runners and wetlands intersected by irrigation channels. This system has a long history of regulated flows for irrigation, stock and domestic water supply but it is also recognised as having high native species richness and diversity, including threatened and endangered fishes, frogs, mammals, and riparian plants.

Environmental watering in the Edward-Wakool system in 2012-13

Watering options and flow-dependent ecological objectives for 2012-13 for the mid-Murray region were developed by CEWO (2012). Based on the resource availability and the catchment conditions, the ecological management objectives for the mid-Murray region were expected to be in the moderate to wet range. The objective of the moderate range is to ‘maintain ecological health and resilience to improve the health and resilience of aquatic ecosystems’ and the objective for the wet range is to ‘Improve the health and resilience of aquatic ecosystems’ (CEWO 2012).

The possibility of a wet to moderate resource availability scenario meant that ecological objectives for 2012-13 (CEWO 2012) were expressed in terms of *improving* ecological outcomes. However, above average temperatures and below average rainfall in 2012-13 meant that environmental water largely contributed to *maintaining* ecological outcomes, consistent with a moderate resource availability scenario. Our evaluation takes this into account by acknowledging the contribution of environmental water to maintaining ecological outcomes.

Four instream watering actions occurred in the Edward-Wakool system in 2012-13:

1. **Yallakool Creek October to December 2012 environmental watering action.** A watering action in Yallakool Creek commenced on 19 October and finished on 7 December 2012. The CEWO ecological objective for this event aimed to maintain inundation of habitat for Murray cod nests and maintain the flow until cod eggs could hatch and larvae drift downstream. The Yallakool Creek discharge during this event was held in the range of 360 ML/day to 683 ML/day with a median discharge of 526 ML/day. The total volume delivered was 13,620 ML comprising 10,620 ML of Commonwealth environmental water (CEW) and 3,000 ML of NSW environmental water.

2. **Colligen Creek November to December 2012 environmental watering action.** A watering action under water use option 1 occurred in Colligen Creek to promote golden perch and silver perch spawning (CEWO 2012). This watering action involved the delivery of two freshes. The first fresh commenced on 2 November 2012, reaching a peak of approximately 903 ML/day on 8 November 2012. Following the fresh, elevated base flows were maintained in anticipation of the second fresh. The second fresh commenced on 8 December 2012 and finished on 18 December with the flows reaching a peak of approximately 655 ML/day on 11 December. The total volume delivered was 10,261 ML, including 7,261 ML of CEW and 3,000 ML of NSW environmental water.

3. **Yallakool Creek February 2013 environmental watering action.** A watering action in Yallakool Creek under water use option 1 commenced on 2 February 2013 and finished on 22 February 2013. The fresh increased over 3 days to a peak of 430 ML/d on 13 February 2013. The CEWO (2012) ecological objective for this event was to provide opportunities for small bodied fish (instream generalists) to breed. Specifically, the objective was to test if a small (~30 cm) increase in water level can initiate a spawning response in small bodied fish. Secondary objectives of this action were to test whether or not a small water level rise result in the movement of medium/large bodied fish and/or spawning of golden perch. The volume of CEW delivered to Yallakool Creek for this action was 1,796 ML.

4. **Yallakool Creek and Colligen Creek March to April 2013 environmental watering action.** A watering action in Yallakool and Colligen Creeks under water use option 1 commenced on 13 March 2013 and ceased on 5 April 2013. In Yallakool Creek the fresh increased to a one day

duration peak of 563 ML/d on 31 March 2013. In Colligen Creek the fresh increased to a one day duration peak of 499 ML/d on 31 March 2013. It is estimated that 3,750 ML of CEW was delivered to Yallakool Creek, and 4190 ML to Colligen Creek. The total volume of CEW delivered was 4,192 ML to Yallakool Creek, and 5,074 ML to Colligen Creek. The CEWO (2012) objective of these watering actions was to test whether a spawning response could be achieved in small bodied fish from a 30 cm rise in water levels in autumn. A secondary objective was to test whether or not a small water level rise would result in the movement of medium/large bodied fish during autumn.

Monitoring of responses to environmental watering

The Edward-Wakool system consists of several distributary rivers with regulators controlling inflows. This facilitates a rigorous assessment of the responses to environmental watering through comparisons between rivers receiving environmental water (treatment rivers) and rivers in close geographic proximity not receiving environmental water (serving as controls). In 2012-13 environmental water was delivered to Colligen Creek and Yallakool Creek (treatment rivers), and the Wakool River and Little Merran Creek served as controls (no environmental water).

Sampling was undertaken in four focus rivers; Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek. The Edward River was also sampled to assess the potential source of propagules for the treatment rivers. In addition, 43 sites were sampled throughout the Edward-Wakool system in an annual survey to assess the response of the fish community to environmental watering. An acoustic array established in 2010 to assess fish movement in the Wakool River, Yallakool Ck and Edward River continued to be monitored in 2012-13. In 2011-12 a set of indicators were selected to assess the ecosystem responses to environmental watering in the Edward-Wakool system (Watts et al 2013). The same indicators were monitored in 2012-13, with the addition of in-channel inundation modelling and shrimp abundance (Table i).

Table i. Indicators used to assess ecosystem responses to environmental watering in the Edward-Wakool system in 2012-13 in relation to the ecological objectives as listed in CEWO (2012).

CEWO (2012) ecological objective	**Expected outcomes of environmental watering**	**Related Basin Plan Objective**	**Indicators**
Support habitat requirements of native fish. Support habitat requirements of native aquatic species, including frogs, turtles and invertebrates. Maintain health of existing extent of riparian, floodplain and wetland native vegetation communities.	• Fish condition • Vegetation condition	• Biodiversity	• In-channel inundation modelling • Rapid habitat assessment of aquatic and riverbank vegetation
Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material (e.g. sediment, nutrients and organic matter).	• Biotic dispersal and movement • Sediment transport	• Ecosystem function	• Water chemistry (dissolved organic carbon, total organic carbon, nutrients, carbon characterisation) • Phytoplankton biomass • Biofilm biomass and diversity • Whole stream metabolism
Support breeding and recruitment of frogs, turtles and invertebrates	• Other biota	• Biodiversity	• Frog community composition, abundance and recruitment • Zooplankton abundance, diversity and size composition • Crustacean abundance and proportion of females in berry
Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations	• Hydrological connectivity	• Ecosystem function	• Acoustic tracking of fish
Support breeding and recruitment of native fish.	• Fish reproduction	• Biodiversity	• Larval fish abundance and diversity • Fish recruitment and community composition/abundance • Ageing of larval and juvenile fish • Annual fish survey in main channel and wetland habitats

Ecosystem responses to environmental watering

Environmental watering objective 1 - Support habitat requirements of native aquatic species

- *There was a small increase in inundated benthic area during the environmental watering actions.* The environmental watering in Colligen Creek resulted in an estimated 14% increase in wetted benthic surface area compared to the base flow 200 ML/day scenario. The environmental watering in Yallakool Creek resulted in an estimated 22% increase in wetted benthic surface area compared to the base flow 170 ML/day scenario. *In contrast, the unregulated flows in August 2012 resulted in a considerably larger increase in wetted benthic area.* On the peak of the unregulated flow event on 2 August 2012 the modelled wetted benthic surface area relative to the base flow scenario increased by 47.8% in Colligen Creek and 58.9% in Yallakool Creek.

- *The relationship between discharge and wetted benthic surface area in these rivers is not linear*. It is likely that the relationship between these factors is strongly influenced by in channel geomorphology. *Further modelling is required to determine the optimum discharge for environmental watering* to increase in-channel inundation and create slackwater habitat that will trigger ecosystem responses to environmental flows, but minimise third party impacts

- *There was a significant increase in the percent cover of submerged aquatic vegetation in Yallakool Creek during the environmental watering action in October to December 2012.* The dominant group were Charophytes, which are a type of macroalgae that are similar to water plants, as they grow from the sediment into the water and produce seed-like spores. There was considerable activity of macroinvertebrates and other organisms in the shallow water in this newly established vegetation. The increase in submerged aquatic vegetation was short lived, because when the water level receded in December 2012, at the end of the environmental watering, the submerged aquatic vegetation became fully exposed and was desiccated.

- *The environmental watering actions in Yallakool Creek in February 2013 and in Yallakool Creek and Colligen Creek in March to April 2013 did not result in an increase in aquatic vegetation*. However, these events would have wetted the riverbank and this may have contributed to maintaining or sustaining riverbank plants that would provide habitat during subsequent flow events.

Environmental watering objective 2 - Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material

- *Water temperature was similar at all sites and followed a seasonal trend that is consistent with that recorded in 2011-2012. The dissolved oxygen concentrations remained at acceptable levels throughout the study period*, and no hypoxic blackwater event was associated with any of the environmental watering actions.
- Dissolved organic carbon levels were very similar between all sites, except Little Merran Creek. Elevated DOC in Little Merran Creek from August through to October 2012 indicates greater carbon inputs associated with the unregulated flows during this time.
- *The bioavailable nutrient concentrations, ammonia, filterable reactive phosphorus (nominally phosphate) and* NO_x *(nitrate plus nitrite) did not exceed ANZECC Trigger concentrations,* with the exception of NO_x on one occasion during the large unregulated flows in Little Merran Creek in August 2012 where substantially larger areas of benthic surface area was wetted.
- *The environmental watering in Yallakool Creek and the Colligen Creek did not result in the composition of dissolved organic matter in these rivers becoming substantially different to the other rivers*. *The environmental watering did not stimulate ecosystem productivity by moving nutrients and carbon between the main channel, upper benches and small low commence to flow floodrunners.* This suggests that the areas of in-stream habitat that were re-wet during these flows did not have substantial amounts of accumulated organic material (such as leaf litter) and/or the small in-channel watering actions did not reconnect a sufficient area of upper benches and floodrunners to result in substantial exchange of organic matter and nutrients.
- *The greatest influence on organic matter composition and concentration over the study period were the unregulated flows in August and September 2012, where all rivers had elevated organic matter compared to May 2012*. Organic matter inputs associated with these unregulated flows did not result in a blackwater event, due to the low water temperature at that time of year.
- *The delivery of environmental freshes in the Yallakool and Colligen rivers had no significant effect on phytoplankton densities*. *Environmental watering resulted in higher diversity in biofilms; high diversity is* usually associated with good ecosystem health. *There was a reduced biofilm biomass in rivers that received environmental water compared to the control rivers*. This is consistent with the hypothesis that increased flow variability from in-channel environmental watering will ensure

biofilm biomass in treatment rivers remains below nuisance levels and that biofilm organic biomass will be highest in rivers that have a more constant regulated discharge.

- *Rates of gross primary production and ecosystem respiration in these rivers were typical of lowland streams with good water quality. There was minimal change in rates of GPP and ER after freshes*. Gross primary production is strongly constrained by low bioavailable nutrient concentrations and the freshes were not of sufficient magnitude to entrain higher nutrient concentrations. Similarly, the relative constancy in ecosystem respiration can be attributed to the low and consistent DOC concentrations. These outcomes should be seen as largely positive, as, the existing levels of metabolism are able to support the fish population without the risk of either algal blooms or anoxic events. However, it is unknown whether an increase in production would result in an increase in fish populations and this could be tested by future studies.

Environmental watering objective 3 - Support breeding and recruitment of frogs and invertebrates

- *The watering actions in Colligen and Yallakool Creeks during the 2012 – 2013 sampling period did not increase the abundance of zooplankton, including individual size classes of zooplankton, nor did it appear to stimulate reproduction.* Zooplankton abundance was instead highly seasonal, affected by factors unrelated to flow, such as temperature. It is possible that the magnitude of the watering actions were insufficient to inundate habitat and stimulate productivity thereby increasing abundance and taxonomic diversity of zooplankton. Connectivity and upstream sources may also play a role in zooplankton abundance, as suggested by the similarity in zooplankton abundance of Colligen Creek and Yallakool Creek to the Edward River.
- *The abundance of shrimp during 2012 – 2013 was not significantly different across the four rivers and the timing of shrimp spawning was not influenced by environmental watering*. In fact, Colligen Creek and Yallakool Creek had fewer shrimp overall compared to the control rivers, perhaps as a result of the higher flows reducing the size and availability of slackwaters that are crucial to larval development and juvenile recruitment.
- *There was little response of frogs to environmental watering actions in Colligen and Yallakool Creeks. The highest frog calling activity was observed during September and October 2012* prior to the environmental watering actions when there were inundated backwaters present from larger unregulated flows within the Edward Wakool system. *The limited response of frogs to environmental watering may be due to low availability of slackwater and inundated habitat.*

Environmental watering objective 4 - Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations

- *All four fish species monitored (Murray cod, golden perch, silver perch and carp) displayed increased activity in response to increasing temperature and flow during spring and early summer*. This period of increased movement corresponded with spawning periods for these species. This increased activity did not result in significant displacement movements, but may represent survival related behaviour, such as an increased feeding response.
- Individual displacement for Murray cod ranged from 10 km downstream to 50 km upstream. Golden perch exhibited a displacement range of 150 km downstream to 10 km upstream. Silver perch were highly mobile, undertaking frequent short (<2 km) return movements. Some movement occurred during environmental watering actions, however this pattern of movement was not restricted to these watering actions.

Environmental watering objective 5 - Support breeding and recruitment of native fish

- *The analysis of fish community structure from 2010 to 2013 recorded nine of the 21 species thought to occur in the central Murray region of the Murray-Darling Basin prior to European settlement.* There was high biomass of apex predators and flow specialists in channel habitat and high abundance of flow generalists in wetland habitat.

- *Flooding and subsequent blackwater events in 2010 and 2011 are still having a strong influence on fish community structure.* Following the blackwater events, Murray cod largely disappeared from all but the upstream zone of this system, where in 2010 and 2011 irrigation outfalls were used improve water quality. Flooding in 2010 also triggered the proliferation and widespread recruitment of carp and goldfish throughout the system. There was stronger native fish recruitment and lower alien species recruitment during drought conditions.

- *There has been a small recovery of native fish populations in the system since the blackwater events in 2010 and 2011.* Successive years of environmental water delivery targeted at Murray Cod recruitment in the upper zone have contributed to recovery of the fish community through connection of critical habitat, maintenance of low flow refuges and providing conditions to promote dispersal of individuals to recolonise areas impacted by the blackwater events. *However, the environmental watering in 2012-13 did not trigger widespread recruitment that is necessary for population growth*.

- *There was no evidence of golden and silver perch spawning as a result of the environmental watering actions.* No large scale increase in displacement movements by golden perch were detected, however, there was an increase in the proportion of tagged fish moving during all three environmental watering events. It is not known if any of these movements were spawning related behaviour because most adult golden perch migrated downstream outside the larval monitoring zone. No eggs or larvae of silver or golden perch were found during or immediately after the watering actions. Furthermore no young-of-year were collected from any of the focus rivers or fish community sampling sites.

- *There was no evidence that the nursery and larval dispersal conditions of Murray cod were enhanced as a result of the October to December 2012 Yallakool Creek watering action***.** The watering action was provided to maintain the water level moving through Yallakool Creek during the Murray cod breeding season. Adult Murray cod did not utilise Yallakool Creek in response to environmental water delivery. Furthermore, although Murray cod larvae and young-of-year recruits were found in the Yallakool Creek, they were not in significantly greater numbers than the rivers that did not receive environmental watering actions.

- *Spawning and recruitment of carp gudgeon, one of the five small bodied fish species found in the Edward-Wakool system, benefited from the November 2012 environmental watering action.* Spawning activity increased, with greater numbers of larvae found after the November environmental water action compared to the other rivers. The number of recruits was also significantly higher in Yallakool Creek and Colligen Creek during and immediately after the November environmental watering actions compared to rivers that did not receive environmental water. However, the number of recruits in the Edward River, the source of the environmental water, was equally high or higher than those found in Yallakool and Colligen Creeks at this time. Therefore, it is not possible to determine if the increase in number of recruits in Yallakool and Colligen Creek is due to increased recruitment within these rivers, or is due to more recruits moving, or being transported, into these systems from the Edward River.

Synthesis of findings

The possibility of a wet to moderate resource availability scenario meant that ecological objectives for 2012-13 (CEWO 2012) were expressed in terms of *improving* ecological outcomes. However, above average temperatures and below average rainfall in 2012-13 meant that the magnitude and duration of 2012-13 environmental watering actions largely contributed to *maintaining* ecological outcomes, consistent with a moderate resource availability.

The 2012-13 environmental watering generally contributed to the maintainence of the habitat of native aquatic species. There was a short-term improvement in submerged aquatic habitat in Yallakool Creek from October to December 2012. More work is needed to model the extent of in-channel inundation under a range of flow scenarios to assist the planning of future watering actions.

The overarching conclusion from the assessment of water chemistry and stream metabolism is that throughout the entire period, encompassing base flow and freshes in all streams, water quality posed no threat to these aquatic ecosystems. At no stage did the dissolved oxygen concentration fall below 4 mg O_2/L, which may then threaten viability of invertebrates and fish communities.

There was no response of zooplankton, shrimp or frogs to the environmental watering actions in 2012-13. Under the objective to ‘Improve the health and resilience of aquatic ecosystems’ we would expect to observe higher abundances and evidence of breeding of zooplankton, shrimp and frogs, but this was not achieved. The main reason for the absence of the predicted response appears to be because critical habitat (slackwaters and inundated vegetation) was not created during watering actions. The environmental freshes did not reach sufficient discharge to introduce nutrients and stimulate zooplankton production, nor create slackwater habitat for frogs and shrimp.

Overall there was little effect of the environmental watering on the spawning and recruitment of native fish community of the Edward-Wakool system. Of the ten native fish species occurring in the system, we found that nine spawned during 2012-13, however, only one species (carp gudgeon) had a significant spawning response to the October to December 2012 environmental watering actions.

In summary, in 2012-13 there were a small number of significant responses to the October to December 2012 in-channel environmental watering, however, some of the expected benefits of environmental watering were not observed. In contrast, there were almost no significant responses to the February, March and April 2013 environmental watering actions.

Environmental watering recommendations

The ecological objectives for environmental water use in the Edward-Wakool system in 2012-13 were expressed in terms of *improving* ecological outcomes (CEWO 2012). However, above average temperatures and below average rainfall in 2012-13 meant that environmental water largely contributed to *maintaining* ecological outcomes, consistent with a moderate resource availability scenario. Better alignment of the timing, magnitude and duration of environmental watering is required to achieve these objectives.

Recommendations relating to the timing of environmental watering

1. *To achieve the objective of 'improving the health and resilience of aquatic ecosystems', environmental watering actions under water use option 1 should be targeted during spring and early summer.* This is the time of year when the greatest benefit for spawning and recruitment of most aquatic species can be realised. An additional benefit of undertaking environmental watering in spring and early summer is that it is less likely to cause water quality issues than environmental watering undertaken in late summer or early autumn when water temperatures are higher and the concentration of dissolved oxygen is lower. Furthermore, delivery of environmental water in spring or early summer may be more straightforward to implement given existing operational constraints, as it can be difficult for river operators to meet all license holders water needs in the Edward-Wakool system during late summer and early autumn during periods of high consumptive demand.

2. *Environmental watering actions under water use option 2 (Edward-Wakool system refuge habitat) can be implemented at any time of the year to avoid damage to key assets and provide refuge during hypoxic blackwater events.* If there is a high likelihood of a blackwater event, the ecosystem can benefit from environmental watering to provide refuges that have a higher concentration of dissolved oxygen. This option was implemented in 2011-12.

3. *Decisions involving the timing of environmental watering should consider the water temperature at the proposed time of of the environmental watering actions because it will strongly influence the success of fish spawning, the risk of hypoxic blackwater events and rate of ecosystem productivity.*

Recommendations relating to the magnitude of environmental watering

4. *To achieve the objective of 'improving the health and resilience of aquatic ecosystems' under water use option 1, the magnitude of environmental watering freshes should be larger than environmental flow actions delivered in 2012-13.* In contrast to the small or absent responses to environmental watering in Yallakool Creek and Colligen Creek in 2012-13, the larger magnitude unregulated flow events in August and September 2012 inundated a significantly larger area of riverbank and triggered an increase in river productivity. Better ecological outcomes could be achieved through delivery of environmental freshes of sufficient magnitude to inundate low lying benches and backwaters and create shallow water habitat and slackwaters and inundate riverbank vegetation. *Additional modelling of inchannel inundation should be undertaken during the planning of environmental watering actions to assist with optimisation of flow magnitude to help achieve watering objectives and maximise the creation of shallow inundated areas.* It is possible that for a given volume of environmental water, a better ecological outcome could have been achieved in 2012-13 by delivery of fewer larger freshes rather than several smaller freshes, however this hypothesis needs to be tested in an adaptive management context

5. *Smaller magnitude freshes (such as those delivered in 2012-13) can be delivered to achieve the ecological objective of 'avoid damage', or to 'provide capacity for recovery or maintain health'.* When smaller magnitude watering actions are being planned and implemented it is important that realistic watering objectives are set for each watering action.

6. *A comprehensive community engagement program will be required to facilitate the delivery of larger environmental freshes to the Edward-Wakool system.* The in-channel inundation modelling, mentioned in recommendation 4, will enable scenarios to be presented to stakeholders prior to implementation, which will help identify and minimise risks and serve to inform and engage stakeholders in the planning process.

Recommendations relating to the duration of environmental watering

7. *The duration and shape of the hydrograph of environmental watering events should be carefully planned to avoid rapid rates of recession to minimise stranding of aquatic biota and desiccation of newly established submerged plants*. For example, future watering actions could include a longer, gradual recession to ensure a portion of newly established submerged habitat remains inundated and has the opportunity to increase in area following the flow recession. This will also

ensure that organisms utilising the inundated shallow areas have sufficient time to return to in-channel habitats and avoid stranding.

8. *There is a need to consider multiple objectives when setting the duration of environmental watering events.* For example, the objective of the environmental watering event in Yallakool Creek from October to December 2012 was targeted for Murray cod but was also of sufficient duration that it resulted in a significant increase in aquatic vegetation that was not observed during the shorter duration environmental watering events in Colligen Creek or Yallakool Creek later in the year.

General recommendations for environmental watering

9. *The quality of source water should be carefully considered prior to each environmental watering action as it will influence the outcome of environmental watering*. The quality of source water for environmental watering actions in the Edward-wakool system (e.g. the Edward River or Mulwala canal) can vary considerably. For example, on occasions when there is considerable overbank flooding in the Murray catchment, the water in the Edward River or the Mulwala canal may carry high dissolved carbon loads (Watts et al 2013).

10. *Decisions around the timing of environmental watering should consider the antecedent hydrological conditions because they can strongly influence the success of subsequent environmental watering actions.*

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1. INTRODUCTION

This report documents the monitoring and evaluation of Commonwealth environmental watering in the Edward-Wakool system in 2012-13. It provides details of the environmental objectives of the watering actions, study design, indicators, methodology, and an assessment of ecosystem responses to environmental watering with respect to the objectives set by the Commonwealth Environmental Water Office. A summary of stakeholder consultation and community involvement in the project is also provided. Results and conclusions from the monitoring and evaluation underpin recommendations for future environmental watering in this system.

2. BACKGROUND

Assessment of ecosystem responses to in-channel environmental flows

The regulation of the world's major river systems is a threat to global biodiversity (Nilsson et al. 2005; Poff et al. 1997; Ward et al. 2001; Poff et al. 2007). Under natural flow regimes, riverine systems are a complex mosaic of habitats which vary across space and time with changes in water volume, velocity and flooding duration (Stanley et al. 2010; Ward et al. 2001). Periods of high flows (flood pulse) inundate river benches, fill backwaters and small anabranches and raise ground water levels, which can lead to the creation of groundwater ponds along river margins (Poff et al. 1997; Ward et al. 1999). Furthermore, periods of low flow result in slow-flowing or still water, which can provide habitat for taxa sensitive to higher flow velocities (Bogan and Lytle 2007; Hazell et al. 2003). Under river regulation, these periods of very high and very low flows are reduced, with subsequent declines in the availability of habitat types linked to these flow conditions (Ward et al. 1999).

Growing awareness of the impacts of river regulation has led to increased interest in the delivery of environmental water to restore the ecological function of regulated river systems (e.g. Poff 2009; Arthington et al. 2010). Two major types of environmental watering are overbank flows, that inundate wetlands and floodplains, and instream flows that are contained within the channel. Internationally, there have been a few high-profile examples of the monitoring and evaluation of instream environmental flows, such as the experimental flood downstream of the USA's Glen Canyon Dam in the mid-1990s (e.g. Speas 2000; Shannon et al. 2001; Valdez et al. 2001) and more recently in 2009 (Cross et al. 2011), and an experimental flood in the Spöl River in Switzerland

(Robinson et al. 2003, 2004). In Australia instream flows have historically been used to disperse algal blooms and other contaminants (e.g. Maier et al. 2004; Mitrovic et al. 2003). Prior to 2009 there were few examples in Australia where environmental water was used to create instream pulsed flows (Watts et al. 2009a).

Water managers need a means of evaluating the success of environmental watering, however our understanding of flow-ecology relationships is limited, especially in large and complex floodplain systems, which hinders our attempts to manage these systems (Poff and Zimmerman 2010). There are only a small number of empirical studies testing the mechanisms whereby changes to the river flow regimes drive changes in key population processes and ecological functions (Arthington et al*.* 2006; Arthington et al*.* 2010). In the case of fish, changes in natural flooding regimes may be associated with reduced recruitment success (Humphries et al*.* 2002), changes in movement patterns (Tonkin et al*.* 2008a) and increased densities of exotic fish species (Gehrke and Harris 2001). Changes to aquatic macroinvertebrate communities (Grubbs and Taylor 2004; Sheldon et al*.* 2002; Vallania and Corigliano 2007) and biofilm production (Ryder 2004) also occur following the simplification of flow regimes. The impacts of changes of flow regime on other riverine taxa, such as frogs, are poorly understood (Kingsford et al. 2010).

Since 2010, instream freshes have been delivered to several river systems in the Murray-Darling Basin. The ecosystem benefit of instream watering actions is not well understood and is being assessed through monitoring and evaluation programs. In addition, water managers require information on the most appropriate timing, duration and magnitude of flows to assist the adaptive management of future flow events. The monitoring of in-stream environmental watering in the Edward-Wakool system in 2012-13 will provide an assessment of the ecosystem responses in this system, as well as provide information to inform decisions on the timing, duration and magnitude of flows in this system

The Edward-Wakool system

The Edward-Wakool system is a large anabranch system of the Murray River main channel. The system begins upstream of the Barmah choke, and travels northwest through a series of river red gum forests before discharging back into the Murray River downstream of Kyalite (Figure 1). It is a complex network of interconnected streams, ephemeral creeks, flood-runners and wetlands including the Wakool River, Yallakool Creek, Colligen-Niemur Creek and Merran Creek.

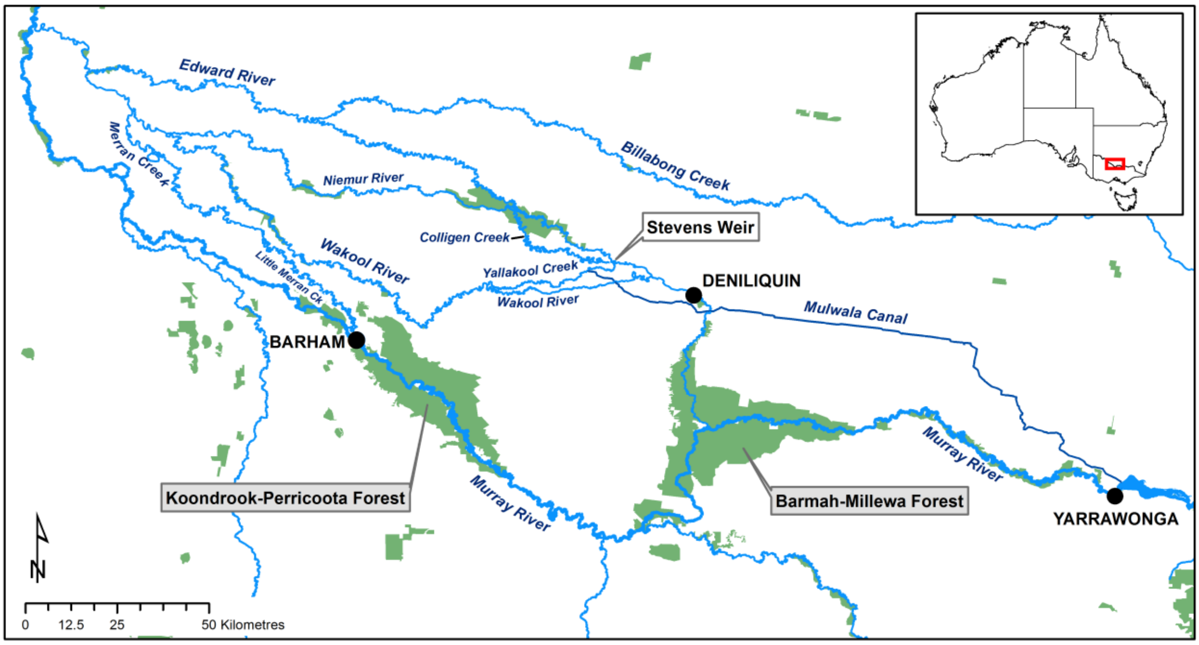


Figure 1. Map showing the main rivers in the Edward-Wakool system.

The Edward-Wakool system is considered to be important for its high native species richness and diversity including threatened and endangered fish, frogs, mammals, and riparian plants. It is listed as an endangered ecosystem, as part of the 'aquatic ecological community in the natural drainage system of the lower Murray River catchment' in New South Wales (*NSW Fisheries Management Act 1994*). This system has abundant areas of fish habitat, and historically had diverse fish communities which supported both commercial and recreational fisheries.

Like many areas of the Murray-Darling Basin, the Edward-Wakool anabranch system has suffered from the effects of river regulation, migration barriers and degradation of water quality. Water regimes within the Edward-Wakool River have been significantly altered by river regulation (Green 2001; Watkins et al. 2010), with changes to the timing and volume of flows. Natural flows in the river system would have been high in spring and very low in summer and autumn. River regulation is likely to have resulted in changes in water velocities, the availability of in-channel habitat types, and ecosystem processes and functions. These problems were manifested in a fish kill event in 2007-08 which resulted in a loss of many hundreds of native fish, including large individuals of the iconic Murray cod.

Between February 2006 and September 2010 there were periods of minimal or no flow in the Edward-Wakool system (Figure 2) due to severe drought conditions. A number of large natural flow events occurred in the Edward-Wakool system between September 2010 and March 2011 coinciding with heavy rainfall in the catchment (Figure 2). Commonwealth environmental water has been delivered to the Edward-Wakool system since 2010.

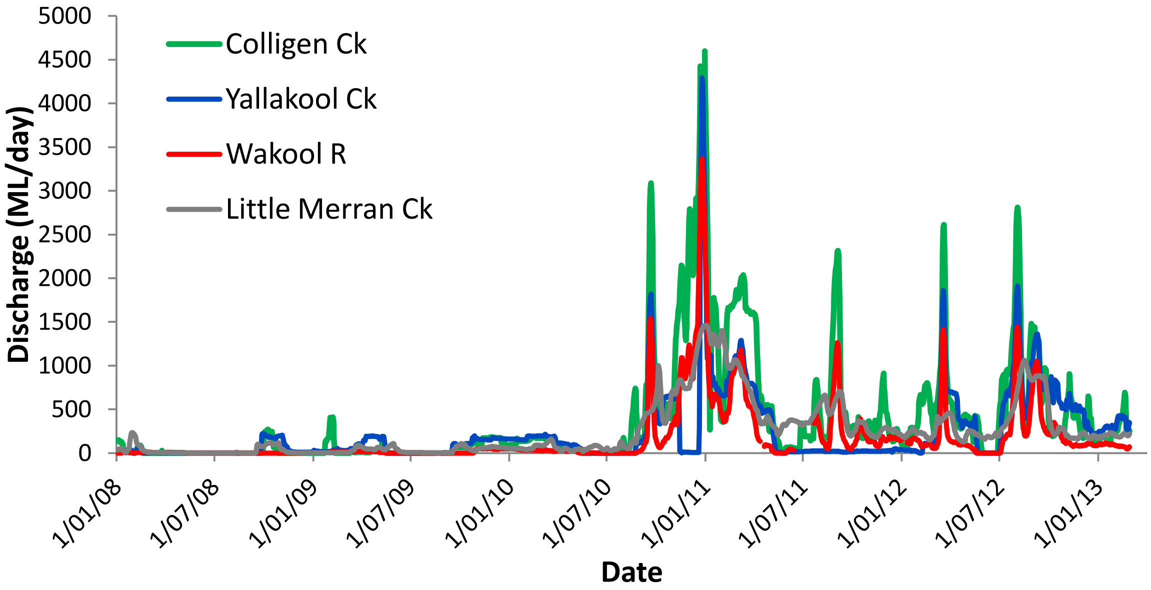


Figure 2. Daily discharge between 01/01/2008 and 28/02/2013 in four rivers in the Edward-Wakool system: Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek. Daily discharge data was obtained from NSW Government water information website (NSW Office of Water, 2012) for four gauging stations: Colligen Creek regulator (409024), Wakool River offtake regulator (409019), Yallakool Creek offtake regulator (409020), and the Little Merran Creek gauge at Franklings Bridge (409044).

A preliminary fish monitoring program was established by NSW Department of Primary Industries (DPI) in 2010 to provide information on native fish populations in the system. The work involved establishing long-term fish monitoring sites throughout the Edward-Wakool system that have been sampled for three consecutive years. In addition, an array of acoustic receivers was established and a population of tagged fish has been maintained in the Edward-Wakool system to monitor behavioural responses to environmental watering. In 2011-12, Charles Sturt University, Monash University and the Murray CMA monitored ecosystem responses to environmental watering in the Edward Wakool system (Watts et al. 2013) focussing on four rivers: Colligen Creek, Yallakool Creek, Wakool River, and Little Merran Creek. The project involved comparing ecosystem responses in rivers that received environmental water to those in rivers that did not receive environmental water. The current project 'Monitoring the ecosystem responses to Commonwealth environmental water delivered to the Edward-Wakool river system, 2012-13' follows on from the previous projects by bringing together the long-term fish monitoring, fish movement and ecosystem monitoring.

3. COMMONWEALTH WATER USE OPTIONS AND FLOW-DEPENDANT ECOLOGICAL OBJECTIVES 2012-2013

Water use options and flow-dependent ecological objectives for 2012-13 for the mid-Murray region were developed by the CEWO (2012), taking into account likely resource availability and the catchment conditions. During the 2011-12 water year, the mid-Murray region experienced relatively wet conditions with significant rainfall and floodplain inundation occurring during late summer and early autumn, and the rainfall in the catchment from 1 July 2011 to 30 June 2012 was greater than the seasonal average (CEWO 2012).

Consistent with the Basin Plan, the 'Framework for determining Commonwealth environmental water use' outlines ecological objectives for environmental watering under a range of water resource availability scenarios (Table 1). The ecological objectives that guided environmental watering under the range of possible circumstances expected in 2012-13 are outlined in Table 2. Based on the resource availability and the catchment conditions, the ecological management objectives for the mid-Murray region were expected to be in the moderate to wet range. The ecological objective of the moderate range is to 'maintain ecological health and resilience' and the ecological objective for the wet range is to 'improve the health and resilience of aquatic ecosystems' (CEWO 2012).

Three water use options relevant to the Edward-Wakool system are listed in Table 3. As these options focus on the delivery of in-channel flows, the ecological objectives for this system focus on breeding, recruitment and habitat requirements of native fish and other aquatic organisms, as well as in-channel ecosystem functions.

Table 1. Ecological and management objectives for environmental water use under different resource availability scenarios. (From CEWO 2012)

	Extreme Dry	**Dry**	**Moderate**	**Wet**	**Very Wet**
Ecological watering objectives	Avoid damage to key environmental assets	Ensure ecological capacity for recovery	Maintain ecological health and resilience	Improve the health and resilience of aquatic ecosystems	Build future capacity to support ecological health and resilience

Table 2. Planning and resource availability scenarios to assist with water use options, with arrow showing the expected resource availability in the mid-Murray region as at 1 July 2012. (From CEWO 2012)

Resource availability scenario as at 1 July 2012	Possible Inflows in 2012-13	Probability of exceedance based on historical inflows for the catchment	Likely resource availability scenarios 2012-13 for the given inflows	Relevant ecological objective for environmental watering
Wet-Moderate	Very high inflows (very wet)	10 %	Very wet	Build future capacity to support ecological health and resilience
	High inflows (wet)	25 %	Wet	Improve the health and resilience of aquatic ecosystems
	Moderate inflows (moderate)	50 %	Moderate	Maintain ecological health and resilience
	Low inflows (dry)	75 %		
	Very low inflows (very dry)	90 %	Dry	Ensure ecological capacity for recovery

Table 3. Water use options and flow-dependent ecological objectives as listed in CEWO (2012) for the mid-Murray system that are relevant for the 2012-13 monitoring program in the Edward-Wakool system.

Option	Site	Flow-dependent ecological objectives
Option 1 - Edward Wakool River system fish freshes A number of freshes throughout the water year. Current planning allows for freshes in spring / early summer and autumn.	Edward River downstream of Stevens Weir, Wakool River, Yallakool Creek, Colligen Creek-Niemur River	• Support breeding and recruitment of native fish. • Support habitat requirements of native fish. • Support habitat requirements of native aquatic species, including frogs, turtles, invertebrates, etc. • Maintain health of existing extent of riparian, floodplain and wetland native vegetation communities. • Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations.
Option 2 - Edward-Wakool River system refuge habitat Dependent on the need to provide refuge habitat which is contingent on catchment conditions. Hypoxic blackwater is most likely to occur from Nov onwards, as water temperatures increase	Edward River, Wakool River, Yallakool Creek, Colligen Creek, Niemur River	• Support habitat requirements of native fish. • Support habitat requirements of native aquatic species, including frogs, turtles, invertebrates, etc. • Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material (e.g. sediment, nutrients and organic matter). • Support ecosystem functions that relate to longitudinal connectivity (i.e. connectivity along a watercourse) and lateral connectivity (i.e. connectivity between the river channel, wetlands and floodplain) to maintain populations.
Option 4 - mid-Murray freshes Unregulated peaks are most likely to occur in spring, however could occur at any time.	Edward River, Wakool River, Yallakool Creek, Colligen Creek-Niemur River	• Support breeding and recruitment of native fish, frogs, turtles, invertebrates etc. • Support habitat requirements of native fish, frogs, turtles, invertebrates etc. • Maintain health of existing extent of riparian, floodplain and wetland native vegetation communities. • Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material (e.g. sediment, nutrients and organic matter). • Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations.

4. ENVIRONMENTAL WATERING IN 2012-13

Up to 60 GL of Commonwealth environmental water was made available for use in the Edward-Wakool River System during 2012-13 (CEWO 2013). Commonwealth environmental water was delivered in conjunction with water supplied by the New South Wales Government. Commonwealth environmental water was used to provide several in-stream freshes delivered between spring 2012 and autumn 2013. Target creeks and rivers included the Edward and Wakool rivers, and Yallakool and Colligen Creeks (CEWO 2013). The Commonwealth environmental water will help build on the outcomes of environmental water provided to the Edward-Wakool River System in previous years.

The possibility of a wet to moderate resource availability scenario meant that ecological objectives for 2012-13 (CEWO 2012) were expressed in terms of *improving* ecological outcomes. However, above average temperatures and below average rainfall in 2012-13 meant that environmental water largely contributed to *maintaining* ecological outcomes, consistent with a moderate resource availability scenario. Our evaluation takes this into account by acknowledging the contribution of environmental water to maintaining ecological outcomes.

Four instream watering actions occurred in the Edward-Wakool system in 2012-13 (Figure 3).

1. **Yallakool Creek October to December 2012 environmental watering action.** A watering action in Yallakool Creek under water use option 1 commenced on 19 October 2012 and finished on 7 December 2012. The CEWO (2012) ecological objective for this event was aimed at maintaining inundation of habitat for Murray cod nests and maintaining the flow until cod eggs could hatch and larvae drift downstream. The Yallakool Creek discharge during this event was held in the range of approximately 360 ML/day to 683 ML/day with a median discharge of 526 ML/day (Figure 3). The total volume delivered was 13,620 ML comprising 10,620 ML of Commonwealth environmental water and 3,000 ML of NSW environmental water allocation.

2. **Colligen Creek November to December 2012 environmental watering action.** A watering action under water use option 1 occurred in Colligen Creek to promote golden perch and silver perch spawning (CEWO 2012). This watering action involved the delivery of two freshes between 2 November 2012 and 17 December 2012. The first fresh commenced on 2 November 2012, reaching a peak of approximately 903 ML/day on 8 November 2012 (Figure 3). Following the fresh, elevated base flows were maintained in anticipation of the second fresh. Due to operational considerations, the second fresh was delayed, and elevated based

flows were reduced and ceased by the end of November. The second fresh commenced on 8 December 2012 and finished on 18 December with the flows reaching a peak of approximately 655 ML/day on 11 December (Figure 3). The total volume delivered was 10,261 ML, including 7,261 ML of CEW, and 3,000 ML of NSW environmental water.

3. **Yallakool Creek February 2013 environmental watering action.** A watering action in Yallakool Creek under water use option 1 commenced on 2 February 2013 and finished on 22 February 2013. The fresh increased over 3 days to a peak of 430 ML/d on 13 February 2013. The CEWO (2012) ecological objective for this event was to provide opportunities for small bodied fish (instream generalists) to breed. Specifically, the objective was to test if a small (~30 cm) increase in water level can initiate a spawning response in small bodied fish. Secondary objectives of this action were to test whether or not a small water level rise resulted in the movement of medium/large bodied fish and/or spawning of golden perch. Note that a fresh in Colligen Creek in February 2013 (Figure 3) was not part of this environmental watering action but was due to an water order rejection. The volume of CEW delivered to Yallakool Creek for this action was 1,796 ML.
4. **Yallakool Creek and Colligen Creek March to April 2013 environmental watering action.** Watering actions in Yallakool and Colligen Creeks under water use option 1 commenced on 13 March 2013 and ceased on 5 April 2013. In Yallakool Creek the fresh increased to a one day duration peak of 563 ML/d on 31 March 2013 (Figure 3). In Colligen Creek the fresh increased to a one day duration peak of 499 ML/d on 31 March 2013 (Figure 3). It is estimated that 3,750 ML of CEW was delivered to Yallakool Creek, and 4190 ML to Colligen Creek. The total volume of CEW delivered was 4,192 ML to Yallakool Creek, and 5,074 ML to Colligen Creek. The CEWO (2012) ecological objective for these watering actions was to test whether a spawning response could be achieved in small bodied fish from a 30 cm rise in water levels in autumn. A secondary objective was to test whether or not a small water level rise would result in the movement of medium/large bodied fish during autumn.

A steering committee led by the CEWO contributed to the planning and delivery of these flows. Participants included representatives of CEWO, NSW OEH, Murray CMA, State Water Corporation, NSW Office of Water, MDBA River Murray Operations, and scientists from NSW DPI Fisheries and Charles Sturt University. Teleconferences were generally bi-weekly in the months preceding the watering event, and weekly during each event. Preliminary results from the monitoring program were provided during these teleconferences to help inform the watering decisions and delivery of Commonwealth environmental water and water supplied by the New South Wales Government.

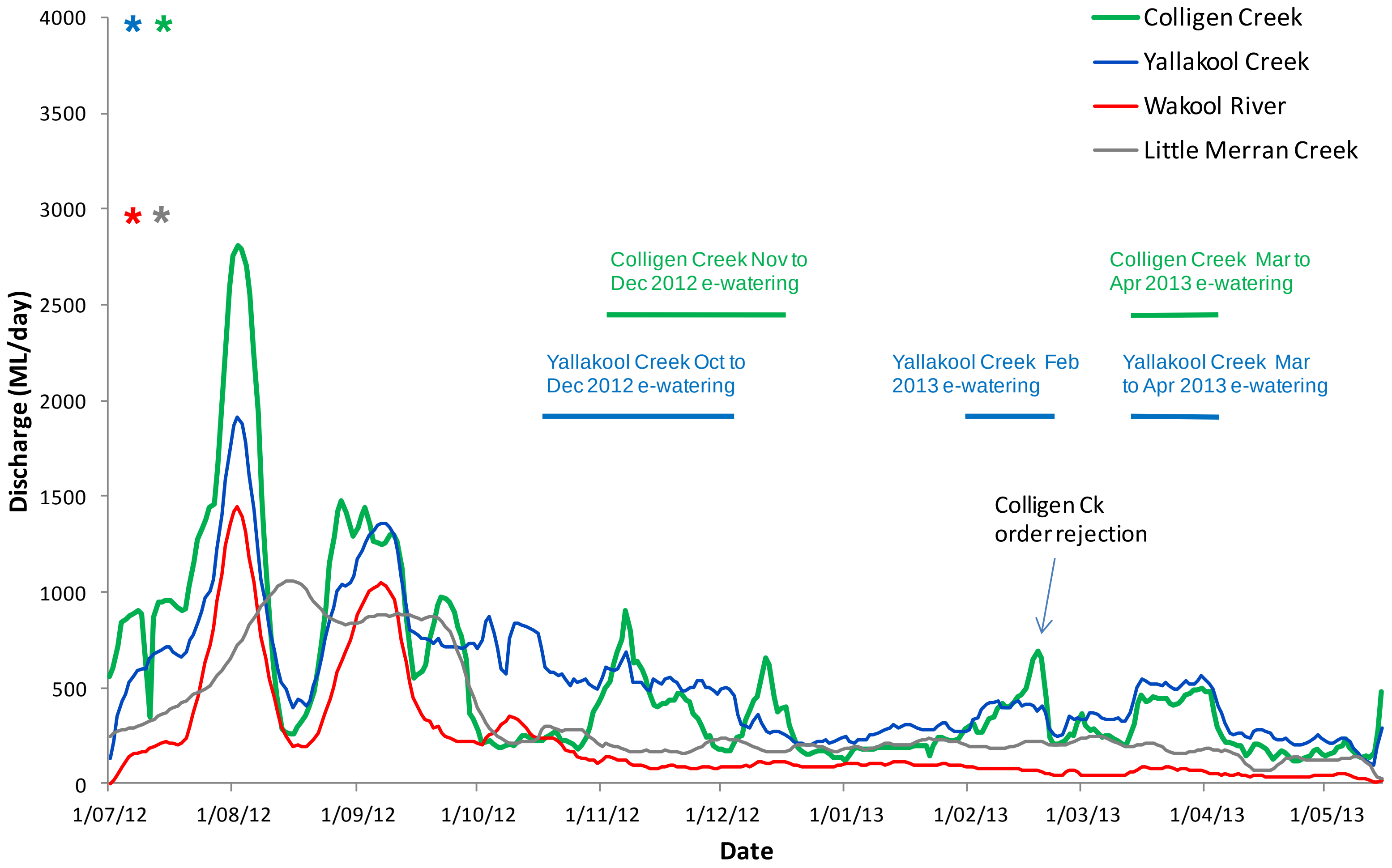


Figure 3. Daily discharge (ML/day) between 1/7/12 and 15/5/13 in Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek. Timing and duration of environmental watering in Yallakool Creek and Colligen Creek in 2012-13 is shown with coloured bars. The star symbols on the y axis indicate estimated bankfull discharge in each river.

5. MONITORING DESIGN AND LOCATION OF SAMPLING SITES

The monitoring of ecosystem responses to environmental watering in the Edward-Wakool system in 2012-13 was undertaken as follows:

1. Focus rivers – Monitoring under watering option 1

The nature of the Edward-Wakool system provides a unique opportunity to assess responses to in-channel environmental watering. In this system there are several distributary rivers with regulators that control inflows. Under watering option 1 (Table 3), environmental water can be delivered as freshes via regulators to Colligen Creek, Yallakool Creek and the Wakool River from the Edward River (Figure 4). This facilitates a rigorous assessment of the responses to environmental watering through comparisons between rivers receiving environmental water ('treatment' rivers) and rivers in close geographic proximity not receiving environmental water ('control' rivers). The study reaches in each focus river ranged from 3 to 5 km in length. These focus reaches were also sampled to assess ecosystem responses to environmental watering in 2011-12 (Watts et al. 2013).

In 2012-13 environmental water was delivered to Colligen Creek and Yallakool Creek (treatment rivers), and the Wakool River and Little Merran Creek served as controls (no environmental water). The Edward River was sampled to assess the potential source of propagules for the treatment rivers (Figures 4, 5). A detailed description of sampling design and data analysis for each indicator is presented in section 5. When linking the monitoring design to the CEWO (2012) ecological objectives, the expected responses are presented in Table 4.

Table 4. Expected ecosystem responses in treatment and control rivers in response to environmental watering in the Edward-Wakool system in 2012-13.

Resource availability scenario:	CEWO (2012) ecological objective:	Expected ecosystem response in treatment' rivers:	Expected ecosystem response in 'control' rivers:
Moderate	"Maintain ecological health and resilience"	No change relative to antecedent conditions (eg. No change in abundance and recruitment of aquatic organisms, water chemistry and habitat availability)	Reduced ecological health and resilience relative to antecedent conditions as a result of not receiving environmental water. (eg. reduced abundance and recruitment of aquatic organisms, reduced habitat availability)
Wet	"Improve the health and resilience of aquatic ecosystems"	Improved ecological health and resilience (eg. greater abundance and recruitment of aquatic organisms, improved water chemistry and increased habitat availability)	No change or reduced ecological health and resilience relative to antecedent conditions as a result of not receiving environmental water.

2. Focus rivers- Monitoring under watering option 2

Under watering option 2 (Table 3) there is the opportunity to deliver environmental water to the Edward River and Wakool River via escapes from the Mulwala canal. To facilitate assessment of environmental watering from the Wakool escape in the event of a blackwater event, sampling in the Wakool River was undertaken upstream and downstream of the Wakool escape (Figure 4) and in the Mulwala canal as a potential source of propagules (Figure 4). Samples from downstream of the escape were processed immediately and were used in the analysis of watering option 1. As no environmental water was delivered from the Mulwala canal to the Wakool River under watering option 2 in 2012-13, the samples from the canal and the upstream Wakool River site were not required to assess watering and are hereafter not referred to in this report.

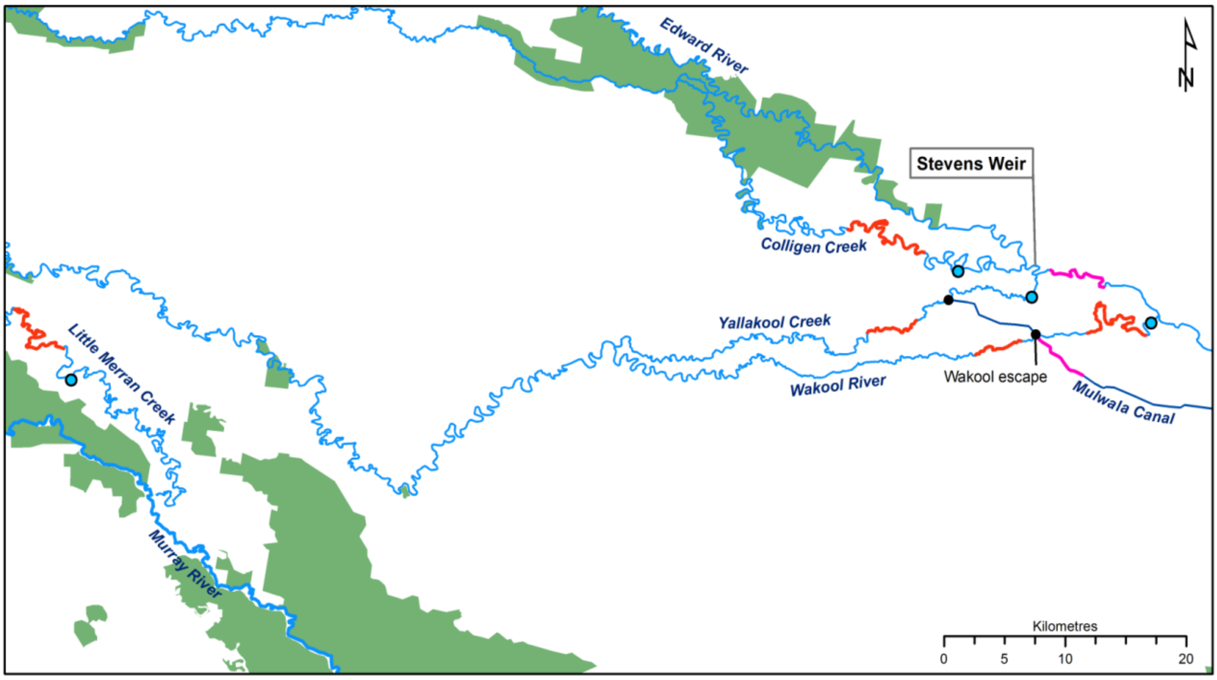


Figure 4. Location of four focus rivers for the assessment of ecosystem responses to environmental watering in the Edward-Wakool system (shown in red). The Edward River and Mulwala canal (shown in pink) were sampled as potential sources of propagules.

Colligen Creek Yallakool Creek

Wakool River Little Merran Creek

Figure 5. Photos of the four focus rivers; Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek.

3. Whole of system assessment

A total of 43 sites were sampled throughout the Edward-Wakool system (Figure 6) to assess the response of the fish community to environmental watering. The sampling included 30 sites that were established in 2010 and 7 sites established in Werai Forest in 2011 (Figure 7). A selection of the sample sites are shown in Figure 8. An acoustic array established in 2010 to assess fish movement in the Wakool River, Yallakool Ck and Edward River continued to be monitored in 2012-13 (Figure 9).

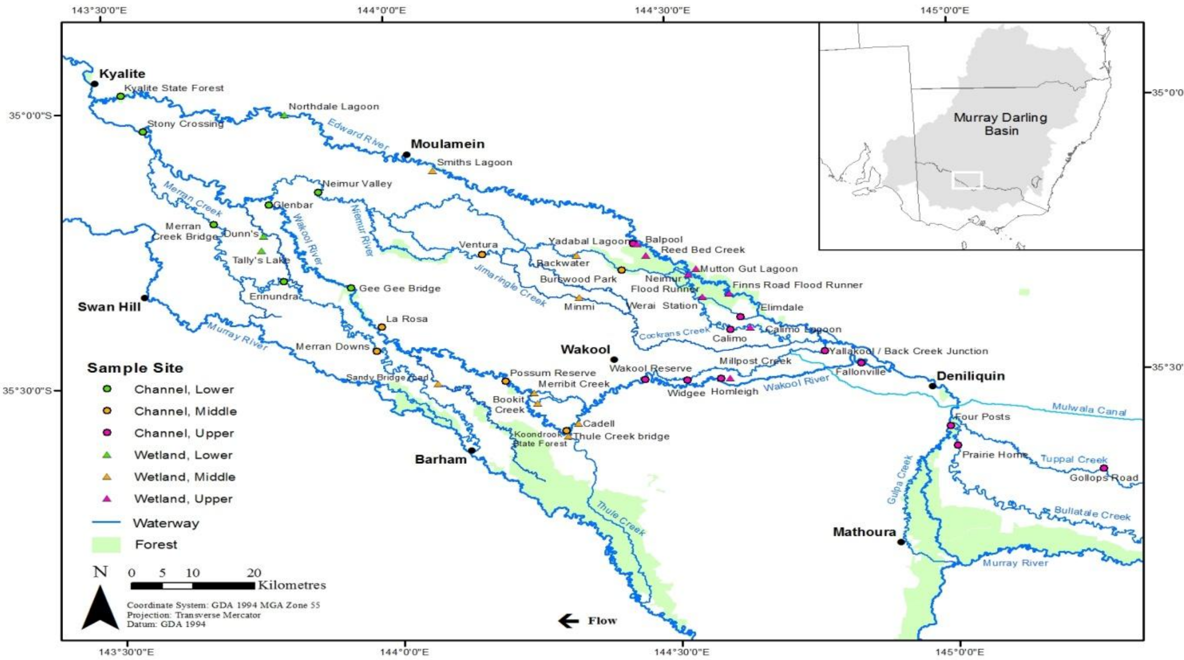


Figure 6. The Edward-Wakool River system highlighting the location of 43 monitoring sites for fish community monitoring. Major forests are shown in green.

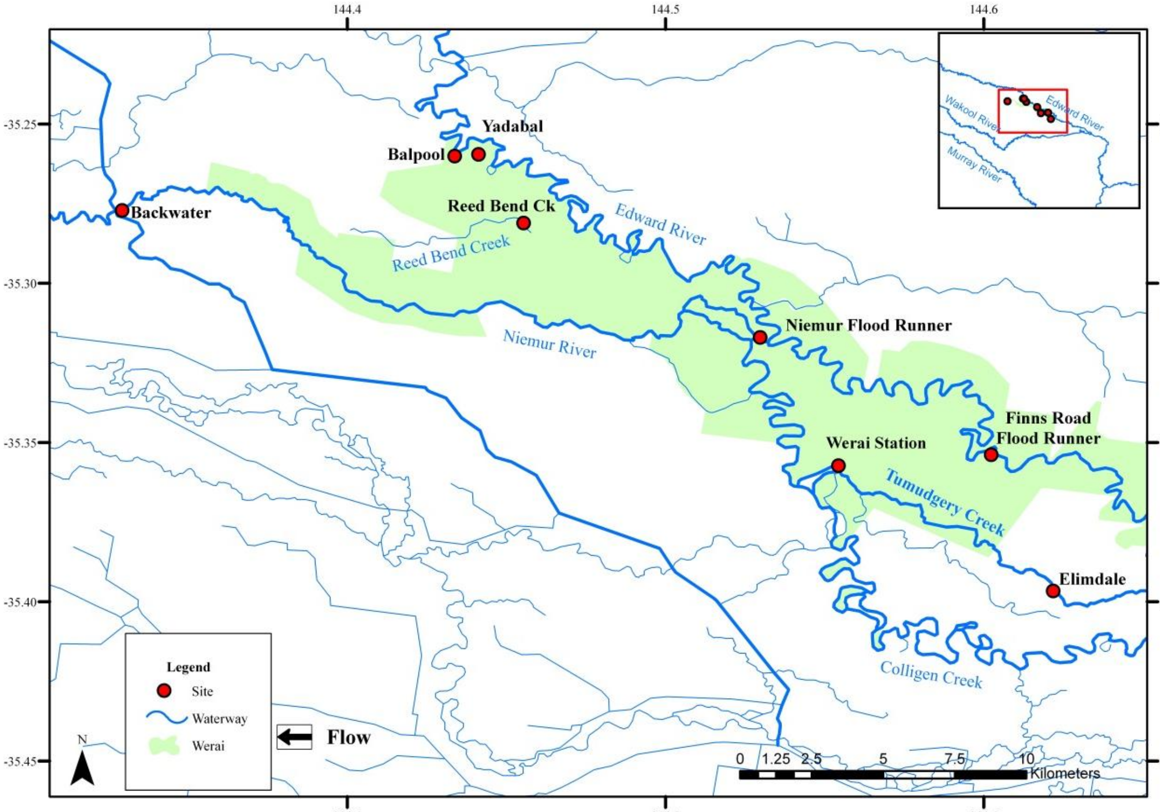


Figure 7. Map of the Werai Forest showing the location of the survey sites.

Widgee, Yallakool Creek

Ventura, Neimur River

Merribit, Merribit Creek

Merran Creek Bridge, Merran Creek

Thule Creek Bridge, Thule creek

Dunn's, Coobool Creek

Figure 8. A selection of the 43 sites sampled for the annual fish community assessment across the study area.

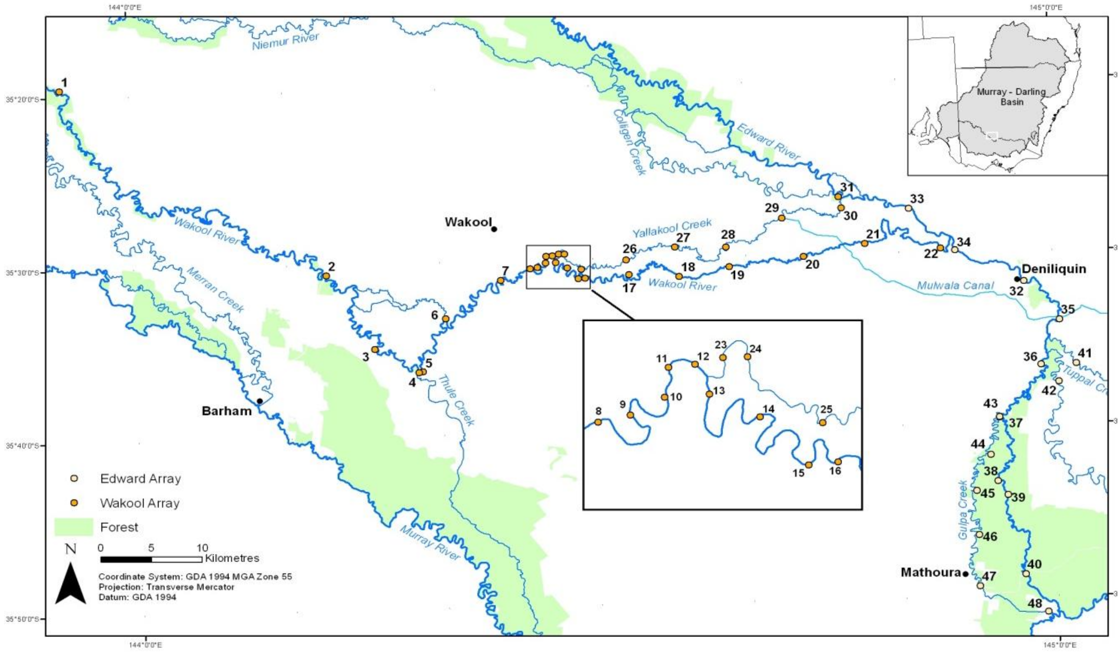


Figure 9. Overview of acoustic receiver array used to detect fish movements in response to environmental water delivery in the Edward-Wakool system. The array was established to detect movements in the Wakool-Yallakool River (orange) and also the upper Edward system (yellow). Detailed coverage of the original tagging location at the Wakool-Yallakool junction is enlarged for clarity.

6. INDICATORS

As it is not possible to measure every ecosystem response to environmental watering, a selection of potential indicators was assessed. The best indicators are those where there is a high level of predictability in response to environmental changes. Cairns et al. (1993) suggest that a good indicator should also be relatively cheap and quick to measure, repeatable and sensitive to environmental change. Furthermore, numerous authors have stated the most important attribute of an indicator is that it can be quantitatively validated, requiring the reliability of the data and what the response indicates to be unambiguous (Fairweather 1999).

In 2011-12 a set of indicators were selected to assess the physical, chemical or biological responses to environmental watering in the Edward-Wakool system (Watts et al. 2013). As these indicators were considered to be appropriate for assessment of the flow-dependent ecological objectives identified in CEWO (2012), the indicators monitored in 2011-12 were monitored again in 2012-13. The indicators selected for the assessment of environmental watering in the Edward-Wakool system are tightly linked with the CEWO (2012) ecological objectives for the proposed water use options for this system (Table 5). These indicators reflect the in-channel focus of the water use options for the

Edward-Wakool system; the majority of indicators focus on assessment of breeding, recruitment and habitat requirements of native fish and other aquatic organisms and in-channel ecosystem functions. The expected responses of these indicators to watering objectives, if the ecological objectives are met, are outlined in Table 4.

The frequency of sampling and locations where the indicators were monitored are listed in Table 6. Some indicators were monitored continuously via logging equipment and others were sampled fortnightly or monthly. Some indicators can be assessed quickly and provide real time information to water managers allowing them to modify the watering regime to achieve the watering objective.

Table 5. Monitoring indicators used to assess ecosystem responses to environmental watering in the Edward-Wakool system in 2012-13 in relation to the ecological objectives as listed in CEWO (2012).

CEWO Ecological objective	Watering option	Indicators
Objective 1: Support habitat requirements of native fish. Support habitat requirements of native aquatic species, including frogs, turtles and invertebrates. Maintain health of existing extent of riparian, floodplain and wetland native vegetation communities.	1, 2, 4	• Inundation modelling • Rapid habitat assessment of aquatic and riverbank vegetation
Objective 2: Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material (e.g. sediment, nutrients and organic matter).	2, 4	• Water chemistry (Dissolved organic carbon, total organic carbon, nutrients) • Carbon characterisation) • Phytoplankton biomass • Biofilm biomass and diversity • Whole stream metabolism
Objective 3: Support breeding and recruitment of frogs, turtles and invertebrates	4	• Frog community composition, abundance and recruitment • Zooplankton abundance, diversity and size composition • Crustacean abundance and proportion of females in berry
Objective 4: Support ecosystem functions that relate to longitudinal connectivity (i.e. connectivity along a watercourse) and lateral connectivity (i.e. connectivity between the river channel, wetlands and floodplain) to maintain populations	1, 2, 4	Longitudinal connectivity • Acoustic tracking of fish
Objctive 5: Support breeding and recruitment of native fish.	1, 4	• Larval fish abundance and diversity • Fish recruitment and community composition/abundance • Ageing of larval and juvenile fish • Large-scale fish survey of main channel and wetland habitats

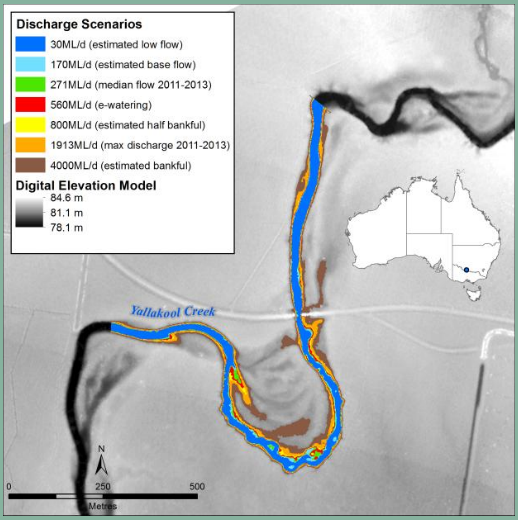
Table 6. Summary of location and frequency for sampling of indicators

		Study sites/reaches			
Ecological Objective	**Indicators**	**Focus rivers:** **Colligen Ck** **Yallakool Ck** **Wakool R** **Little Merran Ck**	**Source rivers:** **Edward R** **Mulwala canal**	**Acoustic array sites in Wakool R, Yallakool Ck and Edward R**	**43 sites throughout Edward-Wakool system**
1. Support habitat requirements of native aquatic species	Inundation modelling	Modelled for range flow scenarios			
	Rapid habitat assessment of aquatic and riverbank vegetation	monthly			
2. Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material	Water chemistry (Dissolved oxygen, light, temperature)	continuous	continuous		
	Water chemistry spot measures	fortnightly	fortnightly		annually
	Water chemistry (carbon, nutrients)	fortnightly	fortnightly		
	Whole stream metabolism	continuous			
	Phytoplankton	fortnightly	fortnightly		
	Biofilms	monthly			
3. Support breeding and recruitment of frogs, turtles, invertebrates	Zooplankton	fortnightly	fortnightly		
	Shrimp	fortnightly	fortnightly		
	Crayfish and Yabby				annually
	Frogs	monthly			
4. Support ecosystem functions that relate to longitudinal and lateral connectivity	Fish movement			continuous	
5. Support breeding and recruitment of native fish.	Fish larvae and eggs	fortnightly	fortnightly		
	Fish community structure				annually
	Fish recruitment				annually

7. RESPONSES OF INDICATORS AGAINST ENVIRONMENTAL WATERING OBJECTIVES

7.1. Objective 1: Support habitat requirements of native aquatic species

7.1.1. Inundation modelling



Key findings

- *The environmental watering in Colligen Creek resulted in an estimated 14% increase in wetted benthic surface area compared to the base flow 200 ML/day scenario. The environmental watering in Yallakool Creek resulted in an estimated 22% increase in wetted benthic surface area compared to the base flow 170 ML/day scenario.*
- *The unregulated flows in August 2012 resulted in a considerably larger increase in wetted benthic area than the environmental watering*. On the peak of the unregulated flow event on 2 August 2012 the modelled wetted benthic surface area relative to the base flow scenario increased by 47.8% in Colligen Creek and 58.9% in Yallakool Creek.
- *The relationship between discharge and wetted benthic surface area in these rivers is not linear*. It is likely that the relationship between these factors is strongly influenced by in channel geomorphology.
- *Further modelling is required to determine the optimum discharge for environmental watering* to increase in-channel inundation and create slackwater habitat that will trigger ecosystem responses to environmental flows, but minimise third party impacts.

Background

Understanding the extent of riverbank inundation under different discharge scenarios is essential to describe changes in wetted benthic surface area and shallow water habitat during environmental watering actions. Inundation modelling can also assist the interpretation of other indicators, such as nutrients, river metabolism, and emergence of zooplankton from riverbank sediments. Remote sensing is a useful method for estimating the extent of inundation under different flow scenarios because it provides results more cheaply and efficiently than ground based survey methods. Previous studies modelling river flow and floodplain inundation have been undertaken for wetlands on the Darling River (Shaikh et al. 2001), and floodplains on the Murrumbidgee River (Frazier et al.2003) and the River Murray (Overton 2005; Overton et al. 2006). These studies have generally focussed on estimating floodplain inundation during overbank flows. Methods employed include optical satellite image analysis, radar remote sensing and of landsat TM (Townsend and Walsh 1998; Shaikh et al. 2001; Frazier et al. 2003; Overton et al. 2006).

The use of digital elevation models to create a floodplain surface that can be inundated under different discharge scenarios may not give the best representation of floodplain inundation, because even small impediments on a predominantly flat floodplain can affect the models. However, in a system such as the Edward-Wakool system where environmental watering is contained within the channel, the use of digital elevation models to create flow path assessments below bankfull is an appropriate approach to compare the extent of riverbank inundation under different discharge scenarios. The inundation models can also serve as a tool to help predict the likely outcome of different flow management options on patterns of riverbank inundation.

Methods

Discharge scenarios were modelled for the four focus reaches; Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek. Each reach was represented within the hydraulic model using a digital elevation model (DEM) supplied by the Murray Catchment Management Authority-NSW. Inundation modelling was undertaken by Marine Solutions. Digital elevation models derived from LiDAR survey can be used for detailed flow path assessments. However, unless carefully checked, small scale artefacts can remain during the conversion of mass point clouds to surface models. Artefacts remaining within DEMs can impede normal flow and impact the results of hydraulic models. Several significant artefacts were removed from the Little Merran Creek and Colligen Creek DEMs to ensure normal stream flow was not impeded. Artefacts were removed by identifying erroneous elevation values and integrating corrected values directly into the elevation surface using a process of data fusion. To account for vegetation in each reach the surface friction coefficient (Manning's n) within the model was set to a value of 0.05 with the exception of the Yallakool Creek site where a value of 0.04 was deemed more appropriate.

Six discharge scenarios were modelled for each river ranging from low flow to estimated bank-full flows (Table 7). The exceptions were Yallakool Creek where an additional environmental watering scenario was modelled and Little Merran Creek where only three scenarios could be successfully replicated because the LiDAR survey was undertaken when discharge was approximately 200ML/day, so low flow and base flow scenarios could not be modelled in this system. In Colligen Creek the environmental watering scenario was the same as estimated half bankfull. Discharge values were converted from ML/day to $m^3.sec^{-1}$ and supplied to the model as static flow values.

Each scenario was modelled assuming an initial dry starting condition with no residual water in the system with the exception of Little Merran Creek reach where it was identified that a base flow of 200 ML/day was present when the DEM was captured. All scenarios were run until stable state flow was achieved whereby the instantaneous flow rate at the downstream boundary condition stabilised and matched the upstream inflow value. The exception was scenario 6 for the Wakool River reach where a steady state flow could not be achieved without a loss from the system into the Edward River. Under the 3000 ML/day discharge a stable state flow of approximately 2820 ML/day was recorded at the downstream boundary condition for the Wakool site with 180 ML/day escaping into the Edward River. Inflow values were provided to the upstream boundary condition as a static value and did not vary over the duration of a model run. Discharge scenarios were modelled using the 2D grid implementation of Eonfusion Flood (Myriax Software) with model outputs post-processed using the GIS functionality of Eonfusion (Myriax Software).

Upon reaching stable state flow, an extent output from the model was captured representing the spatial coverage of the water surface. Within each cell of the extent the water depth and surface elevation were captured allowing a 3D surface of the stream bed underlying the water surface to be constructed. The wetted benthic surface area covered by the water surface was then calculated using the derived 3D surface. Post-processing, including surface area calculations, was achieved using Eonfusion (Myriax Software), Quantum GIS and made distributable using Google Earth.

Table 7. Summary of discharge scenarios modelled for the four focus reaches; Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek.

Scenarios	**Discharge ML/day**			
	Wakool	**Yallakool**	**Colligen**	**Merran**
1 - low flow (estimated)	25	30	30	
2 - base flow (estimated)	50	170	200	
3 - median flow (2011-2013)	110	271	314	230
Environmental watering		560	800	
4 - half bank (estimated)	500	800	800	500
5 - maximum daily discharge (2011-2013)	1442	1913	2808	1062
6 - bankfull (estimated)	3000	4000	4000	

Results and discussion

The estimates of wetted benthic surface area (Table 8) and inundation maps (Figure 10) illustrate that estimated low flows, estimated base flows and the calculated median flow for 2011-2013 were constrained within the river channel. There was a small increase in wetted benthic surface area from the low flows to the median flow scenario. In Colligen Creek the environmental flow of 800 ML/day resulted in a 14% increase in wetted benthic surface area from the base flow 200 ML/day scenario. In Yallakool Creek the environmental flow of 560 ML/day resulted in a 22% increase in wetted benthic surface area from the base flow 170 ML/day scenario.

Figure 10 demonstrates that the wetted benthic surface area during the maximum daily discharge scenario experienced in 2011-2012 during high unregulated flows was considerably higher than the wetted area during the base flow or environmental flow scenarios. The models estimate there would be a considerable further increase in wetted benthic surface area during a bankfull flow, however this type of flow event did not occur during the study period.

Table 8. Estimates of wetted benthic surface area under a range of discharge scenario in the four focus reaches; Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek.

Scenarios	**Wetted surface area (m^3)**			
	Wakool	**Yallakool**	**Colligen**	**Merran**
1 - low flow (estimated)	42,196	33,896	43,257	-
2 - base flow (estimated)	43,587	38,237	48,292	-
3 - median flow (2011-2013)	46,222	41,858	49,982	160,908
Environmental watering		46,726	55,110	
4 - half bank (estimated)	58,547	49,734	55,110	242,902
5 - maximum daily discharge (2011-2013)	200,455	60,789	71,337	542,584
6 - bankfull (estimated)	264,109	86,368	84,820	-

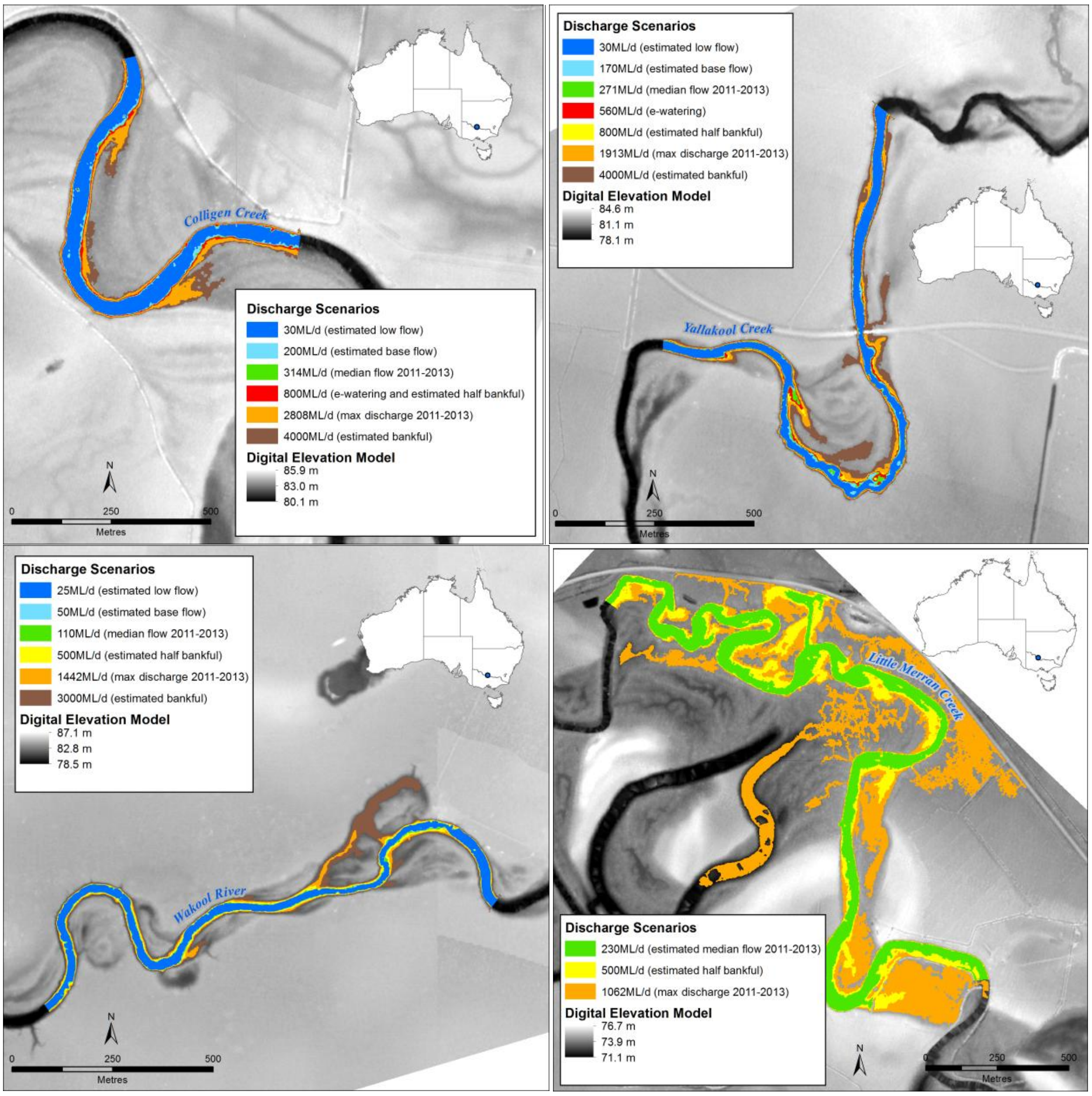


Figure 10. Maps showing representation the spatial coverage of the water surface under different discharge scenarios

Figure 11 shows that the relationship between discharge and wetted surface area in these rivers is not linear. It is likely that the relationship between these factors is strongly influenced by geomorphology. The modelling has demonstrated that a fresh of a given discharge in one river may not result in the same increase in wetted benthic surface area in another river. For example, an increase from 200 ML/day to 800 ML/day in Colligen Creek resulted in a modelled 14% increase in wetted benthic area, whereas a similar increase from 170ML/day to 800 ML/day in Yallakool Creek resulted in a modelled 30% increase in wetted benthic area (Table 8). There is a large increase in estimated wetted surface area with increasing discharge in Little Merran Creek.

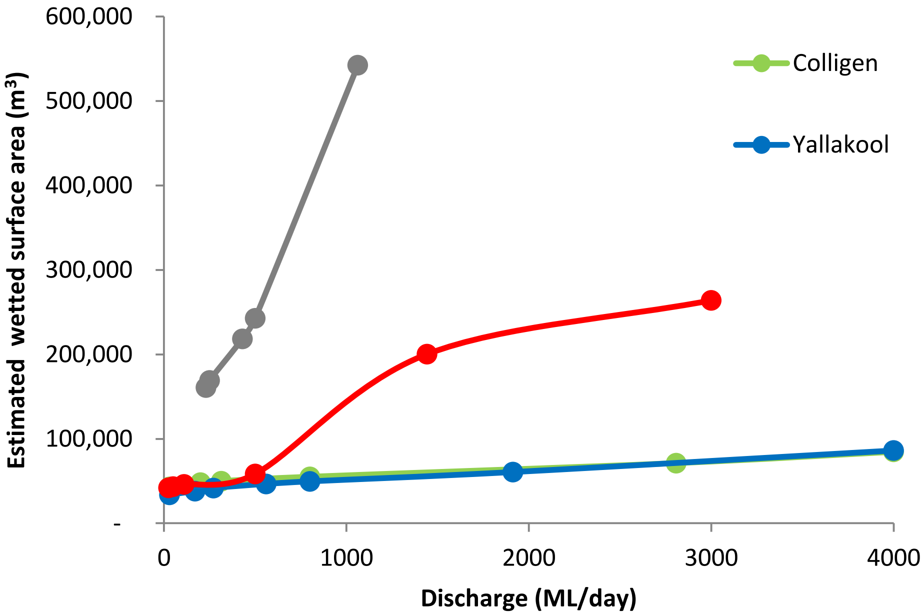


Figure 11. Modelled relationship between discharge (ML/day) and wetted surface area (m^3) for study reaches in Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek.

In summary, the inundation modelling results suggest that the environmental watering in Yallakool Creek and Colligen Creek increased the inundated benthic area in these systems, but the increase was small relative to increases for unregulated events or bankfull discharge. As the data presented here are for only a small section of each river, it is important to consider that the relationship between discharge wetted benthic surface area will be reach specific and that these relationships need to be examined over a longer river distance in the study rivers. This would facilitate better planning for the magnitude and duration of environmental watering events.

These results have important implications for in-channel environmental watering actions. It may be more appropriate to examine the relationship between inundation area and ecosystem responses to in-channel flows rather than focussing on relationships with daily discharge data, as has commonly been the practise. In-channel hydrodynamic modelling under different flow scenarios can be used to:

i) better understand the relationship between in-channel flows and ecosystem responses,

ii) predict the consequences of in-channel flows on biota and ecosystem functions, and

iii) facilitate better planning and management of the future in-channel environmental flows. The modelling can help managers determine the optimum discharge to increase the inundation to produce ecosystem responses to environmental flows, but with minimal third party impacts.

7.1.2.Habitat assessment

Key findings

- *There was a significant increase in the percent cover of submerged aquatic vegetation in Yallakool Creek during the environmental watering action in October to December 2012.* The dominant group were Charophytes, which are a type of algae that are similar to water plants, as they grow from the sediment into the water and produce seed-like spores. There was considerable activity of macroinvertebrates and other organisms in the shallow water in this newly established vegetation.
- *The increase in submerged aquatic vegetation was short lived, because when the water level receded in December 2012, at the end of the environmental watering, the submerged aquatic vegetation became fully exposed and was desiccated.*
- *The environmental watering actions in Yallakool Creek in February 2013 and in Yallakool Creek and Colligen Creek in March to April 2013 did not result in an increase in aquatic vegetation*. However, these watering events would have wetted the riverbank and this may contribute to maintaining riverbank plants that would provide habitat during subsequent flow events.

Background

Riverbank vegetation and aquatic vegetation plays an important role in river ecosystems and provides habitat for fish, invertebrates, frogs and birds (Roberts and Marston 2011). The cover and composition of aquatic vegetation can determine the availability of oviposition sites for macro invertebrates and calling and spawning locations for frogs (Wassens et al. 2010) and support wetland food webs and zooplankton communities (Warfe and Barmuta 2006). Furthermore, the response of aquatic and riverbank vegetation following a flow event can assist understanding the response of other biological indicators.

Riverbank plant survival and growth is affected by the frequency and duration of inundation (Toner and Keddy 1997; Johansson and Nilsson 2002). Frequent inundation can delay reproduction of (Blom and Voesenek 1996), whilst long duration of inundation can reduce growth (Blom et al. 1994; Johansson and Nilsson 2002). Favourable soil moisture and nutrient conditions created by a receding flood can encourage rapid recovery and root and shoot development and many plants, including emergent macrophytes and riparian understorey herbs, often germinate on flood recession (Nicol 2004; Roberts and Marston 2011). Differences in seasonal patterns of inundation within a single year can result in different survival, growth and reproduction responses of riverbank and aquatic plant species (Lowe 2002).

The aim of the monitoring was to assess habitat responses to environmental watering in two zones: 1. Aquatic vegetation within 5 m of water adjacent to the waters edge – representing shallow inundated terrestrial vegetation or submerged and emergent aquatic vegetation, and 2. Riverbank vegetation in a 5 m transect adjacent on the riverbank to the waters edge. This riverbank vegetation becomes inundated aquatic habitat when water levels rise during instream freshes.

Hypothesis

The percent cover of aquatic vegetation and riverbank vegetation will be higher in rivers receiving environmental water than in those not receiving environmental water. Environmental flows of longer duration will result in greater response than those having a short duration.

Methods

A rapid habitat assessment was undertaken once per month at the four focus rivers (Colligen Creek, Wakool River, Yallakool Creek and Little Merran Creek) over the eight month survey period (September 2012 to April 2013). Overall river characteristics were recorded including surrounding land use, general assessments of the surrounding vegetation communities, soil type, continuity of fringing vegetation, percent open water and percent inundated vegetation cover. Inundation levels and extent from watering events were monitored using photopoints. The photo points were set up at sites within each focus river to assess inundation of key features such as point bars and benches.

Three sites within each focus river were surveyed monthly between September 2012 and April 2013. One hundred (100) metre long transects that ran along the water's edge of the river channel were surveyed to monitor changes in the percent cover of terrestrial and aquatic vegetation over time. Each transect was ten metres in width, which allowed for five metres on the riverbank side to represent terrestrial riverbank vegetation and 5m within the water representing submerged and emergent aquatic vegetation plus , in some cases, inundated riverbank vegetation. Measurements of percent cover along each 100m transect were taken visually at 5 m intervals. The riverbank transect was was classed as grasses (tall and short), herbs (tall and short), logs and litter, and bare ground. Aquatic vegetation was classed as tall emergent, short emergent, broadleaf emergent, attached floating, or submerged and the percent cover of each class was recorded.

Results and discussion

Aquatic habitat assessment

There was a different response in each river in terms of aquatic vegetation cover and diversity over the survey period (Figure 12). Hydrological conditions, such as water depth and stability of water levels (Casanova and Brock 2000), and channel geomorphology can both strongly influence aquatic vegetation community and structure (Brock et al. 2006; Thoms et al. 2006).

The aquatic vegetation cover in Little Merran Creek and Wakool Rivers (control river) remained relatively constant over the survey period (Figure 12). There was a trend towards and increase in the percent cover of tall emergent aquatic plants slightly over time in the second control, Wakool River, but this was not statistically significant due to high variation among replicates.

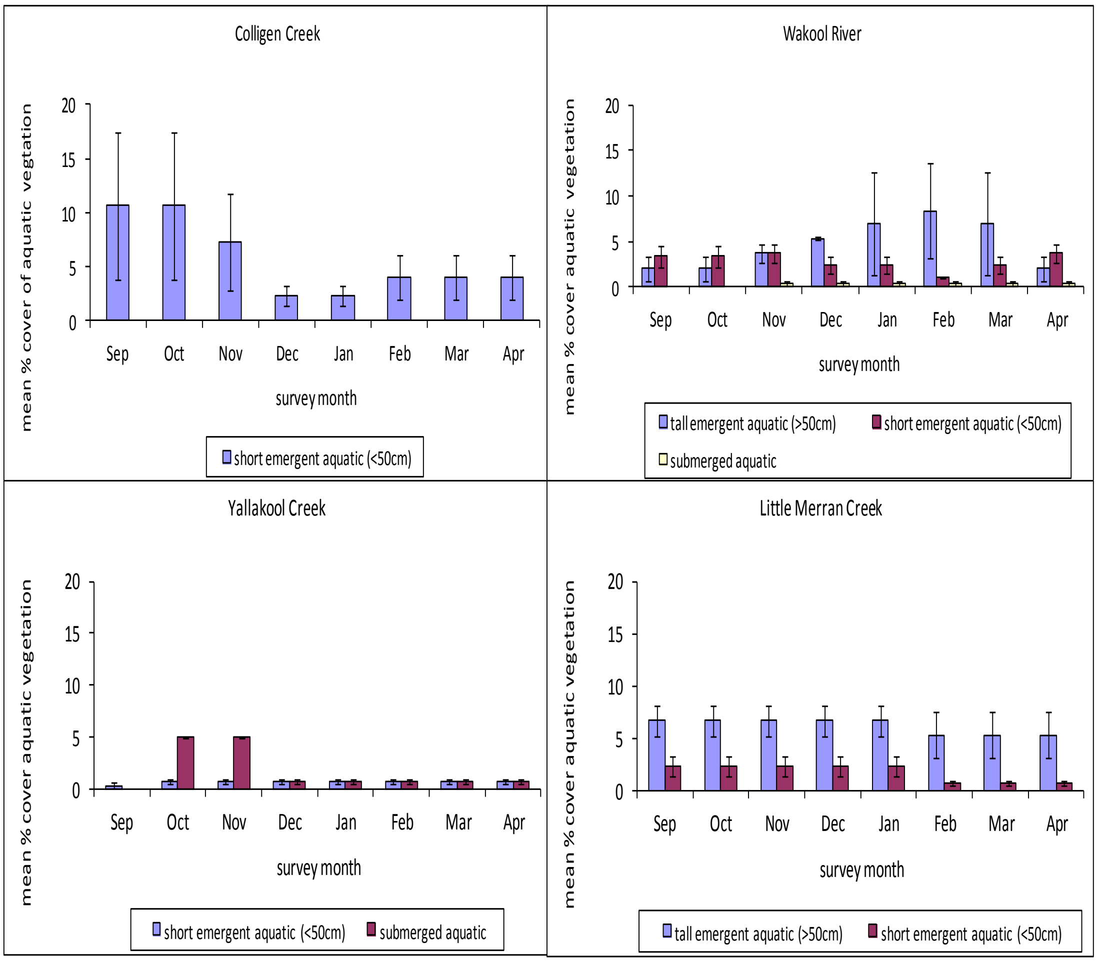


Figure 12. Mean percent cover (± 1SE) of aquatic vegetation cover observed at focus rivers between September 2012 and April 2013.

There was a significant response of submerged aquatic vegetation in the Yallakool Creek October to December 2012 environmental watering action (Figure 12). Submerged aquatic vegetation cover was very low in September 2012, but increased during October and November 2012 (Figure 12). The dominant group were Charophytes, which are a type of algae that are similar to water plants, as they grow from the sediment into the water and produce seed-like spores. The duration of inundation provided the opportunity for submerged vegetation (in particular Characeae *sp*) to increase in area in the shallow water zone that was created during the environmental watering. There was a visible increase in the activity of macroinvertebrates and other organisms in the shallow water zone in this newly established vegetation. The increase in submerged aquatic vegetation was short lived, because the water level receded in December 2012 at the end of the environmental watering, and the percent cover of submerged aquatic vegetation decreased as the banks became exposed and the aquatic vegeration was desiccated (Figure 13). Although Yallakool Creek received additional environmental freshes from March to April 2013, no increase in aquatic vegetation cover was observed as a result of` these shorter environmental watering events (Figure 12; Figure 13). These observations support the hypothesis that environmental watering of longer duration will result in greater response in aquatic vegetation than those having a short duration.

A higher cover of inundated short emergent vegetation, comprised mostly of grasses and rushes, was present in Colligen Creek between September and October 2012 compared to the other focus rivers (Figure 12). The higher cover of aquatic vegetation in Colligen Creek during the November to December 2012 environmental watering action appeared to be due to riverbank plants becoming inundated during the environmental watering, not necessarily due to an increase in production of aquatic vegetation. When the watering action concluded in mid December 2012, the wetted areas receded and dried and several rushes, sedges and short herbs were identified on the drying/dry banks which likely emerged from the receding environmental fresh. The Colligen Creek March to April 2013 environmental watering action resulted in a slight (but non-significant) increase of inundated vegetation (Figure 12).

Figure 13. Exposed bank of Yallakool Creek during environmental freshes during survey period (September 2012 and April 2013): where a) spring, (b) summer and (c) autumn

Riverbank vegetation

Riverbank vegetation consisted of the four vegetation classes (tall and short grass and tall and short herbs) at each river (Figure 14). Colligen Creek contained the highest cover of tall grasses (~20% cover) and tall herbs (~10% cover), whereas Yallakool Creek and Wakool River both contained the highest short herb cover (~35% and ~15% respectively) (Figure 14). Little Merran Creek had the least percent cover of riverbank plants of all the focus rivers.

Riverbank vegetation was similar across all rivers and did not change substantially during the survey period, regardless of whether the river received an environmental fresh or not (Figure 14). The lack of change in vegetation cover may be, in part, because only minor inundation of the riverbank occurred at the study reaches during the environmental watering (Table 8, Section 7.1.1). The study area also experienced extreme mean maximum temperatures consistently above 40 degrees between November 2012 and February 2013, as well as very low rainfall (4.4 mm in January) (Figure 15). Combined with limited riverbank inundation, it is not surprising there was little change in the cover and community compositions of vegetation within the focus rivers.

Riverbank vegetation productivity and structure can be influenced by hydrological conditions such as frequency, duration, magnitude and timing of events (Casanova and Brock 2000; Kehr et al. 2013; Robertson et al. 2001) and understorey vegetation community composition can vary in response to wet and dry periods (Reid et al. 2011). The inundation of riverbank vegetation following larger flow events may be important for the Edward-Wakool system, as increased plant productivity can contribute to carbon and nutrient dynamics in aquatic and terrestrial ecosystems (Sims and Thoms 2002) and provde habitat for a range of organisms.

Grazing by domestic livestock can also influence riparian vegetation (Robertson, 1997; Robertson and Rowling 2000) and evidence of pugging and grazing by sheep, cows and horses was observed during the survey period at all focus rivers. Thus, the relatively low cover of riverbank vegetation at the study sites may be, in part, due to grazing which can reduce plant biomass (Lunt et al. 2007; Reid et al. 2011; Robertson and Rowling 2000) and species diversity in flood prone areas (Robertson 1997). Grazing can also alter plant community composition by advantaging species, such as grasses, that respond positively to grazing (Landsberg et al. 2002). Although grazing impacts were not monitored during this study, the impacts of grazing by domestic livestock should be considered in future monitoring.

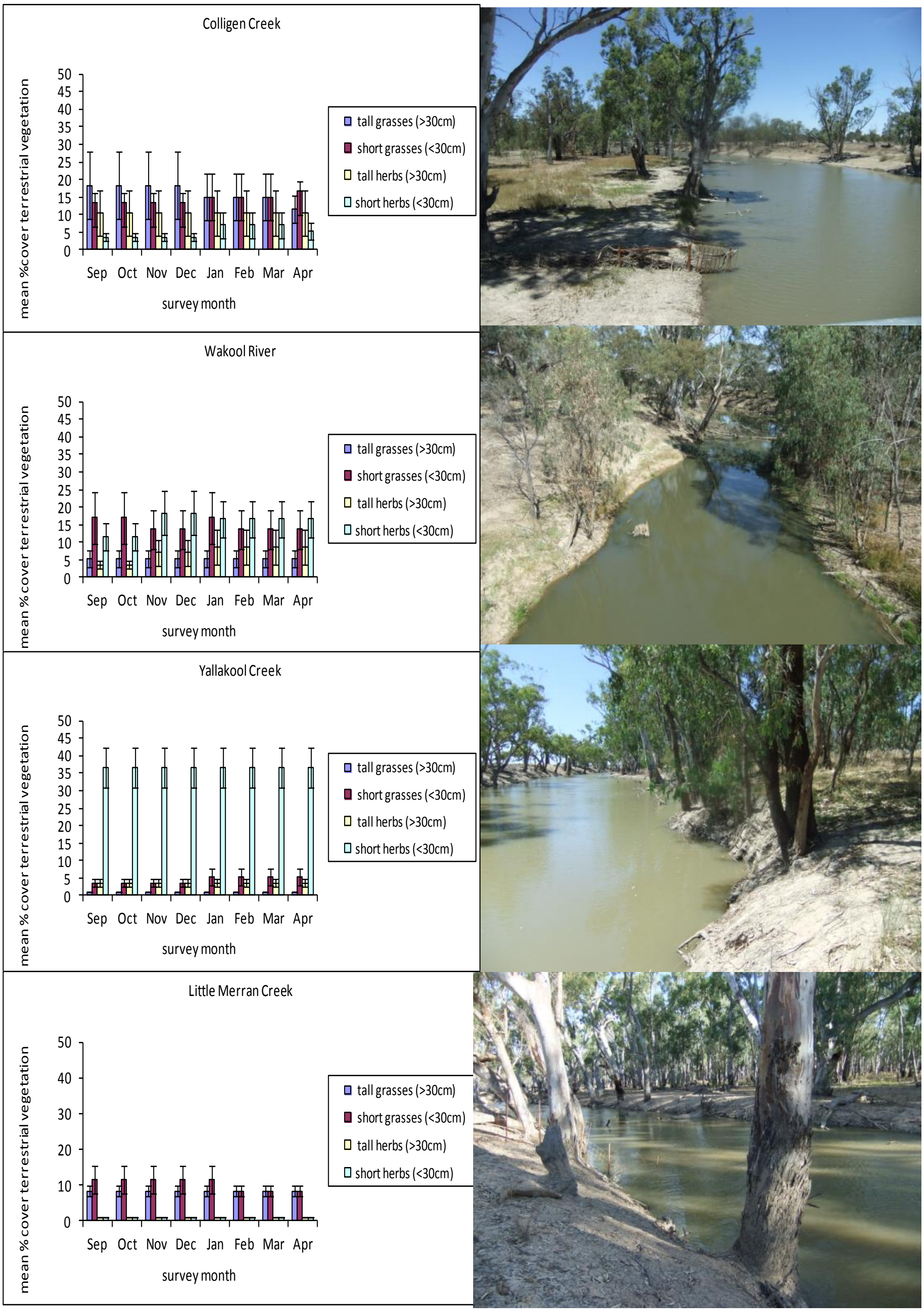


Figure 14. Mean percentage cover of terrestrial and fringing vegetation observed, and accompanying photograph representing typical cover at each focus reach.

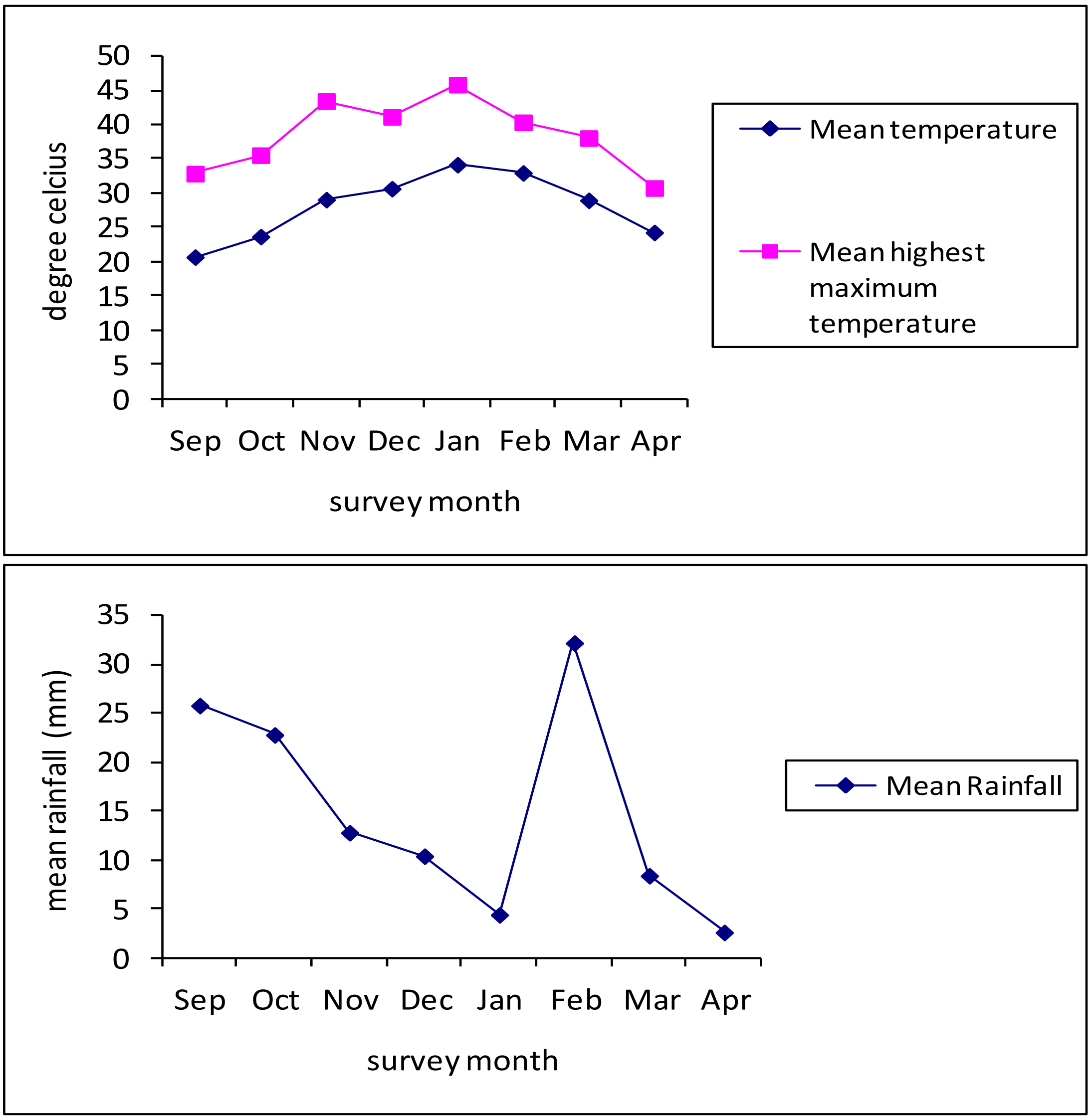
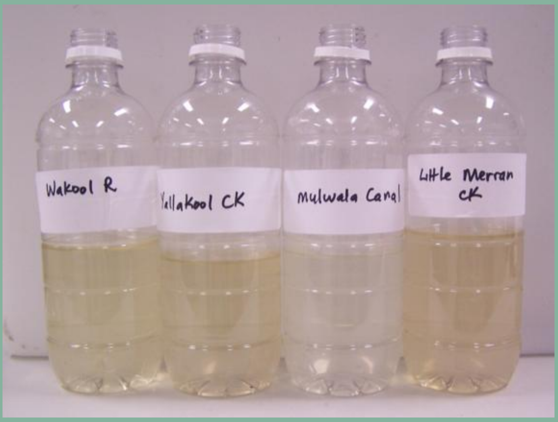


Figure 15. Weather experienced during the survey period (September 2012-April 2013): a) mean monthly maximum temperature and mean temperature; b) mean monthly rainfall

7.2. Objective 2: Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material

7.2.1. Water quality and chemistry



Key findings

- *Water temperature was similar at all sites and followed a seasonal trend that is consistent with that recorded in 2011-2012*.
- *The dissolved oxygen concentrations remained at acceptable levels throughout the study period*, indicating that no hypoxic blackwater event was associated with any of the environmental watering actions.
- *The environmental watering actions did not trigger an increase in DOC.* Dissolved organic carbon levels were very similar between all sites, except Little Merran Creek. Elevated DOC in Little Merran Creek from August through to October 2012 indicates greater carbon inputs associated with the unregulated flows during this time.
- *The bioavailable nutrient concentrations, ammonia, filterable reactive phosphorus and NO_x (nitrate plus nitrite) did not exceed ANZECC Trigger concentrations,* with the exception of NO_x on just one occasion during the large natural flows in the Little Merran Creek in August 2012 where substantially larger areas of benthic surface were wetted.
- *The environmental watering did not stimulate ecosystem productivity by moving nutrients and carbon between the main channel, upper benches and small low commence to flow floodrunners.* This is probably due to the small magnitude of increase in wetted benthic area during the environmental watering (see 7.1.1)

Background

A range of parameters can be measured as indicators of water quality in river systems and many of these are directly or indirectly influenced by environmental watering. Parameters such as dissolved oxygen and temperature will directly influence the suitability of the water for aquatic organisms, such as fish. These may be influenced by flow through changes in water volume, turbulence and through indirect processes, such as alterations in rates of bacterial metabolism and photosynthesis. Nutrients and organic matter concentrations may be influenced by flow, either by dilution or through inputs associated with water contacting parts of the channel or floodplain which were previously dry and which have stores of nutrients and carbon in both plant materials and the soil (Baldwin 1999; Baldwin and Mitchell 2000). Inputs of these substances may have a positive influence on the river community through the stimulation of productivity and increased food availability for downstream communities (Robertson et al. 1999), however, excessive inputs can result in poor water quality through the development of problem algal blooms or blackwater events resulting in very low dissolved oxygen concentrations (Howitt et al. 2007; Hladyz et al. 2011). This project aims to assess changes to water quality in response to alterations in flow and to consider changes in both the quantity and type of organic matter present in the system.

Hypotheses

- Environmental watering is expected to stimulate ecosystem productivity by moving nutrients and carbon between the main channel, upper benches and small low commence to flow floodrunners.
- Environmental watering is not expected to trigger blackwater events in these systems.

Methods

Water temperature and dissolved oxygen were logged every ten minutes at two sites in each of the four focus river reaches, with loggers located approximately 3-5 km apart. Data were downloaded and loggers calibrated approximately once per month, although on some occasions it was a longer period if high flow events made it difficult to retrieve loggers. Light and depth loggers were deployed at the commencement of the 2012-13 monitoring period and data downloaded on a monthly basis.

Water quality parameters (temperature (^{o}C), specific conductivity (mS/cm), dissolved oxygen (%), pH, and turbidity (NTU)) were also measured as spot recordings, fortnightly at two sites within each river.

Water samples were collected from four sites within each river reach to assess for:

- Total Organic Carbon (TOC)
- Dissolved Organic Carbon (DOC)
- Nutrients (Ammonia (NH_3), filtered reactive phosphorus (FRP), dissolved nitrate + nitrite (NOx), Total nitrogen (TN) and Total phosphorus (TP))

DOC and nutrient samples were filtered on-site using 0.2 μm membrane filters. Samples were frozen and organic carbon samples refrigerated for transport to Monash University for analysis. Nutrient analysis (by Flow Injection Analysis) and organic carbon analysis (high temperature conversion to CO_2 followed by infra-red detection) were undertaken by the National Association of Testing Authorities (NATA) accredited laboratory at the Monash University Water Studies Centre using accredited Quality Assurance protocols, thereby ensuring the integrity of data and analysis procedures.

An asymmetrical BACI (before-after, control-impact) (Underwood 1991) statistical design was used to test the effect of specific environmental water actions on water chemistry parameters. Differences in mean values between control/impact rivers and before/during/after environmental watering were evaluated statistically for each watering action using 2-way mixed effects analysis of variance (ANOVA). Because there were multiple sampling times used to represent before, during and after environmental flows, and multiple rivers used as 'control' and sometimes 'impact' rivers, sampling trip (random effect) was nested within Period (fixed effect, three levels: before, during and after), and river (random effect) was nested in Treatment (fixed effect, two levels: control rivers, impact rivers). Impact rivers received environmental freshes, while Control rivers were those that did not receive environmental water. For this analysis particular interest is in the Period x Treatment interaction term, which indicates a significant effect of the environmental watering action. Visual assessment of plots, grouped by Period and Treatment, were used to confirm if the significant interaction term was positively or negatively associated with the environmental watering action.

In November, two watering actions took place, one in Yallakool Creek and the other in Colligen Creek. For the Yallakool Creek November fresh, we compared the two sampling times Before (3 October, 17 October 2012) with the three times During (31 October 2012, 14 November, 28 November 2012) and two times After (12 December, 29 December 2012) the fresh (Table 9). Here, the Yallakool Creek was the 'Impact' river, and the Wakool River and Little Merran Creek the 'Control' rivers. The Colligen Creek November 2012 fresh was delivered as two distinct pulses and so the BACI analyses were run on each pulse separately. For the first pulse, we compared one sampling time before (17 October 2012) with two times during (31 October, 14 November 2012) and one time after (28 November 2012) the fresh. For the second pulse, we compared one sampling time before

(28 November 2012), with one time during (12 December 2012) and one after (29 December 2012) the pulse. Here, Colligen Creek was the 'Impact' river, and the Wakool River and Little Merran Creek the 'Control' rivers (Table 9).

Yallakool Creek received a second fresh in February 2013, and here we compared two sampling times before (9 January 2013, 23 January 2013) with two sampling times during (5 February 2013, 20 February 2013), with one sampling time after (6 March 2013) (Table 9). Yallakool Creek was the Impact river, and the Wakool River and Little Merran Creek the Control rivers. In March 2013, Colligen and Yallakool Creeks received freshes of similar magnitude, duration and 'shape'. The similar hydrographs in both Impact rivers meant we were able to perform an ANOVA with both Yallakool and Colligen Creeks used as Impact Rivers, and Wakool River and Little Merran Creek as Controls. Before the fresh was represented by one sampling time (6 March 2013), during by two sampling times (20 March 2013, 3 April 2013), and after by one sampling time (17 April 2013) (Table 9). The null hypothesis was that water chemistry parameters in the rivers which received environmental water were not significantly different to the control rivers.

Table 9. Summary of dates and rivers used to detect changes in water chemistry parameters (this section), phytoplankton (section 7.2.3), zooplankton (section 7.3.1) and larval fish densities/abundances (section 7.5.1) for the individual 2012-2013 watering actions. A'BACI' style 2-way nested ANOVA was used.

Water action	**Treatment**		**Period (before-during-after)**	**date**
	(impact river/s)	**(control river/s)**		
Yallakool River Nov 2012 watering action	Yallakool	Wakool, Merran	before	1-5 Oct 2012 15-19 Oct 2012
			during	29 Oct-2 Nov 2012 10-14 Nov 2012 26-30 Nov 2012
			after	10-14 Dec 2012, 27-30 Dec 2012
Colligen River Nov 2012 watering action - fresh #1	Colligen	Wakool, Merran	before	15-19 Oct 2012
			during	29 Oct-2 Nov 2012 12-16 Nov 2012
			after	26-30 Nov 2012
Colligen River Nov 2012 2 watering action - fresh #	Colligen	Wakool, Merran	before	26-30 Nov 2012
			during	10-14 Dec 2012
			after	27-30 Dec 2012
Yallakool River Feb 2013 watering action	Yallakool	Wakool, Merran	before	7-11 Jan 2013 21-25 Jan 2013
			during	4-8 Feb 2013 18-22 Feb 2013
			after	4-8 Mar 2013
Yallakool and Colligen Rivers March 2013 watering actions	Yallakool, Colligen	Wakool, Merran	before	4-8 Feb 2013
			during	18-22 Mar 2013 2-5 Apr 2013
			after	15-19 Apr 3013

Results and Discussion

The data collected by the loggers was used to calculate daily average dissolved oxygen concentrations (Figure 16) and temperature (Figure 17) for each of the rivers from September 2012 to April 2013. Water temperature at all sites was similar and followed a seasonal trend that is consistent with that recorded in 2011-2012 (Watts et al. 2013) The dissolved oxygen concentrations remained at acceptable levels throughout the study period, indicating that no hypoxic blackwater event was associated with any of the environmental watering actions.

Spot water quality measurements taken in August 2012, prior to the loggers being installed, indicated that dissolved oxygen concentrations were above 7.6 mg/L at all sites, and the only values below 8 mg/L were recorded in Little Merran Creek on 3 August 2012. The water temperature during this period was 9 ^{o}C and the measured oxygen concentrations were well below saturation, indicating elevated respiration associated with unregulated flows at this time. Little Merran Creek was most affected, but the concentrations remain well above the level at which fish are expected to be seriously impacted (Gehrke et al, 1993).

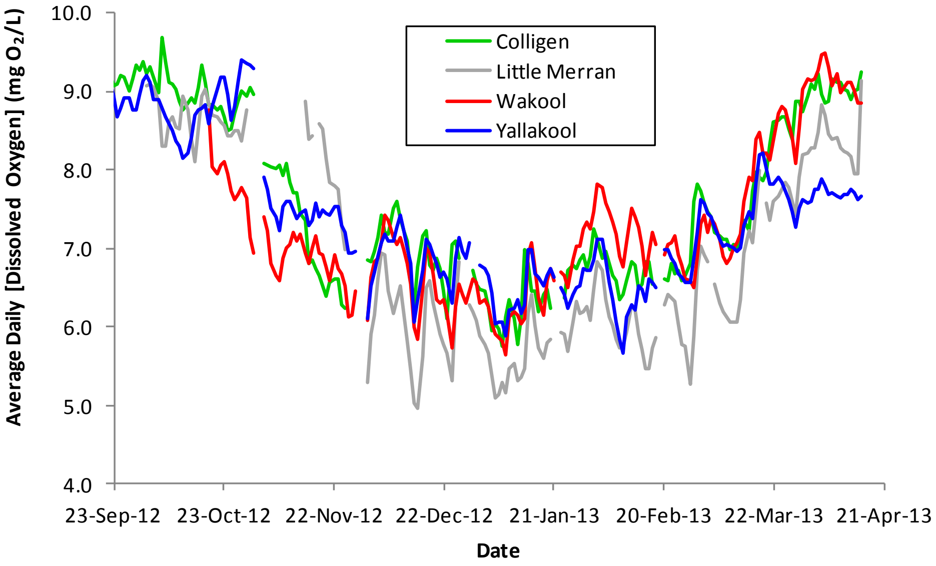


Figure 16. Average Daily Dissolved Oxygen concentrations (mg O_2/L). Data were collected continuously at 10 minute intervals over the period 23/9/2012 to 15/4/2013. Wakool data prior to 17/4/2012 were not included as the sensor was out of the water.

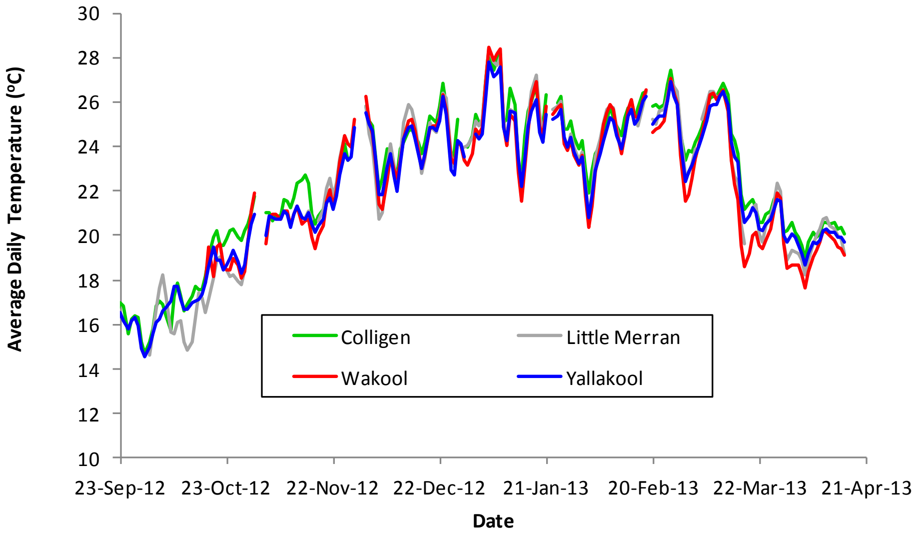


Figure 17. Average Daily Water Temperature (^{o}C). Data was taken from the DO loggers which were collecting data continuously at 10 minute intervals over the period 23/9/2012 to 15/4/2013. Wakool data prior to 17/4/2012 were not included as the sensor was out of the water.

Dissolved organic carbon levels (Figure 18) were very similar between all sites, except Little Merran Creek, on most sampling dates. Elevated levels recorded in the Edward River on 17 October 2012 are not consistent with other water quality parameters (see section 7.2.2). Elevated DOC in Little Merran Creek from August through to October 2012 is consistent with the lower DO concentrations noted above and indicates greater carbon inputs associated with the unregulated higher flows during this time. While the DOC in Little Merran Creek was higher than in the other rivers, at no time did the DOC concentrations reach the levels observed during the unregulated flow event that occurred in March and April 2012 (Watts et al. 2013). After the unregulated flow event DOC concentrations returned to the normal base range (2-4 mg/L) observed in this system. Particulate organic carbon concentrations (Figure 19) remained low throughout the study period and high levels of variability were observed (as might be expected in this concentration range).

The ANZECC (2000) water quality guidelines do not provide trigger levels for total organic carbon and dissolved organic carbon, and this reflects the expectation that there will be large variation in the 'normal' concentrations of organic carbon between ecosystems and also in the chemical and biological reactivity of the mixture of organic compounds making up the DOC and TOC at a particular site. Trigger levels are concentrations of key water quality parameters designed to provide guidance for ecological protection. Where it has been determined that the measured concentration of one or more parameters in the water body exceed this level (or for some parameters, fall outside the given range) the trigger levels are designed to 'trigger' further investigation to establish whether the concentrations are causing harm in that system. Exceedance of a trigger level is not an absolute indicator of ecological harm. Given the variable make-up of organic carbon, and the possible range of ecological responses to this mixture, a trigger level for this parameter would not be appropriate. However, trigger levels are provided for a number of nutrients and these are discussed below.

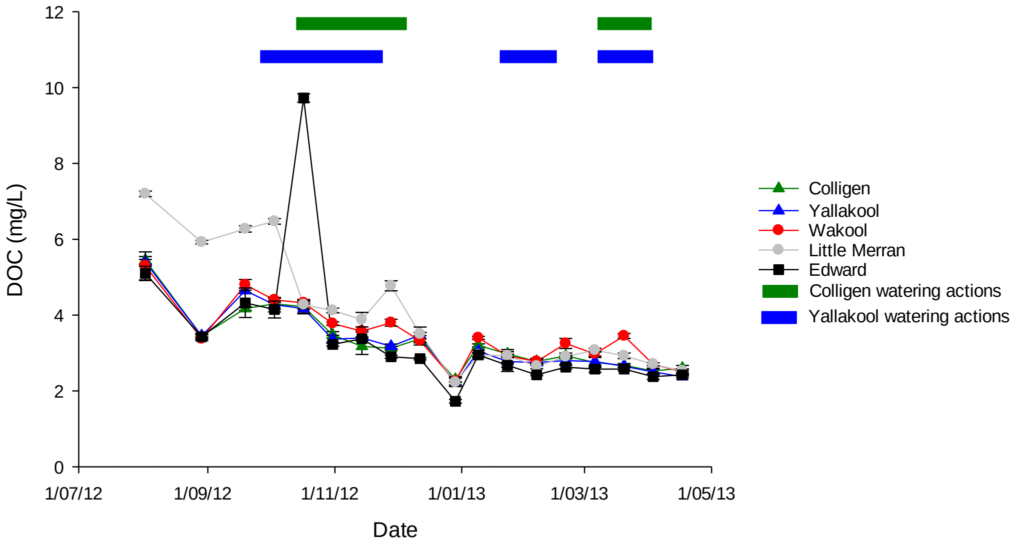


Figure 18. Dissolved Organic Carbon **(**DOC) concentrations in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4)

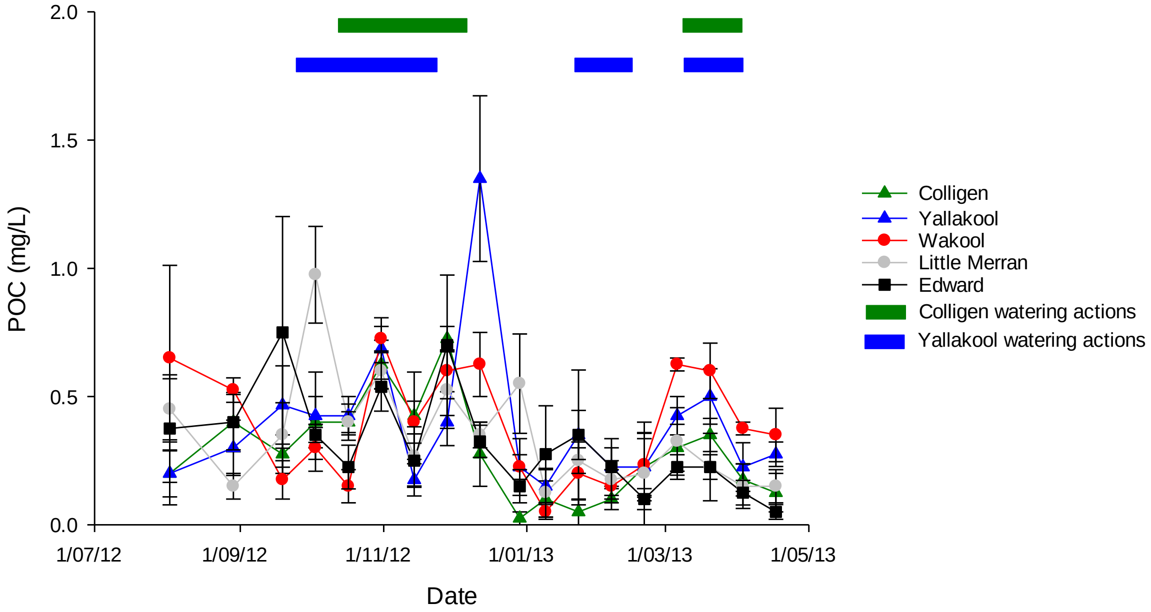


Figure 19. Particulate Organic Carbon (POC) concentrations in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4).

While there were no large scale changes in DOC or POC concentrations associated with the environmental watering actions, a small number of significant interactions were detected using the BACI statistical analysis (Table 10, Figure 20). The analysis has detected differences in the pattern of changing concentrations over time, rather than a clear separation of average concentrations between the treatment and control rivers during the watering action. The only significant interactions between control-impact rivers and before-during-after treatments occurred on the second fresh in Colligen Creek in November. The DOC concentration in the Colligen increased very slightly during this watering action while the control rivers had decreasing concentrations. It should be noted that Colligen Creek started with a slightly lower DOC concentration than the control rivers and only rose to an equivalent concentration during the watering action –the during and after concentrations of DOC are equivalent for the three rivers. No significant interactions were detected for Yallakool Creek. The only significant interaction for the POC data (p=0.041), was associated with a decrease in POC during the same flow event. As this pattern was also not noted in Yallakool Creek and the concentrations are both low and variable, the impact of flow at the scale of these watering actions was minimal.

Table 10. Statistical results for 2 way mixed-effects Analysis of Variance (ANOVA). A significant interaction between the two fixed factors: Period (before, during, after) and CI (control rivers, impact rivers) indicates that mean POC or DOC (mg/L C) in Impact Rivers was significantly different to changes that occurred over the same period of time within the Control Rivers. Significant interactions highlighted in bold print.

Environmental flow	DOC/POC	Main effect	d.f	F-test	p-value
Nov 2012 – Colligen Creek fresh #1					
	DOC	Period (B-D-A)	2,42	4.006	0.025
		CI (C-I)	1,42	2.740	0.105
		Period*CI	2,42	2.928	0.064
	POC	Period (B-D-A)	2,39	0.667	0.518
		CI (C-I)	1,39	1.38	0.246
		Period*CI	2,39	0.46	0.633
Nov 2012 – Colligen Creek fresh #2					
	DOC	Period (B-D-A)	2,29	110.622	0.003
		CI (C-I)	1,1	1.136	0.450
		Period*CI	**2,29**	**16.064**	**<0.001**
	POC	Period (B-D-A)	2,29	6.776	0.003
		CI (C-I)	1,1	2.768	0.344
		Period*CI	**2,29**	**3.569**	**0.041**
Nov 2012 – Yallakool Creek fresh					
	DOC	Period (B-D-A)	2,78	6.123	0.003
		CI (C-I)	1,78	0.997	0.321
		Period*CI	2,78	0.949	0.391
	POC	Period (B-D-A)	2,77	0.118	0.888
		CI (C-I)	1,77	0.153	0.696
		Period*CI	2,77	1.224	0.299
Feb 2013 – Yallakool Creek fresh					
	DOC	Period (B-D-A)	2,54	0.339	0.714
		CI (C-I)	1,54	1.139	0.290
		Period*CI	2,54	0.175	0.840
	POC	Period (B-D-A)	2,53	4.220	0.019
		CI (C-I)	1,53	4.667	0.497
		Period*CI	2,53	0.357	0.701
Mar 2013 – Yallakool & Colligen Creek freshes					
	DOC	Period (B-D-A)	2,58	0.809	0.450
		CI (C-I)	1,58	9.941	0.002
		Period*CI	2,58	1.458	0.241
	POC	Period (B-D-A)	2,58	0.534	0.589
		CI (C-I)	1,58	0.125	0.724
		Period*CI	2,58	0.373	0.690

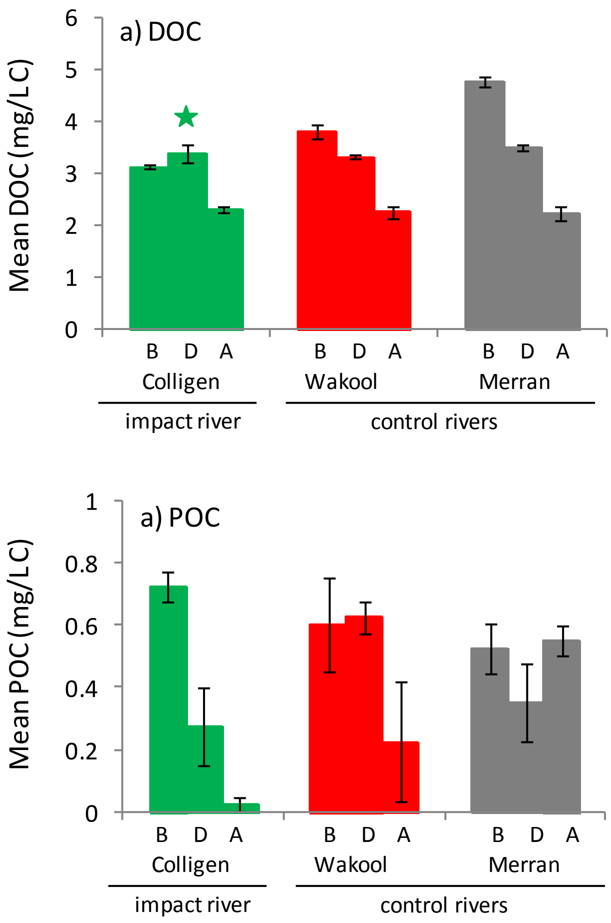


Figure 20: Mean concentration (±1SE) of dissolved organic carbon (DOC) and particulate organic carbon (POC) present in the Edward-Wakool system before, during and after the second environmental flow 'fresh' in Colligen Creek in November 2012. The Wakool River and Little Merran Creek did not receive environmental water, and were used as controls. Planned comparisons with significant interactions between control-impact rivers (impact river; Yallakool Creek, control rivers; Wakool River, Little Merran Creek) and Period (before, during, after) are marked with an asterisk, indicating that mean DOC changed significantly in Colligen Creek during the environmental watering.

Concentrations of Total Nitrogen (Figure 21) and NO_x (nitrate plus nitrate) (Figure 22) also indicate that the unregulated flow in August 2012 resulted in a different response in Little Merran Creek than in the other rivers. Both TN and NO_x were elevated in Little Merran Creek relative to the other rivers over this period, while there is no clear pattern in the ammonia data (Figure 23). The environmental watering actions have not impacted on the nitrogen concentrations in either of the impact rivers, relative to the control rivers.

While the TN concentrations at times exceed the ANZECC (2000) trigger value for lowland rivers of 500 µg/L (0.5 mg/L) (Figure 21), the bioavailable forms of nitrogen are well below the trigger values (with the exception of NO_x in Little Merran Creek at the beginning of August). This pattern is repeated with the concentrations of P - while the Total P concentrations frequently exceed the trigger value of 50 µg/L (0.05 mg/L) (Fgure 24), the filterable reactive P (the more bioavailable fraction) is well below the trigger value of 20 µg/L (Figure 25). A slight increase in bioavailable P occurs in early August 2012 and is greatest in Little Merran Creek, but FRP is not impacted by any of the environmental watering actions. Total P concentrations in November and December indicate that when freshes are being delivered in Colligen and Yallakool Creeks, a decrease in Total P is observed in these two rivers and the Edward River and, while the control rivers retain higher concentrations. This effect was not repeated with freshes later in the season.

The ANZECC Water Quality Guidelines recommend that in order to minimize the risk of algal blooms and other adverse outcomes in lowland rivers in south-eastern Australia, the bioavailable concentrations of ammonia, FRP and NO_x should be below 20, 20 and 40 µg/L respectively (Figure 26). The median concentrations were nearly an order of magnitude lower than these guidelines. The occasional high NO_x values in Little Merran Creek coincided with a natural higher flow event in August-September 2012.

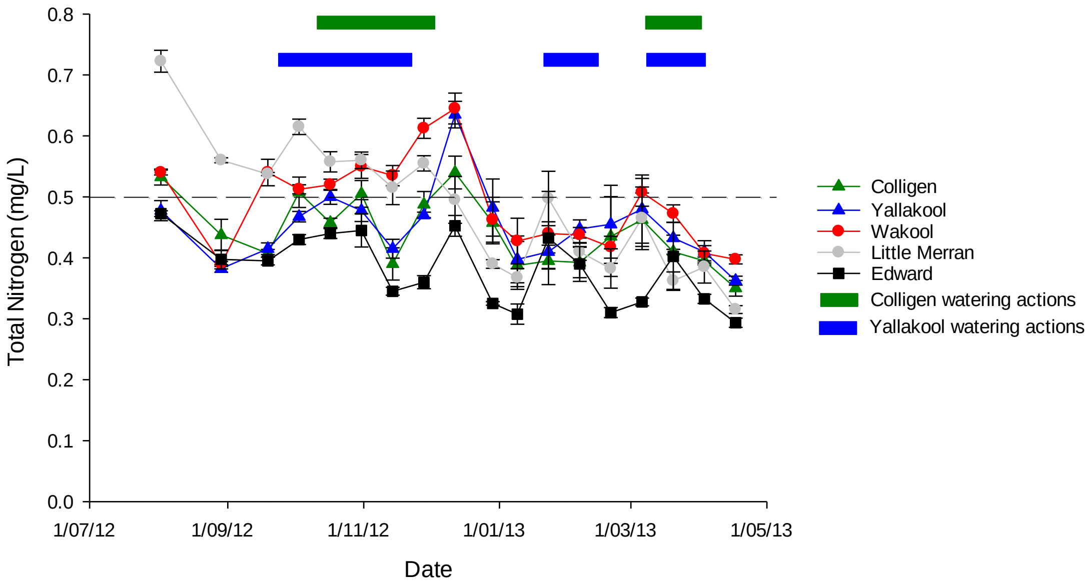


Figure 21. TN Average Total Nitrogen Concentrations in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4). The dashed line indicates the ANZECC (2000) trigger level for this nutrient.

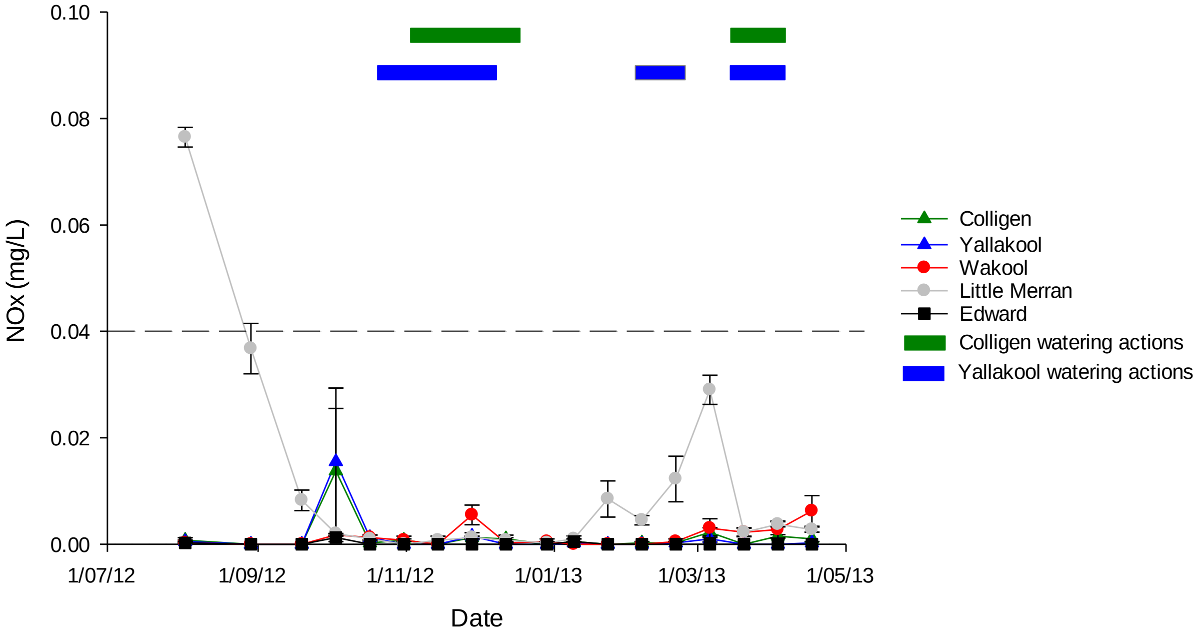


Figure 22. Average NO_x (Nitrate and Nitrite) concentrations in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4). The dashed line indicates the ANZECC (2000) trigger level for this nutrient.

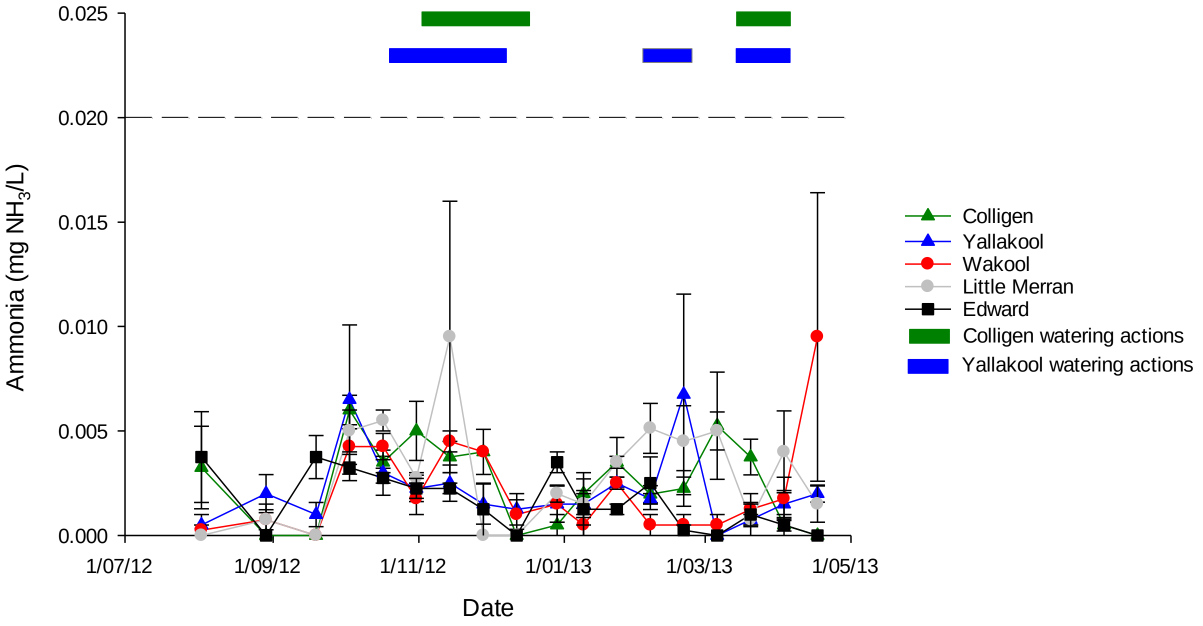


Figure23. Average ammonia concentrations (error bars: 1 s.d., n=4) in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4). The dashed line indicates the ANZECC (2000) trigger level for this nutrient.

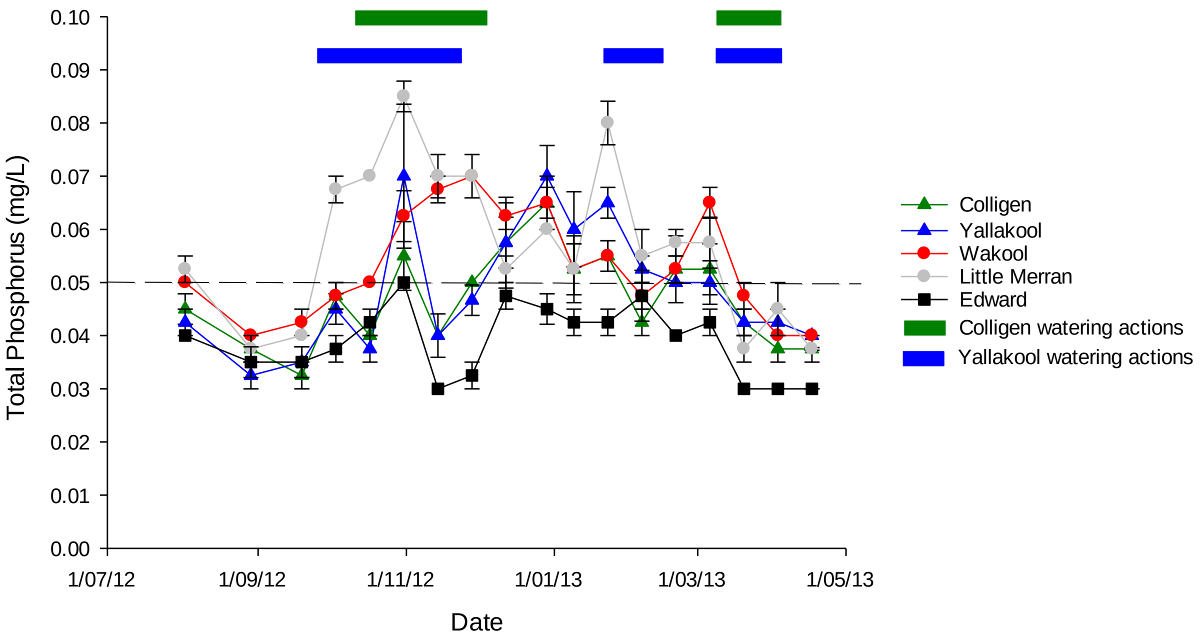


Figure 24. Average Total Phosphorus Concentrations (error bars: 1 s.d., n=4) in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4). The dashed line indicates the ANZECC (2000) trigger level for this nutrient.

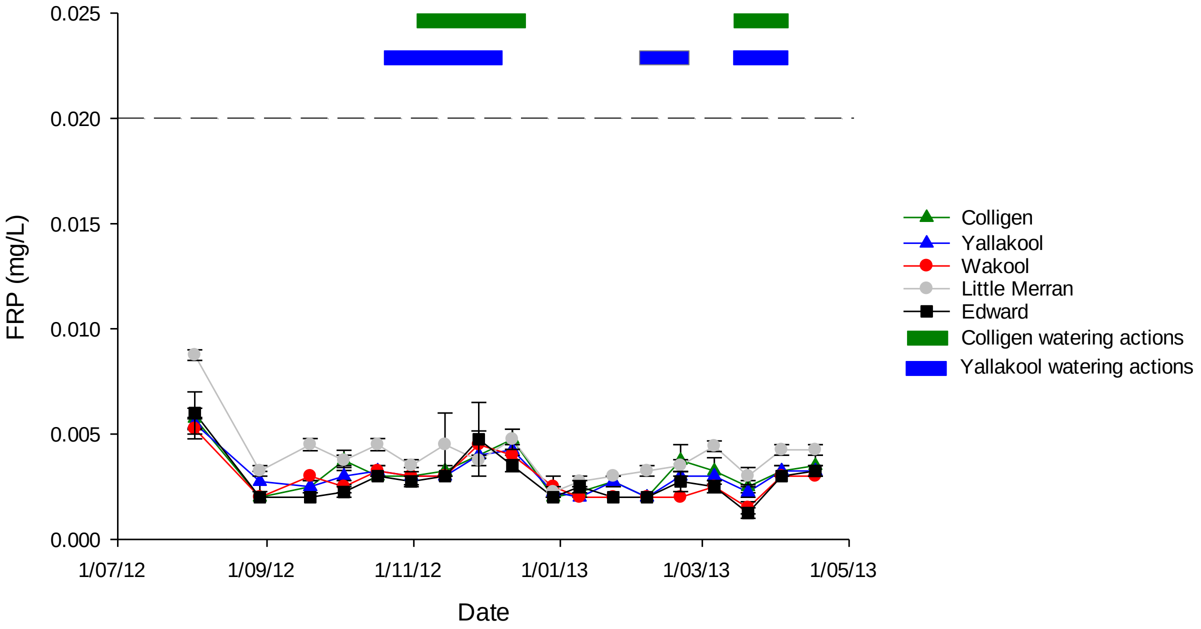


Figure 25. Average filterable reactive phosphorus concentrations (error bars: 1 s.d., n=4). in water from Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and the Edward River between July 2012 and May 2013. Blue and green lines represent the start and finish dates of environmental watering. (error bars: 1 s.d., n=4). The dashed line indicates the ANZECC (2000) trigger level for this nutrient.

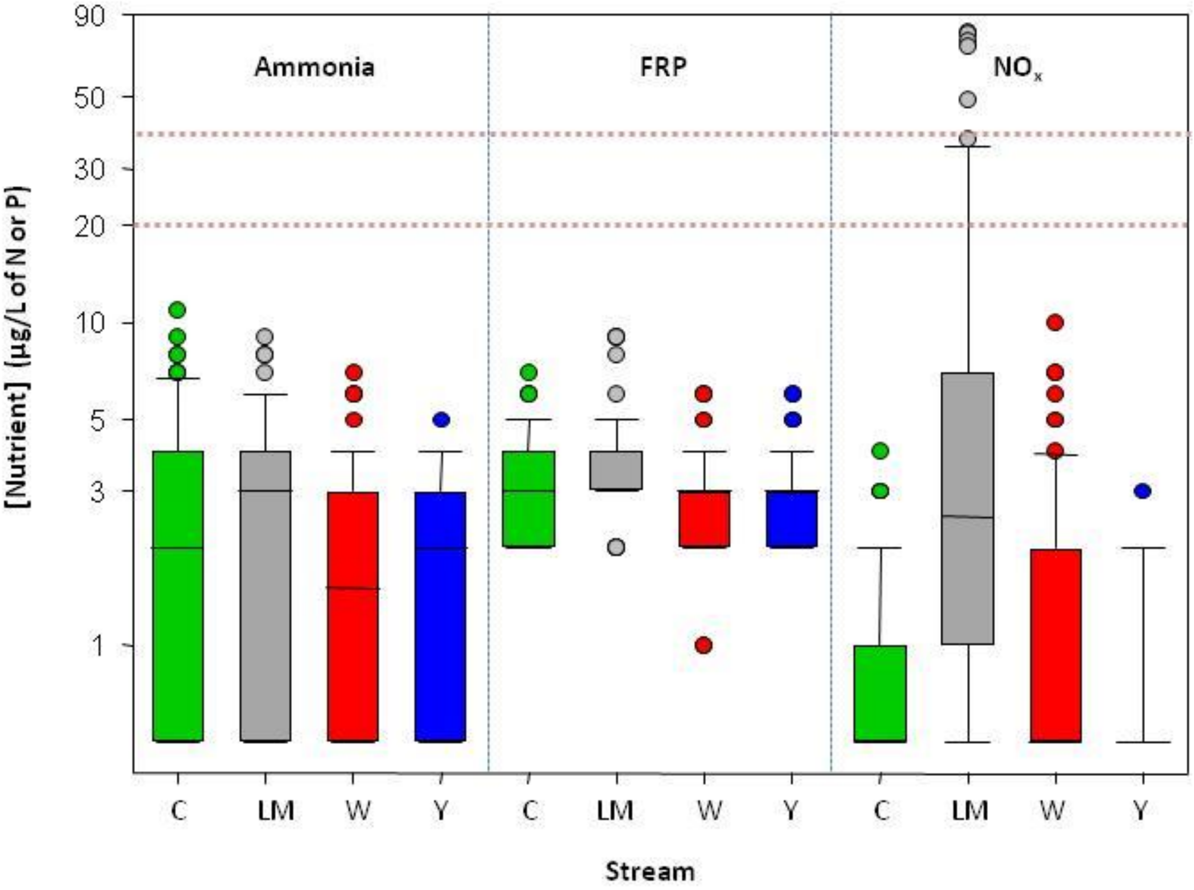
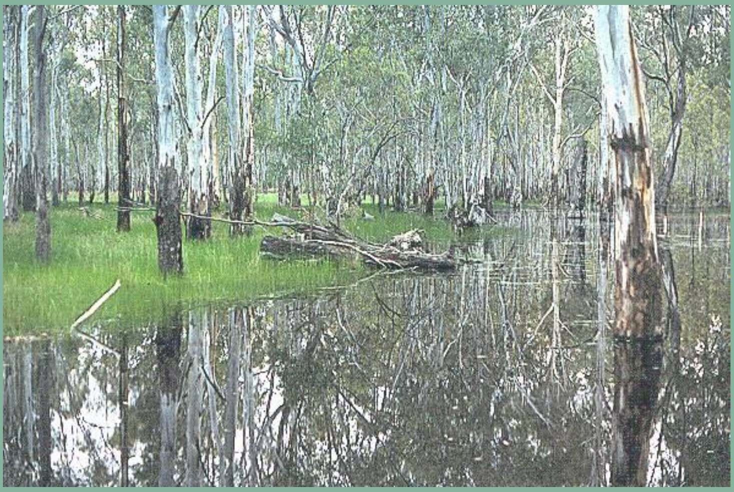


Figure 26. 'Box and Whisker Plot' Summary of bioavailable nutrient concentrations in the four study rivers: C = Colligen Creek, LM = Little Merran Creek, W = Wakool River, Y = Yallakool Creek. Note that the concentration axis is on a logarithmic scale for ease of presentation. All concentrations less than the detection limit of 1 μg/L were plotted as 0.5 μg/L. The boxes represent the data range 25th to 75th percentile, with the 'middle' line in the box being the median. The "whiskers" indicate 10th and 90th percentiles in the data. Outliers are shown as circles. The two dashed horizontal lines represent the ANZECC (2000) Trigger Values for FRP and Ammonia (20 μg/L) and NO$_x$ (40 μg/L).

7.2.2. Organic matter characterisation



Key findings

- *The environmental watering in Yallakool Creek and the Colligen Creek did not result in the composition of dissolved organic matter in these rivers becoming substantially different to the other rivers*. This suggests that the areas of in-stream habitat that were re-wetted during these flows did not have substantial amounts of accumulated organic material (such as leaf litter) and the small in-channel watering actions did not reconnect sufficient area of upper benches and floodrunners to result in substantial exchange of organic matter and nutrients. The in-channel environmental watering did not result in blackwater event.
- *The greatest influence on organic matter composition and concentration over the study period were the unregulated flows* in August and September 2012, where all rivers had elevated organic matter compared to May 2012. Organic matter inputs associated with these unregulated flows did not result in a blackwater event, due to the low water temperature at that time of year.
- Over the period from August to early October 2012, the organic matter content of Little Merran Creek was higher than all other sample sites. This may be due to the longer period of elevated flows in this river, the different water source (Murray River), and the greater areas of new riverbank being wetted in Little Merran Creek than the other study rivers.

Background

Australian riverine ecosystems can be heavily reliant on both algal and terrestrial dissolved organic matter for microbial productivity and can be limited by dissolved organic carbon concentrations (Hadwen et al. 2010). While water quality guidelines (ANZECC 2000) include trigger values (concentrations of concern) for a number of water quality parameters such as chlorophyll a, nutrients, dissolved oxygen and pH, no guidelines are given for organic matter and aquatic environments are expected to have quite varying dissolved organic matter concentrations.

Organic matter is made up of a complex mixture of compounds from a diverse range of sources. Microbial communities do not respond to all types of organic matter in the same way (Baldwin 1999; O’Connell et al. 2000; Howitt et al. 2008) although it has been shown that bacterial communities can respond to changes in organic carbon source quite rapidly (Wehr et al. 2002). The very large, complex type of organic matter referred to as humic substances has been shown to be less available to bacterial communities than simpler non-humic carbon (Moran and Hodson 1990) although this can be altered over time with exposure to ultraviolet light (Howitt et al. 2008).

One way of examining the mixture of organic substances present is to measure the spectroscopic behaviour of the substances i.e. to study which wavelengths of light they absorb, and to examine which wavelengths of light they emit (fluoresce) in response to this absorption of incoming light (Dahlen et al. 1996; Mobed et al. 1996; Baker and Spencer 2004; Howitt et al. 2008). Both absorbance and fluorescence spectra are used to examine the organic matter in this study. As a general guide, absorbance at longer wavelengths indicates larger, more complex organic matter (Bertilsson and Bergh 1999). Absorbance at a particular wavelength may be increased by increasing concentration of organic matter or a change in the type of organic matter.

The consideration of changes in both the quantity and type of organic matter present in the system allows for a more detailed examination of the hypotheses around the movement of organic carbon and the creation of blackwater events.

Hypothesis

If environmental watering facilitates inundation of extensive new sources of organic matter it would result in changes to the spectroscopic responses of the organic matter through alteration of the mixture of compounds that make up dissolved organic matter.

Methods

Samples for organic matter characterisation were collected fortnightly from August 2012 until early May 2013. Four samples were collected from each river reach on each sampling date. On each sampling trip the samples were collected in the following sequence: Little Merran Creek, Yallakool Creek, Wakool River (day 2), Colligen Creek, Edward River, (day 3). All graphs are labelled with the date indicating day 3 (usually Wednesday) of the relevant sampling trip. Water samples were filtered through a 0.2 μm pore-sized membrane at the time of sampling and then stored on ice until returned to the laboratory and then analysed within a 2 days of returning from the field. Absorbance scans were collected using a Varian Cary 4000 instrument across a wavelength range of 550 nm to 200 nm (green through to ultraviolet) with a 1 nm step size. Absorbance is a measure of light absorbed by the sample and is a logarithmic scale. An absorbance of 1 indicates that only 10% of the light of that wavelength is transmitted through the sample. Fluorescence scans were collected using a Varian Eclipse spectrofluorometer scanning both emission and excitation wavelengths to give an excitation-emission matrix (EEM). Excitation wavelengths were scanned from 200 to 400 nm with a 10 nm step size and for each excitation wavelength, emission of light at 90^{O} to the source was recorded from 200 nm to 550 nm with a 1 nm step size. Fluorescence results were corrected for sample absorption and plotted as contour plots (Howitt et al., 2008). To correct for drift in the instrument zero position, each contour plot was scaled by subtracting the average emission intensity across the range 200-210 nm for an excitation of 250 nm from all fluorescence intensities (effectively setting this region of the contour plot to zero on all plots).

An example of a contour plot is shown in Figure 27. The contour plots have the excitation wavelength (light shone into the sample) on the y-axis. On the x-axis is the emission wavelength (light given off by the sample). The intensity of the fluorescence (how much light is given off, corrected for absorbance by the sample) is represented by the colours of the contour plot, with more intense fluorescence represented by the blue end of the scale. The two blue diagonal lines are artefacts of the technique and will be present in all samples- key data is found between these two lines.

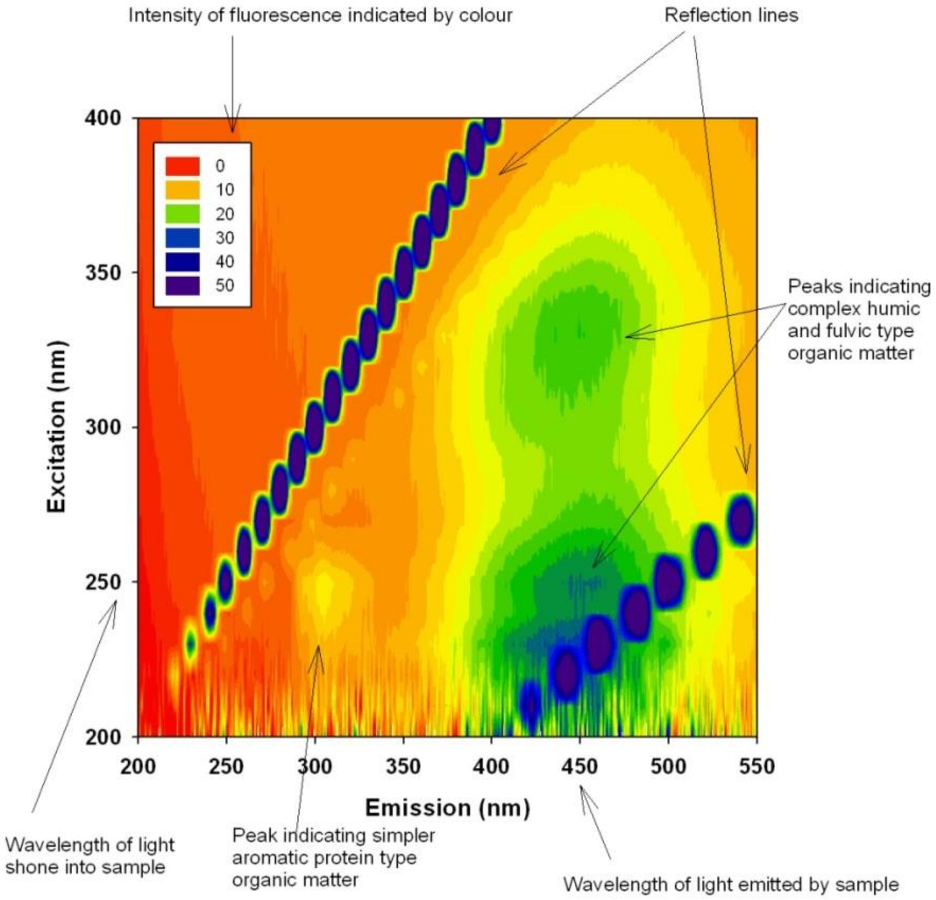


Figure 27. Sample excitation emission contour plot indicating key features of the data. (from Watts et al. 2013)

Results and discussion

Absorbance scans of representative water samples from each sampling trip are presented in Figure 28. The greatest variation between replicates for each river is expected at 200 nm and the range was less than 10% of the measured absorbance on most occasions (up to 20% during higher flows in August but commonly 2-5% later in the season). An exception was a sample from Little Merran Creek on the 14 November, where the shape of the scan indicated sample contamination and the sample was excluded. In general, this data indicates that the greatest influence on organic matter composition and concentration over the study period were the unregulated flows in August and September 2012, where all rivers had elevated organic matter compared to May 2012 (Watts et al. 2013). Over the period from August to early October 2012, the organic matter content of Little Merran Creek is clearly higher than that found at all the other sample sites. This reflects differences in the hydrograph (the elevated flows are spread over a longer period for the Little Merran), the spatial coverage of the water surface and the water source. The organic matter loading in the Little Merran Creek is influenced by overbank flows from the Murray River into the Koondrook-Perricoota

Forest upstream of the offtake and scenario modelling also indicates that the flows at this time resulted in considerably more new riverbank being wetted in Little Merran Creek than for the other study rivers. Throughout the study period the absorbance scans for the Wakool River, Colligen Creek and Yallakool Creek generally match those of the Edward River, where overbank flows through the Barmah-Millewa Forest can be a source of organic matter upstream. Shortly after Little Merran Creek returned to base flows in mid-October the absorbance results become fairly consistent with those of the other study sites. The in-channel pulsed flows in both the Yallakool Creek and the Colligen Creek do not result in the absorbance scans at these sites becoming substantially different to the other sites, suggesting that the areas of in-stream habitat that were re-wet during these flows did not have substantial amounts of accumulated organic material (such as leaf litter).

Representative fluorescence scans are shown in Figure 29a, 29b and 29c. Fluorescence spectroscopy is a more sensitive technique for the characterisation of organic matter and so the difference between the Little Merran organic matter and the other river sites is evident for longer following the unregulated flows in August and September. The fluorescence analysis indicates that by early December 2012 the amount and type of organic matter at all sites is very consistent. Consistent with the absorbance results presented above, there is no evidence that the freshes in the Colligen and Yallakool resulted in changes in the organic matter load or the composition of the mixture of compounds making up the fluorescent dissolved organic matter. There are increases in the aromatic protein region of the spectrum in all rivers during December and these persist through to March 2013, but this appears to be a seasonal effect and the increased signal in this region is consistent with that observed throughout most of the 2011-2012 study period (Watts et al. 2013). Increases in fluorescence in this region of the spectrum may indicate the presence of a more bioavailable fraction of organic matter and may be associated with breakdown of humic and fulvic components by sunlight (Howitt et al. 2008). Peaks in this region have also been associated with bacterial metabolism (Elliott et al. 2006), and have been found in marine environments (Coble 1996) where their presence is likely of algal origin. The patterns observed in the absorbance and fluorescence results are consistent with those observed in 2011-2012 (Watts et al. 2013) - the organic matter profiles become complex and the rivers are different from each other during periods of high flow where overbank flow may occur for individual river sites or for the source rivers upstream, but long periods of in-channel flow result in a reduction and simplification of the organic matter signals and there is consistency across the rivers.

The absorbance and fluorescence results are consistent with the dissolved organic carbon results, which indicate that the dissolved carbon loading in the rivers fed by the Edward River are generally consistent with each other and the source water, irrespective of the flow conditions in the individual rivers. The higher carbon loading in Little Merran Ceek at the beginning of the study period is reflected in all three data sets. The exceptions to the correlations between the data sets are the small reduction in DOC at the end of December which is not evident in the spectroscopic analyses and the very high DOC result in the Edward River on 17 October 2012. This dramatic increase in DOC at this site is not reflected in the absorbance scans, where the Edward is indistinguishable from the connected rivers, which is surprising as a dramatic increase in even quite simple organic matter would be expected to affect at least the very shortest wavelengths in these scans. There is a very slight increase in humic and fulvic signature in the Edward on this date, but an increase in DOC from normal floodplain sources to the concentrations recorded here would be expected to result in higher fluorescence in this region. Increases in DOC at this site have not been accompanied by increases in nutrients, as might be expected with overbank flows and was seen in Little Merran data in August. There is no evidence of a flow-on effect to the connected rivers on this or the following sampling date. In the absence of a highly localised input of extremely simple dissolved organic compounds (which would be expected to be reflected in a dip in dissolved oxygen at the site), it seems likely that the DOC samples for this site may have been contaminated in some way.

The small in-channel watering actions did not reconnect a sufficient area of upper benches and floodrunners to result in substantial exchange of organic matter and nutrients. The hypothesis that environmental watering would stimulate ecosystem productivity by moving nutrients and carbon between the main channel, upper benches and small, low commence-to-flow floodrunners was not adequately tested through a substantial increase in wetted area and was not upheld for the in-channel watering actions in 2012-13.

It was expected that dissolved organic carbon and particulate organic carbon levels would remain relatively unchanged following in-channel environmental watering. The environmental watering was not expected to trigger blackwater events in these systems. These hypotheses were supported- in-channel environmental watering did not result in large inputs of dissolved or particulate organic matter and no blackwater event was observed. Organic matter inputs associated with the unregulated flows in August did not result in a blackwater event, primarily due to the low water temperature at this time of year.

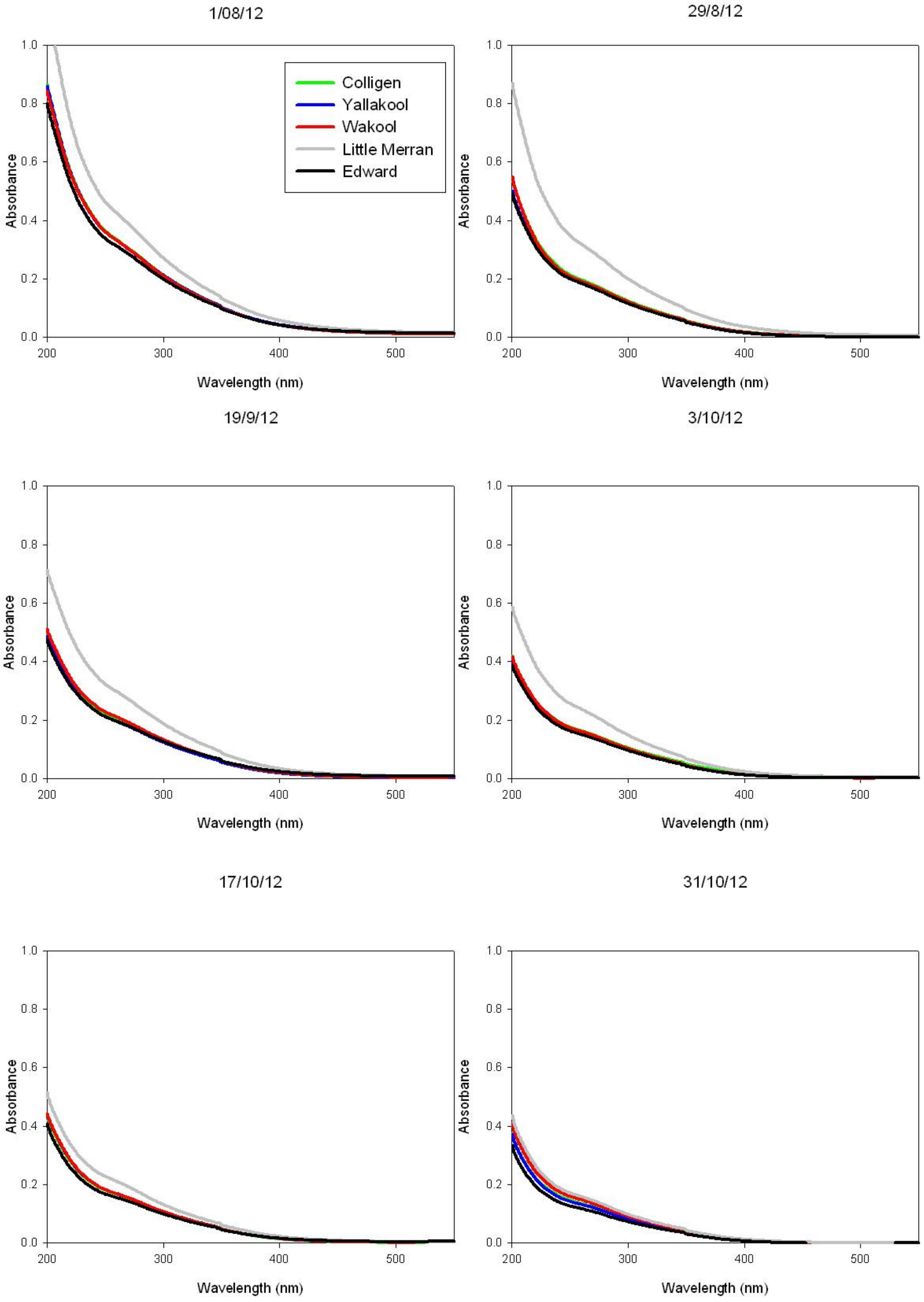


Figure 28a. Absorbance scans of water samples (1 August 2012 to 31 October 2012)

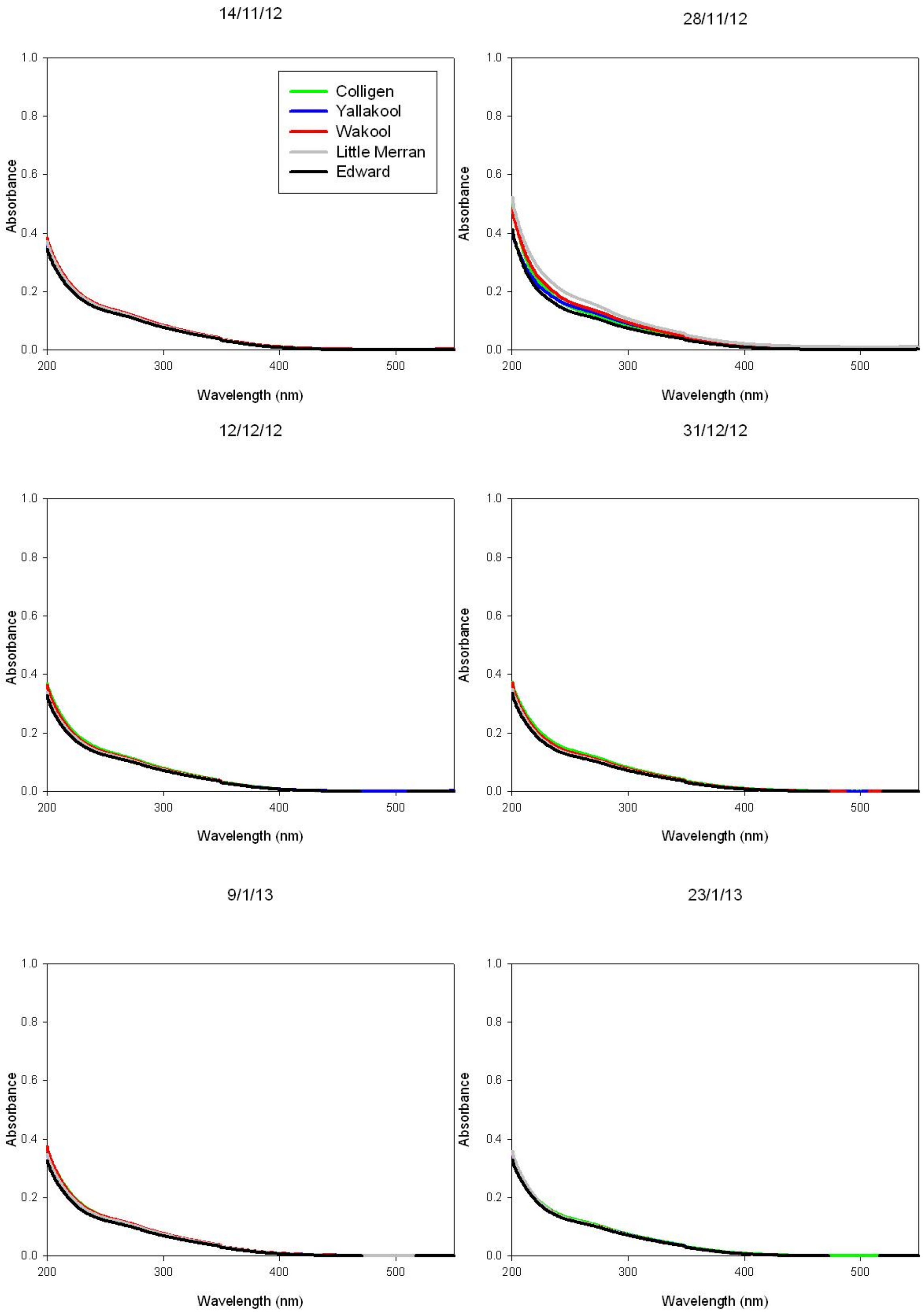


Figure 28b. Absorbance scans for water samples (14 November 2012 to 23 January 2013)

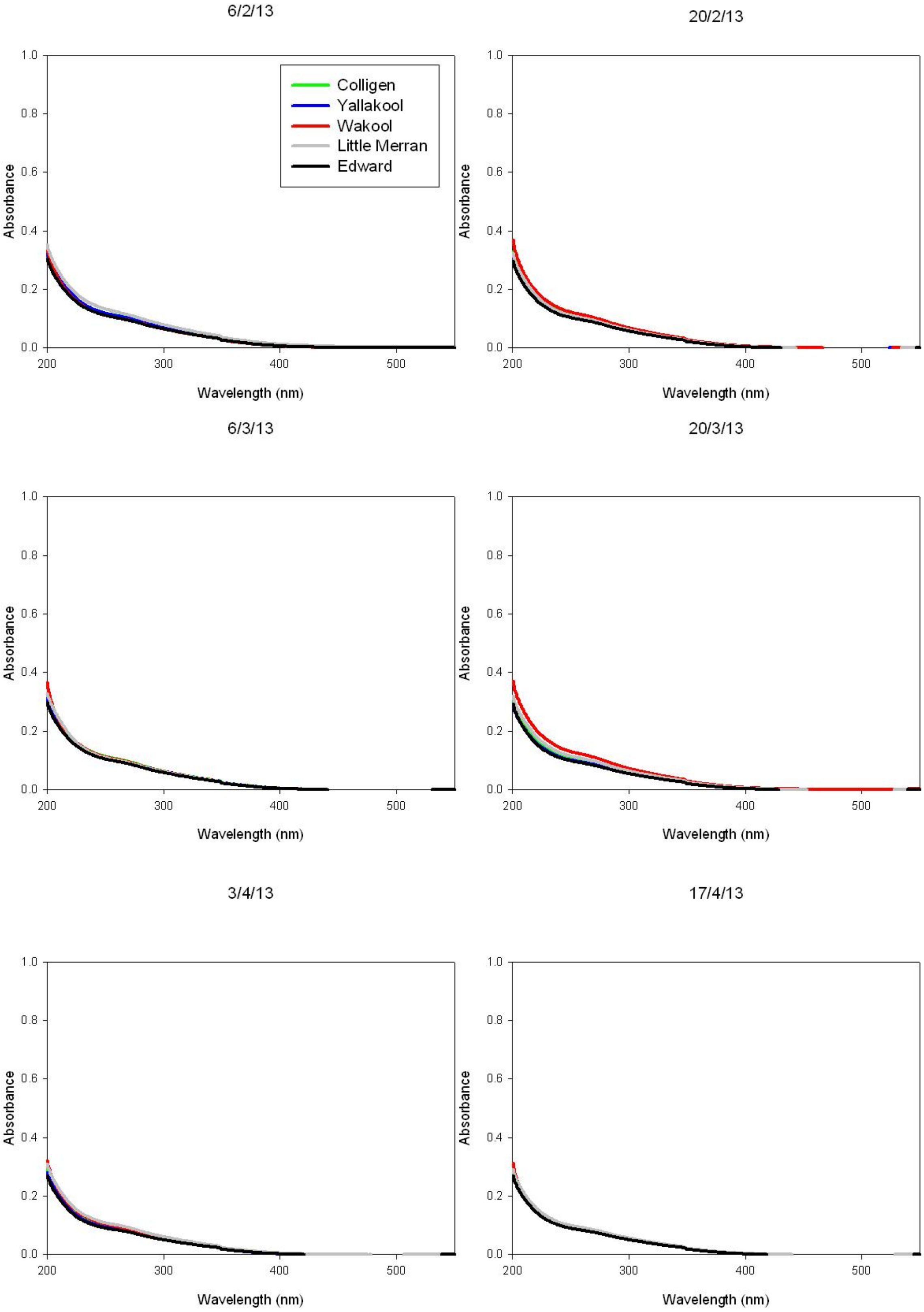


Figure 28c. Absorbance scans for water samples 6 February to 17 April 2013

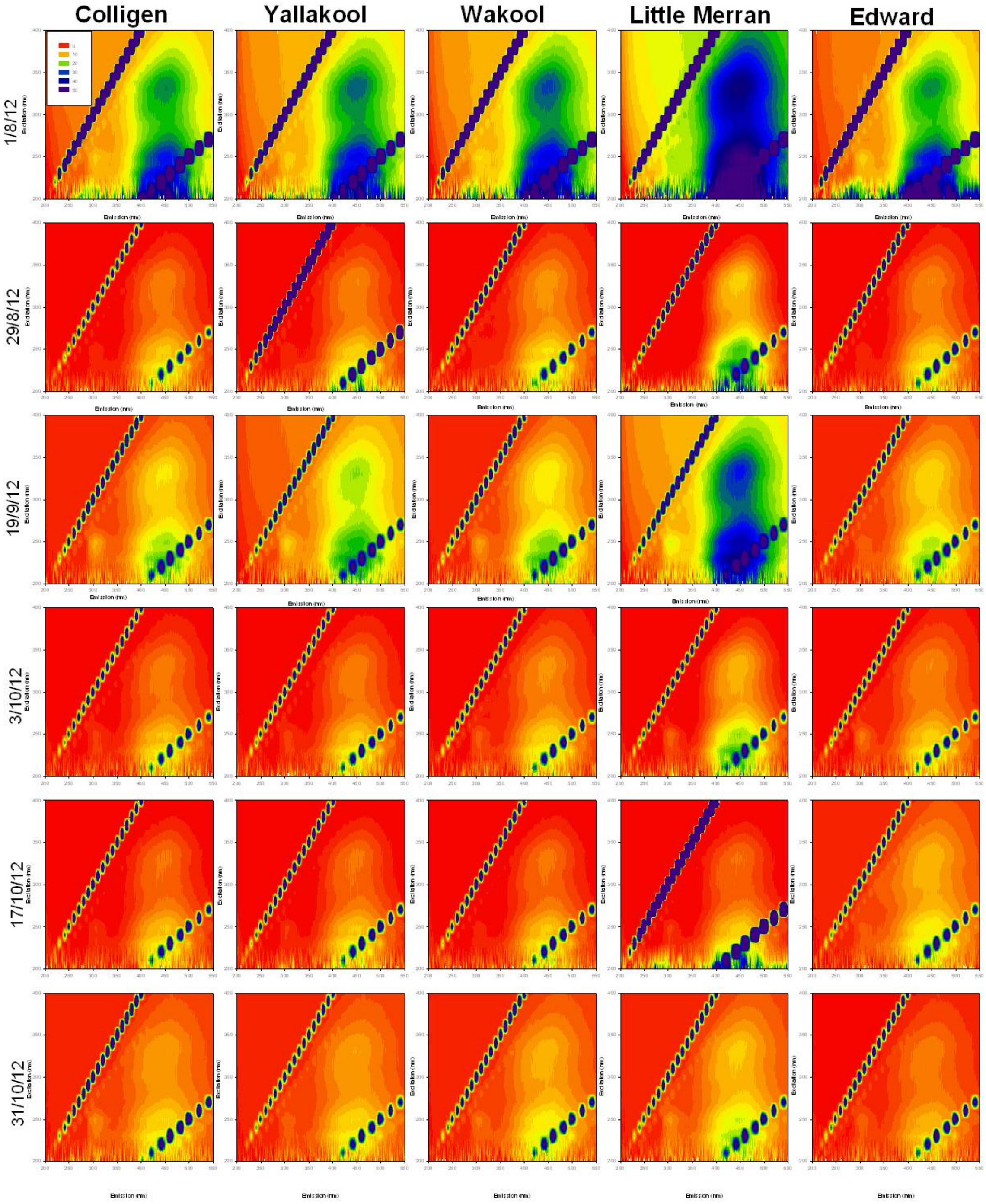


Figure 29a: Fluorescence scans for water samples collected between August and October 2012.

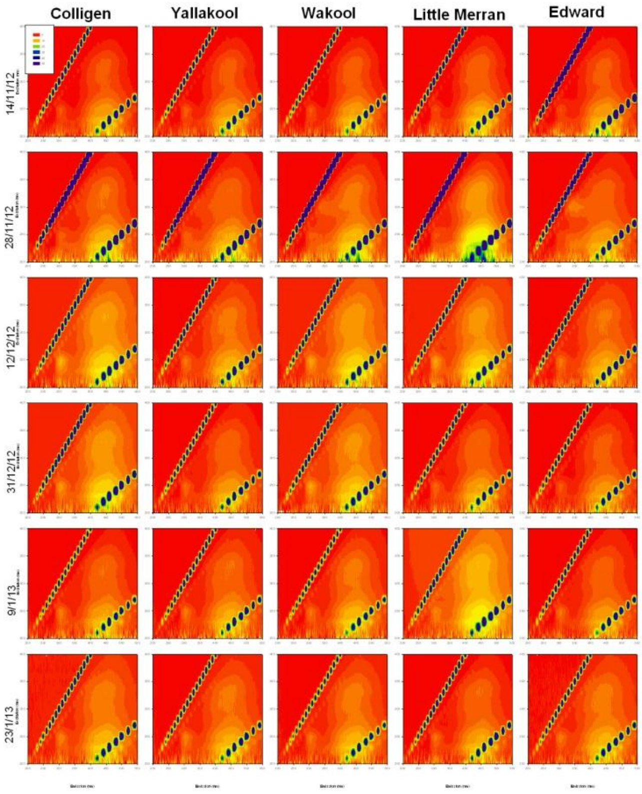


Figure 29b. Fluorescence scans- November 2012 to January 2013.

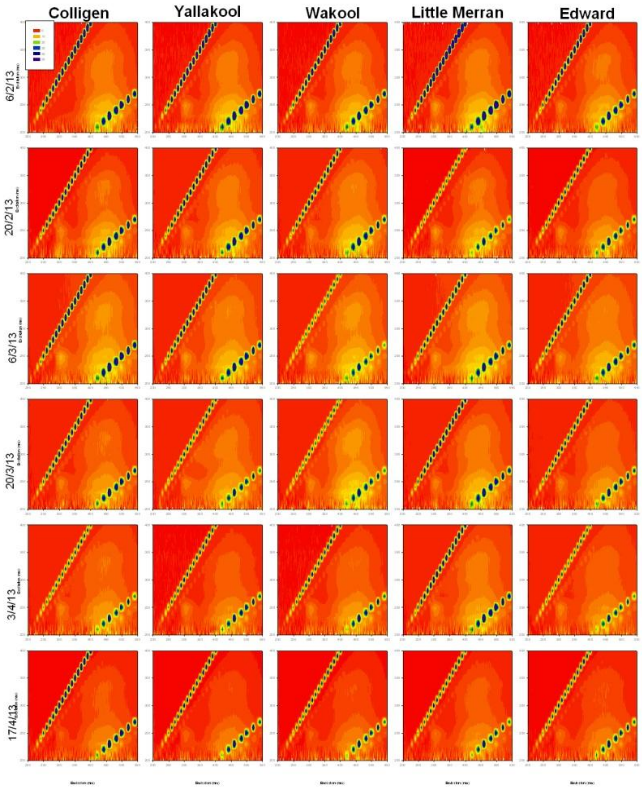


Figure 29c. Fluorescence scans: February to April 2013.

7.2.3. Phytoplankton biomass

Key findings

- *The delivery of environmental freshes in the Yallakool and Colligen rivers had no significant effect on phytoplankton densities* during the November 2012 Yallakool watering action, the February 2013 watering action, or the March 2012 Yallakool and Colligen watering actions.

- In general, phytoplankton chlorophyll-a concentration in all rivers increased from August 2012 through to April 2013, with a reduction in all rivers recorded in May 2013 as water temperature started to decrease.

Background

Phytoplankton are photosynthetic organisms that live in the water column of water bodies. They are a major energy source in standing and slow-flowing water bodies where they are primarily fed upon by zooplankton. They are also an important source of organic carbon through production of dissolved carbon compounds and through settling out to the bottom of rivers and lakes, contributing to sediment particulate organic matter. Algal growth depends on the availability and supply of the nutrients nitrogen and phosphorus, light and warm water temperatures.

Phytoplankton are potential indicators of ecological responses to environmental flows because they proliferate under low flow conditions, potentially outcompeting biofilms for light and nutrients (Carter 2011). Phytoplankton can be influenced by flow through changes in water volume, turbulence and through indirect processes, such as change in bioavailable nutrients and turbidity (influencing light penetration into the water body).

Studies examining the relationship between flows and phytoplankton have tended to focus on the issue of controlling algal blooms. The success of pulsed flows to disperse algal blooms has been demonstrated in the Murray Darling Basin (e.g. Sherman et al. 1998; Webster et al. 2000; Maier et al. 2004; Mitrovic et al. 2003; Bormans et al. 2005). The provision pulsed flow has been recommended as best-practice for controlling algal blooms (Edgar and Davis 2007) and has become part of the operating protocols for many systems including the Murray River. For example, in February 1999, water in addition to operational requirements was released from storages along the Murray and Murrumbidgee rivers to flush a blue-green algae bloom between Euston and Wentworth on the Murray River.

Hypothesis

The environmental watering will result in an initial decline in phytoplankton biomass, due to the enhanced turbidity from that fresh causing light limitation of photosynthesis. Following that initial decline, phytoplankton biomass will increase to a higher than pre-fresh levels due to the influx of nutrients. Environmental watering is not expected to trigger an algal bloom in these systems.

Methods

Five 500 mL water samples were collected from each river reach fortnightly on each sample date to determine the biomass of phytoplankton in the water column. Water was filtered through a GFC-50 0.5 µm pore-sized filter and the filter papers frozen until processing.

Chlorophyll-*a* concentrations were determined using the phaeophytin/acidification method where the total amount of pigment (chlorophyll plus phaeopigments) is determined in an methanol extract by spectrophotometry. The same sample is then acidified, and the chlorophyll degrades to phaeopigment, which is then measured spectrophotometrically. The chlorophyll-a concentration can then be determined from the difference in the two absorbance readings following Ritchie (2006).

Samples were placed in 10 mL of 90% methanol containing 150 mg magnesium hydroxide carbonate, extracted for 18 hours at 4°C, transferred to a 70°C water bath and boiled for two minutes. Samples were centrifuged at 4500 rpm for three minutes and optical densities at 750 and 666 nanometres measured pre- and post-acidification (1 M HCl) using a UV/Visible Spectrophotometer.

An asymmetrical BACI (before-after, control-impact) (Underwood, 1991) statistical design was used to test the effect of specific 2012-2013 environmental water actions on phytoplankton biomass in the Edward-Wakool system. Differences in mean densities of phytoplankton between control/impact rivers and before/during/after environmental freshes were evaluated statistically for each watering action using 2-way mixed effects analysis of variance (ANOVA). Because there were multiple sampling times used to represent before, during and after environmental flows, and multiple rivers used as ‘control’ and sometimes ‘impact’ rivers, sampling trip (random effect) was nested within Period (fixed effect, three levels: before, during and after), and river (random effect) was nested in Treatment (fixed effect, two levels: control rivers, impact rivers). Impact rivers received environmental freshes, while Control rivers were those that did not receive environmental water. For this analysis particular interest is in the Period x Treatment interaction term, which indicates a significant effect of the environmental watering action. Visual assessment of mean (±1SE) biomass plots, grouped by Period and Treatment, were used to confirm if the significant interaction term was positively or negatively associated with the environmental watering action. A summary of dates and rivers used to detect changes in phytoplankton biomass is presented in Table 9 (section 7.2.1). The null hypothesis was that mean phytoplankton densities in the rivers which received environmental water were not significantly different to the control rivers.

Results and discussion

Throughout the study planktonic chlorophyll-*a* concentrations were similar to levels reported for other lowland rivers (Reynolds and Descy 1996). The concentrations observed in the Edward-Wakool system were slightly higher than ANZECC (2000) trigger levels (Figure 30), but not of concern as they were similar to levels measured in the Murray River near Yarrawonga (Howitt et al. 2004).

The delivery of environmental freshes in the Yallakool and Colligen rivers had no significant effect on phytoplankton densities during the November 2012 Yallakool watering action, the February 2013 watering action, or the March 2012 Yallakool and Colligen watering actions ($p>0.05$, Table 11). Missing data for the sampling trip 26-30 November 2012 meant that the November 2012 Colligen watering action could not be statistically analysed. In general, phytoplankton chlorophyll-a concentrations increased from August 2012 through to April 2013, with a reduction in all rivers recorded in May 2013 as water temperature started to decrease.

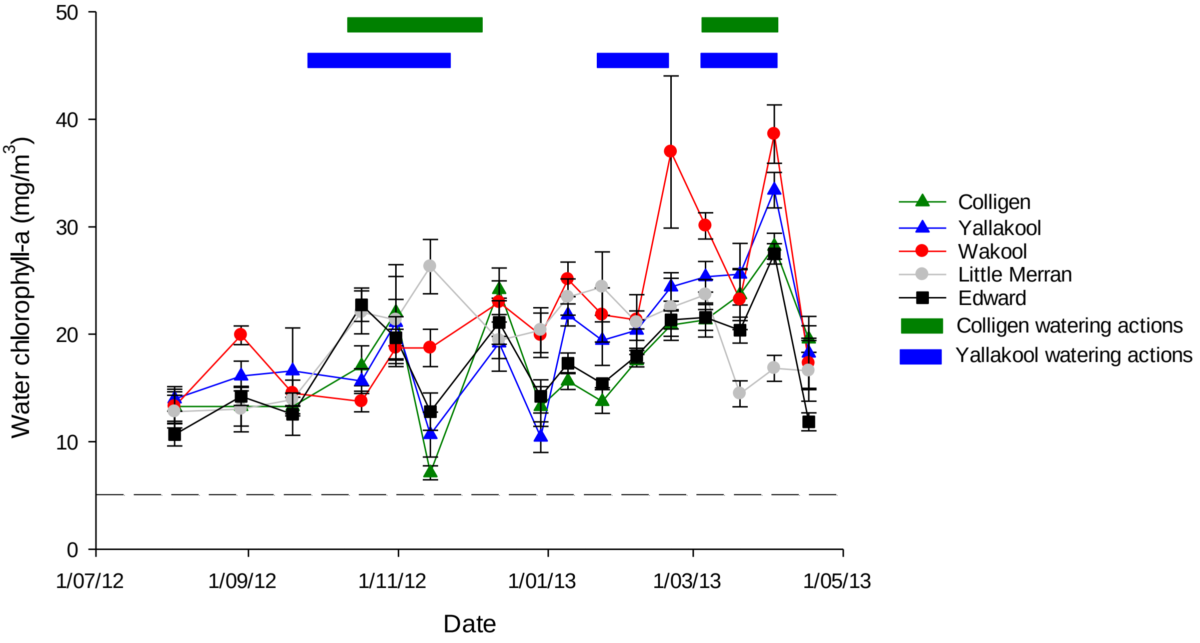


Figure30. Phytoplankton chlorophyll-*a* concentrations (mg/m^3) ± 1 SE in water from Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek between July 2012 and May 2013. Blue and green bars represent the start and finish dates of environmental watering.

Table 11: Results of 2-way mixed-effects Analysis of Variances, comparing mean phytoplankton biomass (ug/L) between control/impact rivers (CI), before, during and after (Period) the 2012-2013 environmental watering actions. A significant interaction between the two fixed factors indicates that the mean phytoplankton biomass within 'Impact' rivers was significantly different to changes that occurred over the same period of time within the Control Rivers. There were no significant interactions.

Environmental watering action	Main effects	d.f	F-test	p-value
Nov 2012 – Yallakool River watering action				
	Period (B-D-A)	2,69	0.290	0.748
	CI (C-I)	1,69	3.450	0.067
	Period*CI	2,69	0.166	0.857
Feb 2013 – Yallakool River watering action				
	Period (B-D-A)	2,54	0.207	0.813
	CI (C-I)	1,54	0.505	0.480
	Period*CI	2,54	0.036	0.965
Mar 2013 – Yallakool & Colligen River watering actions				
	Period (B-D-A)	2,72	0.749	0.476
	CI (C-I)	1,72	0.139	0.709
	Period*CI	2,72	1.113	0.339

7.2.4. Biofilm biomass and composition

Key findings

- *Environmental watering resulted in higher diversity in biofilms.* A high diversity of biofilms usually indicates good ecosystem health. Taxa richness in the month old biofilm growths ranged from five in the Wakool River (control) during April 2013 to a maximum of 15 taxa in Yallakool Creek (received environmental water) in March 2013. Yallakool Creek also had the highest richness during February 2013.

- *There was significantly higher relative biovolume of early successional taxa (diatoms) in rivers that received environmental water* (Colligen Creek and Yallakool Creek) and a higher percentage abundance of green algae in the one month old biofilms in the control rivers (Little Merran Creek and the Wakool River).

- *There was a reduced biofilm biomass in rivers that received environmental water compared to the control rivers*. This is consistent with the hypothesis that increased flow variability from in-channel environmental watering will ensure biofilm biomass in treatment rivers remains below nuisance levels and that biofilm organic biomass will be highest in rivers that have a more constant regulated discharge.

Background

Biofilms (also known as periphyton) are a combination of bacteria, algae and fungi that grow on submerged surfaces (e.g. wood, rocks, sediment) in aquatic systems. They are a major instream source of carbon in river systems and provide food and habitat for a range of organisms.

The biomass and productivity of biofilms are influenced by light, nutrients, temperature and availability of substratum (Peterson 1996; Burns and Ryder 2001) and disturbances such as grazing, changes in water level and flow velocity (Stevenson 1996). Disturbance by flood events is one of the most important regulators of spatial and temporal variability in benthic communities of streams (Davis and Barmuta 1989), with shifts in benthic algal community structure and function being well documented (e.g. Biggs et al. 1999; Uehlinger et al. 1996). Previous studies in lowland river reaches in the Murray-Darling Basin (e.g. Ryder 2004) and faster flowing upland systems (e.g. Watts et al. 2006, 2008, 2009b) have shown that regulated flow regimes with reduced variability in discharge can result in reduced productivity, reduced diversity, and cause the biomass of biofilms to increase to levels that become a nuisance (over 100 mg/m^2; Quinn 1991). Pulsing flows and increasing variability in flow (towards that in unregulated conditions) is a way to reduce biofilm biomass and improve river health. Pulsed flow events have been shown to reset biofilms (Watts et al. 2005, 2008, 2009b), which can have a positive effect on the instream ecosystem by reducing the biomass of biofilm and enabling early successional algae (e.g. diatoms) to become established, facilitating a shift in the biofilm community towards that of a reference stream (Watts et al. 2008; 2011).

Biofilms are excellent indicators of ecological responses to inchannel environmental watering because they respond to flow changes in a time frame (days to weeks) that is appropriate for flow management (Burns and Ryder 2001). The benefits of resetting biofilms through the delivery of in-channel environmental flows include:

- To promote of early successional algal taxa (e.g. diatoms) and higher biofilm diversity. A high diversity of biofilms usually indicates good ecosystem health.
- To contribute to nutrients and particulate organic matter in the water column, thus providing an important food resource for downstream communities
- To reduce in the nuisance of a high algal biomass of biofilm growing on the beds of rivers to avoid it increasing to levels unacceptable to the public. Quinn (1991) recommended that “the seasonal maximum cover of stream or river bed by periphyton as filamentous growths or mats (greater than about 3 mm thick) should not exceed 40% and/or biomass should not exceed 100 mg chlorophyll-a /m2”.

Hypotheses

- Environmental watering is expected to increase flow variability in these systems, increasing diversity in riverine biofilms, in particular increasing the relative biovolume of early successional taxa (eg diatoms).
- Increased flow variability from in-channel environmental watering will ensure biofilm organic biomass in these systems remains below nuisance levels. Biofilm organic biomass will be highest in rivers that have a more constant discharge.

Methods

Blocks of red gum wood (10 x 8 x 2 cm, 232 cm^2) were established at sites in five focus river reaches in October 2012. The biofilm redgum blocks could not be deployed prior to this time due to high flows in Yallakool Creek. Five blocks were suspended on metal racks mounted on star pickets (Figure 31) and placed in the photic zone at a known height relative to river discharge. This enabled biofilms to colonise the blocks and be sequentially harvested at regular and opportunistic intervals to compare the response of biofilm attributes to hydrological and water quality conditions.

Figure 31. Redgum blocks prior to deployment for assessment of bioilms, and b). Redgum block with biofilm established on it.

There were two biofilm treatments a) blocks set out each month and harvested the following month representing newly colonised biofilm and b) blocks set out at the beginning of the project (16 October 2012) and harvested at the end of the project (30 April 2013), representing long-term standing stock. Five 'one month old' blocks were harvested each month and five 'standing stock' blocks were harvested at the end of the study period. For some sample dates there are missing data because the water levels dropped and the blocks were out of water. Biofilm blocks for that month were removed but not processed as they would not represent the true growth over that period.

The following biofilm attributes were assessed:

- Chlorophyll *a* (algal biomass)
- Organic biomass and organic matter percent (percent of total weight)
- Biofilm algal species composition (biodiversity) and relative biovolume of major algal groups

The biofilm from each redgum block was scrubbed into 200 mL of distilled water using a soft nailbrush. Sub-samples were removed from the 200 mL residue (biofilm slurry) for determination of chlorophyll-a, and a 20 mL sample collected for the assessment of taxonomic composition and stored in Lugols solution (Figure 32). Using GC-50 0.5 µm filter papers, a recorded amount of the solution was filtered, the filter paper dried at 80°C for 24 hours, weighed, combusted for four hours at 500°C and reweighed. All samples were weighed to four decimal places and converted to dry weight and ash free dry weight/organic biomass. Percent organic matter was calculated as the proportion of AFDW to DW and converted to a percentage to standardise across sites and dates. Chlorophyll-a in biofilm samples was determined as described in the phytoplankton methods section 7.2.3.

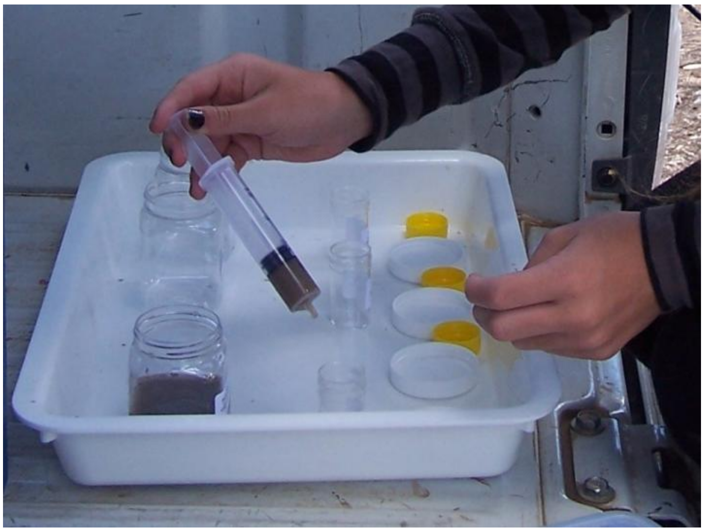


Figure 32. A 20 mL sample of the biofilm residue is collected for the assessment of taxonomic composition

A one-factor ANOVA was undertaken to test if algal biomass and organic biomass from standing stock biofilms was significantly different across the 4 rivers at the end of the monitoring period. When significant differences were indicated, *post hoc* pairwise comparisons were undertaken to determine differences between the rivers.

The average discharge, median discharge and coefficient of variation of discharge was calculated for each sample date from when the redgum block was deployed to when it was sampled. The number of days that blocks were in the water ranged from 21 to 35 days, with most samples being deployed for 28 days. The relationship between biofilm attributes and these flow variables was examined using Pearson's correlations. Correlations between the one month biofilm biomass and independent variables (mean discharge, mean coefficient of variation of discharge (CV), and median discharge) were conducted using Spearman Rank correlations for parameters that were not normally distributed and Pearson's correlations where data were normally distributed. Scatterplots were constructed for correlations which were significant between independent and dependent variables. All univariate analyses were conducted using the software package SPSS Statistics v20.

Results and discussion

A total of 41 taxa were found in the one-month-old biofilm growths and this was considerably higher than the 25 taxa found in the standing stock biofilms. There were 12 and 10 Green taxa, and 13 and 9 Cyanobacteria taxa in the monthly growths compared with the standing stocks, respectively. There were only 6 diatom taxa collected in the standing stock samples compared with 15 in the monthly growths, indicating that many of the diatom species are early successional taxa.

The taxa richness in the standing stock growths ranged from three in Colligen Creek during March 2013 to a maximum of 10 taxa in Yallakool Creek in January and February 2013. The filamentous green alga Oedogonium was the most prolific taxa (10 – 95%) at all sites. Spirogya was also persistent (>50%) in both the Wakool River and in Yallakool Creek in March and April 2013. In the standing stock biofilms the branching filamentous Cyanobacterium, Haplosiphon was the dominant Cyanophyte. Similarly in the Wakool River during March 2013 Haplosiphon made up 40% and during April made up 100% of the biofilm. The majority of Haplosiphon species grow in stagnant or very slow flowing waters, usually clear and with water plants (Komárek 1992). It is also a good nitrogen fixer.

Taxa richness in the monthly algal growths ranged from five in the Wakool River (control) during April 2013 to a maximum of 15 taxa in Yallakool Creek (received environmental water) in March 2013. Yallakool Creek also had the highest richness during February 2013. This is consistent with the expectation that there would be a higher diversity of biofilms in the reaches that had higher variation in discharge. In the one month old biofilms, the branching filamentous Cyanobacterium, Haplosiphon was found at >10% in Colligen Creek and Little Merran Creek in January and March 2013. It was also >50% of the biofilm during March 2013 in the Wakool River and >25% at Yallakool Creek in

November 2012. The cosmopolitan filamentous Diatom *Aulocoseira granulata* was ubiquitous in the Wakool River during November 2012 and in Yallakool Creek during January 2013.

There was significantly higher percentage abundance of green algae in the one month old biofilms in the control rivers (Little Merran Creek and Wakool River) compared to the treatment rivers (Colligen Creek and Yallakool Creek) and consequently a higher relative biovolume of early successional taxa (diatoms) in the treatment rivers (Figure 33). This is consistent with studies demonstrating a positive relationship between diversity in biofilms and flow variability (Ryder 2004). There was increasing algal biomass (chlorophyll-a) in the Wakool River with each subsequent sample of one month old biofilms (Figure 33). This is noteworthy, because new blocks were established each month, so the biomass had accumulated over approximately one month, and does not reflect a build up of biomass over an extended period of time. The response may be due to increasing water temperature and decreased turbidity in the low discharge conditions in the Wakool River. Algal biomass in this system did not reach nuisance levels (100 mg/m2; Quinn 1991). In contrast, the organic biomass was variable across systems, with no obvious pattern among rivers (Figure 34). The highest organic biomass was observed in Little Merran Creek (control river) in March 2013 (Figure 34).

Algal biomass was significantly correlated with mean discharge ($r = -0.425$, $p = 0.043$, n-23) (Figure 35) and organic biomass of one month old biofilms was significantly negatively correlated ($r = -0.438$, $p = 0.028$, n-25) with the coefficient of variation of discharge (Figure 35). These negative correlations demonstrate that the higher discharge and higher variation in discharge during the environmental watering can help to prevent excessive growth of biofilm as can occur under constant flow regimes.

The biomass of standing stock biofilms was compared in April 2013 after growing on the blocks since establishment in October 2012. The organic biomass in April 2013 was significantly higher in the Wakool River (control)(62.8 g/m^2± 16.6) than in Yallakool Creek (19.1 g/m^2± 2.5) or Colligen Creek (25.9 g/m^2± 2.6). Similarly, the algal biomass was significantly higher in Wakool River (22.1 mg/m^2± 1.8) compared to Yallakool Creek (15.1 g/m^2± 21.5) or Colligen Creek (14.6 g/m^2± 2.6). This is consistent with the hypothesis that increased flow variability from in-channel environmental watering will ensure biofilm biomass in treatment rivers remains below nuisance levels and that biofilm organic biomass will be highest in rivers that have a more constant discharge.

In summary, environmental watering resulted in higher diversity in biofilms, in particular an increase in the number and percent abundance of early successional taxa (eg diatoms). There was also a reduced biofilm biomass in rivers that received environmental water compared to the control rivers that had a more constant discharge.

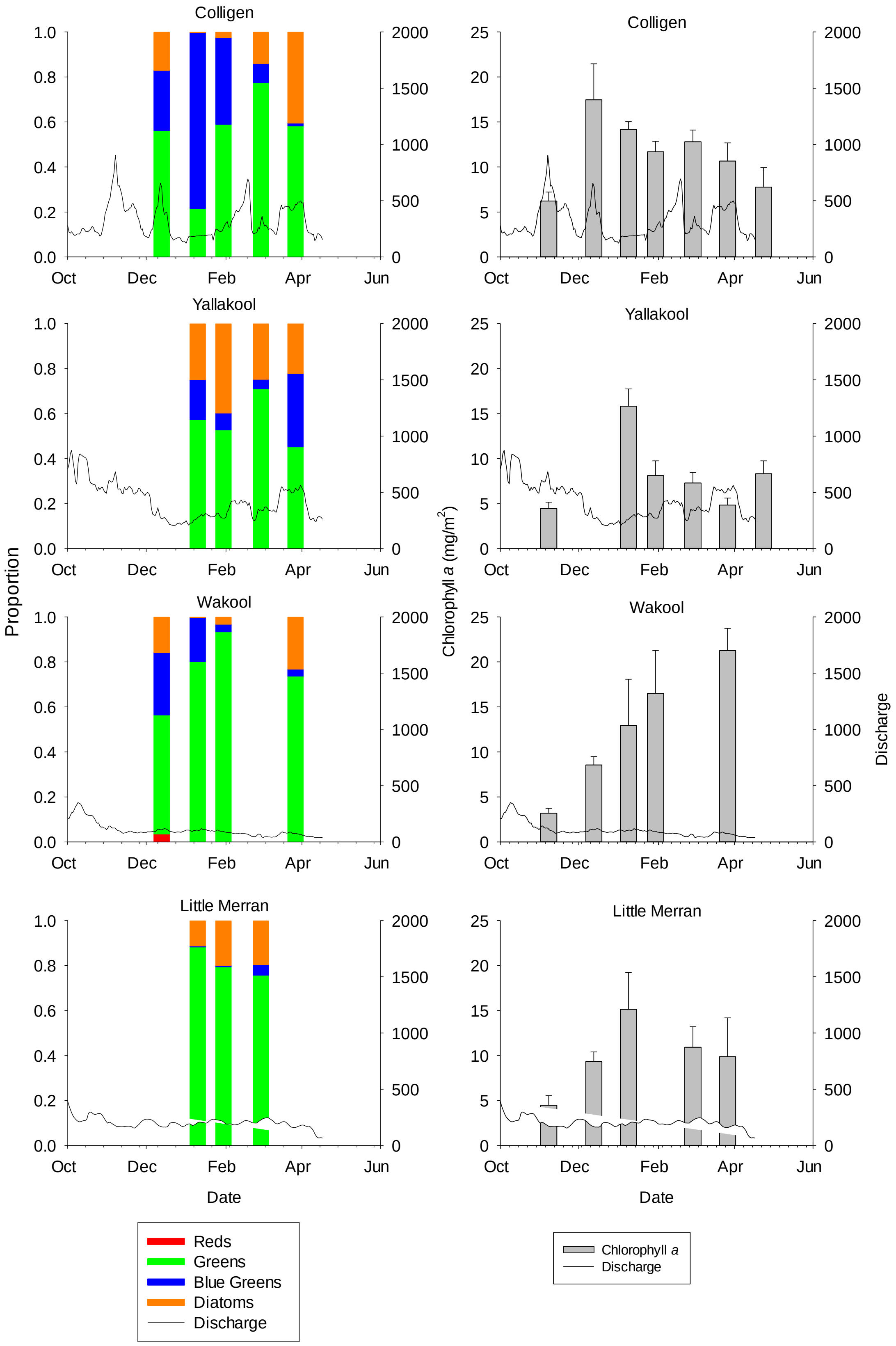


Figure 33. Relative percentage abundance (as biovolume) of algal divisions and algal biomass (chlorophyll-a mg/m^2 ±1SE) from one month old biofilms from Colligen Creek, Yallakool Creek and Wakool River from December 2012 to April 2013.

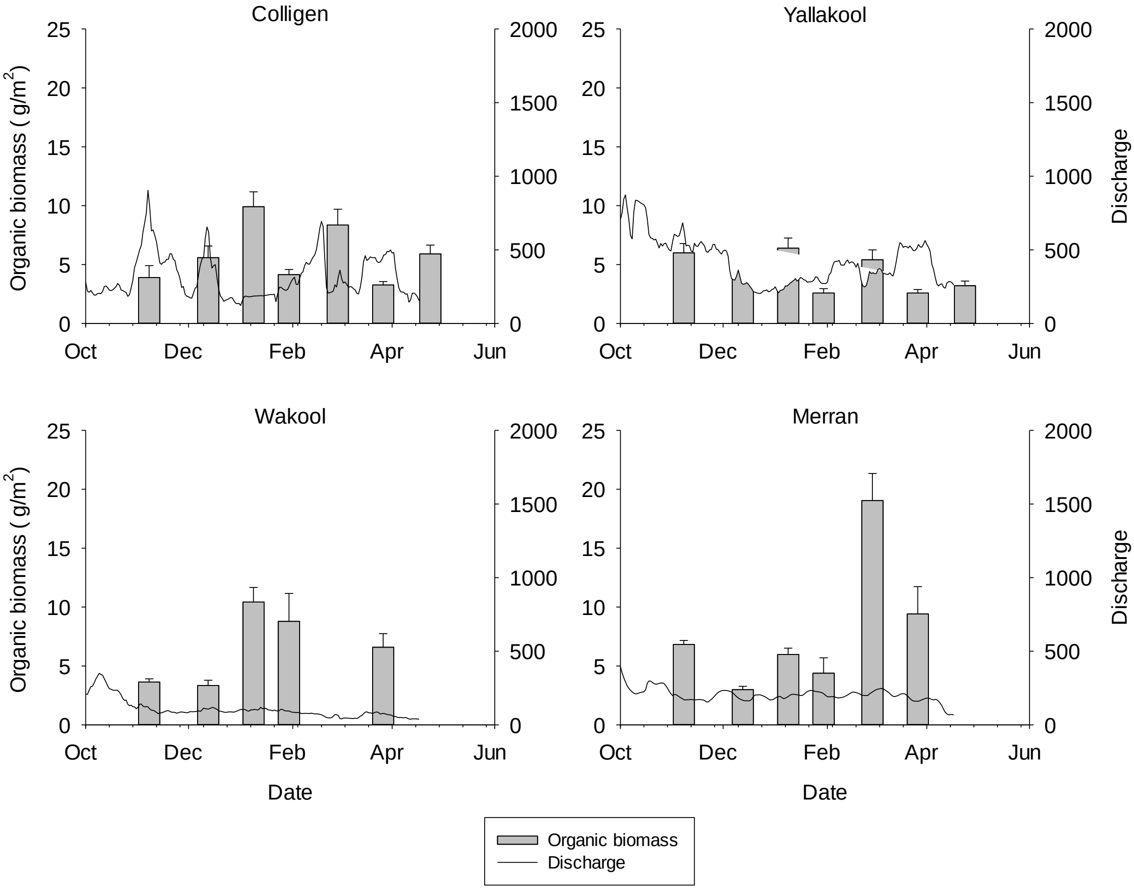


Figure 34. Organic biomass (g/m^2± 1 SE) for 1 month old biofilm grown on redgul blocks suspended below the water surface at Colligen creek, Yallakool Creek, Wakool River and Little Merran Creek.

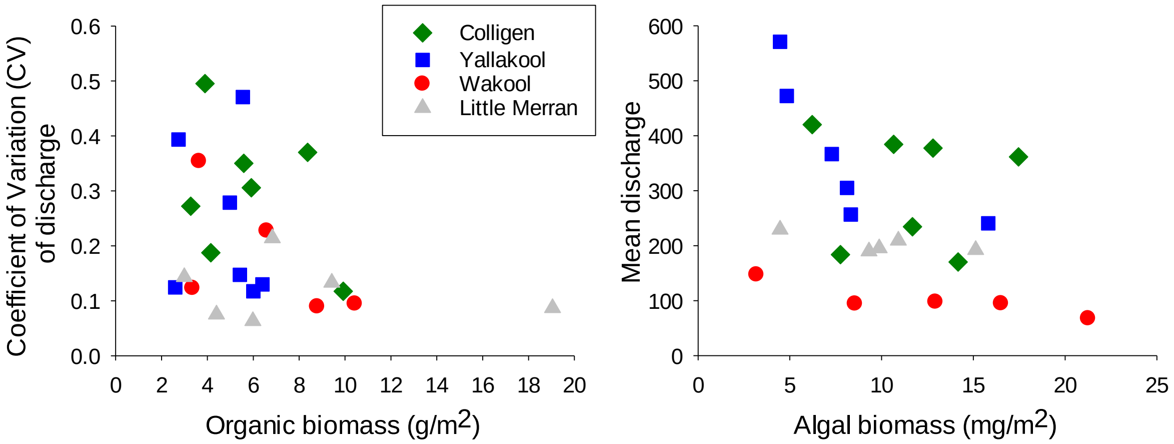


Figure 35. Scatter plots showing statistically significant relationships between biofilm biomass and flow variables across the study rivers.

7.2.5. Whole stream metabolism

Key findings

- *Rates of gross primary production (GPP) and ecosystem respiration (ER) in these rivers were typical of lowland streams with good water quality.*

- *There was minimal change in rates of GPP and ER after environmental watering*. Gross primary production is strongly constrained by low bioavailable nutrient concentrations in this system. The environmental freshes were not of sufficient magnitude to entrain higher nutrient concentrations from rewetting the floodplain. Similarly, the relative constancy in ER can be attributed to the low and consistent DOC concentrations. These outcomes should be seen as largely positive, as, the existing levels of metabolism are able to support the fish population without the risk of either algal blooms or anoxic events. However, it is unknown whether an increase in production would result in an increase in fish populations and this could be tested by future studies.

Background

Whole stream metabolism measures the production and consumption of dissolved oxygen gas ('DO') by the key ecological processes of photosynthesis and respiration (Odum 1956). Healthy aquatic ecosystems need both processes to generate new biomass (which becomes food for organisms higher up the food chain) and to break down plant and animal detritus to recycle nutrients to enable growth to occur. Hence metabolism is a means of assessing the energy base underpinning aquatic foodwebs. The relationships between these processes are shown in Figure 36.

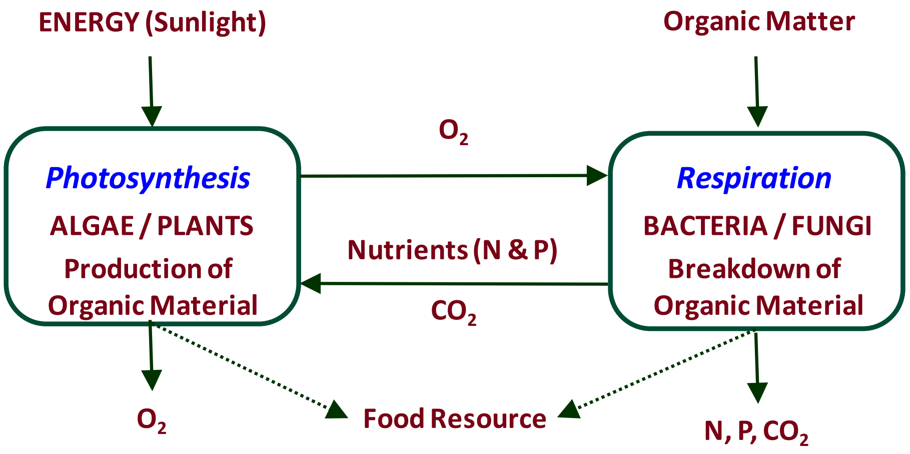


Figure 36. Relationships between photosynthesis, respiration, organic matter, dissolved gases and nutrients

If the rates of these processes are too low, this will limit the amount of food (in the form of bacteria, algae and water plants) available to organisms that consume these entities. This limitation will flow on up through the food web and constrain the populations of larger organisms including fish and amphibians. Rates are expected to vary on a seasonal basis as warmer temperatures and more direct, and longer hours of, sunlight contribute to enhancing primary production. Warmer temperatures and a supply of organic carbon usually result in higher rates of ecosystem respiration (Roberts et al. 2007).

Most concern arises when rates of these processes are too high. Greatly elevated primary production rates usually indicate probable algal bloom conditions (or excessive growth of plant species, including duckweed and azolla). Several deleterious effects can then occur – blocking of sunlight penetration into the water which can kill off other submerged plants, possible production of potent algal toxins and large swings in diel DO (over the course of 24 hours). Although photosynthesizing algae can produce extremely high concentrations of DO during daylight hours, overnight, elevated respiration rates can drive the DO concentration very low, even to the point of anoxia (no dissolved oxygen in the water). Also, when an algal bloom eventually collapses, the very large biomass of labile organic material is then respired, often resulting in anoxia for extended periods. Very low (or no) DO in the water can be fatal to many organisms resulting in fish kills and unpleasant odors. Bloom collapse is also often, but not always, coincident with release of algal toxins, hence the water becomes unusable for stock and domestic purposes as well.

After allowing for seasonal variability, sustainable rates of primary production for a given system will primarily depend on the characteristics of the aquatic ecosystem being considered. Streams with naturally higher concentrations of nutrients (e.g. arising from the geology), especially those with very open canopies (hence lots of sunlight access to the water) will have much higher natural rates of primary production than forested streams, where rates might be extremely low due to heavy shading and low concentrations. The important point is that the ecology of each stream will develop based (partly) on the rates of these fundamental processes. Habitat availability, climate and many other factors also influence food web structure and function. Uehlinger (2000) demonstrated that freshes with sufficient stream power to cause scouring can ‘reset’ primary production to very low rates which are then maintained until biomass of primary producers is re-established.

Hypotheses

The following hypotheses were developed specifically related to the possible impact of freshes on the rates of stream metabolism and were partially derived from the work of Vink et al. (2005) on impacts of irrigation releases on the middle reaches of the Murrumbidgee River and from the predictions of the ‘Flood Pulse Concept’ (Junk et al. 1989):

i) A fresh will result in an initial decline in Gross Primary Production (GPP) due to the enhanced turbidity from that fresh causing light limitation of photosynthesis.

ii) Following that initial decline, GPP will increase to a higher rate than pre-fresh levels due to the influx of nutrients.

iii) Similarly, there will be an initial decline in Ecosystem Respiration (ER) followed by a rapid increase as more labile organic carbon is introduced into the stream channel.

Methods

Rates of primary production and respiration can readily be estimated by continuously recording DO, temperature and ambient light (known as *photosynthetically active radiation* or PAR) over periods of many months under a range of meteorological conditions and flows (Grace and Imberger, 2006). Rates are calculated for each day so that the influence of flow, cloud cover and season can be assessed. A typical daily DO trace is shown in Figure 37. The figure illustrates how DO changes in a sinusoidal pattern over 24 hours, with DO increasing after sunrise, reaching a peak in later afternoon (due to photosynthesis throughout daylight hours) and then declining overnight, as ecosystem respiration (which consumes DO) continues 24 hours a day. Estimation of these rates must also include one additional factor – reaeration, which is the physical movement of DO across the air water interface. If the water contains less DO than it can hold (100% saturation), such as overnight when respiration consumes DO, then more oxygen will diffuse from the atmosphere into the water.

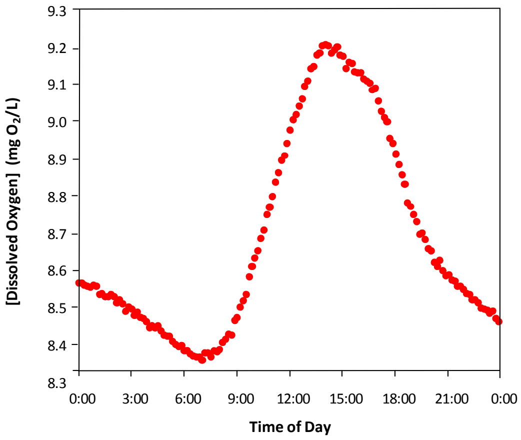


Figure 37. Typical Dissolved Oxygen Concentration profile over a 24 hour period. This data was from the Wakool River on 18th September, 2011.

Battery-powered ZebraTech data sondes (DO Loggers) were deployed mid-stream, with one sonde at either end of each of the four focus reaches (typically separated by 3-5 km). Each sonde was set to measure and log DO and water temperature every 10 minutes. Approximately every month, data were downloaded from the sondes, which were then recalibrated to ensure high quality data. PAR data were also recorded every 10 minutes on loggers placed in open fields near each of the stream sites. The metabolic parameters, gross primary production and ecosystem respiration, plus the reaeration rate were calculated for each day using the daytime regression method described by Atkinson et al. (2008). The DO data for Little Merran Creek for the most of November 2012 was not included in the analysis as the data sonde was out of the water. Only data where the diel curve reasonably matched the shape shown in Figure 37, and the inverse modeling approach produced excellent fits to these diel curves, were included in the subsequent analysis of the metabolic rates.

Statistical analyses were performed to examine the hypotheses. As daily metabolic rates are affected by weather and water temperature, the potential impact of environmental watering was assessed by determining whether there was a change in the *difference* in daily rates between the impacted streams (Yallakool or Colligen Creeks) and the control streams (Wakool River or Little Merran Creek). For example, the 'pre-fresh' difference in GPP between Yallakool Creek and Wakool River was calculated each day. This 'before' data was then compared to the daily 'post-fresh' differences in GPP in the same two streams. The Student t-test was used to test for statistically significant differences (at $p < 0.05$) between the before and after data sets assuming that the Shapiro-Wilk normality requirement was met in the two data sets (at $p < 0.05$). If normality conditions were not met, then a Mann-Whitney Non-Parametric Rank Sum Test was performed.

Results

The rates of both primary production and respiration remain relatively constant over the entire study period (Figures 38 to 41). There are some longer term (seasonal) trends where both primary production and respiration rates increased during summer due to warmer water temperatures, and in the case of primary production, more hours of sunshine. At no stage between September and early March did any of the loggers record very low dissolved oxygen concentrations (< 20% DO saturation), even during and immediately after the freshes. Elevated flows did lead to a decrease in %DO maxima each day by 0-20% and also the same magnitude decline in the minimum %DO. Consequently, the environmental watering in November and February did not constitute a black water event. This finding is consistent with the organic matter characterization results (section 7.2.2). Although these flows connected with some low-lying backwater areas, they did not spread out onto the floodplain, remain for days-weeks and then return, which is the most common scenario for development of 'black water' (high dissolved organic carbon and very low or no dissolved oxygen).

Table 12 provides summary data for the gross primary production (GPP) and ecosystem respiration (ER) rates for each river. The table lists the lowest and highest rates found, plus the median value. The P/R ratio refers to the balance between primary production rates and ecosystem respiration rates. A value for this ratio of < 1 indicates that more organic carbon is being consumed in the study reach than is being produced by primary production (photosynthesis).

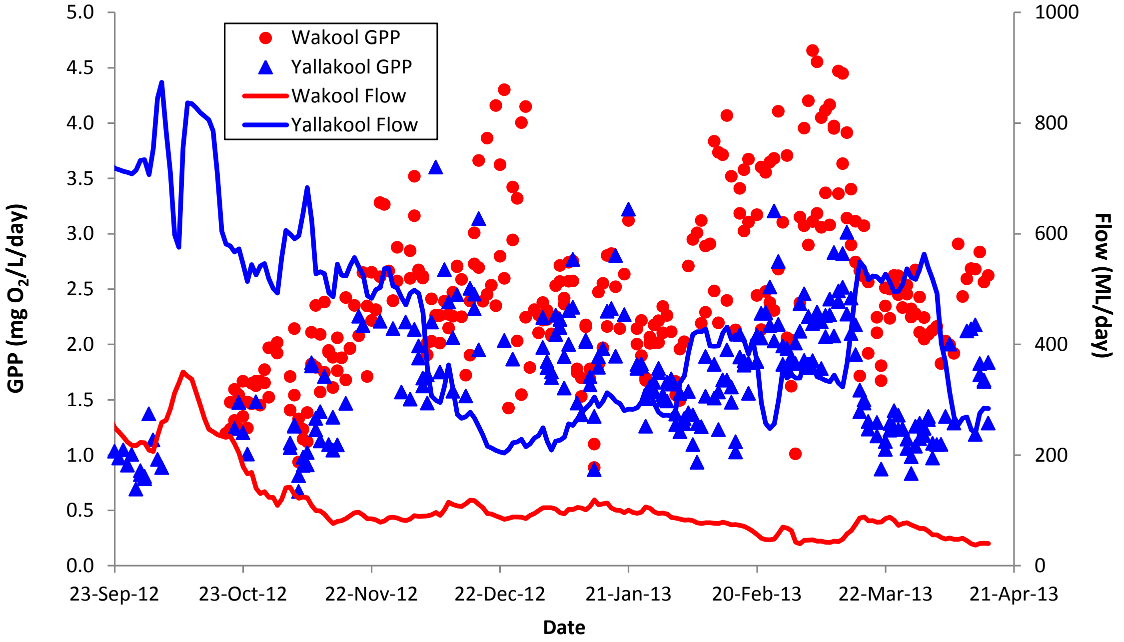


Figure 38. Gross Primary Production (GPP) rates and stream discharge for the Wakool River and Yallakool Creek from September 2012 to April 2013.

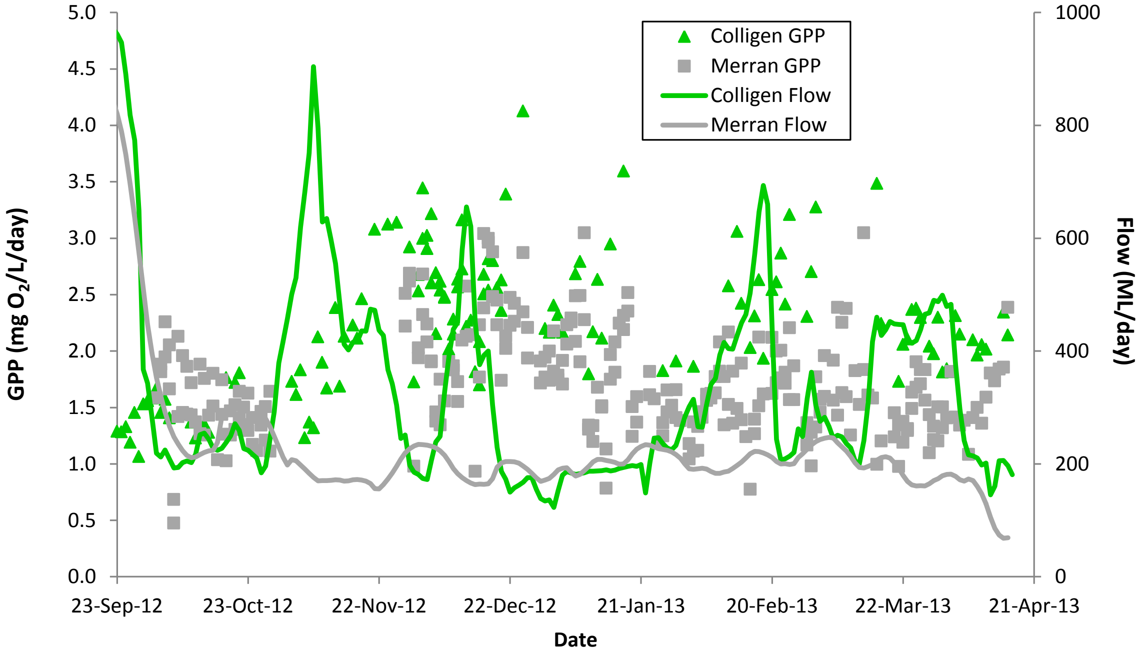


Figure 39. Gross Primary Production (GPP) rates and stream discharge for Colligen and Little Merran Creeks from September 2012 to April 2013. (The Merran loggers were out of the water from 28 Oct to 28 Nov, 2012).

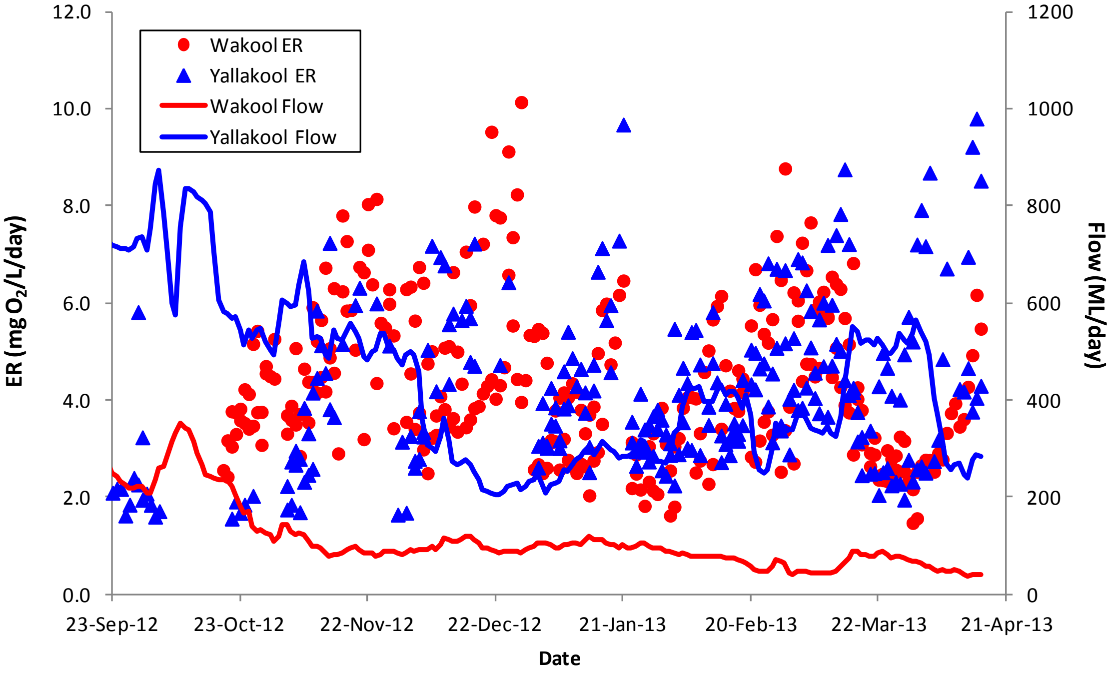


Figure 40. Ecosystem Respiration rates and stream discharge for the Wakool River and Yallakool Creek from September 2012 to April 2013.

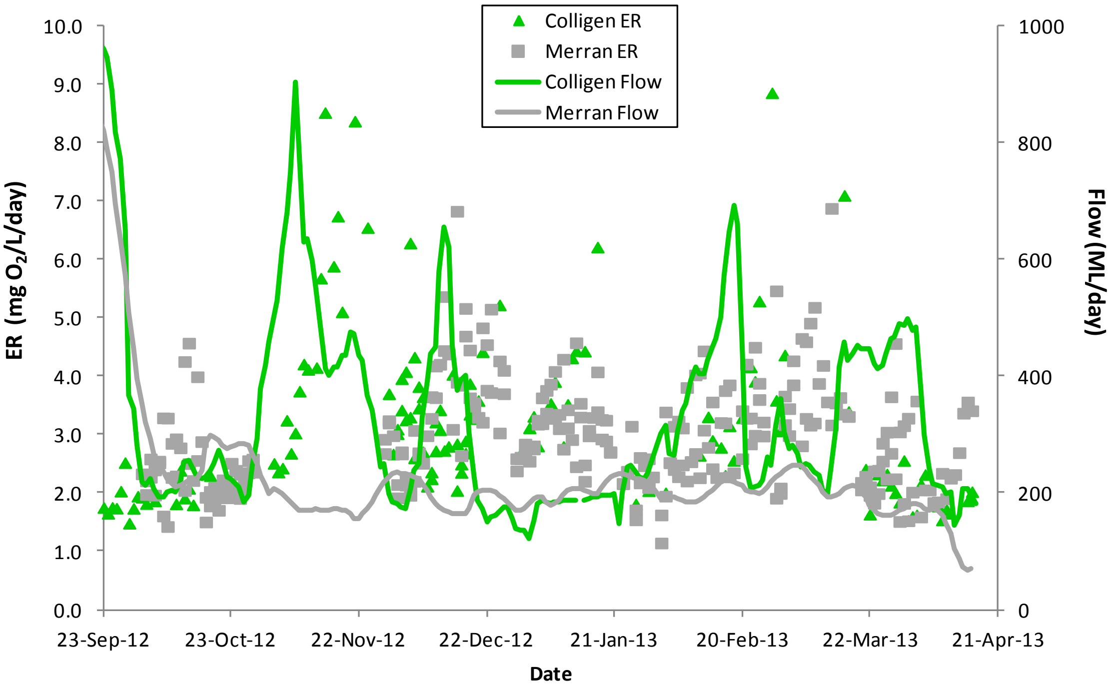


Figure 41. Ecosystem respiration rates and stream discharge for Colligen and Little Merran Creeks from September 2012 to April 2013. (The Merran loggers were out of the water from 28 Oct to 28 Nov, 2012).

Table 12. Summary of primary production (GPP) and ecosystem respiration (ER) rates for the four study rivers (combined upstream and downstream data), September 2012 - April 2013

	Colligen Creek (n = 203)			Little Merran Creek (n = 222)		
	Median	Min	Max	Median	Min	Max
GPP (mg O_2/L/Day)	2.22	1.06	4.45	1.72	0.47	3.05
ER (mg O_2/L/Day)	2.87	1.49	10.00	2.69	1.15	6.89
P/R	0.77	0.25	1.27	0.63	0.19	1.02

	Wakool River (n = 311)			Yallakool Creek (n = 250)		
	Median	Min	Max	Median	Min	Max
GPP (mg O_2/L/Day)	2.38	0.89	6.48	1.68	0.67	3.60
ER (mg O_2/L/Day)	3.86	0.97	10.14	3.86	1.56	9.80
P/R	0.62	0.18	2.60	0.44	0.13	0.96

The gross primary productivity and ecosystem respiration rates in the four study rivers (Table 12) are typical of rates found in non-polluted, slow flowing rivers elsewhere in the world. As was also found during the 2011-12 monitoring, these rates are very similar between the four rivers, with median primary production rates and median respiration rates differing by less than a factor of 2 between the four rivers. This is largely unsurprising given the similarity in land forms, land use, the sizes of the rivers and that they are all in the same region. The median values for the P/R ratio (0.44 – 0.77) indicate that for most of the time, these rivers are net heterotrophic (P/R < 1) implying that there must be another source of organic carbon fuelling respiration. Such carbon additions generally come from further up in the catchment or have fallen in/been washed in from riparian vegetation. It is pertinent to note though that primary production within the stream channel, as measured here by GPP, is also an extremely important contributor to organic carbon supply (which is the food sustaining higher organisms in the food web).

Figure 42 clearly demonstrates that there was also an extremely large variation in GPP at any particular light value. This indicates that light is generally not the major factor limiting primary production in these streams. If that were the case, a much stronger relationship would be expected between Daily PAR and GPP. Hence, this suggests another factor or factors must be contributing to the measured GPP rates. One of these important factors is the biomass of organisms capable of photosynthesizing that are present. These are typically divided into three major groups: the macrophytes, biofilms and floating algae (phytoplankton). Biofilms are likely to be the dominant primary producer in most reaches of these streams, especially in the shallow, marginal zones of the stream where sunlight easily penetrates through the water. In the water column, photosynthesis can only occur when there is sufficient light. When the water is turbid, this limits how far into the water light can penetrate. The point at which photosynthesis is no longer biologically viable is known as the 'euphotic depth', Z_{eu}. This parameter can also be estimated from simple relationships derived from empirical data. As a relationship is not available for these rivers, we used information for the Darling River, a larger, turbid, lowland river in northern NSW (Oliver et al. 1999). This empirical equation is Z_{eu} = 4.6 / (0.04 x Turbidity + 0.73). Table 13 lists the euphotic depth for each river based on the regular, fortnightly measurements of turbidity taken during this project. The euphotic depths indicate that for all reaches deeper than Z_{eu}, insufficient light will reach the sediment surface to allow macrophytes and biofilms to grow. Hence biofilms will be restricted in range to shallower regions on the edges of the stream. This will decrease the overall primary productivity of the rivers.

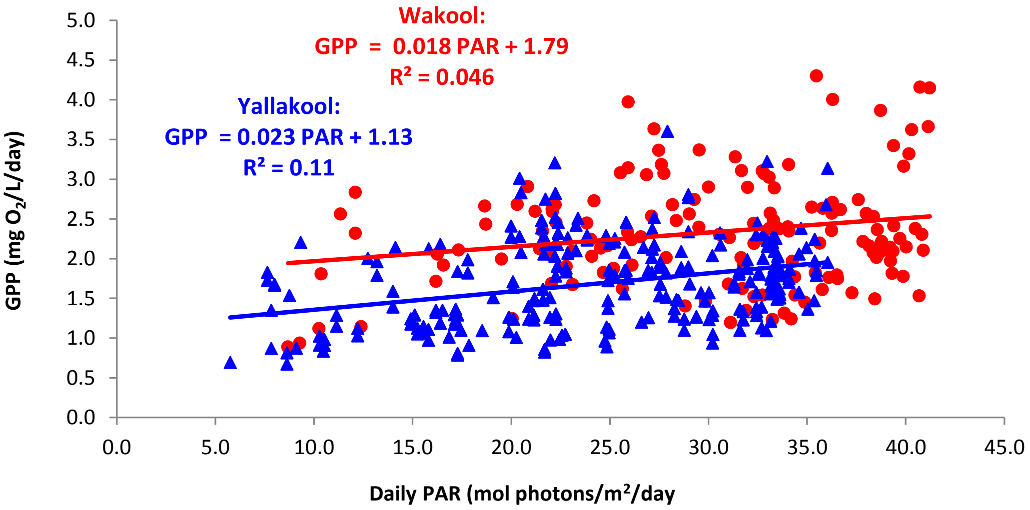


Figure 42. Linear relationships between daily light (as PAR) and gross primary production for the Wakool River and Yallakool Creek. Collated data from September 2012 to April 2013. P-values for the slope term: Wakool < 0.01; Yallakool < 0.001

Table 13. Summary of stream turbidity (NTU) plus the median euphotic depth, Z_{eu}, for the four study rivers, August 2012 - April 2013.

	Colligen	Little Merran	Wakool	Yallakool
Median	60	92	70	70
Min	27	25	36	41
Max	124	168	185	157
Z_{eu} (m)	1.48	1.05	1.30	1.31

It was expected that increasing water temperatures associated with seasonal change would result in faster physiological rates in organisms and hence greater rates of GPP and ER. The relationship between Mean Daily Water Temperature and the Rate of Daily Gross Primary Productivity (Figure 43) confirms this hypothesis. The figure shows data for Colligen Creek, but the other three streams were similar. The scatter of data about the positive trendlines for Little Merran Creek and Wakool River were much greater, indicating that other factors were equally or more important than temperature in determining GPP. Also not shown, but displaying similar positive relationships with Mean Daily Water Temperature, are the rates of Ecosystem Respiration in the four rivers. The slopes and coefficients of variation (r^2) for all eight plots are given in Table 14. All four coefficients of variation (r^2) were low for the positive relationship between ecosystem respiration and mean daily water temperature, indicating again that other factors are probably more important.

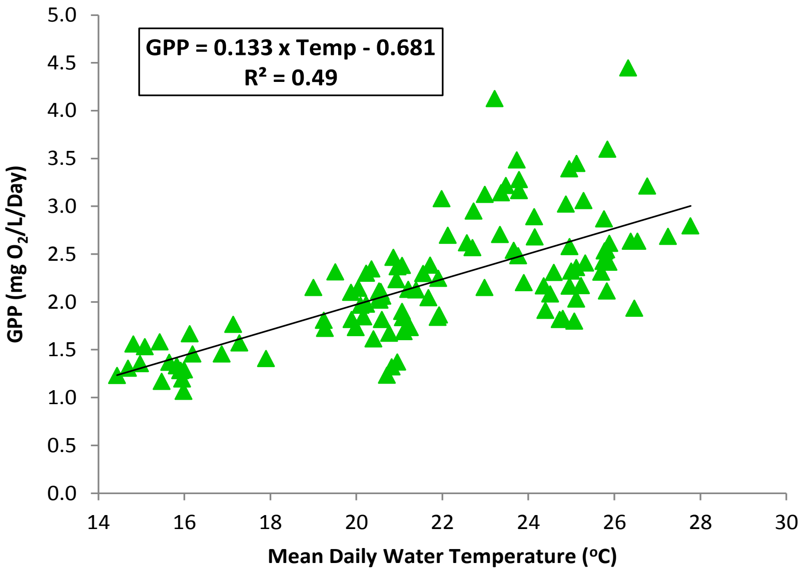


Figure 43. Linear relationship between daily mean water temperature and gross primary production for Colligen Creek. Collated data from September 2012 to April 2013. P-value for slope < 0.001.

Table 14. Temperature Dependence of GPP and ER Rates for the four study streams.

		GPP *vs* Temperature		ER *vs* Temperature	
Stream	**n**	**Slope**	**r^2**	**Slope**	**r^2**
Colligen	203	0.117	0.44	0.184	0.19
Little Merran	265	0.052	0.15	0.114	0.18
Wakool	311	0.106	0.13	0.162	0.072
Yallakool	250	0.116	0.41	0.138	0.058

Note: All slopes were significantly different from 0 (p-value < 0.001)

The temperature dependence of the GPP and ER rates exemplified in Figure 43 and determined by linear regression (Table 14) indicates a potential problem during hot, dry summers. Higher water temperatures automatically mean lower dissolved oxygen solubilities. By itself this is rarely problematic as solubility is 6.4 mg O_2/L at a water temperature of 40^{o}C, well above the ANZECC trigger value of 4.0 mg O_2/L. However, as temperature increases, the net drawdown of O_2 also increases as respiration rates increase more rapidly with temperature than GPP (Table 14). This net drawdown exacerbates the lower O_2 solubility and increases the likelihood of suboxic, even anoxic conditions. Fortunately, physical reaeration by diffusion of O_2 from the atmosphere will partially counteract this potential for developing very low dissolved oxygen concentrations. It is highly pertinent to note that reaeration across the air-water interface is significantly higher from a moving water column than from a still one, especially if the current increases water turbulence. Thus maintenance of even slow flow rates, for example by periodic freshes, is far preferable to standing water in the streams as a means of addressing potential anoxia during hot, dry spells. Note that this is a different scenario to blackwater events, where rapid oxygen drawdown is brought about by direct contact of water with high levels of organic carbon on the floodplain.

Another key factor controlling the rate of photosynthesis is the concentration of nutrients in the water. Nutrients, and in particular nitrogen and phosphorus, are needed to form new cells (carbon, hydrogen and oxygen are also needed but are abundantly available in the water). As was also found previously in 2011-2012, the bioavailable nutrient concentrations in the water column of each of the rivers in this study were very low (see Figure 26 section 7.2.1). In most cases, the nitrate (NO_x) concentration was below the detection limit of 1 µg/L. These very low nutrient concentrations are the major constraint on the overall primary productivity of the rivers. This nutrient limitation means that large algal blooms are unlikely in these streams. As seen regularly with algal blooms in rivers such as those in the Darling River system in northern NSW, high turbidity and hence low light penetration into the water is insufficient to prevent large blooms if nutrient concentrations are high. The high turbidity simply favours species such as the toxic cyanobacteria (blue-green algae) *Anabaena*, which can alter its position in the water column to move to the surface and obtain sufficient light to grow prolifically. The major difference between the rivers in the Edward-Wakool system and those in the Darling and other regions, is the very low nutrient concentrations which greatly limit algal proliferation. Hence management focus in the study region should continue to include nutrient minimization strategies.

When considering possible nutrient limitation, the form of the nutrient is critically important. Measurements of total concentrations of nitrogen and phosphorus include all N and P in that sample. However, only the bioavailable fractions, nominally FRP for phosphorus and the sum of ammonia and NO_x for nitrogen, are in the form readily taken up by primary producers, whereas other N and P species can be relatively recalcitrant (not suitable for assimilation into plant biomass). Table 15 shows several salient features of the nutrient concentrations in the study streams over the period August 2012 to April 2013: i) the median concentrations of the bioavailable forms are extremely low by both national and international standards for lowland rivers; ii) As noted in the Water Quality section of this report (section 7.2.1), the Total P and Total N concentrations are frequently above the ANZECC Water Quality Guidelines; iii) typically only 5-7% of the phosphorus in the water column is bioavailable and just 1% (or less) of the nitrogen is bioavailable. This highlights a general finding in turbid waters, that the use of total nutrient concentrations, rather than bioavailable concentrations, can provide extremely misleading information about the potential of a water body to suffer adverse effects from ostensibly high nutrient levels.

Table 15. Median Bioavailable and Total Nutrient Concentrations and the fraction of Bioavailable P & N in the four study streams.

	Nutrients (µg/L)							
River	**n**	**Ammonia**	**FRP**	**NO_x**	**Total P**	**Total N**	**% Bioavailable P**	**% Bioavalable N**
Colligen	18*	2	3	< 1	40	425	6.0	0.7
Little Merran	18*	3	4	2.5	60	470	6.7	1.2
Wakool	18*	1.5	3	< 1	50	495	5.0	0.5
Yallakool	18*	2	3	< 1	50	440	6.0	0.5

* Sampled on 18 separate occasions with typically 4 replicates in each sample.

Unlike 2011-12, where there was some indication of enhanced rates of GPP and ER after freshes, such behaviour was not strongly evident during 2012-2013, in contrast to the initial hypotheses. It is clear that GPP is strongly constrained by bioavailable nutrient concentrations and that the freshes were not of sufficient magnitude to entrain higher nutrient concentrations (e.g. from rewetting the floodplain). Similarly, the relative constancy in ER can be attributed to the low and consistent DOC concentrations (see Water Quality, section 7.2.1). These outcomes should be seen as largely positive, as, for example, the existing levels of metabolism are able to support the fish population without running the risk of either algal blooms or anoxic events.

The influence of freshes on the rates of GPP and ER has been determined by examining these rates before and after each of these events. The rationale is to isolate the effect of the fresh from weather and water temperature effects, which are assumed to act equally on both the Impact rivers (Yallakool or Colligen Creeks) and the Control rivers (Wakool River or Little Merran Creek). Two of the four sets of results from the Table are presented as Box Plots in Figures 44 and 45: Yallakool Creek with control = Wakool River, and Colligen Creek with control = Little Merran Creek respectively.

Although the statistical analysis summarized in Table 16 indicates that there were statistically significant differences in metabolic rates between the control and impact streams induced by the freshes on several occasions, the direction of the change was variable. Of the nine comparisons of ecosystem respiration (five for Yallakool, four for Colligen), five showed a significant increase in ER in the weeks immediately following the fresh; there were two cases of suppressed ER compared to the control (Feb 2013 fresh in Yallakool Creek with the Wakool River as control and the extended Nov-Dec 2012 fresh in Colligen Creek with Little Merran Creek as the control). Two cases showed no significant difference.

The preponderance of ER enhancement is largely in line with the hypothesis that a fresh would increase ER by introducing new organic matter. The results were less consistent for GPP. Of the nine combinations shown in Table 16, there were four suppressions (in line with the hypothesis related to increased light attenuation), two enhancements and three with no significant difference pre and post fresh. However, by far the most important finding of this statistical analysis is that any suppression or enhancement of GPP or ER was very small; typically limited to within the range ± 2 mg O_2/L/Day as shown in Figures 44 and 45. It was noted earlier that the metabolic rates in these streams are relatively low (but 'normal') compared to other streams around the world and it is apparent that these freshes are sometimes causing small fluctuations in these 'low' rates. Freshes that result in water moving onto the floodplain and then returning to the stream channel may have a much larger impact on gross primary production and ecosystem respiration, but this was unable to be examined during the 2012-13 period as there were no overbank flows.

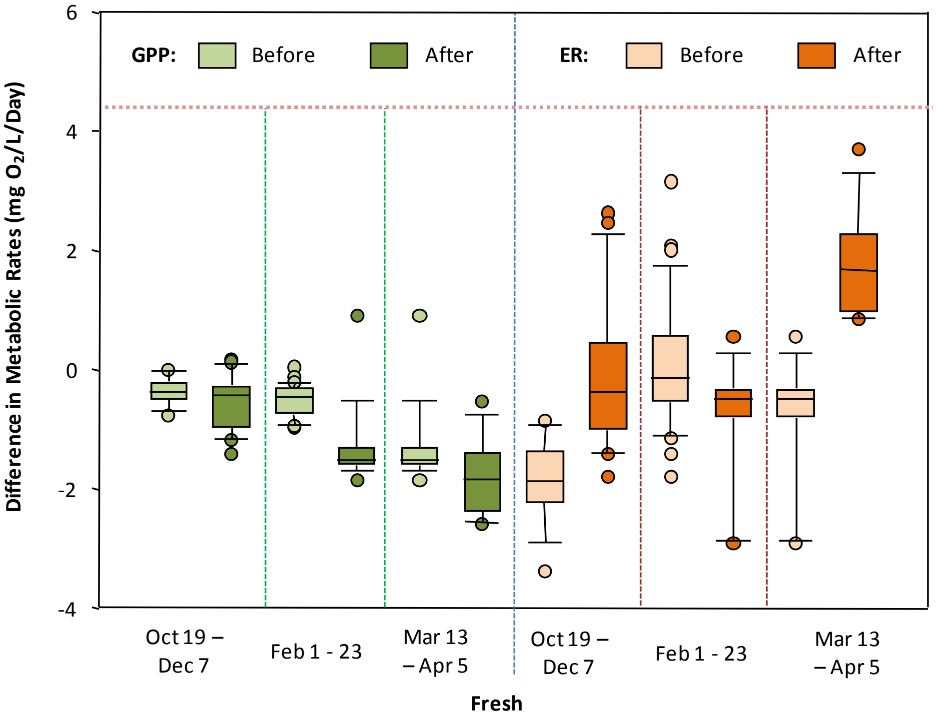


Figure 44. Box plots showing the effect of the three freshes on metabolism in Yallakool Creek. A negative value on the Y-axis indicates that the rate in the control stream (Wakool River) was higher than in Yallakool Creek. The boxes represent the data range 25th to 75th percentile, with the 'middle' line in the box being the median. The "whiskers" indicate 10th and 90th percentiles in the data. Outliers are shown as circles.

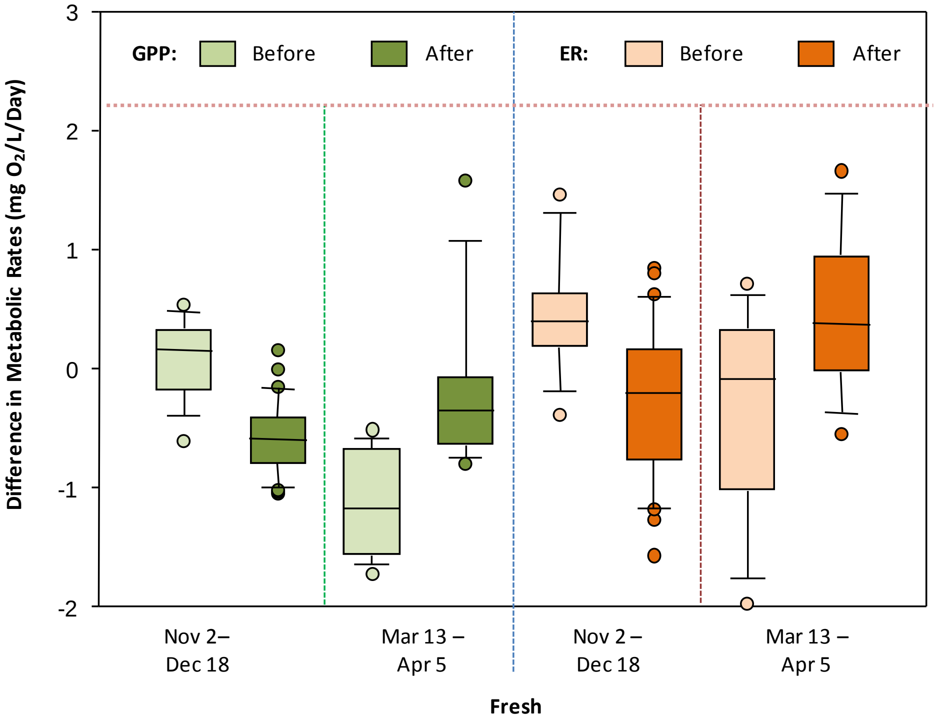


Figure 45. Box plots showing the effect of the three freshes on metabolism in Colligen Creek. A negative value on the Y-axis indicates that the rate in the control stream (Little Merran Creek) was higher than in Colligen Creek. The boxes represent the data range 25th to 75th percentile, with the 'middle' line in the box being the median. The "whiskers" indicate 10th and 90th percentiles in the data. Outliers are shown as circles.

Table 16. Statistical Summary of Effects of Freshes on Stream Metabolism. Green shading = rate enhancement, pink = rate suppression, compared to control.

Stream	**Control**	**Watering Event**	**Data Periods in Relation to Fresh**		Effect on Metabolic Rates*	
			Before	***After***	**GPP**	**ER**
Yallakool	Wakool	19/10/12 to 7/12/12	22/9/12 to 4/10/12	9/12/12 to 6/1/13	T, no sig diff in GPP, p = 0.234, Power = 0.22	T, enhancement of Yallakool ER, p < 0.001, Power = 0.99
		1/2/13 to 23/2/13	1/1/13 to 31/1/13	24/2/13 to 12/3/13	M-W, suppression of Yallakool GPP, p < 0.001	T, suppression of Yallakool ER, p = 0.020, Power = 0.65
		13/3/13 to 5/4/13	24/2/13 to 12/3/13	6/4/13 to 15/4/13	M-W, no sig diff in GPP, p = 0.13	T, enhancement of Yallakool ER, p < 0.001, Power = 1.00
	Little Merran	19/10/12 to 7/12/12	22/9/12 to 4/10/12	9/12/12 to 6/1/13	*no before data*	*no before data*
		1/2/13 to 23/2/13	1/1/13 to 31/1/13	24/2/13 to 12/3/13	M-W, enhancement of Yallakool GPP, p < 0.001	M-W, no sig diff in ER, p = 0.17
		13/3/13 to 5/4/13	24/2/13 to 12/3/13	6/4/13 to 15/4/13	T, suppression of Yallakool GPP, p = 0.006, Power = 0.83	T, enhancement of Yallakool ER, p < 0.001, Power = 1.00
Colligen	Wakool	2/11/12 to 18/12/12	29/9/12 to 21/10/12	20/12/12 to 26/1/13	T, no sig diff in GPP, p = 0.875, Power = 0.053	T, enhancement of Colligen ER, p < 0.001, Power = 0.99
		13/3/13 to 5/4/13	24/2/13 to 12/3/13	7/4/13 to 15/4/13	M-W, suppression of Colligen GPP, p= 0.010	T, no sig diff in ER, p = 0.310, Power = 0.26
	Little Merran	2/11/12 to 18/12/12	29/9/12 to 21/10/12	20/12/12 to 26/1/13	T, suppression of Colligen GPP, p < 0.001, Power = 0.99	T, suppression of Colligen ER, p = 0.0013, Power = 0.92
		13/3/13 to 5/4/13	24/2/13 to 12/3/13	7/4/13 to 15/4/13	M-W, enhancement of Colligen GPP, p = 0.004	T, enhancement of Colligen ER, p = 0.037, Power = 0.57

* ‘T’ = Student's two-tailed t-test; performed if the Shapiro-Wilk normality test was passed (at p = 0.05). ‘M-W’ = Mann-Whitney, Non-parametric Rank Sum Test; performed when normality requirement for t-test not met.

7.3. Objective 3: Support breeding and recruitment of frogs and invertebrates

7.3.1. Zooplankton

http://www.freshwaterlife.org/servlet/CDSServlet?status=ND0xMTg2NyZjdG5faW5mb192aWV3X3NpemU9Y3RuX2luZm9fdmlld19mdWxsJjY9ZW4mMzM9KiYzNz1rb3M~

Key findings

- *The watering actions in Colligen and Yallakool Creeks during the 2012 – 2013 sampling period did not increase the abundance of zooplankton, including individual size classes of zooplankton, nor did it appear to stimulate reproduction.* Zooplankton abundance was instead highly seasonal, affected by factors unrelated to flow, such as temperature.

- It is possible that the magnitude of the environmental watering actions were not sufficient to inundate riverbank habitat and stimulate productivity, thereby we did not observe the expected increase in abundance and taxonomic diversity of zooplankton.

- *Environmental freshes of greater magnitude and duration would likely be needed to elicit a response, particularly flows which increase the extent of riverbank inundation.*

Background

Zooplankton are abundant, widely distributed and a diverse group of organisms which are the foundation of many aquatic food-webs (Lampert 1997). Zooplankton consist of two major groups; rotifers and microcrustaceans (cladocerans, copepods, and ostracods) which are common in the planktonic community, but also occupy benthic and littoral areas (Boon et al. 1990; Shiel 1990). In riverine ecosystems zooplankton are one of the major food sources for larval fish, therefore forming an important link between primary producers and higher trophic levels (King 2005). Fish recruitment is strongly influenced by the availability of food resources as well as flow regime. It is one thing for environmental flows to elicit a spawning response in fish, but if larvae have no food supply, then fish may not exhibit successful recruitment due to high initial mortality.

During the early life stages of fish, natural mortality is high and the availability of appropriately-sized prey is thought to be critical to recruitment success (May 1974). Zooplankton are typically less than 4 mm in size, and rotifers, which tend to dominate the zooplankton community, are generally less than 200 µm while some cladocera taxa reach lengths of 4-6 mm (Shiel 1995). Zooplankton samples are generally collected with a 53 µm net, however further sieving allows separation into size classes which can be directly related to fish larvae with corresponding gape sizes (Masson et al. 2004).

Although zooplankton are not often used in studies of hydrological variation or monitoring of flow impacts, they provide an easy, inexpensive option for monitoring. In addition, their rapid life cycles means they respond quickly to changes in their environment, including altered flow. Hydrology plays an important role in structuring zooplankton communities, primarily flow velocity and water residence time. Different taxonomic groups and life-history stages have varying flow velocity requirements, however it is generally accepted that zooplankton abundance is negatively related to velocity (Vranovský 1995)). High flows impact on zooplankton communities via direct mortality, dislodgement and suppression of reproduction (Ferrari et al. 1989; Rzoska 1978). Low flows and hence longer water residence time is positively related to zooplankton abundance (Basu & Pick 1996). This is thought to be due to the creation of slackwater habitats, which are known to support high densities of zooplankton, higher temperatures, and greater food availability (Reckendorfer et al 1999; Ning et al 2010).

Riverine zooplankton communities are, however, a product of both in-channel community dynamics and upstream sources (Saunders and Lewis 1988). Impoundments, for example, may be significant sources of individual zooplankters, contributing to downstream populations during periods of high flow (Dickerson et al. 2010; Humphries et al. 2013). Floodplain inundation up stream of zooplankton communities can similarly increase abundance and diversity (Ning et al. 2013; Saunders and Lewis 1988).

Zooplankton are responsive to environmental changes in temperature, turbidity, nutrients and other ecological parameters. Different taxonomic groups and life-history stages also have varying flow velocity requirements which, in addition to the previously mentioned characteristics, make them a potential indicator for the assessment of environmental watering. By examining the response of zooplankton to changes in flows, we can better understand the relationship with other components of the ecosystem such as native fish recruitment success.

Hypotheses

In monitoring the response of zooplankton to watering actions in the Edward-Wakool system in 2012 – 2013 we make the following predictions:

1. The abundance of zooplankton and the proportion of egg-carrying zooplankton will decrease during the delivery of freshes, due to high velocity leading to mortality, displacement and the suppression of reproduction
2. The abundance of zooplankton in Colligen and Yallakool Creeks will increase following increases in discharge due to Stevens weir (Edward River) acting as a source of zooplankters.
3. The abundance of zooplankton and the proportion of egg-carrying zooplankton will increase during low flows due to longer water residence time, and higher temperatures.

Methods

Five replicate zooplankton samples were collected at seven river reaches by filtering 50 L of water with a 53 µm plankton net (Figure 46). Samples were preserved in 70% ethanol prior to taxonomic identification in the laboratory. Samples were collected fortnightly between August 2012 and early May 2013.

Figure 46. River water is filtered through a plankton net

In the laboratory zooplankton samples were stained then sieved into four size fractions (53 – 106 µm, 106 – 200 µm, 200 – 500 µm, and 500 µm +). These sizes could then be compared with the gape size of predatory larval fish as described by Masson et al. (2004) which provides an upper limit for larval fish prey size. Zooplankton in each size fraction were then sub-sampled and identified and counted under a stereomicroscope. The main microcrustacean groups (cladocera, copepoda) were counted seperately from rotifers, and the presence of ovigerous zooplankton (individuals carrying eggs or resting stages) were noted. Individual subsamples were counted until a minimum of 200 individuals had been identified or 100% of the sample had been processed. All count data were converted to densities (i.e. animals per litre) prior to analysis.

Preliminary data analysis indicated that there was little effect of dilution on densities of zooplankton, and therefore only density data is presented here. Zooplankton data from flowing waters is sometimes converted to a number which takes into account discharge, (e.g. Transport, which provides an indication of the overall number of animals moving downstream each day, and is influenced by river discharge). However, this approach has been criticised for inflating abundances, and for making assumptions about the movement of zooplankton populations in a riverine system.

To examine differences across rivers, a two-way ANOVA was performed on the main factors of river and date of sampling. When significant differences were indicated, post hoc pair wise comparisons (Tukey's test) were undertaken to determine differences between the factors. An asymmetrical BACI (before-after, control-impact) (Underwood, 1991) statistical design was used to test the effect of specific 2012-2013 environmental water actions on zooplankton density and the proportion of ovigerous zooplankton, as described in Section 7.2.1 and Table 9.

Correlations between the density of zooplankton and independent variables (7 day mean discharge, 7 day mean C.V discharge, dissolved organic carbon (DOC), particulate organic carbon (POC), temperature, turbidity and chlorophyll *a* (Chl*a*) were conducted using the non-parametric Kendall-rank co-efficient. Where data was available, means for the previous 7 days were used to reflect the conditions during the short life cycles of zooplankton (4-5 days for rotifers; Shiel 1995). Scatterplots were constructed for correlations which were significant between independent and dependent variables. All univariate analyses were conducted using the software package SPSS Statistics v20.

Results

Seasonal patterns

Rotifers numerically dominated across all rivers, comprising approximately 88 % of the zooplankton assemblage (cladocera and copepods compromised 7 % and 5 % respectively). Rotifer density varied seasonally, with peaks in late October 2012 and December 2012 / January 2013 across all rivers (Figure 47). Copepod percent contribution peaked in August and September across all rivers. Cladocera on the other hand made up a low proportion of zooplankton across all rivers with the exception of the Little Merran Creek, where they made up a high proportion from January to April (19 – 87 %). A Tukey's test for differences in cladocera density among sites supports this finding (Appendix 1; Tukey's test, P<0.001 for Little Merran vs. all rivers).

Zooplankton in the smallest size class (53 – 106 µm) dominated all rivers, comprising on average 66 % (Figure 48). Seasonal fluctuations in the relative proportions of each size class were variable, however, zooplankton size increased over December 2012 and January 2013 in all rivers. Zooplankton size also increased in the Little Merran Creek in October 2012 and March 2013. Zooplankton greater than 500 µm in size comprised on average only 0.06 % of total zooplankton density and have therefore been excluded from statistical analyses (Figure 48).

A two-way ANOVA indicated that the total density (number of animals per litre) of zooplankton in each river across the 2012 - 2013 sampling period was similar (Appendix 1; Table 17; Figure 49; Two-way ANOVA, DF=4; P >0.05). Indeed, zooplankton density peaked at the same time among all rivers – October 2012 and January 2013. However, zooplankton density in the 53-106 and 200-500 size classes were similar among the Edward, Colligen and Yallakool, but not the Little Merran and Edward Rivers (Tables 18 and 19).

Table 17. Zooplankton density in five rivers in the Edward-Wakool system across the 2012 – 2013 sampling period. Numbers are totals across all sampling dates, with minima and maxima in parentheses.

River	Size class (µm)	Total zooplankton	Rotifers	Cladocera	Copepods
Colligen	53 – 106	15869 (7 - 714)	15525 (7 - 712)	18 (0 - 4)	326 (0 - 26)
	106 – 200	6907 (3 - 387)	6438 (3 - 374)	127 (0 - 19)	341 (0 - 27)
	200 – 500	1040 (0 - 185)	945 (0 - 184)	66 (0 - 22)	28 (0 - 3)
	>500	5 (0 - 2)	4 (0 - 2)	0 (0 - 0)	1 (0 - 0)
Yallakool	53 – 106	15075 (0 - 896)	14879 (0 - 890)	16 (0 - 4)	179 (0 - 10)
	106 – 200	8185 (7 - 496)	7716 (6 - 495)	174 (0 - 12)	293 (0 - 32)
	200 – 500	1176 (0 - 234)	1088 (0 - 233)	60 (0 - 7)	26 (0 - 2)
	>500	6 (0 - 2)	5 (0 - 2)	0 (0 - 0)	1 (0 - 0)
Wakool	53 – 106	18447 (15 - 689)	18089 (14 - 689)	10 (0 - 2)	346 (0 - 50)
	106 – 200	7456 (6 - 556)	7069 (2 - 556)	156 (0 - 10)	229 (0 - 28)
	200 – 500	1588 (0 - 304)	1470 (0 - 303)	83 (0 - 13)	34 (0 - 4)
	>500	128 (0 - 46)	124 (0 - 46)	1 (0 - 0)	3 (0 - 1)
Little Merran	53 – 106	11207 (0 - 466)	10920 (0 - 466)	91 (0 - 40)	195 (0 - 10)
	106 – 200	7132 (9 - 490)	4888 (0 - 325)	1954 (0 - 464)	285 (0 - 26)
	200 – 500	2252 (0 - 388)	467 (0 - 56)	1754 (0 - 388)	28 (0 - 4)
	>500	7 (0 - 1)	1 (0 - 0.1)	4 (0 - 1)	3 (0 - 1)
Edward	53 – 106	16593 (1 - 764)	16281 (1 - 764)	38 (0 - 15)	275 (0 - 38)
	106 – 200	7670 (0 - 371)	7225 (0 - 368)	91 (0 - 12)	353 (0 - 23)
	200 – 500	788 (0 - 70)	689 (0 - 69)	85 (0 - 45)	14 (0 - 2)
	>500	1 (0 - 0)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)

Table 18. Results of post-hoc analysis of zooplankton density for the 53 – 106 µm size class. Numbers indicate significant P values for differences between rivers.

	Colligen	Edward	Little Merran	Wakool	Yallakool
Colligen					
Edward					
Little Merran	<0.001	<0.001			
Wakool	<0.01	<0.001	<0.001		
Yallakool			<0.05	<0.001	

Table 19. Results of post-hoc analysis of zooplankton density for the 200 – 500 µm size class. Numbers indicate significant P values for differences between rivers.

	Colligen	Edward	Little Merran	Wakool	Yallakool
Colligen					
Edward					
Little Merran	<0.001	<0.001			
Wakool	<0.001	<0.001	<0.001		
Yallakool			<0.001	<0.01	

Ovigerous (egg-carrying) zooplankton

Across all rivers and sampling times, a greater proportion of cladocera (15 %) were carrying eggs than rotifers (6 %) and copepods (5 %) (Figure 50). Cladocera egg production peaked in November 2012 across all sites, and again in March and April 2013. Rotifer egg production did not show a clear pattern across time, however, was low in all rivers in December 2012 to January 2013.

There was a significantly greater proportion of ovigerous zooplankton in Little Merran Creek than in Colligen Creek or the Edward River (Two-way ANOVA, DF=4,63; P <0.001; Tukey's test, P<0.001 and P<0.001 respectively), and a significantly lower proportion in the Edward River than the Wakool River and Little Merran Creek (Tukey's test, P<0.05 and P<0.001 respectively). Colligen Creek had a significantly lower proportion of ovigerous rotifers than the Yallakool Creek (Appendix 1; Two-way ANOVA, DF=3; P <0.01; Tukey's test, P<0.05). The Edward River had a significantly lower proportion of ovigerous copepods than all the Wakool River and Yallakool Creek (Appendix 1; Two-way ANOVA, DF=3; P <0.001; Tukey's test, P<0.001 and P<0.05 respectively).

Responses to Yallakool and Colligen Creek watering actions

The watering actions carried out during 2012 – 2013 had no effect on zooplankton density during Colligen Creek November 2012 fresh, Yallakool Creek February 2013 fresh, and the Yallakool Creek and Colligen Creek March 2013 fresh (Appendix 2; Figures 51-55). There was a significant interaction between period (before, during, after) and control/impact for the 106 – 200 μm size class (Two-way mixed effects ANOVA df = 2,97; p = 0.03). This was a result of an increase in abundance during and after the watering action in Yallakool Creek compared to the control rivers. However, this result should be treated with caution given the P value was only just under the significance level of 0.05, and the fact that this watering action coincided with seasonal peaks in zooplankton abundance occurring across all rivers. The watering actions in the Colligen Creek and Yallakool Creek did not significantly stimulate egg production in zooplankton (Appendix 2).

Responses to environmental variables

There were no significant correlations between zooplankton density and the environmental variables (discharge, CV, DOC, POC, temperature, turbidity and chlorophyll a) (Table 20). The proportion of total zooplankton which were ovigerous was positively correlated with DOC and negatively correlated with temperature (Table 20; Figure 56). Ovigerous copepods showed the same pattern.

Table 20. Results of correlations (non-parametric Kendall-rank co-efficient) with environmental data. Statistically significant results are highlighted. * = P<0.01, ** = P<0.001, *** = P<0.0001.

	Density (individuals/L)							***Ovigerous (proportion)***			
	Total zooplankton	**Rotifers**	**Cladocera**	**Copepods**	**53-106**	**106-200**	**200-500**	**Total zooplankton**	**Rotifers**	**Cladocera**	**Copepods**
7 day mean daily discharge (ML/d)	-0.07	-0.07	-0.01	0.12	-0.05	-0.04	-0.18	0.16	0.13	0.07	0.16
7 day CV of daily discharge (ML/d)	0.04	0.09	-0.09	0.14	0.2	-0.06	-0.22	0.07	0.22	-0.06	0.06
DOC (mg/L)	0.05	0.05	0	-0.17	0.16	-0.03	-0.19	**0.46*****	0.29	0.19	**0.44****
POC (mg/L)	0.14	0.13	0.01	-0.09	0.18	0.07	-0.06	0.26	0.24	0.17	0.23
Temperature (°C)	0.15	0.08	-0.06	0.34	-0.02	0.26	0.34	**-0.39****	-0.26	-0.18	**-0.37****
Turbidity (NTU)	0.03	-0.04	0.28	0.04	-0.09	0.12	0.27	-0.05	-0.16	0.1	0.02
Chl*a* (mg/m^3)	0.12	0.06	0.1	0.29	0.06	0.16	0.09	0.12	0.02	0	0.06

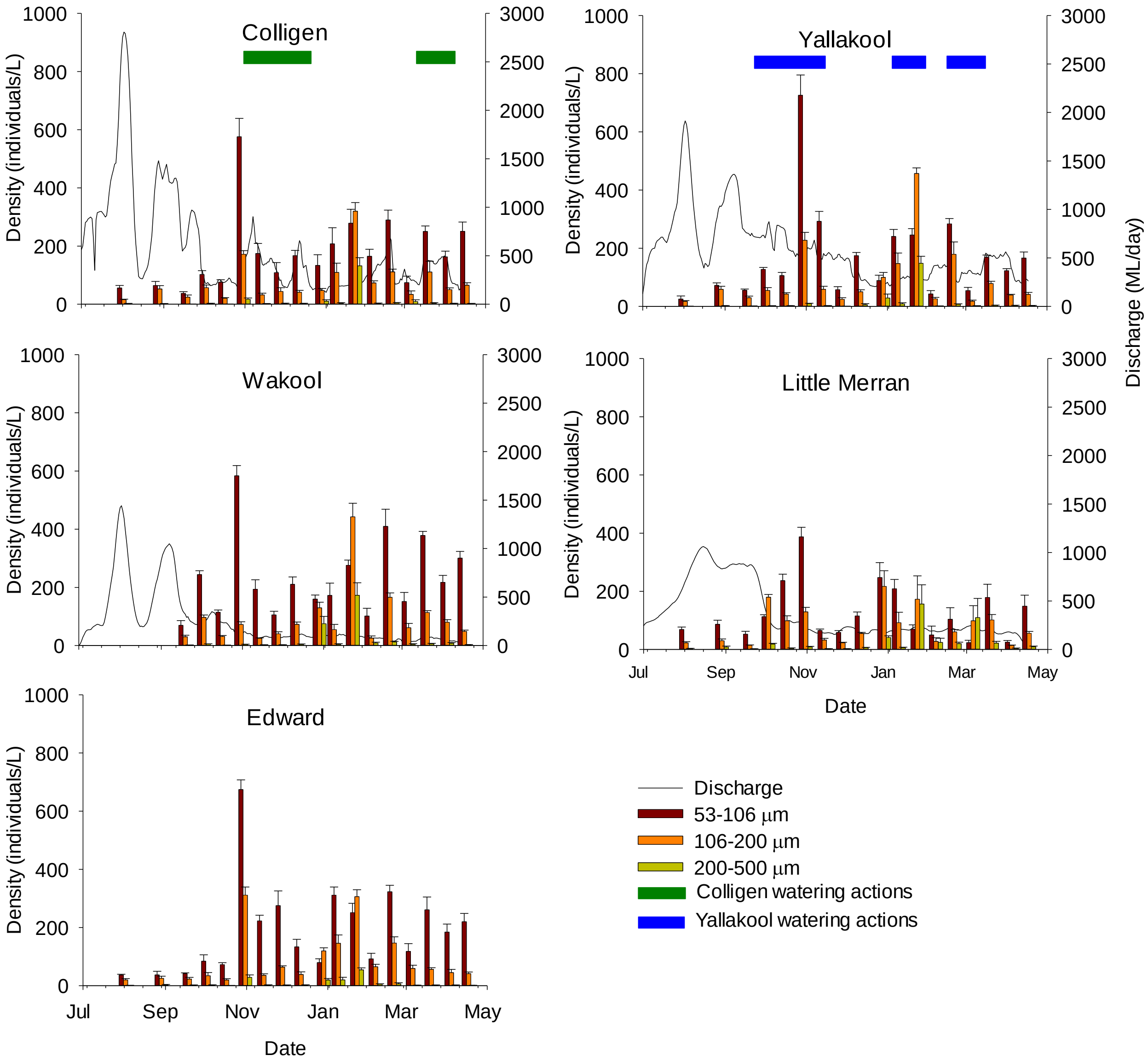


Figure 47. Total zooplankton density within each size class across sites. Horizontal bars indicate duration of watering actions in Colligen Creek and Yallakool Creek.

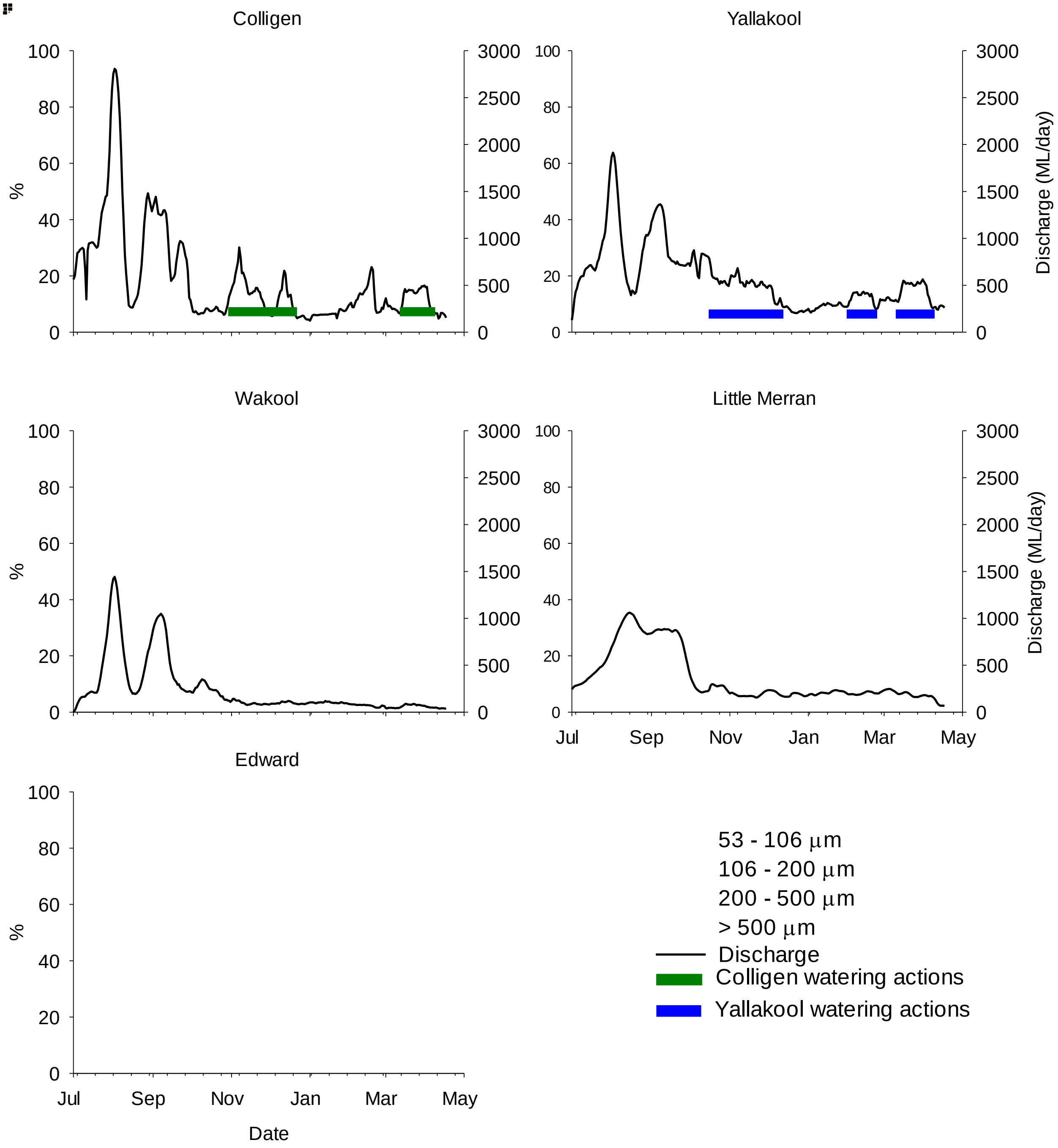


Figure 48. Percent contribution (%) of each zooplankton size class. Note Edward site was located in weir pool, therefore no hydrograph is presented. Horizontal bars indicate duration of watering actions in Colligen Creek and Yallakool Creek.

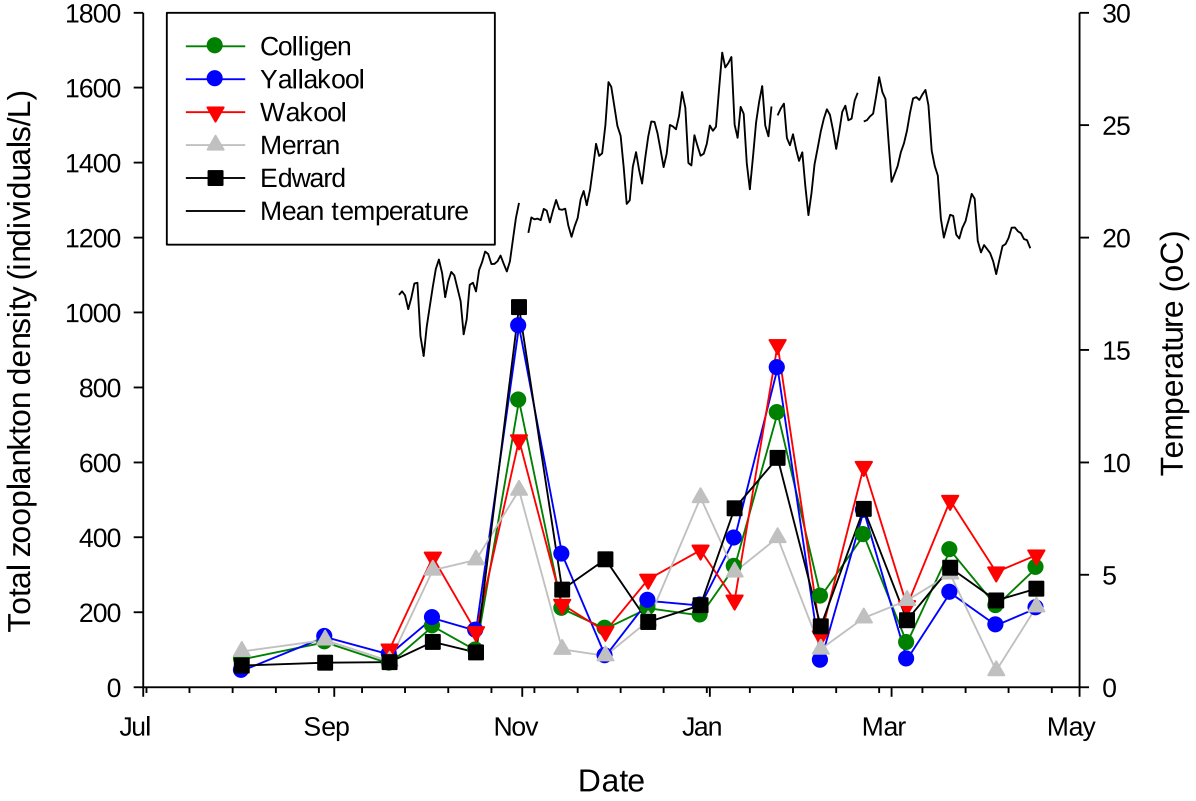


Figure 49. Total density of zooplankton and mean temperature at all sites during the 2012 – 2013 sampling season.

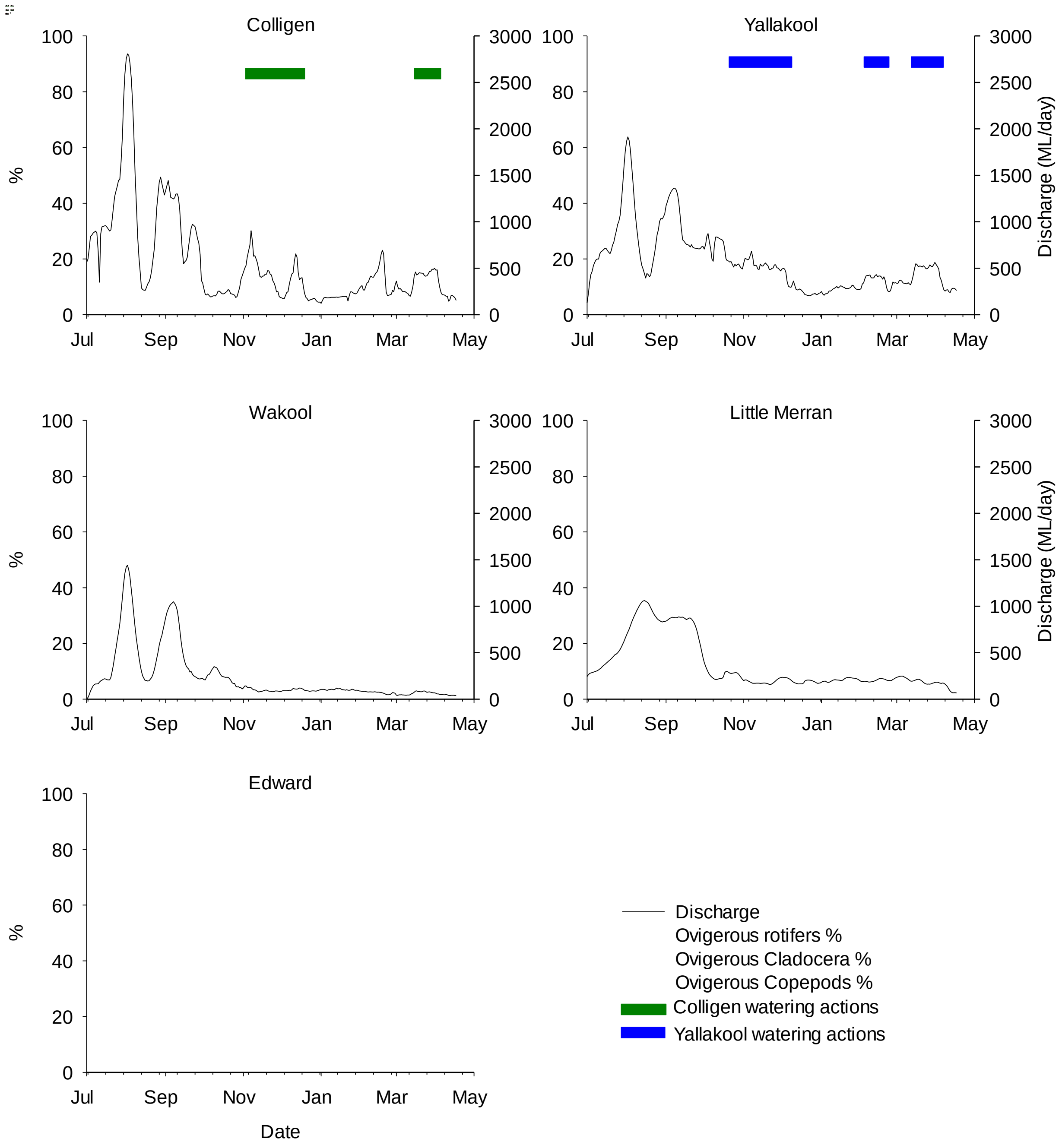


Figure 50. Percent contribution (%) of ovigerous (egg-carrying) zooplankton. Note Edward site was located in weir pool, therefore no hydrograph is presented. Horizontal bars indicate duration of watering actions in Colligen Creek and Yallakool Creek.

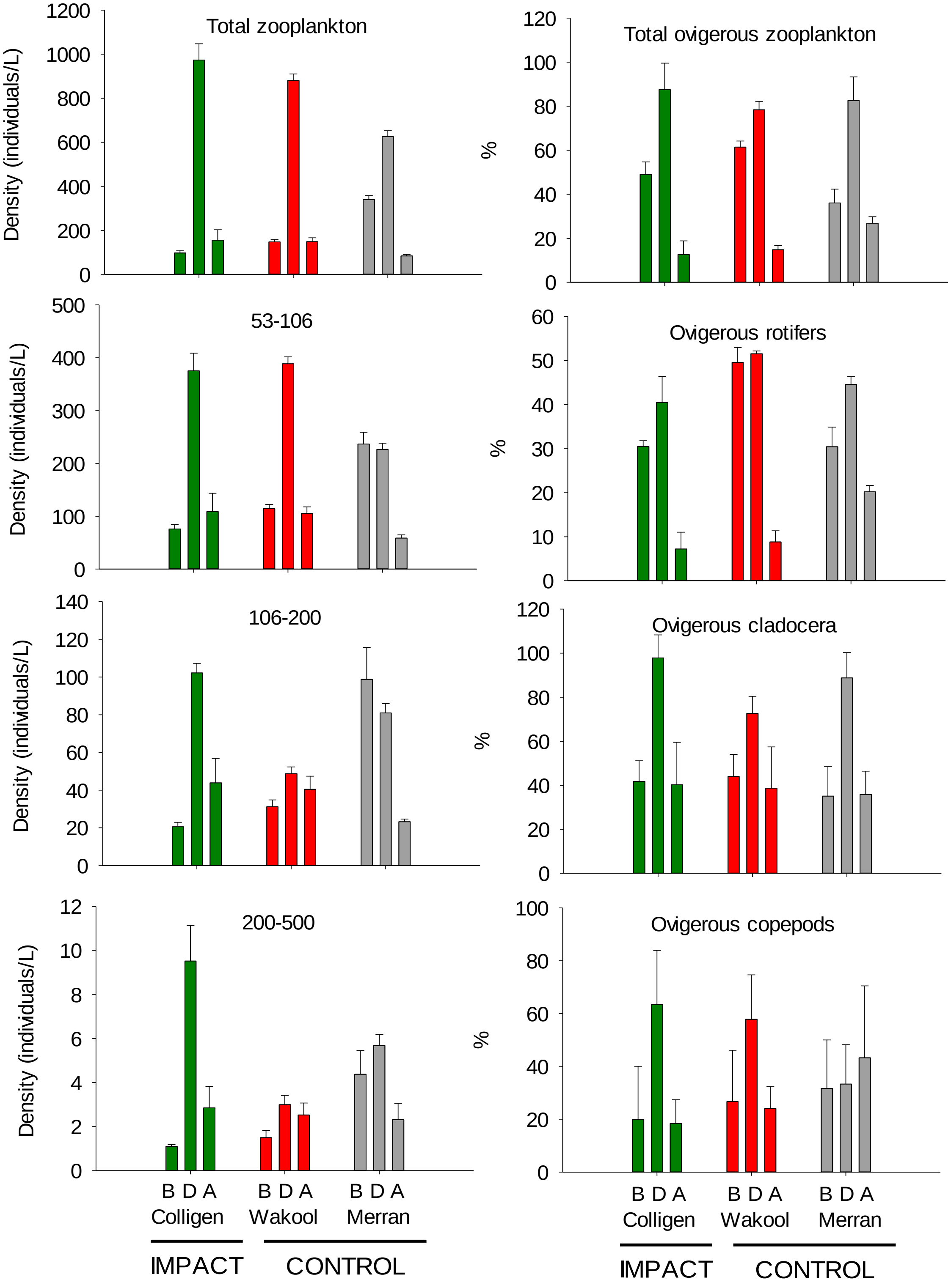


Figure 51. Density of zooplankton and proportion of ovigerous zooplankton before (B) during (D) and after (A) the October to November 2012 fresh in Colligen Creek. The Wakool River and Little Merran Creek did not receive environmental water and were used as controls.

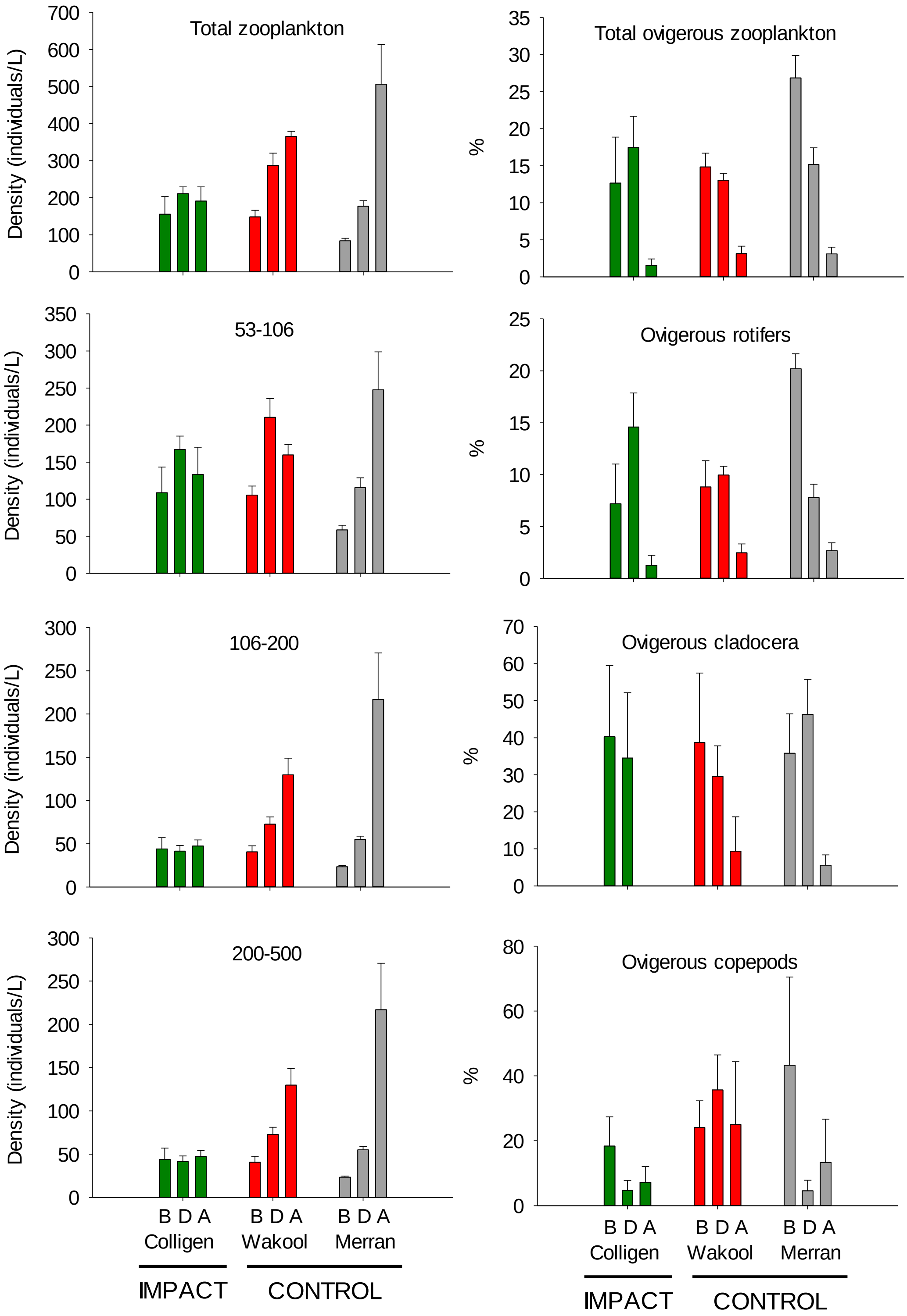


Figure 52. Density of zooplankton and proportion of ovigerous zooplankton before (B) during (D) and after (A) the December 2012 fresh in Colligen Creek. The Wakool River and Little Merran Creek did not receive environmental water and were used as controls.

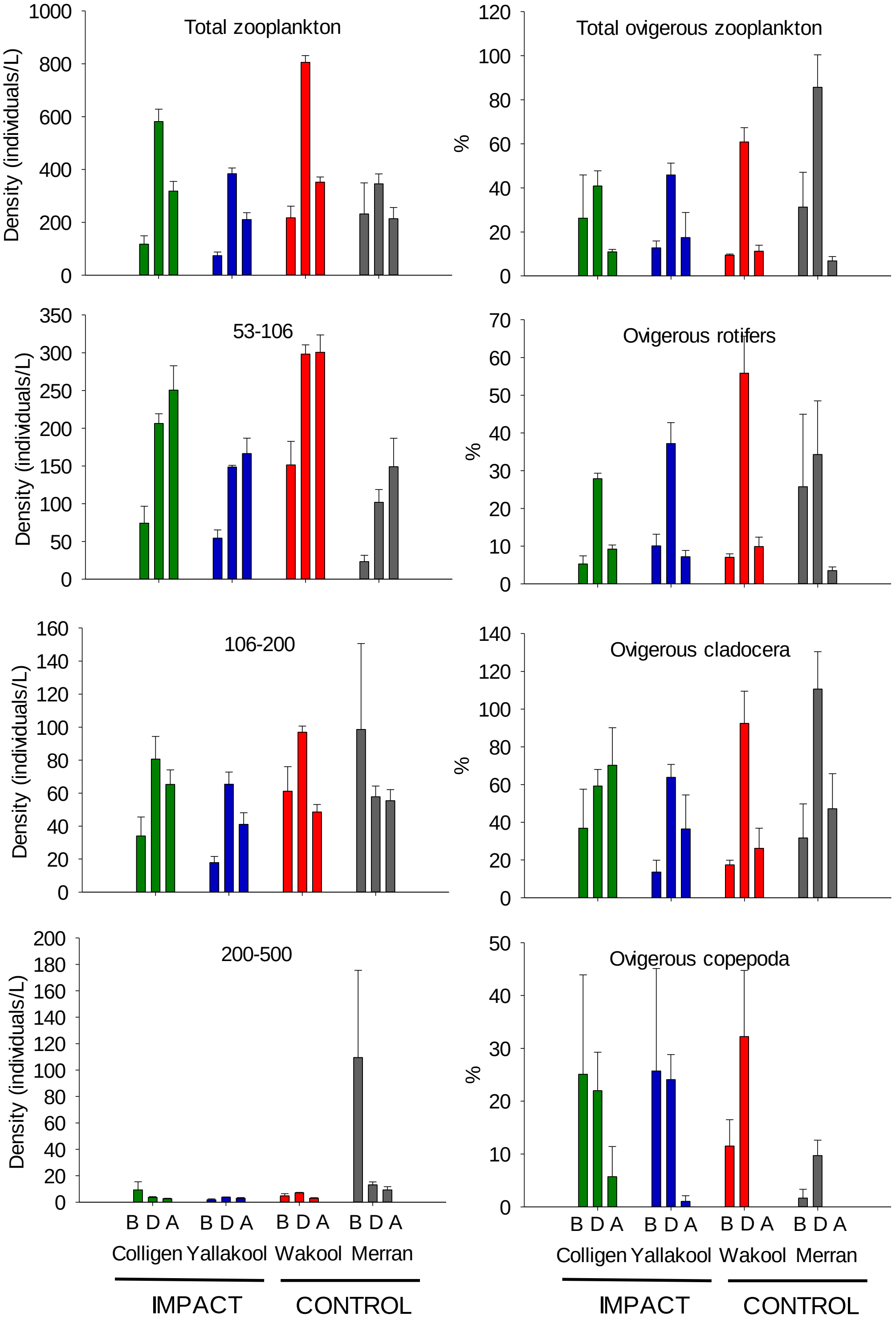


Figure 53. Density of zooplankton and proportion of ovigerous zooplankton before (B) during (D) and after (A) the March/April 2013 fresh in Colligen Creek and Yallakool Creek. The Wakool River and Little Merran Creek did not receive environmental water and were used as controls.

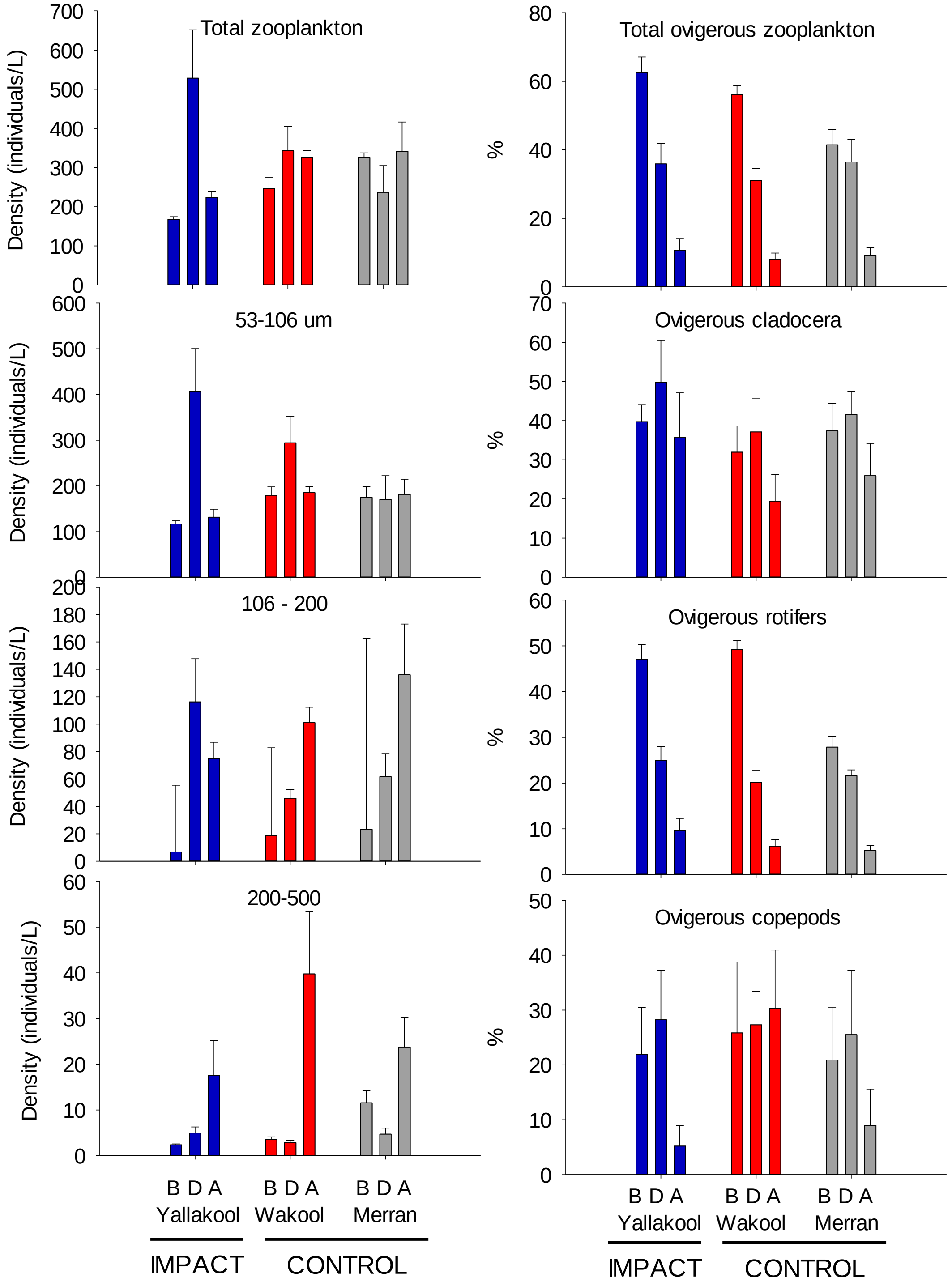


Figure 54. Density of zooplankton and proportion of ovigerous zooplankton before (B) during (D) and after (A) the October/November/December 2012 fresh in Yallakool Creek. The Wakool River and Little Merran Creek did not receive environmental water and were used as controls.

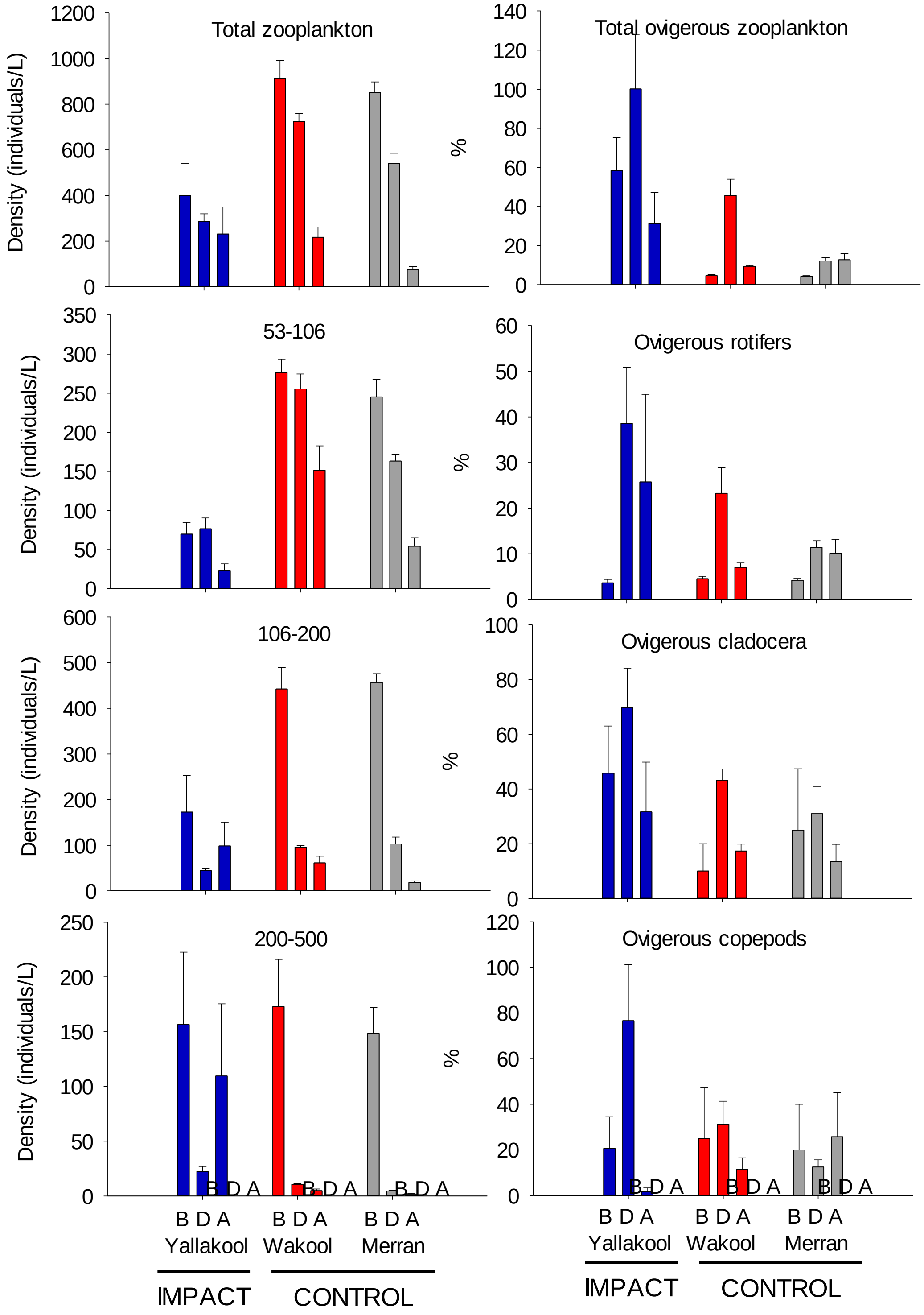


Figure 55. Density of zooplankton and proportion of ovigerous zooplankton before (B) during (D) and after (A) the January/February 2013 fresh in Yallakool Creek. The Wakool River and Little Merran Creek did not receive environmental water and were used as controls.

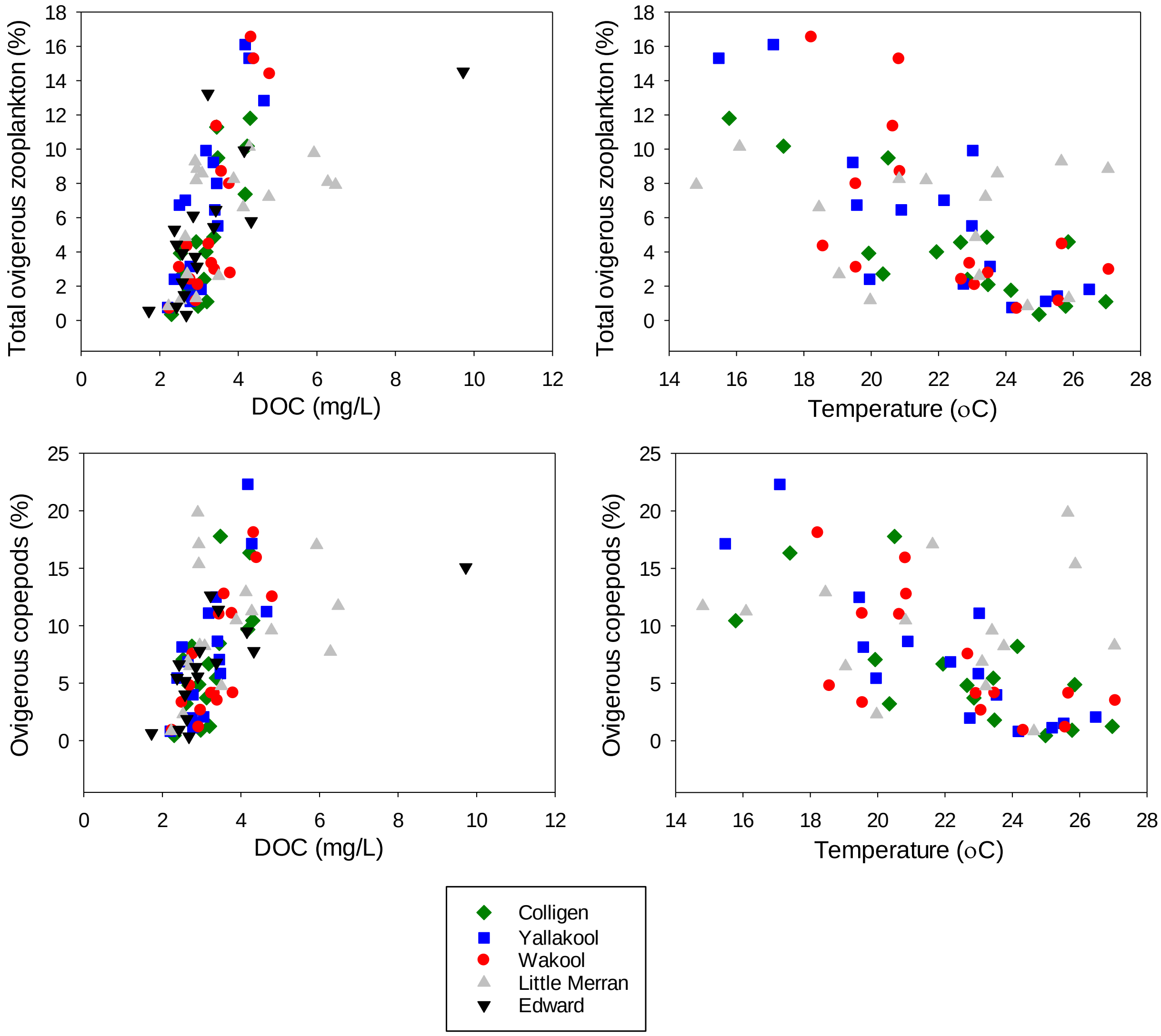


Figure 56. Scatter plots showing statistically significant relationships between zooplankton and environmental parameters across the study rivers.

Discussion

2013 – 2013 watering actions in Colligen Creek and Yallakool Creek

We predicted that the abundance of zooplankton and the proportion of egg-carrying zooplankton would decrease during the delivery of freshes, due to high velocity leading to mortality, displacement and the suppression of reproduction. However, the abundance of zooplankton and the proportion of zooplankton carrying eggs did not change during the watering actions in Colligen and Yallakool Creeks in 2012-13. It is possible that the magnitude and duration of the watering actions in 2012-2013 were insufficient to displace large numbers of zooplankton or suppress reproduction to an extent that we were able to detect the predicted changes. Zooplankton abundances have been found to be negatively correlated with flow during prolonged seasonal rises in discharge in lowland rivers (Saunders and Lewis 1989; Van Zanten and Van Dijk 1994). Zooplankton abundance is also affected by dilution from increasing volumes of water (Basu and Pick 1997), however, there was no evidence of dilution of zooplankton densities here. Instead, changes in abundance of zooplankton in the Edward-Wakool system appeared to be unrelated to the magnitude and duration of flows seen during the 2012-2013 monitoring period.

It is possible that the magnitude of the watering actions in 2012-2013 were insufficient to inundate habitat and stimulate zooplankton productivity in the Edward-Wakool system. Flows sufficient to inundate greater areas of habitat (benches, slackwaters, and floodplain areas) are needed to stimulate emergence from the egg bank and to introduce nutrients and carbon into the system from upstream inundated floodplain areas, leading to greater productivity and potentially increasing abundance and taxonomic diversity (Ning et al. 2010).

Upstream sources vs. in-channel production

Zooplankton populations in riverine systems at any given time are influenced by a combination of in-channel production and individuals transported from connected habitats (Jenkins and Boulton 2003; Wahl et al. 2008). Upstream and adjacent habitats (in-channel and floodplain) can act as a source of individual zooplankters and resting stages (Petts 1984; Vadadi-Fülöp 2013), and upstream habitats such as weirs and dams often develop large zooplankton communities (Havel and Pattinson 2004). In-channel production is a combination of continual turnover of the residing zooplankton population and the hatching of dormant eggs from the egg bank in riverine sediments.

During the 2012- 2013 study period, there was no evidence of a dilution effect during freshes. In addition, the pattern of zooplankton abundance in Colligen Creek, Yallakool Creek and the Edward River were more similar to one another than Little Merran Creek and the Wakool River, suggesting abundances in Colligen Creek and Yallakool Creek may be reflecting upstream (source) abundances in the Edward River. During the 2011-2012 study period, the pattern of zooplankton abundance in Yallakool and Colligen Creeks were similar (Watts et al. 2013). Although the Edward River was not sampled in 2011-2012, it suggests a similar reliance on upstream sources as found in 2012-13. A longitudinal study of zooplankton in the Missouri River, USA, found zooplankton (particularly microcrustacea) assemblages and abundance were significantly influenced by upstream impoundments (Havel et al. 2013). Similarly, Humphries et al. (2013) found zooplankton biomass in the regulated Goulburn River to be strongly influenced by discharge and unrelated to temperature, attributing this pattern to high zooplankton production in the warm and shallow upstream reservoir (Lake Eildon). A longitudinal study on zooplankton abundance is needed to further examine the influence of impoundments on zooplankton dynamics in the Edward-Wakool system.

The proportion of ovigerous rotifers was greatest in the weeks leading up to the late October peak in zooplankton abundance among all rivers, suggesting in-channel production was responsible for the seasonal peak in abundance observed. However, it is possible that the unregulated high flows in late winter/spring 2012 leading up to the monitoring period increased productivity and inundated greater areas of habitat for zooplankton, resulting in the observed peak. Since monitoring only began after these high flows, this is purely speculative.

The role of low flows, nutrients and temperature

Zooplankton had a distinct seasonal pattern across all sites, largely unrelated to flow, with peaks in abundance occurring at the same time (late October and January) across all rivers. The similarity in the timing and magnitude of these peaks suggests that seasonal cues for reproduction in zooplankton were overriding other factors which varied among the rivers, such as flow. This is consistent with the findings of the 2011-2012 monitoring, when increases in zooplankton abundance were consistent among rivers but unrelated to flow (Watts et al. 2013). Temperature is widely known to affect zooplankton abundance (Kobayashi 1997; Reckendorfer 1999), but was unrelated to zooplankton abundance here. Temperature was, however, negatively correlated with the presence of ovigerous zooplankton, and it is possible that the lag effect of a change in rate of reproduction was greater than the 7 day temperature window used for statistical analysis here. Indeed zooplankton biomass in a temperate river has been found to be related to temperature when temperature for the previous month is considered (Humphries et al. 2013).

The persistence of low flow conditions (and hence longer water residence time) in the Wakool for the majority of the study period may have led to increased zooplankton production in that system. Water residence time strongly influenced zooplankton biomass and community structure in a study of the Danube River floodplain, with greater biomass during longer water residence times (Baranyi et al. 2002). Higher abundances of zooplankton in the Wakool in February to April 2013 followed elevated phytoplankton levels and hence food availability. Nutrient limitation has proven to be a significant factor prohibiting zooplankton production in other temperate rivers, with strong positive relationships between chlorophyll *a* and zooplankton biomass (Basu and Pick 1997).

Of all the rivers sampled in the Edward-Wakool during 2012 – 2013, the zooplankton community in Little Merran Creek differed the most from other rivers in terms of taxonomic and size class composition, and abundance. Although total numbers of zooplankton sampled in Little Merran Creek were low compared to the other rivers, zooplankton individuals were generally larger in Little Merran Creek. Zooplankton biomass is therefore likely to have been similar, if not greater, in the Little Merran compared to the other rivers given the larger size of the individuals. In one study of riverine zooplankton, microcrustaceans numerically made up only 2 % of zooplankton abundance but 46 % of biomass (Saunders and Lewis 1988). Although time constraints prevented us from calculating zooplankton biomass, caution must be taken in relating zooplankton abundance to food availability for larval fish in the Little Merran, since the large size of zooplankters may have meant a similar or greater biomass of food to other rivers.

In conclusion, the watering actions carried out in the Edward-Wakool system in 2012 – 2013 appeared to have little influence on zooplankton populations. Seasonal cues (possibly temperature) had a greater influence on zooplankton than the magnitude of watering actions in the Colligen and Yallakool. Low flow conditions in the Wakool River appeared to increase phytoplankton production and consequently, zooplankton abundances. Environmental freshes of greater magnitude and duration would likely be needed to elicit a response, particularly flows which aim to increase the extent of inundation. In addition, the role of upstream storages in shaping downstream zooplankton populations needs to be further explored.

7.3.2. Shrimp

Key findings

- *The timing of shrimp spawning was not influenced by environmental watering*.
- *The two rivers receiving environmental water (Colligen Creek and Yallakool Creek) had fewer shrimp compared to the control rivers*. It is possible that the higher flows reduced the size and availability of slackwaters that are crucial to larval development and juvenile shrimp recruitment. Shrimp recruitment occurs during summer when flows would normally be low under unregulated conditions. Sharp increases in flow may result in higher larvae mortality as a result of displacement and catastrophic drift from slackwater habitats.

Background

Freshwater shrimp are an important component of freshwater ecosystems, particularly with regard to nutrient cycling (Covich et al. 1999; Crowl et al. 2001; March et al. 2001). In the Edward-Wakool system, shrimp often occur in very large numbers and as well as being important to ecosystem function are likely to be an important food source for vertebrates such as native fish. One of the objectives of the watering Option 1 in the Edward-Wakool system is to support ecosystem function and support habitat requirements of native aquatic species, including frogs, turtles, and invertebrates.

In the Murray-Darling Basin there are two atyid species, *Paratya australiensis* and *Caridina mccullochi*, and one palaemonid, *Macrobrachium australiense*. Previous studies have shown that the distribution and abundance of *C. mccullochi* and *M. australiense* may be affected by altered flow regimes, whereas *P. australiensis* seems to be more tolerant (Richardson et al. 2004) This relationship is thought to be linked to the availability of suitable slackwater habitats during their spawning period (Richardson et al. 2004). Slackwaters act as an important nursery habitat for larval shrimp by providing refuges from current and energetic advantages during a key stage of their development (Humphries et al. 2006; Price and Humphries 2010). Flow has the ability to alter the size, availability and permanence of slackwaters (Bowen et al. 2003; Price et al. 2013), thus environmental watering has the potential to affect the recruitment success of shrimp.

The aim of this component of the study was to determine if the environmental watering actions in 2012-2013 influenced the relative abundance of shrimp in the Edward-Wakool system.

Hypothesis

The abundance of shrimp larvae and juveniles in the rivers that received environmental watering will differ from the control rivers that do not receive environmental water. The effects of the environmental watering on shrimp abundance will be influenced by how the environmental freshes influence the availability of slackwaters. Shrimp abundance will be higher in rivers that have more permanent and larger areas of slackwater.

Methods

Shrimp larvae and juveniles were sampled in addition to larval and juvenile fish using quatrefoil perspex traps containing bioluminescent light sticks (see methods in section 7.5.1 and Figure 87). Three traps were set at five sites within each of the four rivers/creeks (15 traps in total per river). Traps were deployed at random along the littoral edge at each site at dusk, and retrieved the following morning (7:00-9:00am). All shrimp collected from the light traps were preserved in 90% ethanol and returned to the laboratory for processing.

Abundances were log10(x+1) transformed prior to statistical analyses when necessary to normalise data and stabilize variances. To test if total abundance was significantly different across the 4 rivers, total abundance was analysed for each species using a one way ANOVA with river as the grouping variable. When significant differences were indicated, *post hoc* pairwise comparisons were undertaken to determine differences between the rivers.

Results and discussion

Abundance of shrimp

A total of 71,249 shrimp were collected over the 2012-2013 spawning season. Of these, 35,007 were *P. australiensis* and 36,242 were *M. australiense* (Table 21). The mean abundance of shrimp was significantly different across the four rivers (ANOVA d.f=3, F-test=4.806, $p<0.05$) (Figure 57). The mean (±SE) number of shrimp sampled from Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek was 574 (±85), 814 (±194), 1205 (±270) and 2576 (±663), respectively. *P. australiensis* were significantly more abundant in Little Merran Creek than in Colligen Creek and Yallakool Creek that both received environmental freshes (ANOVA d.f=3, F-test=4.796, $p<0.05$). Colligen Ceek also had significantly fewer *M. australiense* than Little Merran Creek (ANOVA d.f=3, F-test=3.229, $p<0.05$).

The rivers that received environmental water had fewer shrimp overall compared to the two control rivers. Increased flows can often reduce rather than increase the size and availability of slackwaters crucial to larval development and juvenile recruitment of shrimp (Bowen et al. 2003; Vietz et al. 2013). This is supported by Richardson et al. (2004) who found that *M. australiense* was found in fewer numbers and *C. mccullochi* was absent in sections of the Campaspe River which experienced higher flows in summer for irrigation purposes compared to other sections of that river. Permanence of slack waters was also likely to be affected by watering actions. Price et al. (2013) found that fluctuations in discharge resulted in a reduction in the permanency of slack waters. It is possible that the recruitment success of shrimp may be affected by this, as shrimp recruitment occurs during the normally low flow summer period (Humphries et al. 2006). Sharp increases in flow may result in higher larvae mortality as a result of displacement and catastrophic drift from slackwater habitats (Humphries et al. 2006; Price and Humphries 2010). Indeed, Hancock and Bunn 1997 found that high flow events limited the recruitment of *P. australiensis.*

The magnitude of flow provided by the watering actions in Colligen Creek and Yallakool Creek reached less than half bankfull levels and were likely to have decreased the size and availability of slackwaters. Veitz et al. (2013) found that in the Broken River there was a general decrease in the area of slackwaters as flow increased and slackwaters became available only at the channel margins. Only at near bankfull levels when benches were inundated, do large slackwaters start to develop and result in a general increase in slackwater area. Indeed, the inundation modelling from section 7.1.1 suggests a similar pattern in the Edward-Wakool system (Figure 10, section 7.1.1.) at bankfull flows. Therefore, for shrimp at least, it is likely that the magnitude and timing of environmental flows delivered during the 2012-2013 water actions did not result in an increase in available habitat for shrimp recruitment which is a key objective of the watering actions.

Table 21. Total abundance of shrimp collected with light traps from the four focus rivers in the Edward-Wakool system during 2012-2013.

Species		*Colligen*	*Yallakool*	*Wakool*	*Merran*	Total
Paratya australiensis		4010	3157	8237	19603	35007
Macrobrachium australiense		2919	7598	7653	18072	36242
	total	6929	10755	15890	37675	71249
	(%)	(10)	(15)	(22)	(53)	

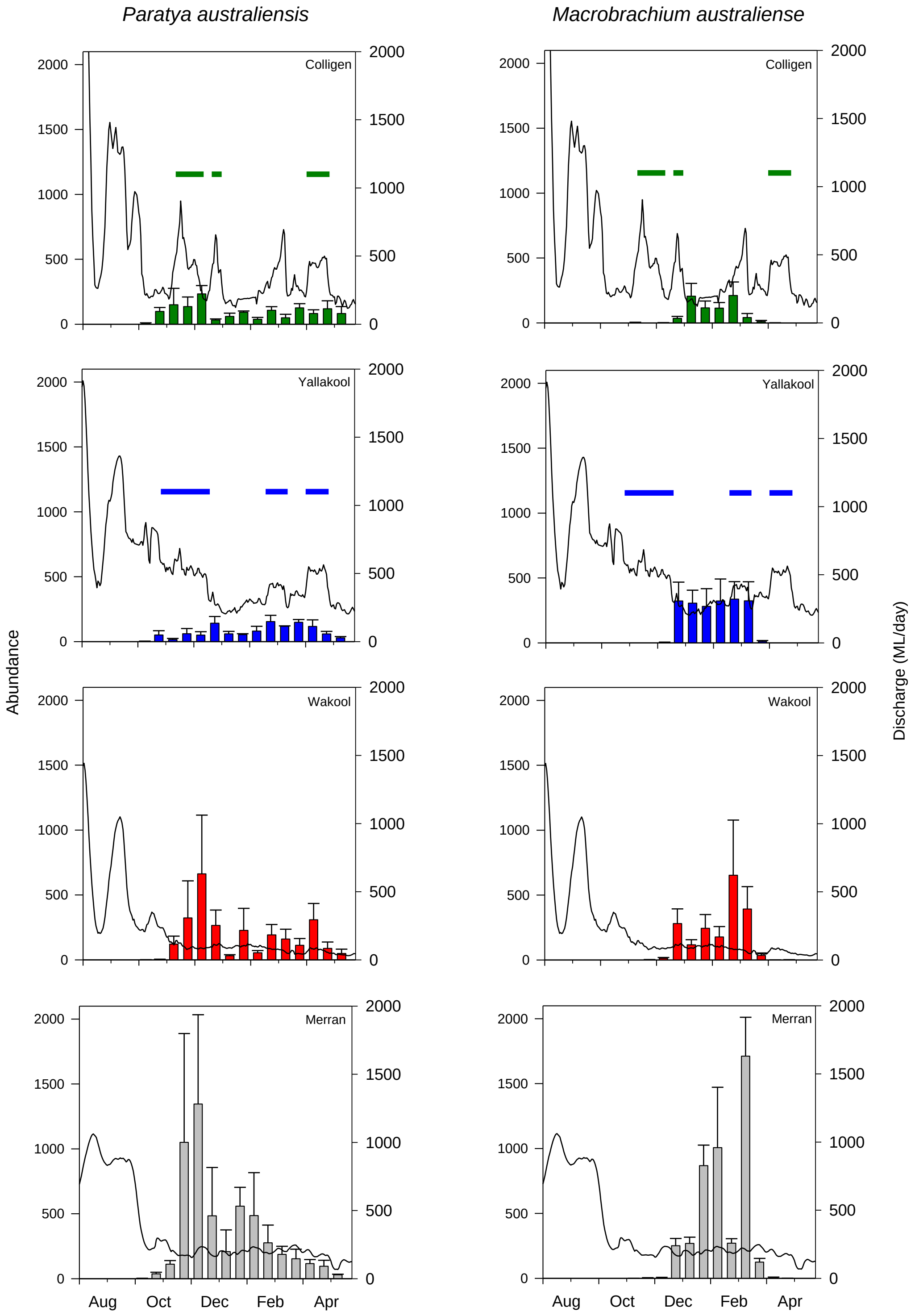
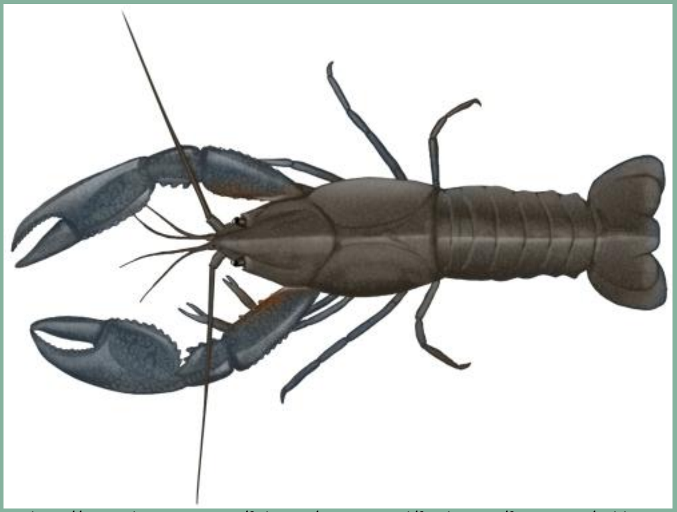


Figure 57. Mean abundance (±1SE) of *P. australiensis* and *M. australiense* in the Edward-Wakool system during 2012-2013. Horizontal bars indicate duration of environmental watering actions in the treatment rivers, Colligen Creek and Yallakool Creek.

7.3.3. Murray crayfish and the common yabby



http://www.dpi.nsw.gov.au/fisheries/recreational/freshwater/fw-species/yabby

Key findings

- *No Murray crayfish were caught between 2010 and 2013*
- Yabbies were found at 32 sites (23 channel, and 9 wetland sites). *There were signifcantly more yabbies caught in 2011 when compared to 2010, 2012 and 2013*. This increased abundanced occurred after large flow event and supports findings by Reid et al. (1997) who analysed yabby catch data over several decades and found increased catch rate correlated with flood levels. One possible explaination for this result is that the input of large quantities of carbon into the system during flooding benefited yabby recruitment and survival.

Background

Two species of freshwater crayfish inhabit the Edward-Wakool system, the Murray crayfish (*Euastacus armatus*) and the common yabby (*Cherax destructor*). Both species are of considerable conservation value but little is known of either species specific flow requirements. The yabby can adapt to a range of different habitats and river conditions, and has a natural range of approximately two million square kilometers, including the Murray-Darling Basin (Unmack 2001). The Murray crayfish has a much more restricted range and is found only in the Murray and Murrumbidgee catchments. Both species have a higher tolerance to changes in water quailty than many native fish species (King et al. 2010). However, abnormal events, such as flow-linked hypoxic water or extended drought, can lead to high population mortality (Morris et al. 2005).

Mckinnon (1995) suggested that the provision of environmental water could lead to a recruitment response in both species. Environmental water delivery could be used to maintain water quality during events such as hypoxic blackwater events, or be used to inundate wetlands where significant populations of yabbies are known to occur. Little is known about the current abundance and distribution of Murray crayfish or yabbies within the Edward-Wakool system. Monitoring was undertaken to determine the relative abundance of these species among habitat types and spatial zones within the system in relation to the flow regime delivered within a watering year. These data are part of the time-series collected during the fish community monitoring program. Responses to individual flow events are not examined, instead long term condition through comparison of changes in abundance over time is made. Catch data from 2012-13 will be compared with abundances collected in previous years to plot change trajectories and related to water regime.

Methods

A total of 37 sites were sampled in 2010, and 43 sites from 2011 onwards (the inclusion of Werai) were sampled annually. Sample sites were stratified between wetland and channel habitats to determine the role these different habiat types in supporting crayfish communities in the Edward-Wakool system. These habitats were further stratified by postion in the system; upper, middle, lower and Werai to capture any potential difference that may occur over a larger spatial scale(Figure 109).

Five baited (with liver) Munyana crab traps (75 cm diameter) were set and retrieved after a minimum of 2 hours at each site (Figure 58). Incidental catches from an additional 10 baited fish traps were also recorded. All crayfish were measured to the nearest mm (carapace length), their sex determined, and if female, the presence or absence of berries (eggs or juveniles) on the underside of the tail recorded.

Changes in abundance among years were analysed using ANOVA. Data were exposed to a variance-stabilising log (x+1) transformation and quantile plots confirmed normalised distributions. A post-hoc Tukey test was applied to determine any specific year that resulted in higher recruitment.

Figure 58. Manyana crab trap (top left), opera house (top right), are used to sample for Murray crayfish (bottom left) and yabbies (bottom right).

Results and Discussion

No Murray crayfish were caught in any year. Recent intensive sampling for crayfish in the Edward-Wakool system also did not record any Murray crayfish in the Wakool River and only low numbers in the Edward River (pers com. Martin Asmus). However, historical information indicates that crayfish were historically widespread and relatively abundant throughout the current study area (Gilligan et al. 2007) with recreational fishers reporting Murray crayfish declines in the system (O'Connor 1986). These data suggest few Murray crayfish persist in the region and that there is little evidence of a recent recovery.

A total of 275 yabbies ranging in size from 11 to 70 mm were sampled over the four years from 2010 to 2013 (Figure 59). They were found at 32 sites; 23 channel, and 9 wetland (Figure 59). There were signifcantly [F (7, 24) = 6.923, P <0.001] more yabbies caught in 2011 when compared to 2010, 2012 and 2013 across all zones (upper, middle, lower and Werai). This increased abundanced occurred after large flow event and supports findings by Reid et al. (1997) who analysed yabby catch data over several decades and found increased catch rate correlated with flood levels. One possible explaination for this result is that the input of large quantities of carbon into the system during flooding benefited yabby recruitment and survival.

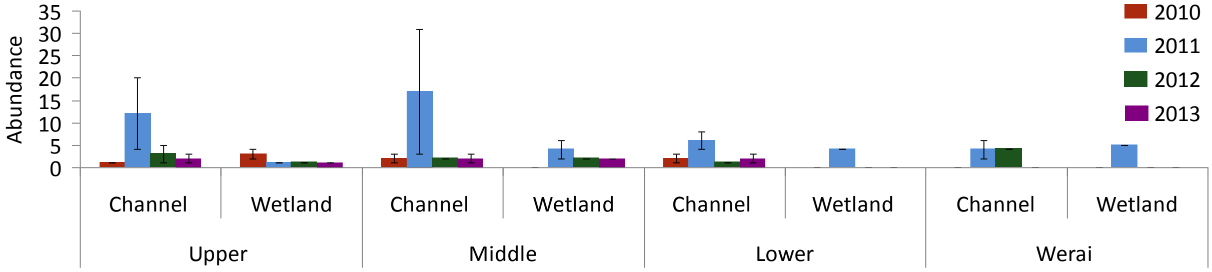


Figure 59. Mean average number of yabbies caught at all sites per section of the river. Red: 2010, blue: 2011, dark green: 2012 and purple: 2013. Error bars show standard error of the means.

In post flood years (2012, 2013), yabby numbers returned to pre-flood abundance. There were no observed differences in abundances between 2010, 2012 and 2013 [F (7,24) = 6.923, P <0.0 [F (7, 24) = 6.923, P <0.001]. One possible explanation for the decline in yabby abundance after 2011 may be changes in food availability. Giling et al. (2009) and McKinnon (1995) reported that flood events can cause a shift in natural food availability (especially aquatic invertebrates), prompting yabbies to relocate in order to fulfil their dietry requirements.

Yabbies were captured in both channel and wetland habitats. While yabbies have a high tolerance to rapid changes in water quality (Hobbs 1981), they prefer wetland habitats (Johnston 2009). The abundance of yabbies in channel sites could indicate that flow regulation and the prolonged drought created a situation where channel habitat was effecetively a series of disconneted pools serving as isolated wetlands. Many other wetlands in the region became totally dry during the drought and may take many years to recover following re-connection. Variability of in-channel flows and periodic reconnection of wetlands to prevent extended drying and to facilitate carbon transfer may benefit yabby populations.

7.3.4. Frogs

Key findings

- Six frog species were recorded at the four rivers between September 2012 and April 2013.
- *The highest frog calling activity was observed during the September and October 2012* surveys prior to the environmental watering actions when there were inundated backwaters present from larger unregulated flows within the Edward Wakool system.
- *There was a minor response of frogs to watering actions in Colligen Creek.* Frog calling increased when flows were sufficiently high to inundate vegetation. However, there was no response to environmental watering evident in Yallakool Creek. *The limited response of frogs to environmental watering may be due to low availability of slackwater and inundated habitat.*
- *Frog recruitment was not observed*, and no tadpoles or egg masses were observed at any river, regardless whether it received an environmental fresh or not.

Background

Riverine frogs have a range of life history strategies which allow them to utilise and occupy riverine habitats. In south-eastern Australia nine frog species are wholly dependent on flowing water for reproduction, (Gillespie and Hines 1999) whilst five others use streams for breeding only under certain flow conditions (Gillespie and Hines 1999; Heard et al. 2006). Many generalist riverine frog species utilise a range of habitats created during overbank events, such as back-waters and ground-water pools (Bateman et al. 2008; Wassens and Maher 2011). Permanently inundated systems may not necessarily support frog breeding due to their high predator densities, rather they may be used as refuge habitats during dry periods (Wassens and Maher 2011). Seasonally or intermittently inundated wetland systems, such as the Edward Wakool system, may be important for breeding by species with longer development times (Wassens et al. 2010; Wassens and Maher 2011) therefore in-channel environmental watering is most likely to lead to an increase in frog abundance and calling activity if intermittent and seasonal habitats are created adjacent to the main channel. For example Wassens and Maher (2011) found that temporary water bodies created during environmental flows were preferentially utilised for breeding over the more persistent habitat within the stream channel.

Hypotheses

- Frog activity (number of individuals calling and observed) will increase in rivers that receive environmental water relative to rivers that do not receive environmental water.
- Frog breeding (by presence of egg masses, tadpoles and metamorphs) will be observed more often in rivers that receive environmental water relative to rivers that do not.

Methods

Tadpoles were surveyed monthly from September 2012 to April 2013 during the day within a 50 m transect at three locations within each focus river using a large D-bottom sweep net (Figure 60). Tadpoles caught in the net were identified to species level if possible and their developmental stage recorded according to Anstis (2002). Once identified, all tadpoles were released at the point of capture.

Three replicate audio and visual surveys were undertaken at each focus river at monthly intervals, to detect distinct calls of resident frog species and record the number of individuals calling. The number of individuals was estimated when calling frog numbers exceeded the ability of the recorder to differentiate between calling individuals. Frogs were surveyed at night along a 200 m long and 5 m wide transect running parallel to the water's edge for 30 minutes on each survey occasion. A spotlight was used to search along the water's edge and within terrestrial habitats. Each individual encountered was identified to species level.

Changes in the abundance of each frog species between focus rivers was assessed using the Kruskal–Wallis one-way analysis of variance by ranks, which is non-parametric equivalent to Analysis of variance. Changes in individual frog species abundance in each focus river over time were measured using the Jonckheere-Terpstra Test for ordered alternatives. Spearman's rho analysis was used to measure the relationships between frog abundance and mean rainfall during the survey period. All Analyses were carried out in SPSS version 20, IBM.

Figure 60. Tadpole surveys using a sweep net at one of the study rivers

Results and discussion

Six frog species were recorded at the four focus rivers over the eight month survey period (September 2012 to April 2013). Little Merran Creek initially supported the highest frog diversity which included the only record of the eastern banjo frog (*Limnodynastes dumerilii*) (Figure 61). Colligen Creek contained the highest number of frog (n=509), respectively followed by Little Merran Creek (n=399), Yallakool Creek (n=170) and Wakool River (n=114) (Figure 61). No frogs listed as vulnerable or endangered under the EPBC Act 1999 were observed or heard calling.

Frog community composition was similar across the rivers over the survey period, with spotted marsh frog (*L. tasmaniensis)* and barking marsh frog (*L. fletcheri*) being the most commonly encountered species, followed by plain's froglet (*Crinia parinsignifera)* respectively (Figure 62). No significant differences between frog abundance occurred in terms of the abundance of the above-mentioned species (Kruskal Wallis Test; p=0.286; p=0.064; p=0.334 respectively). There were significant differences however in *Litoria peronii* numbers between rivers (Kruskal Wallis Test: p=0.000) which was expected, as

Colligen Creek and Little Merran Creek both had higher numbers compared to the other rivers (Figure 61, Figure 63). Eastern froglet (*C. signifera*) numbers were significantly different between rivers (Kruskal Wallis Test: p=0.005) and were also found in higher abundances during September and October 2012 in Colligen Creek than at the other rivers (Figure 61; Figure 63). The eastern banjo frog (*L. dumerilii*) was recorded once only in September 2012 (Figure 61; Figure 63). Our findings are comparable to frog diversity and numbers observed along the Murray River between Lock 15 and the South Australian border during an environmental watering event in 2005-06 (Val et al 2007) and very high compared to the more recent environmental flow event in the Darling Anabranch in 2010-12 which detected only 292 individuals during a three year survey period (Bogenhuber et al. 2013).

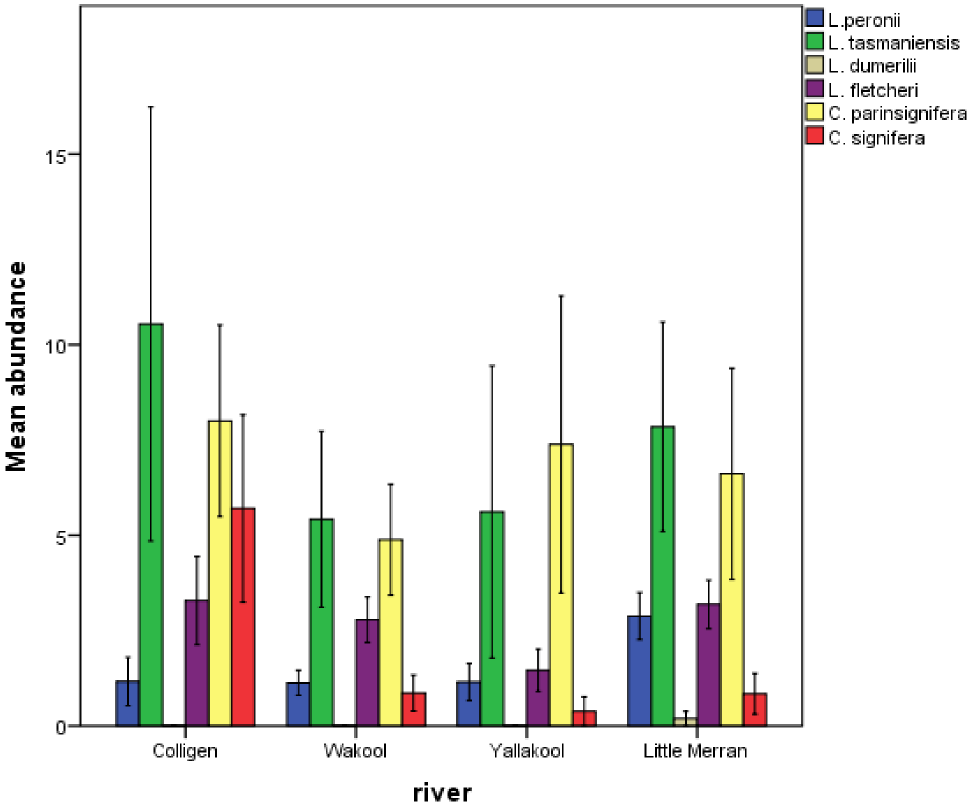


Figure 61. Frog community composition and mean abundance (± 1SE) identified during audio and visual frog surveys within each focus reach between September 2012 and April 2013.

Figure 62. Typical frog species detected during night time surveys at the study reaches. a) Peron's tree frog, b) plain's froglet, c) barking marsh frog and d) spotted marsh frog

Response to environmental watering

It was hypothesised that frog activity (measured as an increase in the number of individuals observed) would increase in Colligen and Yallakool Creeks in response to environmental watering. The most frog activity was observed during the September and October 2012 surveys in all rivers prior to the environmental watering actions. There were inundated backwaters present from larger unregulated flows within the Edward Wakool system that occurred prior to the commencement of the four environmental watering actions, which could have accounted for the large frog numbers and significant differences in abundances of *Litoria peronii* (Kruskal Wallis Test: p=0.000) and *Crinia signifera* (Kruskal Wallis Test: p=0.005) between rivers observed during the September and October 2012 surveys. An increase in activity at this time was also expected as most frogs that were identified are generally actively calling from September and during the warmer months (Wassens and Maher 2011). Subsequent surveys did not detect significant differences across rivers in terms of frog abundance for *L. tasmaniensis (*Kruskal Wallis Test: p=0.286) *L. fletcheri (*Kruskal Wallis Test: p=0.266) or *C. parinsignifera* (Kruskal Wallis Test: p=0.064), as there was a gradual reduction in frog diversity and numbers from December 2012 in all rivers (Figure 63).

Flows during the Colligen Creek November to December 2012 environmental watering action inundated some riverbank vegetation, and concurrent surveys found frog numbers were higher compared with the other rivers (Figure 63). Furthermore, a pair of *L. fletcheri* was observed in amplexus during the November to December 2012 environmental watering action whilst amplexus was not observed at the other rivers throughout the study period. The increase in frog numbers and activity at Colligen Creek could be due to the environmental freshes and a shift into the warmer seasons in which the frogs that were identified are generally more active (Wassens 2011). Little Merran Creek, which received no environmental freshes, also had high calling frog numbers during October (Figure 61), but may have been influenced by climate, with a shift into the warmer seasons in which the frogs identified in this study are generally more active (Wassens 2011).

The Colligen Creek March to April 2013 environmental watering action was a smaller event than the November to December action and did not inundate a significant area of riverbank vegetation (see Section 7.1.2). The frog response to this event was muted compared to the spring fresh in terms of numbers. Frog response during the Colligen Creek March to April 2013 environmental watering action did not differ from frog responses at other focus rivers during that period, thus this event may not have reached sufficient height to inundate suitable breeding habitats or trigger an increase in frog calling activity during this watering action, thus possibly why fewer individuals were observed or heard calling in response to the Colligen Creek March to April 2013 environmental watering action. The frog community in Colligen Creek differed to the other focus rivers, as it contained higher numbers of plain's and eastern froglets (*Crinia spp.*) and spotted marsh frogs (*L. tasmaniensis*) (Figure 63). *Crinia parinsignifera* dominated the frog community at Colligen Creek, and although it was already initially prevalent, its activity did not increase in response to either of the environmental freshes, rather it declined significantly over time during the survey period (Figure 63; Jonckheere-Terpstra Test: p=0.000).

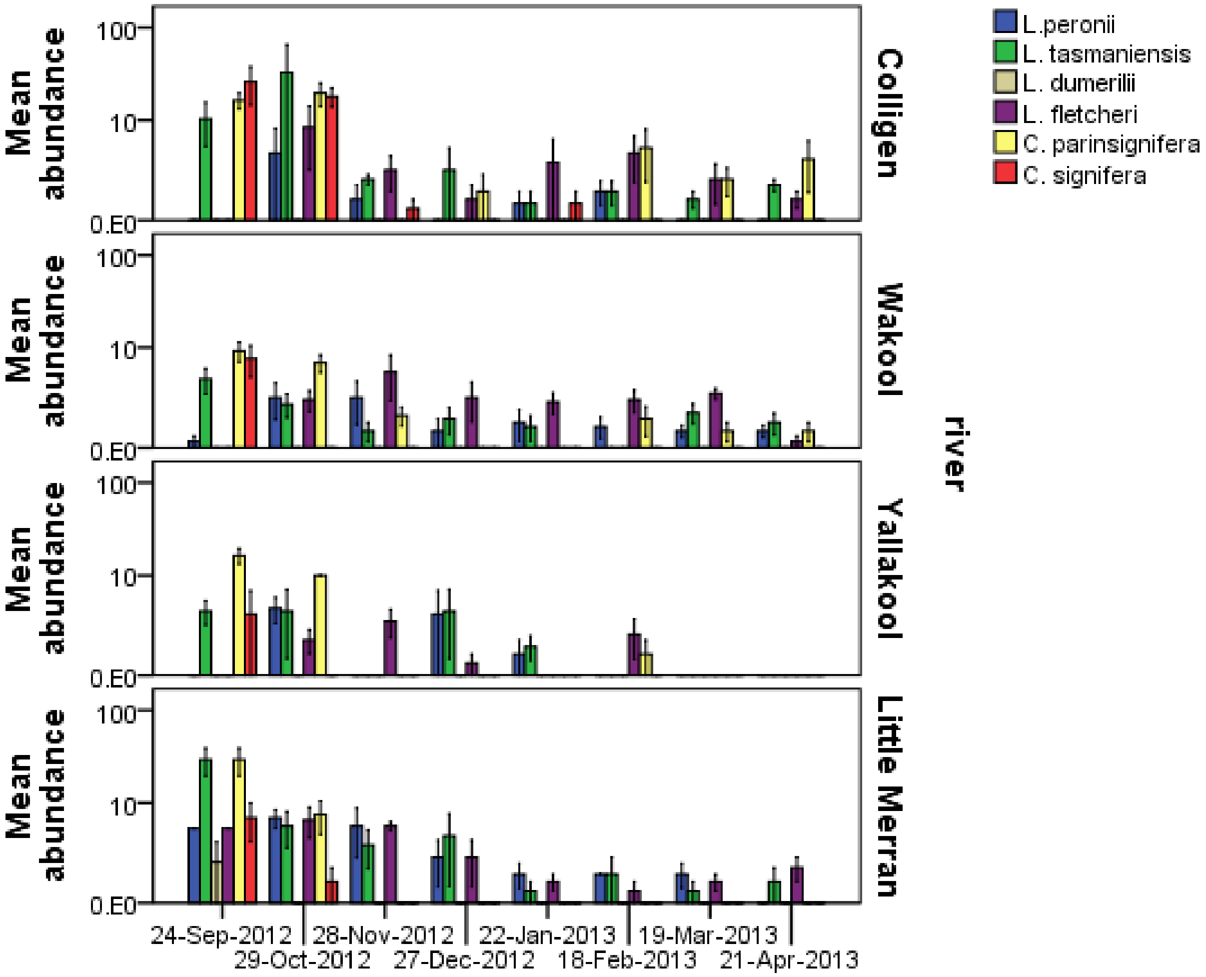


Figure 63. Frog community composition and mean abundance (± 1SE) identified within each focus reach during each survey month (September 2012 and April 2013).

Although there was a slight increase in frog numbers at Colligen Creek during the November to December 2012 and the March to April 2013 environmental watering actions compared to focus reaches that did not receive freshes, they were only significantly different to other rivers in terms of *Crinia signifera* and *C. parinsignifera* from September 2012 to April 2013 (Jonckheere-Terpstra Test: p=0.000). However no significant differences were detected between the other frog species in this river. Furthermore, the Yallakool Creek environmental watering actions resulted in little difference to other focus reaches in terms of increased frog numbers (Jonckheere-Terpstra Test: p=not sig). Frog recruitment was not observed, and no tadpoles or egg masses were observed at any focus river, regardless whether it received an environmental fresh or not.

The small frog response in Colligen Creek and the lack of frog response in Yallakool Creek to the environmental watering actions as well as significant declines in frog abundances (*Litoria peronii, Limnodynastes tasmaniensis*, *L. fletcheri*, *C.sigifera* and *C.parinsignifera*) over time observed in Little Merran Creek (Jonckheere-Terpstra Test: p=0.001; p=.011, p=.030,p=0.009,p=0.009 respectively) and declines for *C. signifera* and *C. parinsignifera* in Colligen Creek and the Wakool River (Jonckheere-Terpstra Test: p=0.000; p=.001 respectively), may be in part, due to the unprecedented hot weather and extremely low rainfall conditions experienced in the region at this time (Figure 15, section 7.1.2). Mean maximum temperatures escalated to 45.9 degrees Celsius and low mean rainfall of 4.4 mm recorded in January 2013 (Figure 15, Section 7.1.2) which is considerably lower than the 30 year January average monthly rainfall which ranges between 25 and 50 mm (BOM, accessed online 5 September 2013). Although rainfall decreased over time during the survey period, along with frog abundances at Colligen Creek, Wakool River and Little Merran Creek (Jonckheere-Terpstra Test: p=0.000), a strong correlation existed between rainfall and abundance for all frogs (Spearman's Correlation; p= <0.005) across all focus rivers when there was better rainfall earlier in the season.

Riverbank vegetation cover and aquatic vegetation cover was generally low in each focus reach (Section 7.1.2), which potentially reduced suitable habitat for frogs. Inundated vegetation is used by frogs as calling and spawning locations (Wassens et al. 2010) and as habitat for adults and tadpoles (Healey et al. 1997). Inundated vegetation also acts as substrate for biofilm which is an important food source for tadpoles. The availability of inundated vegetation may have been low because environmental freshes remained in channel, not reaching half bank full (Section 7.1.1). As a consequence, the lack of newly wetted vegetated habitats may be a key factor influencing the response of frogs to environmental watering.

High predator densities in the main channel from low flows may also inhibit frog breeding activity and could explain why no tadpoles were caught in-channel during the survey period. Exotic predatory fish including the mosquito fish (*Gambusia holbrooki*), known to predate upon tadpoles (Anstis 2002; Ralph et al. 2011) and the European carp (*Cyprinus carpio*), also known to impact frog recruitment (Spencer and Wassens 2009), were present in most rivers. The occurrence of these predators, combined with small environmental freshes, may have resulted in no suitable inundated habitat being available to provide refuge from predators or support breeding and recruitment of frogs (Wassens and Maher 2011).

7.4. Objective 4: Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations

7.4.1. Fish movement

Key findings

- *Murray cod, golden perch, silver perch and carp all displayed increased activity in response to increasing temperature and flow during spring and early summer*. The period of increased movement corresponded with spawning periods for these species.
- Individual displacement for Murray cod ranged from 10 km downstream to 50 km upstream. Overall displacement of Murray cod from the refuge pool was strongly correlated with the hydrograph; 95% of the larger movements occurred between August and November 2012.
- Golden perch exhibited a displacement range of 150 km downstream to 10 km upstream. Peak displacement occurred from mid-September to mid-October 2012. Environmental water delivered to Yallakool Creek from 0ctober 2012 to December 2012 did not result in large scale displacement of the golden perch population, however some individuals moved 5 to 15 km. The environmental watering in March April 2013 resulted in minimal movement by golden perch. Golden perch spent 80% of their time in the refuge pool.
- Silver perch were highly mobile, undertaking frequent short (<2 km) return movements. Some movement occurred during environmental watering actions, however this pattern of movement was not restricted to these flow events. Sliver perch were detected mostly outside the refuge pool, displaying a preference for upstream areas where flow velocity was higher in the Yallakool Creek. By June 2013 after cessation of flows, all fish returned to the refuge pool, demonstrating the importance of this habitat in sustaining native fish in the region.
- Tagged carp displayed periods of large displacing movements interspersed with periods of sedentary behaviour, with most movement occurring between August and November.

Background

Freshwater fish are highly mobile and move in response to several stimuli in order to spawn, disperse and feed (Lucas et al. 2001). Flow is often a major cue for freshwater fish migration and many species move in response to water flows (Agostinho et al. 2007). Delivery of environmental flows is therefore expected to initiate some type of fish movement response in Australian freshwater fish if appropriately timed and managed to benefit target species.

Acoustic tracking is a useful method for obtaining information on fish movements. The process involves implanting a transmitter into a fish, which is then detected by a series of stationary readers installed in a target stream. Acoustic monitoring can provide high resolution spatial information on fish location, and data can be graphically presented to identify movement patterns (Barnett et al. 2010). In the case of environmental water delivery, the strategic placement of acoustic receivers could provide information on timing of movements, distances travelled, residency, correlation with water flow and, potentially, evidence of spawning behaviour or nest site selection. Such information is of great importance to determine both the delivery success of a particular environmental water volume or to inform the planning of future events.

Murray cod spawn between mid-October and late-November (Humphries 2005) independent of flow conditions (Humphries and Lake 2000, King *et al* 2009a), thus increasing available spawning habitat through water delivery is expected to lead to increased spawning success. It is hypothesised that environmental water delivery within the Murray cod spawning season would result in a movement response in adult Murray cod consistent with that of spawning fish. That is, Murray cod would take advantage of increased flows, leaving refuge habitat during the spawning period and returning at the completion. It was hypothesised that environmental water delivery outside of the Murray cod spawning period would facilitate return movements in post spawn Murray cod.

Golden perch and silver perch spawning occurs from early November to March (Roberts et al. 2008; King et al. 2009a). Spawning in these species has been linked to a rise in both water level and temperature (Mallen-Cooper and Stuart 2003) with in-channel spawning evident outside of flood years (King et al. 2009). Both these species are known to undertake large migrations, thought to possibly be spawning related (Reynolds 1983). It was hypothesised that environmental water delivery could stimulate similar movements, consistent with that of spawning fish or movement that serves some other life history strategy. This section reports on the success of environmental water delivery and suggestions for management of future events.

Methods

To monitor fish movement responses in relation to environmental water delivery using acoustic telemetry methods, a series of receivers was deployed along the Wakool River and Yallakool Creek (Figure 64). Fish movement studies focused on the largest refuge pools in the system Wakool Reserve, which is located at the junction of Yallakool Creek and Wakool River. The pool is approximately 5 km long in low flow conditions and provides a valuable low flow refuge. The pool supports a diverse native fish community and is a popular recreational angling location. There is scope to manipulate flows into the pool through controlled operation of irrigation escapes and regulators on the Yallakool Creek and Wakool River.

A linear array of 48 VEMCO VR2W (VEMCO Ltd., Halifax, Nova Scotia, Canada) acoustic receivers was deployed in the Edward Wakool river system in 2010. VR2W acoustic receivers are submerged, single channel (69 kHz), omni-directional receivers which record time, date and identity of acoustic tagged fish swimming within detection range of the receiver units. The array incorporates the Wakool River from Gee Gee Bridge upstream to the Edward River offtake and along the length of the Yallakool Creek. From here the Edward array encompassed the Edward River from Stevens Weir upstream to the Murray River Offtake, the length of Gulpa Creek and the lower reaches of Bullatale and Tuppal creeks (Figure 64). The Edward and Wakool arrays combined provide stream coverage of approximately 430 km with a mean distance of approximately 8 km between receivers. Receiver site locations were strategically selected for approximately equidistant spacing, with additional receivers at key locations such as remnant pools, potential barriers, stream confluences and outer extremities of the study area. Additional receivers were deployed in the remnant junction pool below Wakool Reserve Road Bridge to investigate small scale movement patterns in the refuge pool and to maximise detection of the tagged population during periods of high visitation. The receivers provided continuous monitoring throughout the study period, with data retrieval conducted quarterly and batteries replaced annually.

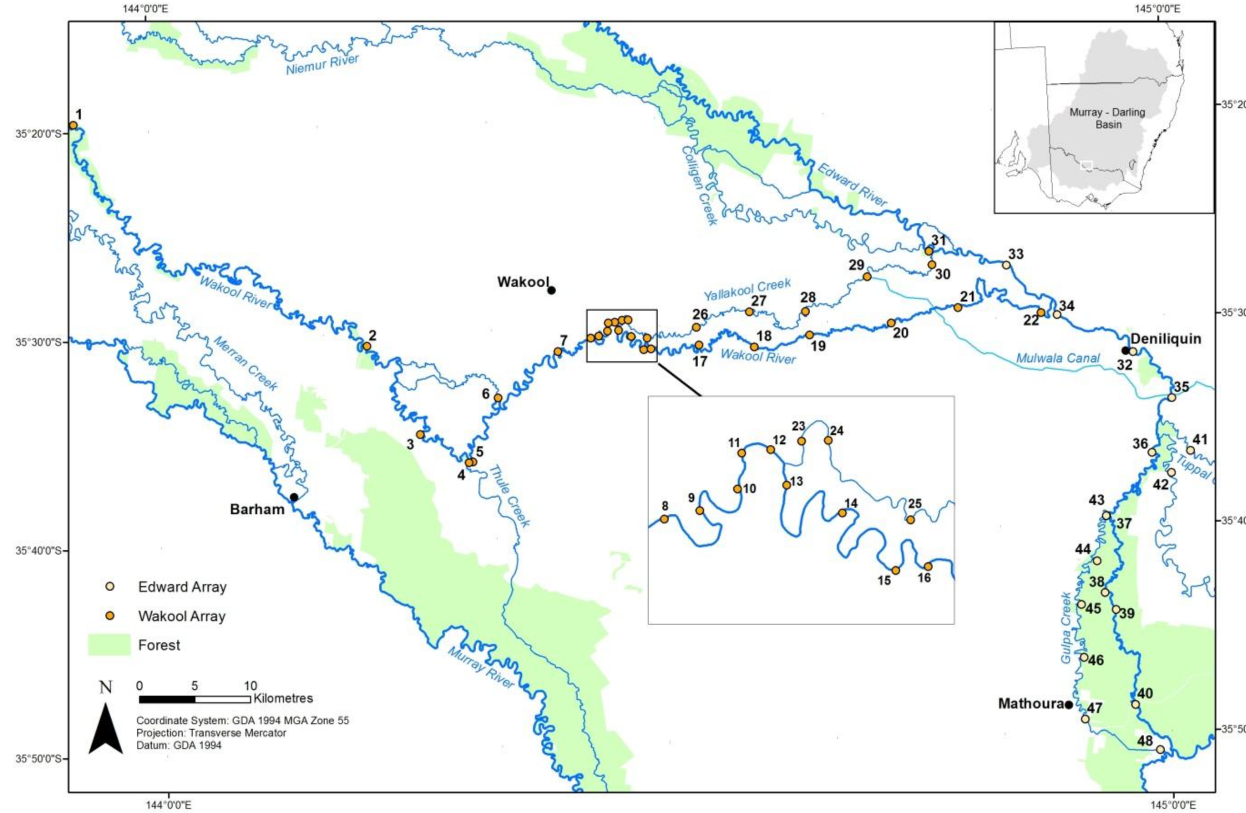


Figure 64. Overview of acoustic receiver array used to detect fish movements in response to environmental water delivery in the Edward-Wakool system. The array was established to detect movements in the Wakool / Yallakool River (orange) and also the upper Edward system (yellow). Detailed coverage of the original tagging location at the Wakool – Yallakool junction is enlarged for clarity.

Electrofishing was used to capture four large-bodied fish species, Murray cod *(Maccullochella peelii)*, golden perch (*Macquaria ambigua*), silver perch *(Bidyanus bidyanus)* and alien common carp *(Cyprinus carpio),* for insertion with an acoustic tag. Captured fish were transferred to a bank-side surgery station and anesthetised in a 200 L oxygenated tank of ambient water containing 50 mgL^{-1} benzocaine (ethyl-p-aminobenzoate) (Sigma Aldrich, Shanghai) in preparation for surgical tag insertion. Surgery followed the procedure described in Butler et al. (2009) and tagged fish were released at the capture site (Figure 65, 66). Where possible, the sex of the fish was determined by examining the gonads through the incision prior to transmitter insertion.

Receiver data was downloaded and stored in a purpose built SQL database. Prior to analyses, single detections were removed (Clements et al. 2005). The detection data were then visualised in Eonfusion software (Myriax Software Pty Ltd) by plotting fish movement on the river network to identify and exclude detections falling out of sequence (false detections). All remaining detection data were plotted on to the river network to generate time series movement video files. Data was then used to calculate a range of movement metrics in Eonfusion for further comparison of fish movement in relation to flow. For each transmitter a range of movement metrics were calculated: travel time, daily displacement, cumulative displacement, directional movement, activity, and location specific water flow. Displacement is a measure of geographical distance moved by an individual. It is represented as either daily displacement, the relocation distance in a single day or as cumulative displacement, the distance of a tagged individual from its release point. From here on the terms daily displacement and displacement (cumulative displacement) are used. Activity refers to the aggregate daily movement of a transmitter fish defined as the total combined up and downstream movement per day. Individual transmitter displacement and mean displacement for each species are examined in relation to flow for the study period. Similarly, mean activity and mean daily displacement are presented in relation to flow.

Recession of flows in the Wakool system leads to a series of remnant pools which provide refuge for the fish community. The study area is centralised around a large refuge pool fed by both the Yallakool Creek and Wakool River. Utilisation of this refuge habitat and the river reaches up and downstream by transmitter fish in relation to flow is examined. Daily visitation fish data were grouped into zones (upstream, downstream and refuge) and the proportion of the tagged population for each species is presented in relation to flow. Upstream habitat utilisation is further investigated with the relative proportion of tagged fish using the upper Wakool River and Yallakool Creek determined.

Since establishment of the array 8,922,086 detections have been recorded from the entire acoustic tagged fish population (n=195). For the purpose of this report, only transmitter fish tagged prior to August 2011 (plus 10 silver perch tagged August 2012) that were detected in the array from 1 August 2012 to 30 June 2013 (334 days) were used in the analysis. During this period 1,845,535 detections from 77 transmitter fish were recorded within the study area (Table 22). Detection data is unreliable from an analysis perspective because data can become dominated by fish that take up residence near a receiver. To overcome this, detection data were transformed to location and movement metrics based on time. All data subsequently presented in this chapter is based on daily detection data.

Separate tagging events in August 2010, August 2011, August 2012 and March 2013 resulted in the capture, tag and release of 162 fish: 60 Murray cod *(Maccullochella peelii)*, 52 golden perch (*Macquaria ambigua*), 16 silver perch *(Bidyanus bidyanus)* and 34 alien common carp *(Cyprinus carpio)*. All fish were collected from the remnant pool occurring below the junction of the Wakool River and Yallakool Creek.

Correlations between total river discharge and other factors (daily displacement, habitat use and upstream movement) were explored using the Spearman Rank Test function in Microsoft Excel. Analyses sought to determine whether specific aspects of fish behaviour were linked to hydrograph. A BACI design was not appropriate as there was limited spatial coverage in the array due to budgetary and logistical constraints with the array established in a zone most likely to hold native fish populations and receive water. Further statistical analysis was not conducted given the data presented in this report represents a period of one year.

Table 22. Summary of transmitter fish (*n*), mean length (± s.e.) (total length for Murray cod and golden perch; fork length for silver perch and carp) and weight (± s.e.) detected during the study period.

Species	***n***	**Length (mm)**	**Range (mm)**	**Weight (g)**	**Range (g)**
Golden Perch	19	421 ±14	330-536	1,447 (±158)	643-2933
Murray Cod	25	546 ±22	400-900	2,759 (±478)	689-11,732
Silver Perch	12	308 ±16	212-376	460 (±69)	132-894
Carp	21	497 ±18	313-636	2,764 (±335)	629-6,656

Figure 65. Top left; Murray cod having transmitter inserted. Top right; Surgery to insert tags. Bottom left; Chris Smith downloads an acoustic receiver at Yallakool Creek. Bottom right; A VR2W acoustic receiver rigged for deployment next to a V13-1x A69 acoustic tag.

Figure 66. Tagged species; clockwise from top left; Murray cod, golden perch, carp, silver perch.

Results and discussion

Murray Cod movement in response to environmental watering

Individual displacement for Murray cod over the study period ranged from 10 km downstream to 50 km upstream, with 18 km being the maximum displacement recorded by an individual in a single day. 68% of tagged Murray cod moved greater than 5 km, of which 95% undertook these movements from August to November 2012. 24% of tagged Murray cod moved distances greater than 10 km from the refuge pool. The general behaviour exhibited by tagged Murray cod involved periods of rapid upstream movement between August to November 2012 followed by a stationary period of approximately 1 to 4 weeks, followed by a return movement to the Wakool reserve pool. A long period (December 2012 to July 2013) of small localised movement, mostly within the refuge pool, followed this period (Figure 67, WMV file).

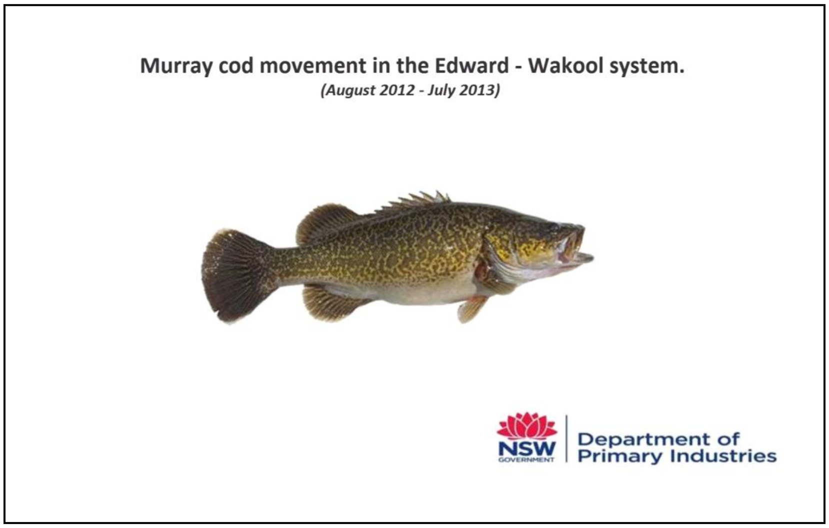


Figure 67. . Murray cod movement in the Edward – Wakool system. (Microsoft Word 2010). Time series movement of Murray cod is presented in relation to flow is available at this website. http://www.csu.edu.au/research/ilws/research/SRAs/Water/Water_projects%20-EdwardWakool.htm Discharge (ML/D) for the Wakool River and Yallakool Creek is shown on the left hand side of the video.

Overall displacement of Murray cod from the refuge pool was strongly correlated with the hydrograph (Spearman: R=0.9, $P < 0.001$, with an R^2= 0.812) (Figure 68), with the average displacement of the tag population increasing upstream with flow (Figure 69). Upstream displacement occurred largely during higher flows but peak activity and displacement behaviour occurred from August to November 2012 and coincided with the increased discharge in both the Wakool River and Yallakool Creek.

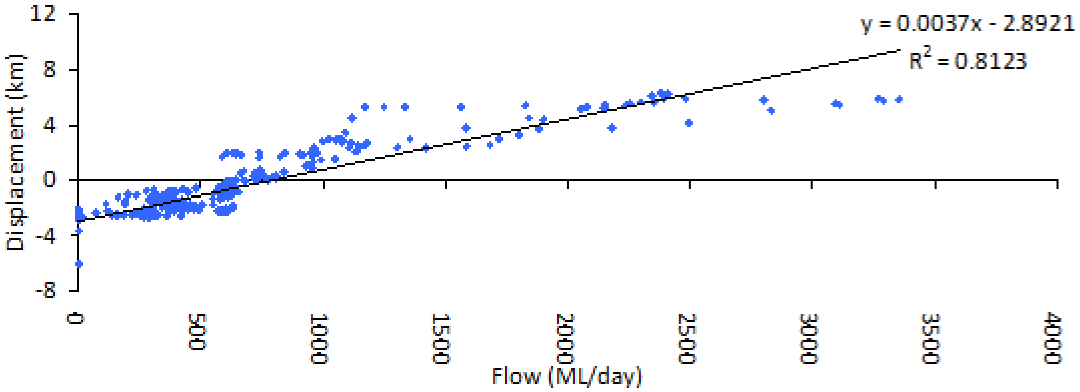


Figure 68. Murray cod displacement correlated with flow.

Sixty percent of tagged Murray cod moved upstream of the refuge pool (Figure 70) in response to environmental watering between October and December 2012. The timing and duration of these movements are consistent with the behaviour of spawning Murray cod (Koehn & Harrington 2006) and may indicate fish used the freshes to take advantage of new habitat and actively seeking out suitable breeding partners or nesting habitat. Fish undertaking upstream movements during August to November 2012 predominantly moved into the Wakool River, with less than 5% of upstream moving individuals utilising Yallakool Creek (Figure 71). A reason for this preference for the Wakool River is unknown but does highlight the importance of maintaining habitat in this reach. By early January 2013, most Murray cod had returned to the tagging location and performed only localised movements within the pool at Wakool reserve. Environmental water delivery in Yallakool Creek from October to December 2012 was targeted at maintaining inundation of habitat for Murray cod nests prior to hatch. While tagged cod displayed a preference for the upstream Wakool River reach that did not receive a fresh, environmental water delivery in Yallakool Creek may have provided benefits to cod in Yallakool Creek, for example facilitating the return movements of fish to following spawning and nesting.

Murray cod movement was also monitored during the environmental watering in Yallakool Creek and Colligen Creek between February and April 2013. Murray cod exhibited increased activity during these freshes but all tagged fish remained within the refuge pool (Figure 70). Even though no displacement occurred at this time, the increased activity of fish during these flows may indicate other behavioural responses, such as are occurring within the refuge habitat during the environmental watering, increased feeding or access to newly inundated habitat.

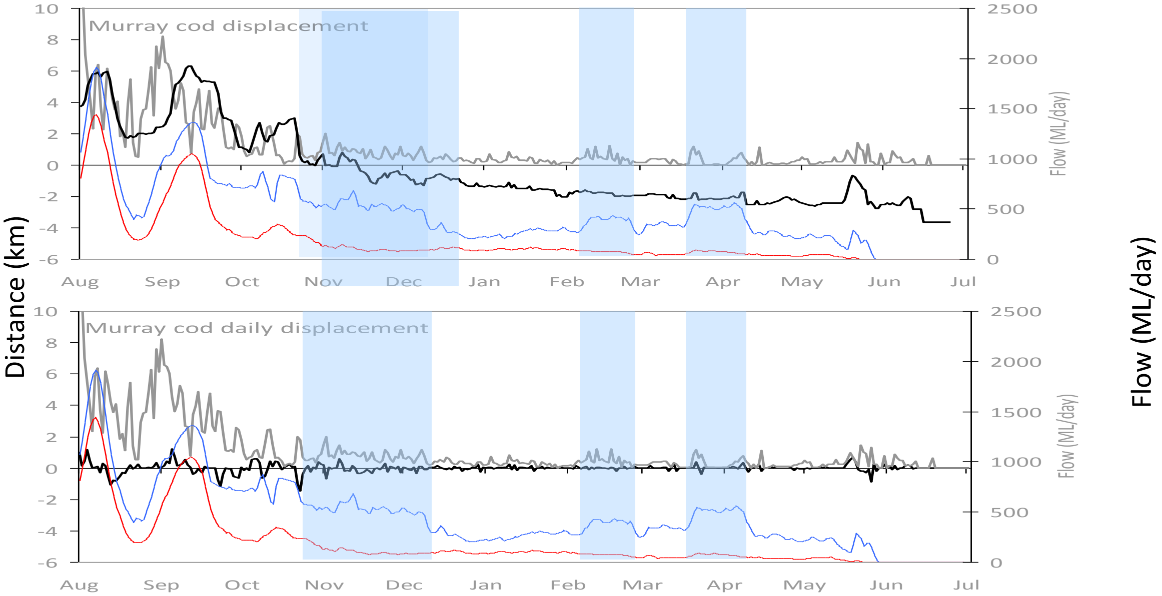


Figure 69. Displacement (top) and daily displacement (bottom) (left axis, black) in km for Murray cod, in relation to activity (left axis, grey) and flow (right axis) (Yallakool Creek flow -Blue, Wakool River –red). Environmental water periods shown with the blue shading.

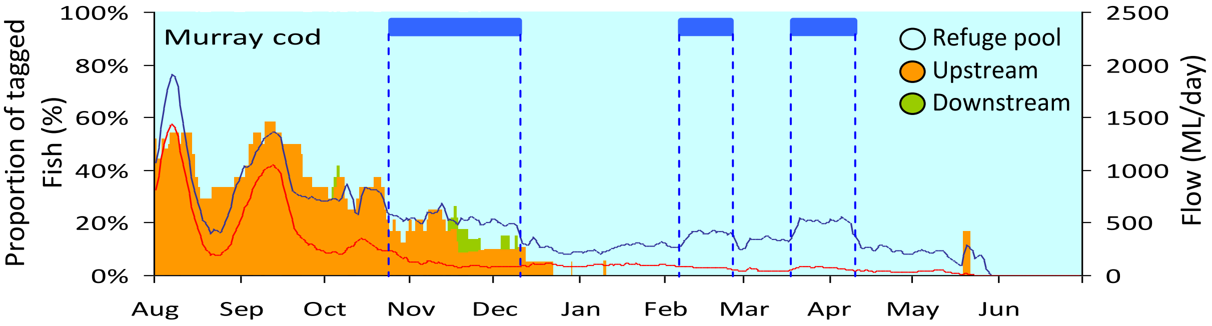


Figure 70: Daily habitat selection of Murray cod shown as the tagged proportion upstream, downstream and in the refuge pool (left axis) – in relation to the flow (right axis) (Yallakool Creek flow -Blue, Wakool River –red). Environmental water delivery is highlighted by blue bars.

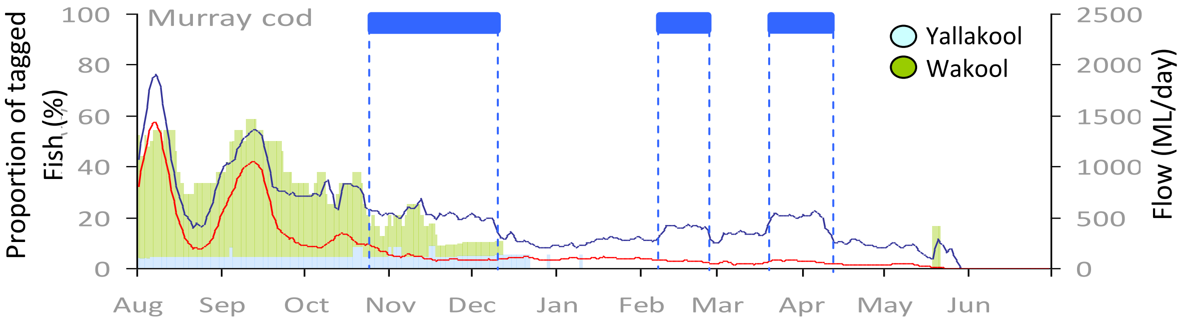


Figure 71. Upstream habitat selection by Murray cod (left axis) in relation to flow (right axis), (Yallakool Creek flow -Blue, Wakool River red). Environmental water periods shown with the blue boxes.

Golden Perch movement in response to environmental watering

Golden perch exhibited a displacement range of 150 km downstream to 10 km upstream, with a maximum daily displacement of 38 km recorded by an individual. Peak displacement for golden perch during the study period occurred from mid-September to mid-October 2012 (Figure 72). Displacement occurred on larger flows with overall displacement modestly correlated with flow (Spearman: R=0.5, $P < 0.001$, with an $R^2=0.8$) (Figure 73). Movements during this time are known to coincide with spawning behaviour in the nearby Barmah-Millewa forest (King et al. 2009).

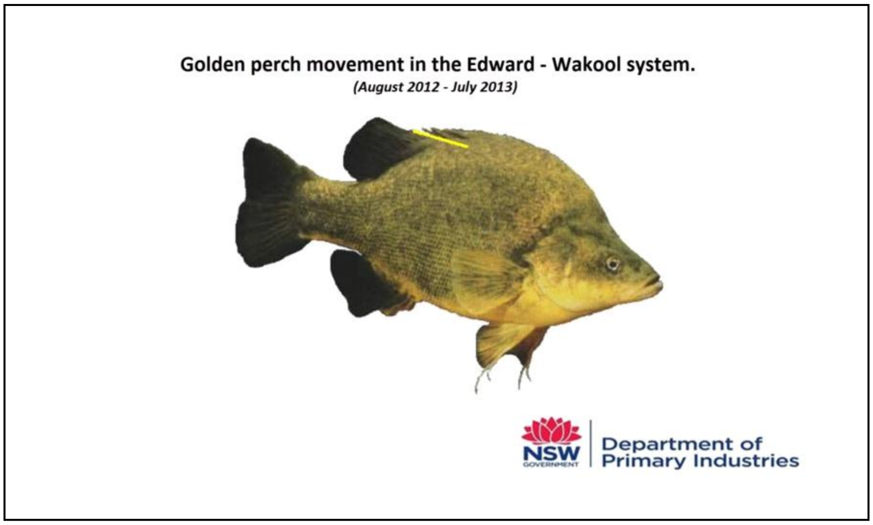


Figure 72. Golden perch movement in the Edward – Wakool system. Time series movement of golden perch is presented in relation to flow is available at this website. http://www.csu.edu.au/research/ilws/research/SRAs/Water/Water_projects%20-EdwardWakool.htm Discharge (ML/D) for the Wakool River and Yallakool Creek is shown on the left hand side of the video.

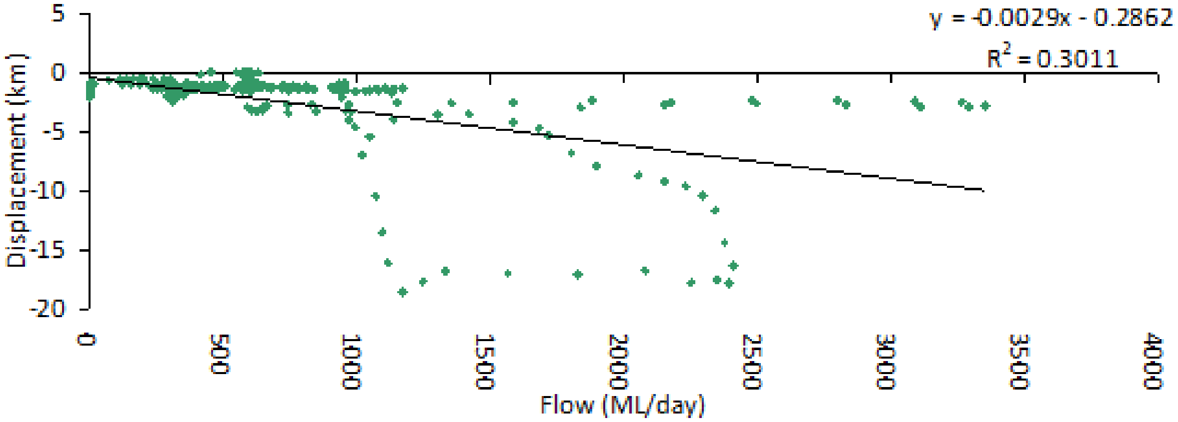


Figure 73. Displacement of tagged golden perch correlated with flow.

During higher flows, golden perch movements were mostly downstream. Disparity between the upstream and downstream movement is supported by long term movement data for golden perch in the Wakool study area. Overall population displacement has varied from predominantly upstream to downstream in years following a hypoxic blackwater event (unpublished data). The results demonstrate that golden perch are able to travel large distances over relatively short timeframes and were able to make use of new habitat when inundated.

Between August and October 2012 all tagged individuals had an observed displacement of greater than 5 km per day and many of these movements were greater than 10 km (37% of the tag population) during the same period. Large scale displacement (> 10km) was observed more frequently over multiple days but most of these movements occurring between August and October (Figure 74). Between October 2012 and July 2013 overall displacement decreased substantially but individuals that made large scale movements (40 – 150 km) eventually returned to the capture location.

Environmental water delivered to Yallakool Creek (19 0ctober 2012 to 7 December 2012) did not result in large scale displacement for the golden perch tag population as a whole, however some individuals made displacement movements of 5 to 15 km. Subsequent deliveries of environmental water (Yallakool Creek 2 February 2013 to 22 February 2013, 13 March 2013 to 5 April 2013) resulted in minimal increases in activity by golden perch, and displacement movements were typically less than 3 km.

From August 2012 to early January 2013 up to 45% of tagged golden perch were recorded outside the refuge pool (Figure 75). The preference for downstream movement only occurred between August 2012 and January 2013. Fish leaving the refuge pool between the months of February and June 2013 chose to move only upstream. For fish moving upstream, a clear preference was shown for the Wakool River during August 2012 to January 2013, but fish moved into the Yallakool Creek from February to June 2013 (Figure 76). These upstream movements by golden perch into the Yallakool were strongly correlated (Spearman; R=0.8, $P < 0.001$, with an $R^2=0.5171$) with flow in the creek, including the environmental watering (Figure 77). This activity and other small scale movements may indicate that golden perch are utilising these increases in flow to feed both within and outside the refuge pool.

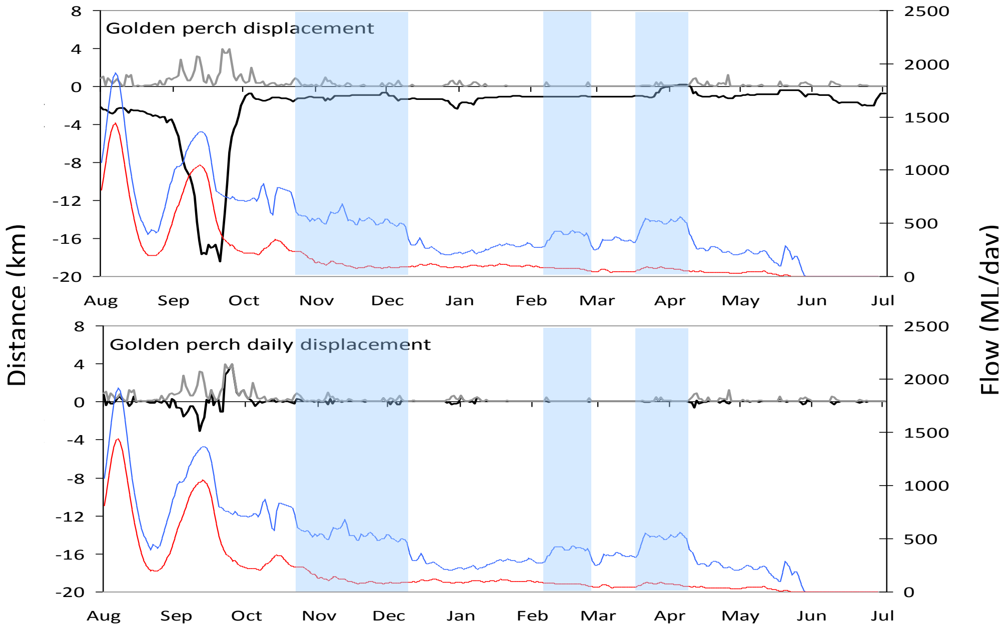


Figure 74. Displacement and daily displacement (left axis, black) in km of golden perch in relation to activity (left axis, grey) and flow (right axis). Environmental water periods shown with the blue shading.

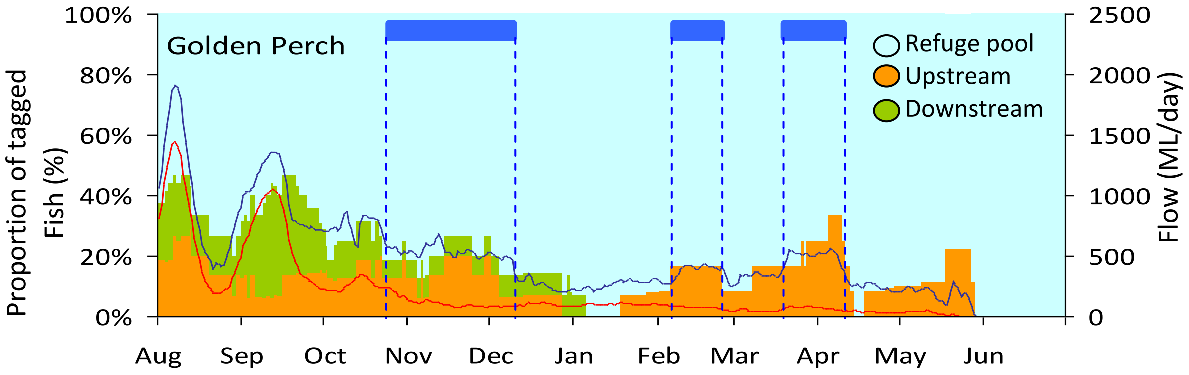


Figure 75. Daily habitat selection of Golden perch shown as the tagged proportion upstream, downstream and in the refuge pool (left axis) – in relation to the flow (right axis) (Yallakool Creek flow -Blue, Wakool River –red).

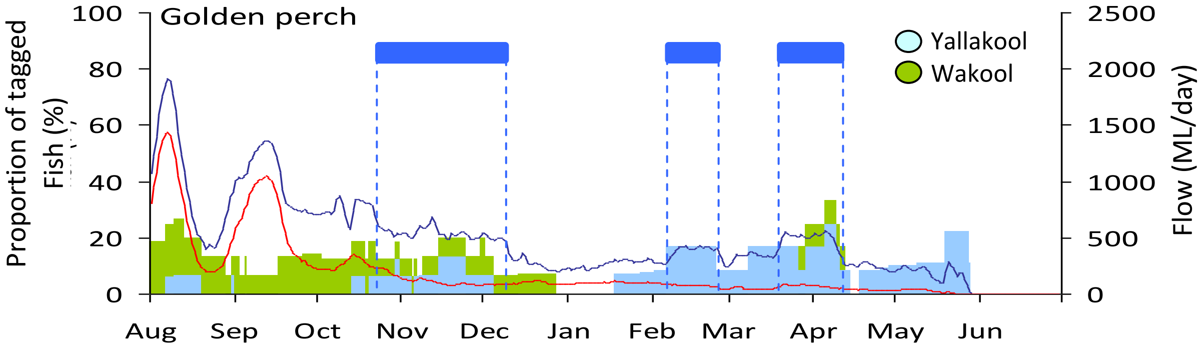


Figure 76. Proportion of tagged golden perch (left axis) upstream of the refuge pool in the Wakool (green) and Yallakool (blue) in relation to the flow (right axis). Yallakool environmental water delivery shown by the blue bars.

Movement data indicated golden perch return to the refuge pool after periods of displacement, spending 80% of their time in this habitat. This display of strong site fidelity is consistent with other studies (Crook 2004; O'Connor et al. 2005), similarly the opportunistic use of available habitat observed in this study by golden perch was also observed by Koster et al. (2012). During extended drought, environmental water delivery should be used for the maintenance of viable golden perch source populations throughout the Wakool. The strong preference for returning to particular refugia should be considered vital for continued survival of golden perch. Environmental flow delivery should therefore be provided at the appropriate time to maintain drought refugia and encourage movement outside the refuge pool for emigration, feeding and recruitment.

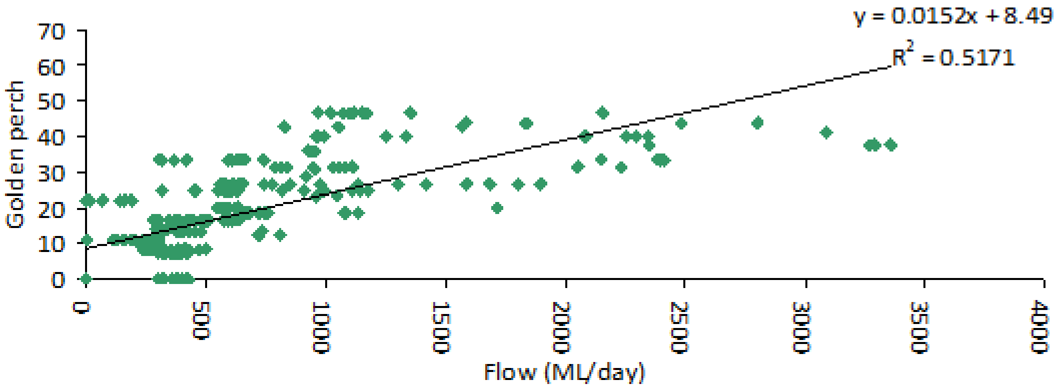


Figure 77. Proportion of upstream displacing tagged golden perch correlated with Yallakool creek flow.

Silver perch movement in response to environmental watering

Silver perch were highly mobile, undertaking frequent short distance (<2 km) return movements throughout the study (Figure 78 and wmv file). Three individuals made large scale (>30 km) displacing movements, ranging from 72 km downstream to 66 km upstream. 42 km was the maximum daily movement recorded by an individual. During the study period individuals amassed movements of more than 1100 km (mean 290 km). Displacement movements greater than 5 km were exhibited by 73% of tagged silver perch, however in contrast to data from 2010 to 2012 (unpublished) only a small percentage (27%) of tagged fish recorded displacements greater than 10 km from the refuge. The targeted silver perch tagging event on 2 October 2012 to boost low number of silver perch remaining in the study area may explain this difference. Few individuals remained in the study area prior to this tagging event.

Movement during environmental watering actions was evident with several silver perch making displacement movements during delivery; however this pattern of movement was not restricted to these flow events with other displacement movements occurring through the year (Figure 79).

Sliver perch were detected mostly outside the refuge pool (63% of all tagged fish) displaying a preference for upstream habitat found in this zone 56% of the time (Figure 80). Silver perch that moved upstream occupied areas where flow velocity was higher in the Yallakool Creek (Figure 81). By June 2013, on cessation of flows in both the Yallakool Creek and Wakool River all fish returned to the refuge pool, demonstrating the importance of this habitat in sustaining native fish in the region.

Silver perch are thought to have a strong flooding requirement for successful spawning (Humphries et al. 1999) but more recent work has shown they are also able to spawn within channel (King et al. 2009; Mallen-Cooper and Stuart 2006). However, little is known about their movement behaviour and the role it plays in the survival of the species, and a synthesis of the last three years data with linkages to other ecosystem components (e.g. recruitment) will help with this.

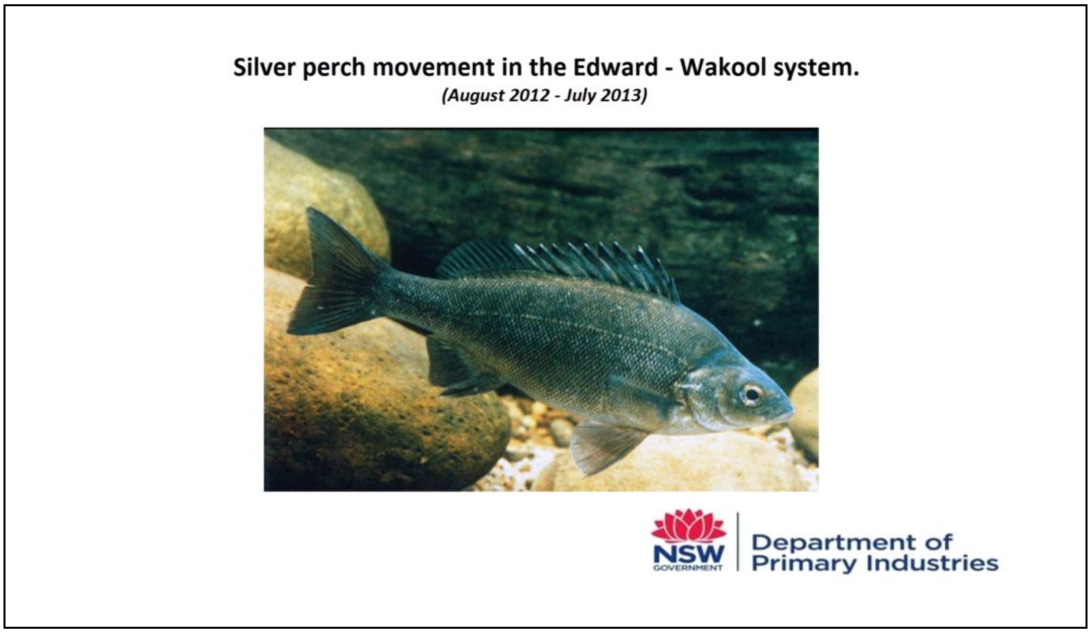


Figure 78. Silver perch movement in the Edward – Wakool system. Time series movement of silver perch is presented in relation to flow is available at this website. http://www.csu.edu.au/research/ilws/research/SRAs/Water/Water_projects%20-EdwardWakool.htm The discharge (ML/D) for the Wakool River and Yallakool Creek is shown on the left hand side of the video.

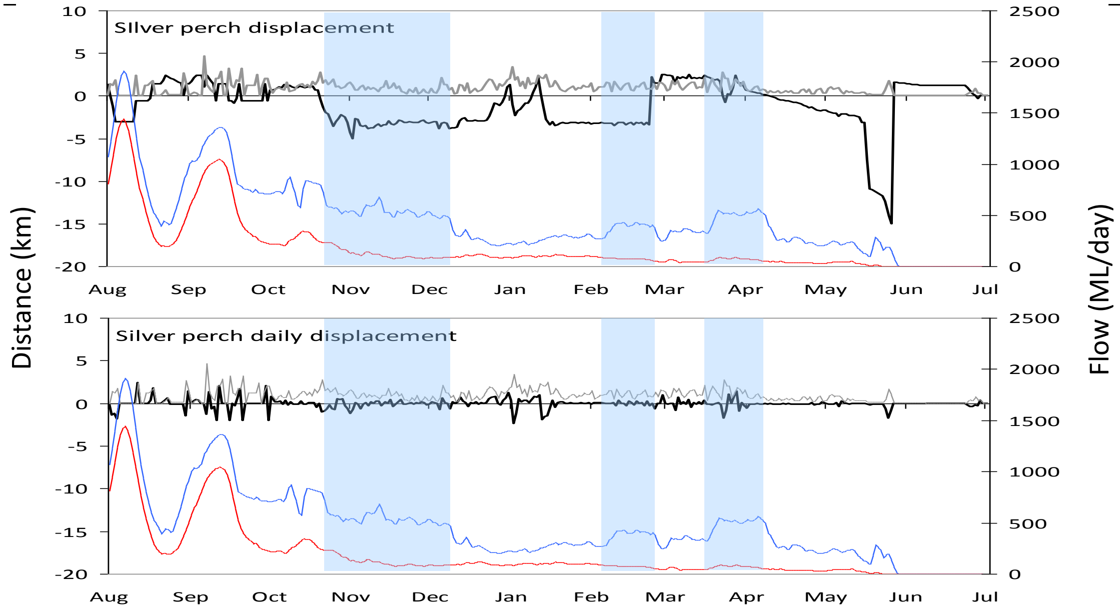


Figure 79. Displacement and daily displacement (left axis, black) in km of silver perch in relation to activity (left axis, grey) and flow (right axis). Environmental water periods shown with the blue shading.

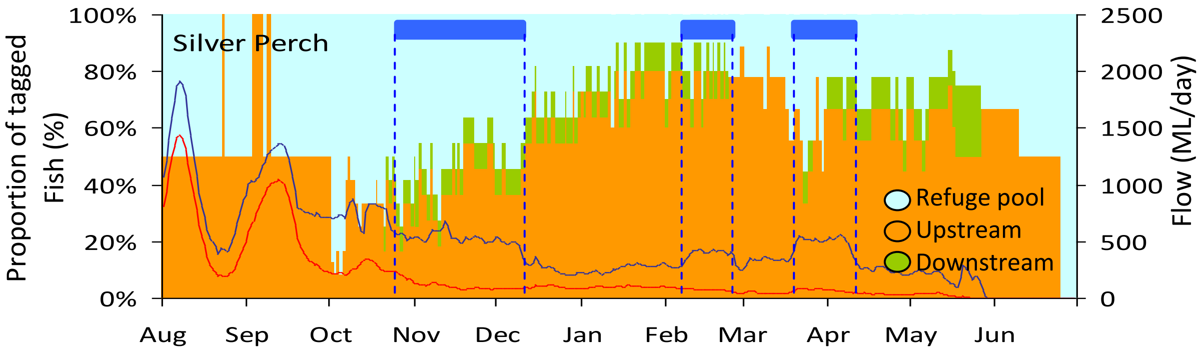


Figure 80. Daily habitat selection of Silver perch shown as the tagged proportion upstream, downstream and in the refuge pool (left axis) – in relation to the flow (right axis) (Yallakool Creek flow -Blue, Wakool River –red). Environmental water delivery is highlighted by blue bars.

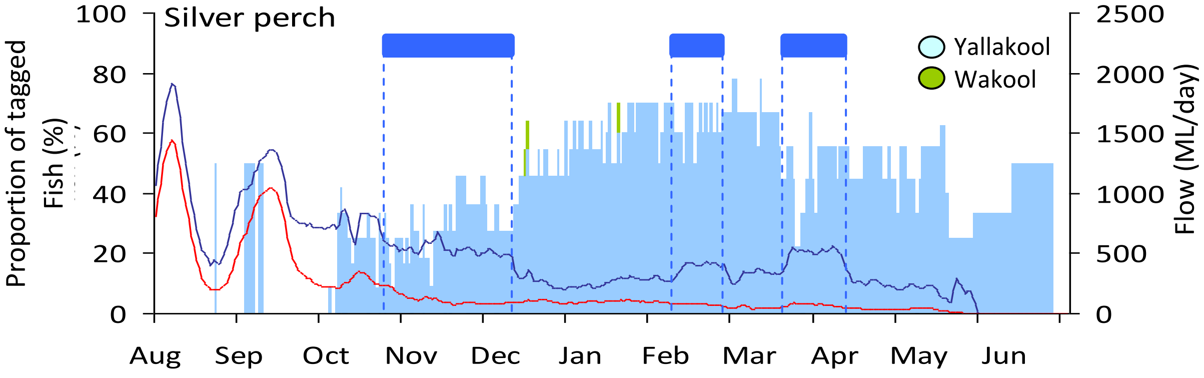


Figure 81. Proportion of tagged silver perch (left axis) upstream of the refuge pool in the Wakool (green) and Yallakool (blue) in relation to the flow (right axis). Yallakool environmental water delivery shown by the blue bars.

Carp movement in response to environmental watering

Tagged carp displayed periods of large displacing movements interspersed with periods of sedentary behaviour (Figure 82 and wmv file). Peak displacement for carp occurred between August and November 2012 coinciding with high flow events; however a small number of individual fish undertaking large scale movements outside of this period resulted in a high mean displacement from January to May. Individual displacements away from the refuge pool in carp ranged between 150 km downstream to 119 km upstream, with a 32 km maximum daily displacement, but these displacements were only exhibited by less than 20% of the overall population. Distances greater than 5 km from the refuge pool were recorded in 76% of tagged carp during the study, with all of these fish undertaking these movements during August to November. Similarly, 67% were recorded at distances greater than 10 km from the refuge pool, with 70% of these movements occurring during the August to November period.

Movements greater than 5 km in a single day were rare, occurring on 1% of the detection days. Similar results were reported in studies by Reynolds (1983) and Stuart and Jones (2006) utilising external tagging data to monitor carp movements but were determined utilising mark recapture techniques which do not account for movement of animals between capture events. The acoustic techniques used in this study provide continuous monitoring without the need to impact behaviour through repeated capture and sampling location bias.

Monitored carp displayed high site fidelity, residing in close proximity to their point of capture or choosing to return to it after short periods of displacement. With the exception of a small number of individuals undertaking large scale movements, carp typically made movements of less than 5 km, spending 64% of their time in the refuge pool (Figure 83). Carp leaving the refuge pool moved in both upstream and downstream directions (Figure 84). Movement during environmental water releases was evident with an increased proportion of carp moving upstream out of the refuge pool into the Yallakool Creek during environmental flow delivery (Figure 85).

Carp recruitment in Australia occurs from mid spring to summer, however may occur as late as autumn and early winter at temperatures between 16 to 32 °C and is influenced by flow regulation (Driver et al. 2005). Manipulation of flow delivery could be utilised in the system to control carp numbers, for example flow manipulation to desiccate eggs, deny access to spawning habitat, or prevent carp leaving areas where they occur in high abundances in the system (e.g. wetland habitats identified during community sampling).

Stuart et al. (2006) reported on the opportunity to trap migrating carp at regulatory structures using carp separation cages installed in fishways. In the Edward Wakool system, fishways currently operate on Colligen Creek Regulator, Yallakool Creek offtake, Edward River offtake, Gulpa Creek offtake and Steven's Weir providing opportunity for utilisation of this technology to control carp, in collaboration with environmental water delivery. The most effective timing, magnitude, duration of flows for trapping at regulatory structures could be implemented utilising the movement data collected in this study to maximise resources. However, the results indicate that such control techniques may only target the highly mobile carp and the local population inhabiting the immediate downstream area (5 to 10 km) of the structure. Nevertheless, understanding the movement strategy of carp throughout the system is a critical step in controlling future dispersal on environmental water allocations. Any opportunities to limit dispersal should be explored and included into annual water planning processes.

Figure 82. Common carp movement in the Edward – Wakool system. Time series movement of common carp is presented in relation to flow in a video available at this website. http://www.csu.edu.au/research/ilws/research/SRAs/Water/Water_projects%20-EdwardWakool.htm. The discharge (ML/D) for the Wakool River and Yallakool Creek is shown on the left hand side of the video.

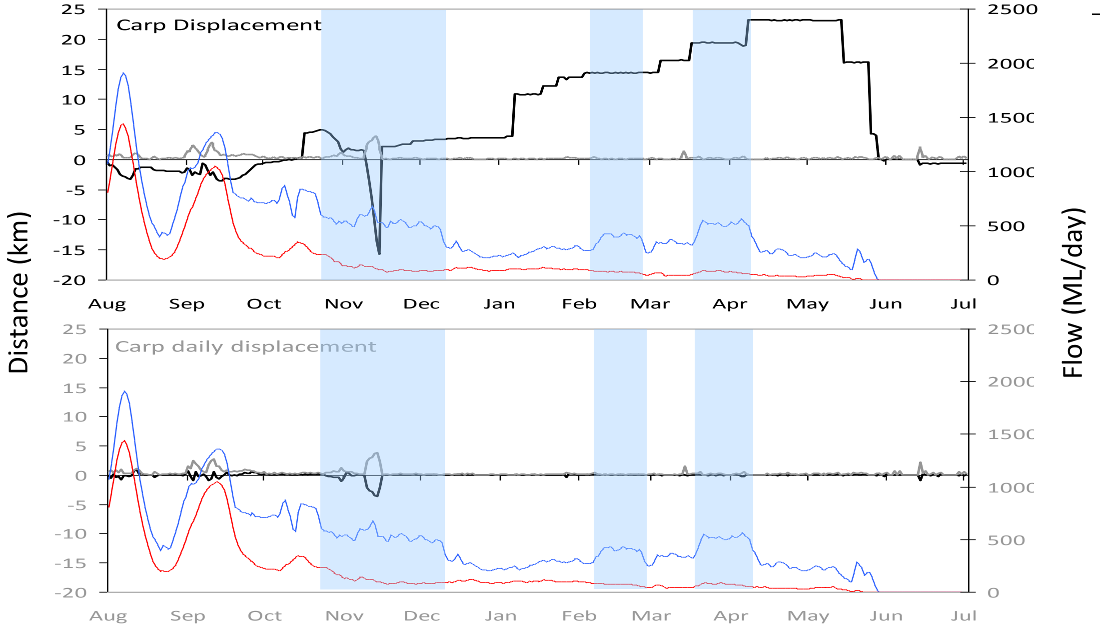


Figure 83. Displacement and daily displacement (left axis, black) in km for carp, in relation to activity (left axis, grey) and flow (right axis). Environmental water periods shown with the blue shading.

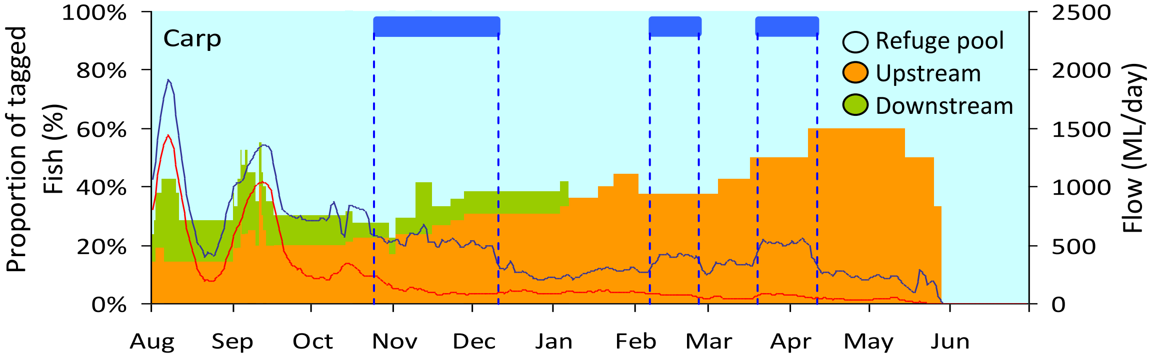


Figure 84. Daily habitat selection of carp shown as the tagged proportion upstream, downstream and in the refuge pool (left axis) in relation to the flow (right axis) (Yallakool Creek flow -Blue, Wakool River –red). Environmental water delivery is highlighted by blue bars.

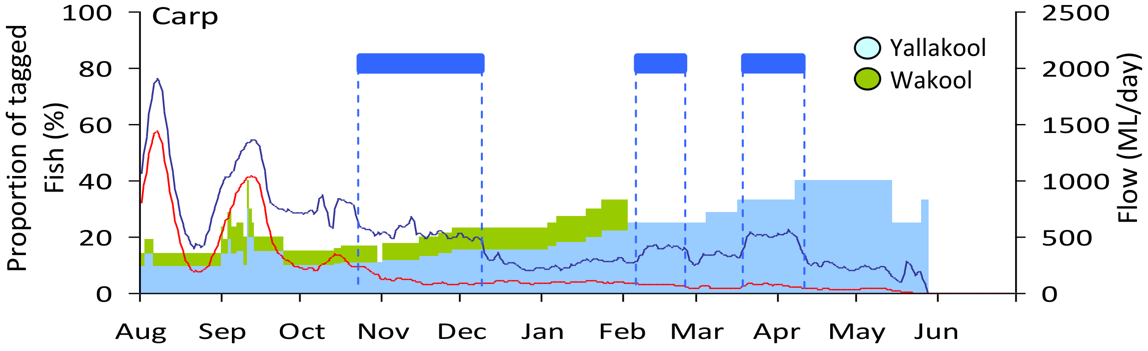


Figure 85. Proportion of tagged carp (left axis) upstream of the refuge pool in the Wakool (green) and Yallakool (blue) in relation to the flow (right axis). Yallakool environmental water delivery shown by the blue bars.

General trends in fish movement

All four monitored fish species displayed increased activity and displacement in response to increasing flow during spring and early summer. This period of increased movement corresponded with different spawning periods for many of these fish. Once fish moved away from the tagging location, there was a period of absence, but the vast majority of fish returned to the Wakool reserve pool. These observations suggest two important management issues. Firstly, the Wakool reserve is an important refuge pool in the region and is used by all fish species during periods of low flow. When flow declines in Yallakool creek and the Wakool River, available fish habitat declines, leaving small shallow pools with varying connectivity, with the Wakool reserve forming the largest in both size and depth and may drive much of the site fidelity observed. Secondly, the flow regimes delivered this watering year were sufficient for all tagged fish species to leave the pool and access new habitat, and also to subsequently return when flows ceased. Movement however, differed from previous data in both displacement distance, and the number of fish undertaking these movements was reduced corresponding with reduced flows.

Delivery of environmental water encouraged all species, except Murray cod, to move into Yallakool Creek. Murray cod moved during spring and early summer predominantly into the Wakool River, but this could have been related to habitat differences in the two systems as opposed to water delivery. This pattern of movement was consistent with spawning related behaviour, with cod moving upstream prior to a sedentary period, suggestive of nesting, before returning down river to the refuge pool.

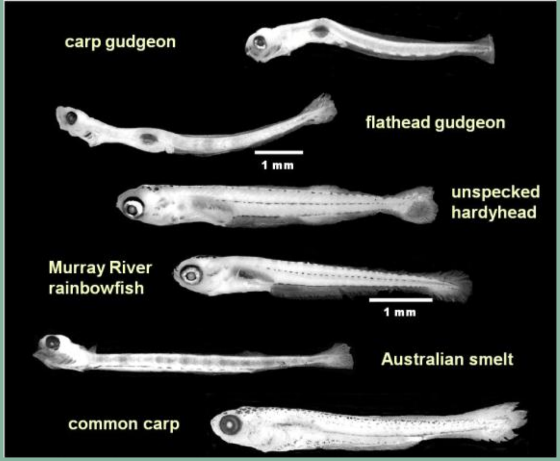
Golden perch made movements consistent with that of spawning behaviour on the unregulated events during September 2012 but no recruitment was detected in community sampling (possibly due to inadequate temperature cues). During environmental watering events, even though temperatures were adequate, movements were not consistent with that of a spawning related behaviour, and this could have been due to inadequate magnitude and duration. Additional work is required to identify the most appropriate flows needed by golden and silver perch.

Silver perch movement did not appear to be spawning related, but the high activity indicates that longitudinal connectivity is important for this species, and that relatively high velocity areas may also be preferred. Environmental water delivery should be focused for this species on maintaining refuge habitat (as with all other native species mentioned) during drought periods, and further testing of relationships with flow through flow manipulation studies.

Carp movement within 2013 did not appear to be directly related to increases in flow or seasonally related to spawning behaviour, even though large unregulated flow events would have inundated spawning habitat such as wetland habitat. Movement during environmental watering did not increase markedly or appear to be spawning related. This movement data suggests that instream flows similar to that delivered to promote Murray cod spawning or flow generalist spawning (CEWO 2012) does not benefit carp recruitment, and even inundation of wetland habitats (unregulated flows) does not always elicit a spawning response. Hence, a positive outcome is that a targeted flow delivery for native fish species did not appear to benefit carp dispersal.

7.5. Objective 5: Support breeding and recruitment of native fish

7.5.1. Fish spawning (abundance of larval fish and eggs)



Key findings

- *Larvae of ten species of fish, of which 8 were native, were found in the Edward-Wakool system over the 2012-2013 spawning season*. Larvae of only one species, carp gudgeon, were found in significantly higher numbers immediately after an environmental fresh when compared to rivers that did not receive environmental water.
- *There was no evidence of golden and silver perch spawning as a result of the environmental watering* freshes delivered to Colligen Creek in November 2012. No eggs or larvae of silver or golden perch were found during or immediately after the watering actions. Although there were fish moving during all three environmental watering events it is not known if any of these movements resulted in spawning because most adult golden perch migrated downstream outside the larval monitoring zone. Future monitoring should assess spawning in downstream sites.
- *There was no evidence that the nursery and larval dispersal conditions of Murray cod were enhanced as a result of the October to December 2012 Yallakool Creek watering action*. **A**lthough Murray cod larvae were found in the Yallakool Creek, they were not in significantly greater numbers than in the rivers that did not receive environmental water. The spawning and drifting of Murray Cod occurred independently of the environmental watering.

Background

The distribution and abundance of Murray-Darling Basin native fish populations have undergone considerable declines since European settlement, and current populations are estimated to constitute just 10% of that which may have occurred historically (Lintermans 2007). One of the major causes of this decline is river regulation, which has reduced the frequency and magnitude of flow extremes and in many regions has altered the seasonality of river flows (Cadwallader 1978). The ecological requirements of freshwater fish are considered to be closely linked to flow, with changes in hydraulic conditions having considerable effect on pre-spawning condition and movement, spawning and subsequent recruitment, as well as the provision of food and habitat resources (Humphries and Lake 2003). To mitigate some of the effects of regulation on the ecology and health of Murray-Darling rivers, the release of environmental flows is becoming a more commonly applied restoration tool, and in the context improving fish populations, is seen as a potential way of enhancing the spawning and recruitment of native fish species (Murray-Darling Basin Commission 2004).

Murray-Darling fish appear to exhibit a diversity of flow requirements, although for many species, knowledge of specific flow requirements for many species is poor (Humphries et al. 1999; Pusey et al. 2004). Humphries et al. (1999) proposed three broad groups for native fish based on their life history, and likely flow requirements for spawning and recruitment. ‘Mode 1’ fish, are characterised as long-lived, large-bodied fish species whose spawning, or magnitude of spawning is associated with flow pulses, such as Golden and Silver Perch; ‘Mode 2’ fish, are characterised as long-lived, larged-bodied fish species whose spawning is independent of flow conditions, but whose recruitment may benefit from flow events (e.g Murray cod, trout cod, riverblackfish); and ‘Mode 3’ fish are short-lived, small-bodied fish that also spawn independently of flow pulses, and may well flourish under low flow conditions due to the warm temperatures and higher food resources that such environments can provide (e.g carp gudgeon, Australian smelt, unspecked hardyhead, flathead gudgeon) (Humphries et al. 1999). Flow-response studies of native fish spawning and recruitment that have been conducted since the modes proposed by Humphries et al. (1999), and in particular those that have focussed on assessing both overbank and large within channel environmental flows, give support to the life history modes proposed, in particular confirming the importance of flow pulses for golden and silver perch spawning and recruitment (Mallen-Cooper and Stuart 2003; Roberts et al. 2008; Zampatti and Leigh 2013), and the independence of species such as Murray cod spawning from flow conditions. Less has been documented on the role of flow in the spawning and recruitment for smaller bodied species however (but see King et al. 2003; Tonkin et al. 2008a; Tonkin et al. 2011).

One of the objectives of the 2012-2013 watering actions in the Edward-Wakool system was to provide spawning outcomes for native fish. For many rivers in the Murray-Darling Basin, including those in the Edward-Wakool system, constraints to water delivery limit the extent to which environmental watering actions can mimic magnitude, timing and duration of the natural flood regime. While large in-channel pulses have been found to promote recruitment in golden perch, silver perch and Murray Cod, less is known of the effect of smaller in-channel pulses on the spawning and recruitment of native fish. Monitoring of the abundance and diversity of larval fish was undertaken through spring and summer of 2012-2013 to detect which species of the Edward-Wakool fish community had spawned, and to assess the effect of small in-channel pulses on spawning (this section) and recruitment (section 7.5.2).

Hypotheses

1) The spawning of some native fish species, as measured by abundance of larvae, will increase either during or following environmental water delivery, compared to nearby rivers not receiving environmental water.

2) Production of larvae will be significantly greater in the rivers receiving environmenental freshes compared to those that do not.

Methods

Eggs, larvae (Figure 86) and juvenile fish were sampled to evaluate the spawning response of fish to specific flow events. Sampling, which involved a combination of quatrefoil light traps and boat trawls, was undertaken fortnightly across five rivers in the Edward-Wakool system from August 2012 to April 2013. Colligen Creek and Yallakool Creek received environmental freshes, the Wakool River and Little Merran Creek did not receive environmental freshes (controls) and the Edward River was included as it was the source of the environmental water delivered to Colligen Creek and Yallakool Creek.

To sample for larval and juvenile fish, three quatrefoil perspex light traps containing bioluminescent light sticks (Figure 87) were set at five sites within each river (15 traps in total per river). Light traps were deployed randomly along the littoral edge at each site at dusk, and retrieved the following morning (7:00-9:00 am). Five by 5 minute boat trawls were undertaken in each river, at one site (Figure 88). The boat trawls, which targeted pelagic eggs, larvae and juveniles entrained in the water column, involved deploying a 500 μm conical drift net (0.5 m mouth) on one side of the boat, and slowly zigzagging across the river in an upstream direction. The amount of water filtered by each trawl was recorded by a flow meter placed within the mouth of the net.

Additional targeted drift net sampling was undertaken in December 2012 and February 2013 to assess if golden perch and silver perch spawned in response to the in-channel freshes delivered to Colligen Creek and Yallakool Creek by the Commonwealth Water holder (Watering actions 2 &3, see section 3). The catch of larvae and eggs caught in Colligen Creek was compared to two rivers (Wakool River and Yallakool Creek) that did not receive environmental water, over three consecutive nights during the peak of the freshes (12-14 December 2012 and 5-7 February 2013). At each river, three conical drift nets were set in the evening (1 hour after last light), and retrieved first thing in the morning. The amount of water filtered by each trawl was recorded by a flow meter placed within the mouth of the net.

Larvae and juveniles collected from the light traps, boat trawls and drift nets were preserved in 90% ethanol and returned to the laboratory for processing. All individuals were identified, measured (SL mm), and their developmental stage recorded according to Serafini and Humphries (2004). Here, we classify larvae within five key developmental stages; protolarvae (with and without yolksac), flexion, postflexion, metalarvae and juvenile/adults. Otoliths were extracted from a subset of Murray cod and carp gudgeon larvae to determine growth rates and hatch dates (section 7.5.2).

The number of fish/eggs collected in drift nets and boat trawls were standardised to the amount of water filtered by the net. Due to the patchy nature of larval fish aggregation, larvae collected in the three light traps at each river site were pooled to make up one larger composite sample, giving five replicate samples per river, per trip. Response variables were log-transformed prior to statistical analyses when necessary to normalise data and stabilize variances. To test if the production of larvae (total abundance) was significantly different across the 5 rivers across the entire spawning season, total larval abundance was analysed for all species (where there was enough data) using a one way ANOVA with river as the grouping variable. When significant differences were indicated, *post hoc* pairwise comparisons were undertaken to determine differences between the Rivers. Where log-transformations failed to normalize species abundance data, a generalized linear model with a poisson distribution was used to test the effect of river instead.

An asymmetrical BACI (before-after, control-impact) (Underwood 1991) statistical design was used to test the effect of specific 2012-2013 environmental water actions on larval abundance in the Edward-Wakool river system. Differences in mean number of larvae between control/impact rivers and before/during/after environmental freshes were evaluated statistically for each watering action using two-way mixed effects analysis of variance (ANOVA). Because there were multiple sampling times used to represent before, during and after environmental flows, and multiple rivers used as 'Control' and sometimes 'Impact' rivers, sampling trip (random effect) was nested within Period (fixed effect, three levels: before, during and after), and river (random effect) was nested in Treatment (fixed effect, two levels: control rivers, impact rivers). Impact rivers received environmental freshes, while Control rivers were those that did not receive environmental water. For this analysis particular interest is in the Period x Treatment interaction term, which indicates a significant effect of the environmental watering action. Visual assessment of mean (±1SE) biomass plots, grouped by Period and Treatment, were used to confirm if the significant interaction term was positively or negatively associated with the environmental watering action.

The dates and rivers used to detect changes in fish larvae is consistent with the approach used to assess water chemistry (Table 9, section 7.2.1). The null hypothesis for all watering actions was that mean larval abundance of native fish species in the rivers which received environmental water would not be significantly different to the control rivers. All statistical analyses were carried out using the freeware R and the R package NLME (R Development Core Team 2013).

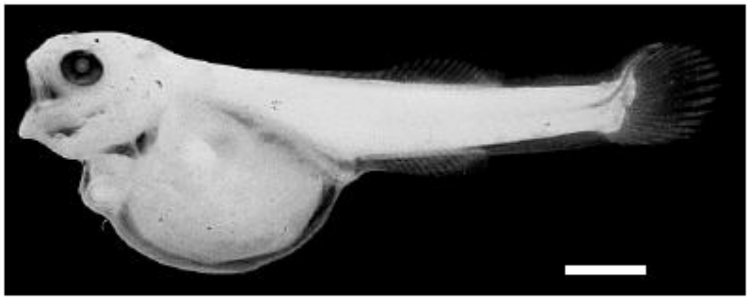


Figure 86. Murray cod larva at six days old. The white bar represents 1 mm. From Serafini & Humphries (2004). Preliminary guide to the identification of larvae of fish from the Murray-Darling Basin

Figure 87. Photos of light traps used to sample larval fish, showing bioluminescent light stick in the centre of the trap

Figure 88. Drift net used to undertake boat trawls for larval fish. This sampling method is undertaken after dark.

Results and discussion

Species abundance and diversity and timing of spawning

A total of 33,044 larval and juvenile fish, representing 11 fish species, were collected in the 2012-2013 monitoring study (Table 23). Of these, 6,203 were larvae, and 26,841 were juveniles/adults.

Eight of the 11 fish species collected as larvae/juveniles were native species. Small-bodied species made up the majority of larvae collected across the five rivers, and were represented by (in order of most to least abundant), Australian smelt (*Retropina semoni*), carp gudgeon (*Hypseleotris* spp.), flathead gudgeon (*Philypnodon grandiceps*), unspecked hardyhead (*Craterocephalus stercusmascarum fulvus*) and Murray River rainbowfish (*Melanotaenia fluviatilis*) (Table 23).

Large-bodied species that were found as larvae were Murray cod (*Maccullochella peelii*), river blackfish (*Gadopsis marmoratus*), silver perch (*Bidyanus bidyanus*) and carp (*Cyprinus carpio*). Of these, Murray cod and carp were the most abundant, with 216 and 193 larvae sampled respectively (Table 23). Quatrefoil light traps, boat-trawls and drift nets caught 98.2% (n=6,088), 1.1% (n=68) and 0.7% (n=47) of the total larval catch, respectively. Because of low catch rates of larvae in drift nets and boat trawls, statistical analyses were limited to light trap catch data.

Table 23. Catch summary of fish *a)* larvae and *b)* juveniles/adults collected with light traps, boat trawls and drift nets from the five rivers in the Edward-Wakool system during 2012-2013.

	Light traps						**Boat trawls**						**Drift nets**			
a) Larvae	*Col.*	*Yal.*	*Wak.*	*Mer.*	*Edw.*	Total	*Col.*	*Yal.*	*Wak.*	*Mer.*	*Edw.*	Total	*Col.*	*Yal.*	*Wak.*	*Total*
Australian smelt	83	130	123	1916	1653	3905	1	1	0	10	5	17	0	0	0	0
carp gudgeon	469	77	19	112	916	1593	12	0	2	8	21	43	37	1	5	43
Murray cod	5	97	69	17	28	216	1	1	0	2	1	5	0	2	2	4
carp	0	1	85	98	9	193	0	0	1	1	0	2	0	0	0	0
unidentified	10	0	1	11	65	87	0	0	0	0	0	0	0	0	0	0
flathead gudgeon	22	1	4	47	11	85	0	0	0	0	0	0	0	0	0	0
Murray River rainbowfish	3	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0
river blackfish	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0
silver perch	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0
gambusia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unspecked hardyhead	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
oriental weatherloach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	**592**	**307**	**304**	**2203**	**2682**	**6088**	**15**	**2**	**3**	**21**	**27**	**68**	**37**	**3**	**7**	**47**
%	10	5	5	36	44		22	3	4	31	40		79	6	15	
b) Juvenile/adults	*Col.*	*Yal.*	*Wak.*	*Mer.*	*Edw.*	Total	*Col.*	*Yal.*	*Wak.*	*Mer.*	*Edw.*	Total	*Col.*	*Yal.*	*Wak.*	*Total*
Australian smelt	230	104	91	814	259	1498	847	201	55	238	293	1634	0	2	1	3
carp gudgeon	6251	2465	88	813	13414	23031	119	19	1	4	21	164	237	112	1	350
Murray cod	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
carp	0	0	2	4	0	6	0	0	0	0	0	0	0	0	0	0
unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
flathead gudgeon	93	4	1	1	37	136	2	0	2	0	1	5	0	0	2	2
Murray River rainbowfish	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
river blackfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
silver perch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gambusia	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0
unspecked hardyhead	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0	0
oriental weatherloach	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0
Total	**6574**	**2573**	**182**	**1634**	**13710**	**24673**	**968**	**221**	**62**	**242**	**316**	**1809**	**237**	**114**	**8**	**359**
%	27	10	1	7	56		54	12	3	13	17		66	32	2	

The contribution of the different developmental stages which made up the larval catch varied across fish species (Figure 89). The majority of Australian smelt collected were proto-larvae (the earliest developmental stage) while carp gudgeon were predominately meta-larvae (the most developed larval stage). Murray cod were only collected as meta-larvae as they are well developed at hatch, and do not have any earlier stages. For the remaining, less common species, relatively similar proportions of proto-larvae, flexion, post-flexion and meta-larvae were sampled.

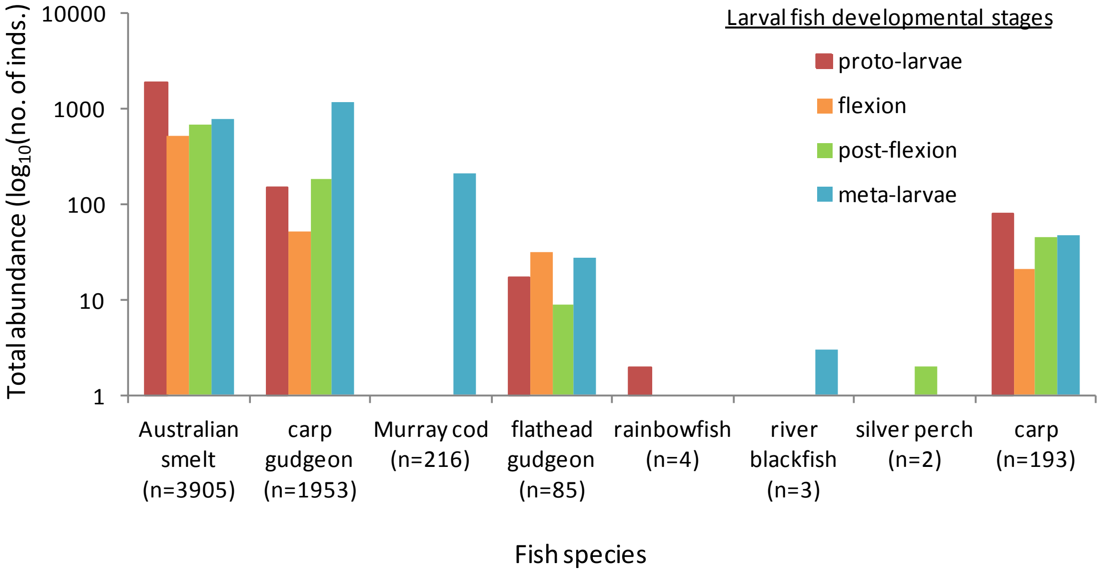


Figure 89. Total abundance of the different developmental stages of larvae caught for each fish species. *n*=the number of larvae collected across entire the sampling season. Note y-axis is on a logarithmic scale.

Seasonal timing in appearance of larval fish

On average, five to six species were found as larvae in each of the rivers (Figure 90). The duration and timing of the spawning period for small-bodied species such as Australian smelt, carp gudgeon and flathead gudgeon was variable, and differed across rivers (Figure 90). Australian smelt were found earliest in the spawning season, and were collected as larvae from September to December 2012 in all rivers. The other abundant species (carp gudgeon, Murray cod, flathead gudgeon and carp) peaked around October to December 2012 (Figure 91). Carp gudgeon larvae were found in highest numbers in Colligen Creek and the Edward River, appearing from November 2012 to April 2013 (Figure 91). Carp gudgeon were less abundant in Yallakool Creek, the Wakool River and Little Merran Creek, and the spawning season in these rivers was shorter compared to Colligen Creek (Figure 90 and 91).

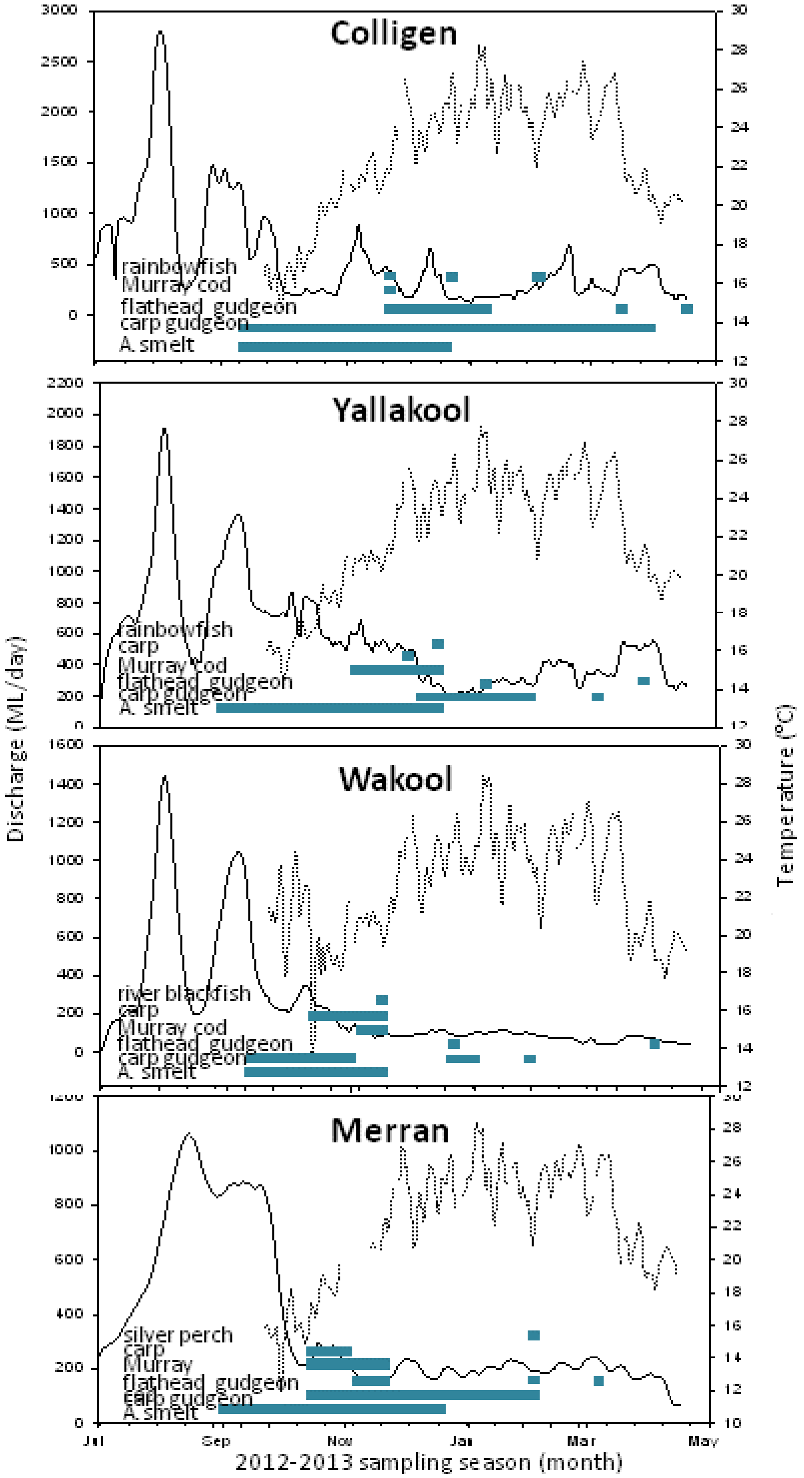


Figure 90. Occurrence (blue bars) of the fish larvae sampled in Colligen Creek, Yallakool Creek, Wakool River and Little Merran Creek from August 2012 to April 2013, along with discharge (solid line) and temperature (dashed line) profiles.

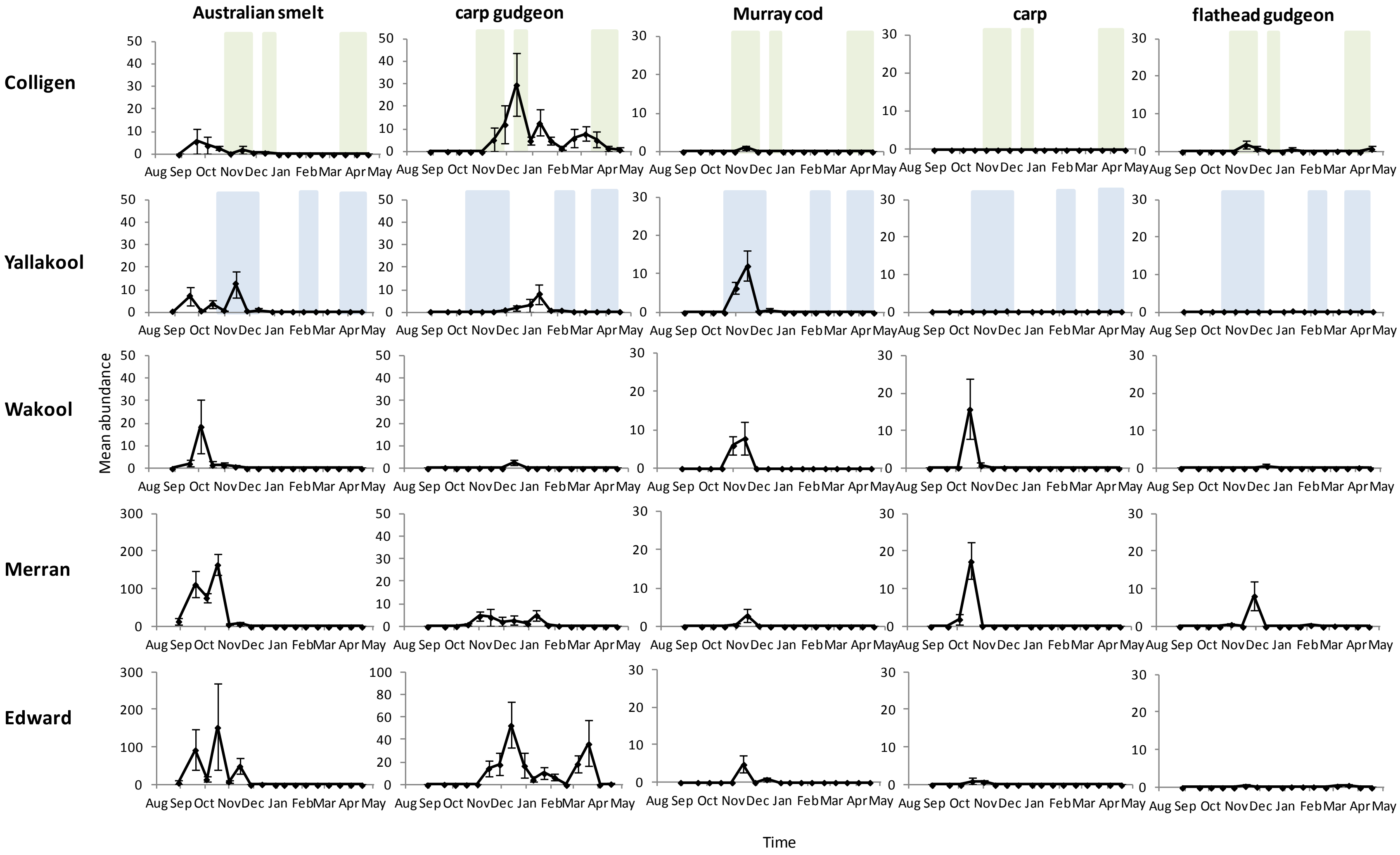


Figure 91. Temporal trends in mean larval abundance (±1SE) of the five most common fish species in the Edward-Wakool system over the 2012-2013 spawning season. Coloured bars represent when Commonwealth environmental water was delivered to Colligen Creek (green bars), and Yallakool Creek (blue bars). The Wakool River and Little Merran Creek did not receive environmental water and the Edward River was the source of the environmental water. Note: Y axes vary.

Environmental watering event-based analysis

November 2012 Colligen Creek watering action targeting golden and silver perch spawning

We found no evidence to indicate that golden or silver perch spawned in response to the two freshes delivered to Colligen Creek in November 2012. No golden perch eggs, larvae or juveniles were found in drift nets, boat trawls or light traps. Two silver perch larvae were collected in Little Merran Creek in January 2013, indicating that some level of spawning had occurred independently of the environmental flows delivered to Colligen Creek.

Of the five most abundant species, carp gudgeon was the only species that were detected to significantly increase in abundance after the first environmental fresh compared with rivers that did not any environmental water at this time (d.f= 2, 54, *F*-test=5.658, p=0.0059) (Figure 92). Abundances of Australian smelt, carp, flathead gudgeon and Murray cod did not significantly increase during or after the first fresh (Figure 92, Table 24). During the second fresh which followed shortly after, carp gudgeon mean abundances increased during the fresh, however site-level variation in abundances meant these observations were not significant (Figure 93, Table 24). Flathead gudgeon larval abundances, which were unaffected by the first environmental fresh, also showed no response to the second fresh (Figure 93, Table 24). Statistical analyses could not be performed for the other fish species due to the low numbers sampled.

November 2012 Yallakool Creek water action targeting Murray cod

Environmental water delivered to Yallakool Creek in November 2012 coincided with the spawning period for Murray cod. Murray cod larvae were found in all 5 rivers, and appeared in the Edward-Wakool system from mid-October through to mid December 2012 (Figure 91). Abundances were greatest in Yallakool Creek and the Wakool River and were in lower abundance in Little Merran Creek, Edward River and Colligen Creek. Numbers of larvae were not significantly greater in Yallakool Creek compared with rivers that did not receive environmental flows (Wakool River and Little Merran Creek) (d.f=2,99, *F*-test=1.423, p=0.245, Table 24). The spawning and drifting of Murray Cod occurred independently of the environmental watering (d.f=2, 99, *F*-test=1.42, p>0.05) (Figure 94). There was no significant change in Australian smelt, carp gudgeon, flathead gudgeon or carp abundances in Yallakool Creek during or after the environmental watering (Figure 94, Table 24).

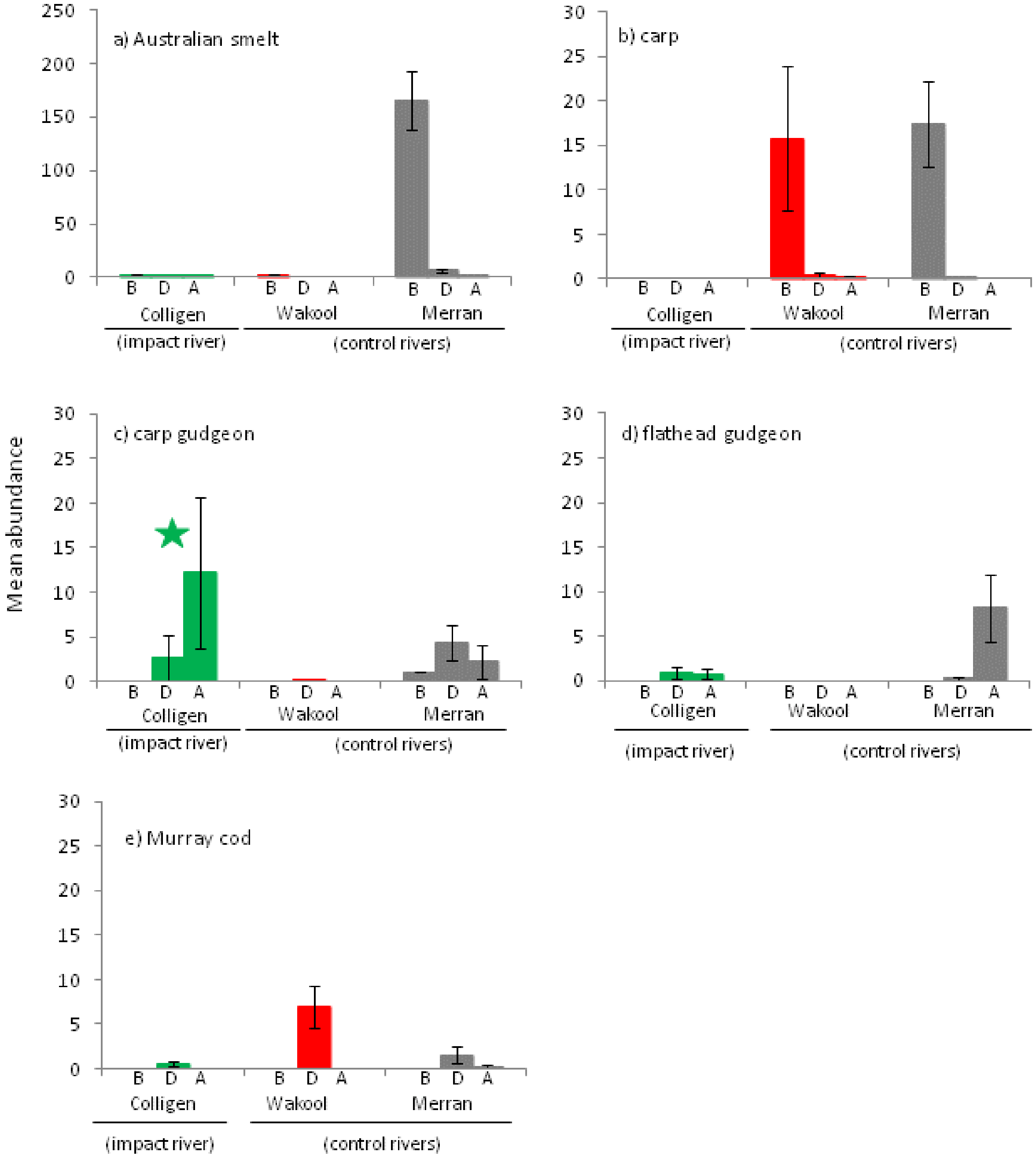


Figure 92. Mean abundance (±1SE) of fish larvae present in the Edward-Wakool system before, during and after the first environmental fresh in Colligen Creek on 2 November 2012. This flow was aimed at eliciting a golden and silver perch spawning response. The Wakool River and Little Merran Creek did not receive environmental water, and were used as controls. Planned comparisons with significant interactions between control-impact rivers (impact river; Colligen Creek, control rivers; Wakool River, Little Merran Creek) and Period (before, during, after) are marked with an asterisk, indicating that mean larval abundance changed significantly in Colligen Creek either during or after the environmental watering.

Table 24. Statistical results for 2 way mixed-effects Analaysis of Variance (ANOVA). A significant interaction between the two fixed factors: Period (before, during, after) and CI (control rivers, impact rivers) indicates that the mean abundance of larval fish within Impact Rivers was significantly different to changes that occurred over the same period of time within the Control Rivers.

Environmental flow	Species	Main effect	d.f	F-test	p-value
Nov 2012 – Colligen Creek fresh #1					
	carp gudgeon	Period (B-D-A)	2,54	1.440	0.245
		CI (C-I)	1,54	0.272	0.603
		Period*CI	**2,54**	**5.658**	**0.006**
	Australian smelt	Period (B-D-A)	2,54	2.117	0.130
		CI (C-I)	1,54	0.322	0.572
		Period*CI	2,54	0.333	0.717
	Murray cod	Period (B-D-A)	2,54	17.92	<0.0001
		CI (C-I)	1,54	0.916	0.342
		Period*CI	2,54	0.295	0.745
	carp	Period (B-D-A)	2,54	32.031	<0.0001
		CI (C-I)	1,54	15.924	<0.0001
		Period*CI	**2,54**	**15.774**	**<0.0001**
	flathead gudgeon	Period (B-D-A)	2,54	1.385	0.259
		CI (C-I)	1,54	0.034	0.853
		Period*CI	2,54	0.579	0.563
Nov 2012 – Colligen Creek fresh #2					
	carp gudgeon	Period (B-D-A)	2,38	1.827	0.174
		CI (C-I)	1,38	16.021	0.155
		Period*CI	2,38	0.0001	0.998
	flathead gudgeon	Period (B-D-A)	2,38	4.921	0.012
		CI (C-I)	1,38	0.112	0.786
		Period*CI	2,38	0.480	0.622
Nov 2012 – Yallakool Creek fresh					
	carp gudgeon	Period (B-D-A)	2,99	4.216	0.017
		CI (C-I)	1,99	0.126	0.723
		Period*CI	2,99	0.774	0.477
	Australian smelt	Period (B-D-A)	2,99	8.143	<0.001
		CI (C-I)	1,99	0.211	0.646
		Period*CI	**2,99**	**3.365**	**0.03**
	Murray cod	Period (B-D-A)	2,99	2.403	0.095
		CI (C-I)	1,99	1.661	0.200
		Period*CI	2,99	1.423	0.245
	carp	Period (B-D-A)	2,99	2.581	0.080
		CI (C-I)	1,99	5.100	0.026
		Period*CI	**2,99**	**5.183**	**0.007**
	flathead gudgeon	Period (B-D-A)	2,99	0.732	0.483
		CI (C-I)	1,99	0.576	0.449
		Period*CI	2,99	0.336	0.69
Feb 2013 – Yallakool Creek fresh					
	carp gudgeon	Period (B-D-A)	2,69	1.512	0.227
		CI (C-I)	1,69	0.550	0.460
		Period*CI	2,69	0.415	0.661
Mar 2013 – Yallakool & Colligen Creek freshes					
	flathead gudgeon	Period (B-D-A)	2,74	1.192	0.309
		CI (C-I)	1,74	0.378	0.540
		Period*CI	2,74	1.933	0.151
	carp gudgeon	Period (B-D-A)	2,74	1.138	0.325
		CI (C-I)	1,74	0.042	0.836
		Period*CI	2,74	2.303	0.107

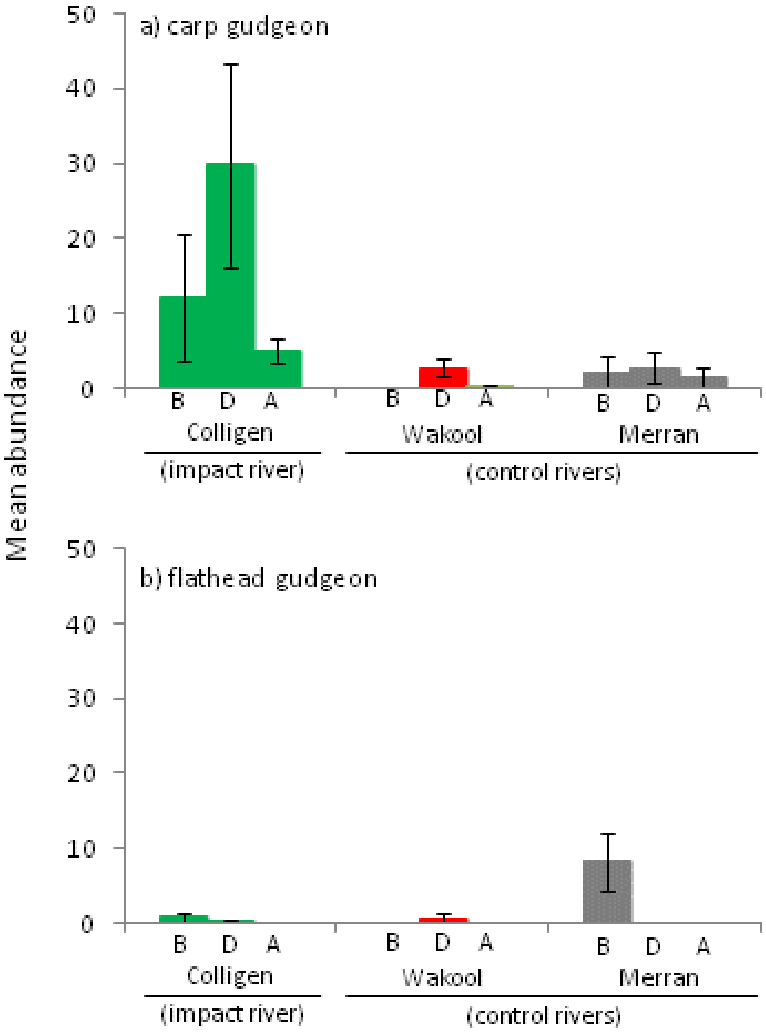


Figure 93. Mean abundance (±1SE) of fish larvae present in the Edward-Wakool system before, during and after the second environmental fresh in Colligen Creek on 31 November 2012. This flow was aimed at eliciting a golden and silver perch spawning response. The Wakool River and Little Merran Creek did not receive environmental water, and were used as controls. There were no significant interactions (see Table 24).

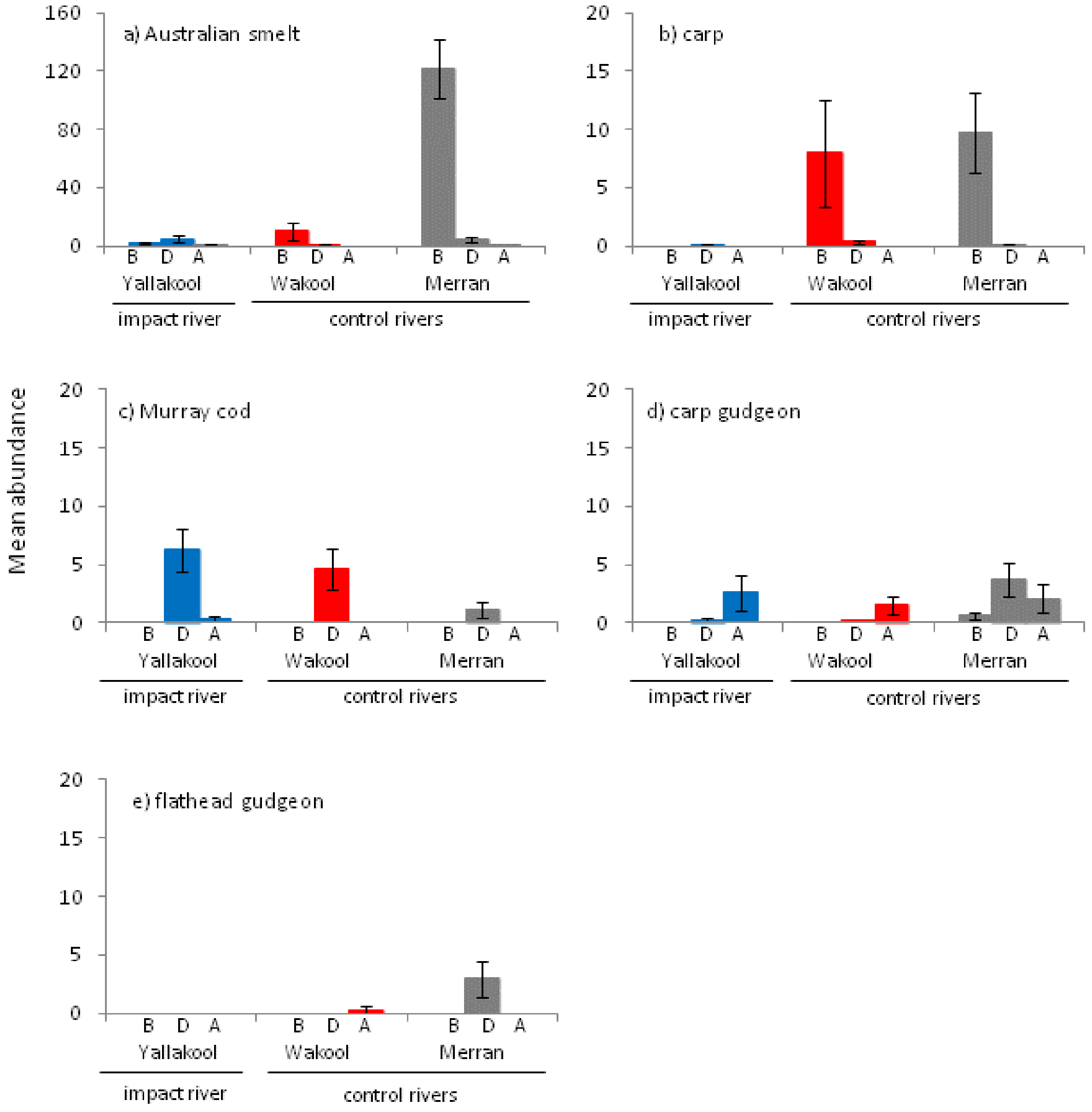


Figure 94. Mean abundance (±1SE) of fish larvae present in the Edward-Wakool system before, during and after an environmental flow in Yallakool Creek in November 2012. This flow was targeted towards maintaining water levels in Yallakool Creek to enhance Murray Cod spawning and dispersal. The Wakool River and Little Merran Creek did not receive environmental water, and were used as controls. There were no significant interactions (see Table 24).

February & March 2013 Yallakool Creek and Colligen Creek watering actions targeting the spawning and recruitment of small bodied fish.

Carp gudgeon and flathead gudgeon were the only species present in Yallakool and Colligen Creeks during the February and March 2013 watering actions in large enough numbers to be plotted visually (Murray River rainbowfish were also present in the system at this time, but only several individuals were collected) (Figures 95 and 96). For the February 2012 Yallakool Creek environmental fresh, statistical comparisons of Before-During-After, and Control-Impact could only be conducted for carp gudgeon. We found no significant change in carp gudgeon abundances either during or after the fresh compared to the 'control' rivers that did not receive environmental water (d.f=2, 69, *F*-test=0.41, p>0.05). Similarly, there was no significant different in carp gudgeon or flathead gudgeon abundances in either Yallakool or Colligen Creeks in March compared to Little Merran Creek and Wakool River, despite receiving sustained environmental freshes (Figure 96, Table 24).

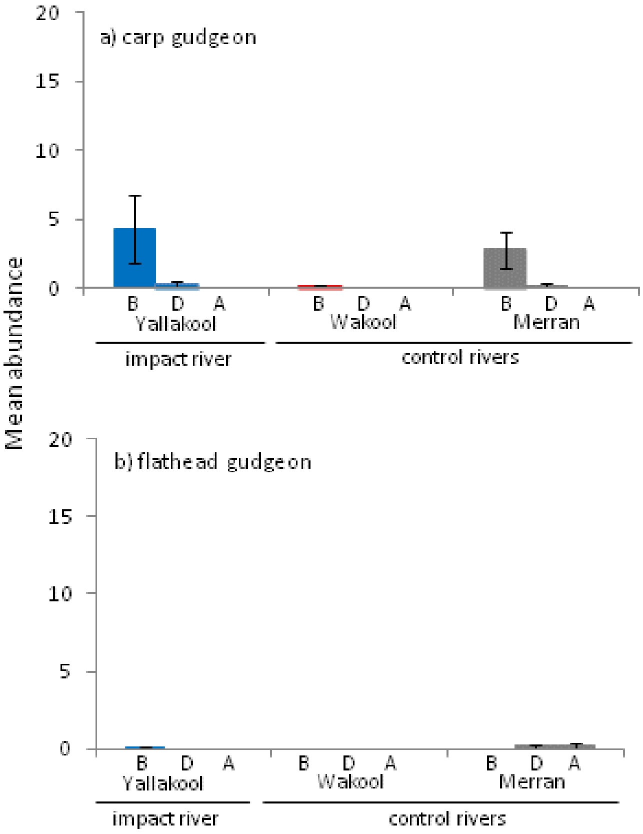


Figure 95: Mean abundance (±1SE) of fish larvae present in the Edward-Wakool system before, during and after an environmental flow 'fresh' in Yallakool Creek in February 2012. This flow was targeted to eliciting a spawning and recruitment response from small-bodied fish. The Wakool River and Little Merran Creek did not receive environmental water, and were used as controls.

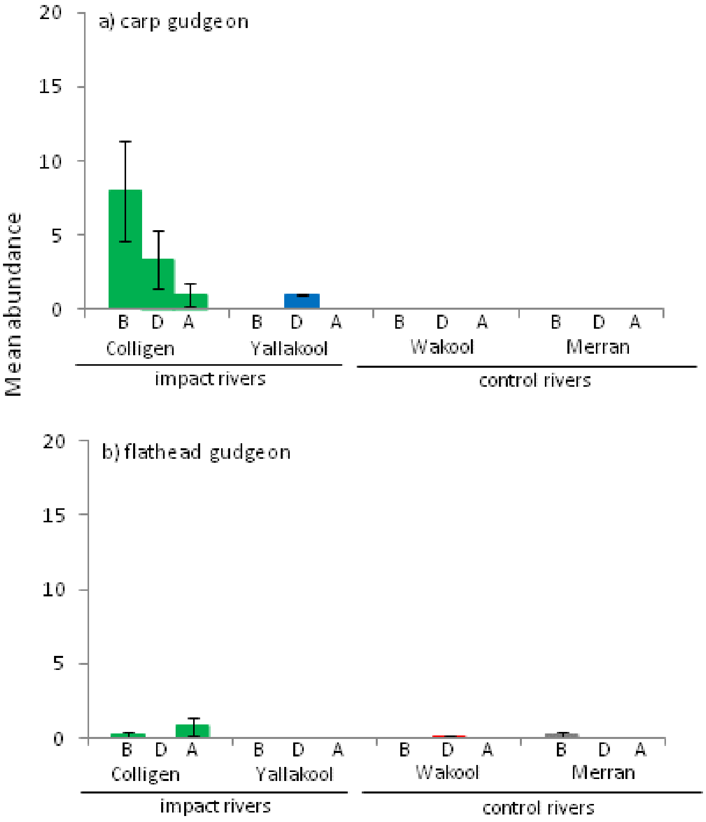


Figure 96. Mean abundance (±1SE) of fish larvae present in the Edward-Wakool system before, during and after two environmental flow 'fresh' which occurred simultaneously in Yallakool Creek and Colligen Creek in March 2013. The Wakool River and Little Merran Creek did not receive environmental water, and were used as controls. There were no significant interactions (see Table 24).

Difference in total larval production across rivers

The abundance of larvae sampled over the entire sampling period was significantly different across the five rivers (d.f=4, *F*-test=32.0, p<0.001). Mean (±SE) numbers of larvae sampled from the Edward River, Little Merran Creek, Colligen Creek, Yallakool Creek and Wakool River were 523 (±118), 438 (±52), 116 (±18), 61 (±13) and 60 (±11), respectively. Australian smelt, the most abundant species, was found in significantly greater numbers in Little Merran Creek and the Edward River weirpool (d.f=4, *F*-test=20.52, p<0.001) (Figure 97), neither of which received environmental freshes. Carp gudgeon larvae were found in significantly greater numbers in Colligen Creek and the Edward River weirpool (d.f=4, *F*-test=17.13, p<0.001), Murray cod larvae were significantly more abundant in the Wakool River and Yallakool Creek (d.f=4, *F*-test=8.325, p<0.001), and flathead gudgeon were significantly more abundant in Little Merran Creek (d.f=4, Wald chi-square=67.24, p<0.001) (Figure 97). In contrast, larval abundances of carp were significantly less in Colligen Creek and Yallakool Creek (d.f=4, Wald chi-square=193.0, p<0.001) (Figure 97).

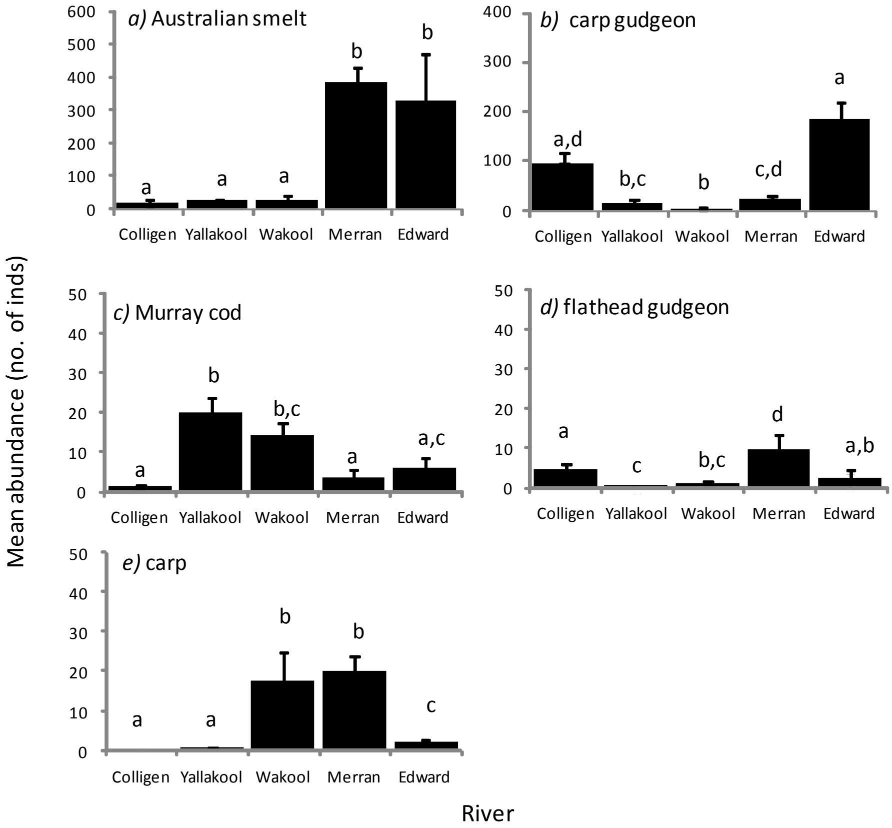


Figure 97. Mean total abundance of larvae for the five most abundant fish species collected in Colligen Creek, Yallakool Creek, Wakool River, Little Merran Creek and Edward River during the 2012-2013 sampling season; *a)* Australian smelt, *b)* carp gudgeon, *c)*Murray cod, *d)* flathead gudgeon and *c)* carp. Y-axes vary. Letters denote homogenous sub-set groups based on Tukey's *post hoc* significance tests.

Summary

Ten species of fish, of which 8 were native, were found to spawn (as indicated by presence of larvae) in the Edward-Wakool system over the 2012-2013 spawning season. Larvae of only one species, carp gudgeon, were found in significantly higher numbers immediately after an environmental fresh when compared to rivers that did not receive environmental water. Although there were six environmental freshes delivered to Yallakool Creek and Colligen Creek combined, this increase in abundance of carp gudgeon was only observed after the Colligen Creek November 2012 fresh.

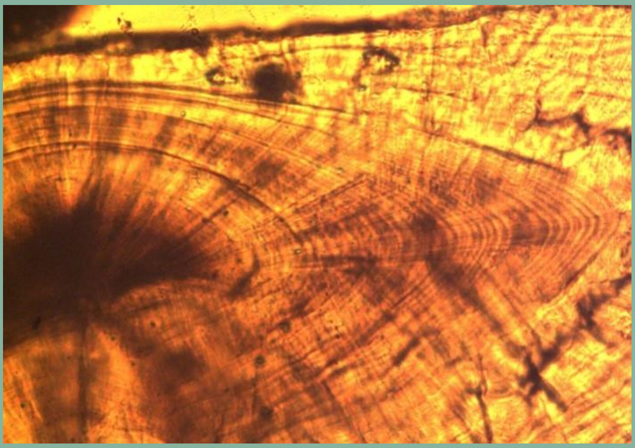
In-channel freshes targeted towards golden and silver perch spawning did not result in eggs or larvae being sampled. The capture of two silver perch larvae in light traps in Little Merran Creek, despite the relatively stable flows prior to their collection, support Mallen-Cooper and Stuart's (2003) findings that this species is able to breed (albeit at low levels) without sustained in channel flow pulses. Further lines of evidence (for example, movement data and the capture of juveniles at the end of the spawning season in April), will help to provide more definitive conclusions as to whether or not these targeted pulses may resulted spawning responses of these two species.

Sustained and stable flows were maintained in Yallakool Creek in November 2012; with the aim to enhance the spawning and recruitment of Murray cod. The number of Murray cod larvae collected in Yallakool Creek was not significantly different to the Wakool River, which had much lower discharge during this time, indicating Murray Cod will spawn in the Edward-Wakool system regardless of flow conditions. These findings confirm the strong body of knowledge that currently shows Murray Cod spawn at peak times in November-December, and regardless of flow conditions (Rowland 1983; Humphries et al. 2005; Koehn and Harrington 2006; King et al. 2009). While the provision of environmental flows may not be beneficial for Murray cod spawning, the role of flow in aiding the dispersal of larvae of some species (e.g Murray cod, golden perch and silver perch) into suitable nursery and juvenile habitats is relatively unknown, but could be advantageous for recruitment success if larvae are dispersed into areas of high food resources (Welcomme 1979). The community sampling conducted in April 2013, will determine whether more Murray cod juveniles were found in Yallakool Creek compared to the control rivers (Wakool River and Little Merran Creek)(see section 7.5.3) and help elucidate whether or not environmental watering may have provided beneficial conditions for recruitment. However, based on results presented in previous sections we expect that increased recruitment in Yallakool Creek is likely to be limited, because of the lack of a significant boost in productivity of food resources, such as zooplankton and shrimp, as a result of the environmental watering actions.

In this study, we used the total abundance of larvae collected across the entire sampling period as an indicator for the magnitude of spawning occurring in the focus rivers. We tested the hypothesis that production of larvae for fish species would be greatest in the rivers receiving environmental freshes (Colligen Creek and Yallakool Creek) compared to those that did not (Wakool River, Little Merran Creek). Out of the three most abundant native species (carp gudgeon, Australian smelt, Murray Cod) we found no evidence to support this hypothesis. Overall, our findings show that the watering actions in the Edward-Wakool system did not significantly influence the spawning behaviour or spawning magnitude of the native fish species present in the system. For flow-dependent species such as golden and silver perch, it is likely that the magnitude and duration of the water actions was not great enough to promote spawning. For flow-independent species such as Murray cod, flow conditions appear unimportant for spawning, and therefore we would not expect an increase in spawning response from this species (Rowland 1983; Humphries 2005; Koehn and Harrington 2006;

King et al. 2009b).For low-flow specialists, such as many of the smaller bodied native fish species, it may be that unless watering actions can provide a significant increase in low flow habitats (such as inundated slackwaters, backwaters and off channel wetlands) for significant periods of time that allow the spawning, hatching and rearing of larvae to take place, then the spawning response of such species in relation to watering actions will also be limited (Humphries et al. 1999).

7.5.2.Fish recruitment



Key findings

- *The number of recruits of carp gudgeon was significantly higher in Yallakool Creek and Colligen Creek during and immediately after the November environmental watering actions compared to rivers that did not receive environmental water.* However, the timing of larval abundance and hatch dates of recruits within the source river (Edward River) also increased over the same period of time. *Thus, it is not possible to determine whether carp gudgeon larvae or recruits were generated from within Colligen and Yallakool Creek, or is due to recruits moving, or being transported, into these systems from the Edward River.*
- Back-calculated larval hatch dates of Murray cod ranged from 16 October to 4 December 2012. There was a small peak in Murray cod larvae hatching around 20 October and a larger peak around 4 November 2012, both near the onset environmental watering actions in Colligen Creek and Yallakool Creek. *However, there were no significant difference in Murray cod hatch dates among control or impacted river reaches for either of the watering actions*. These data support the peer-reviewed literature that spawning of Murray cod is unrelated to river discharge.
- *It was not possible to evaluate whether environmental watering actions influenced recruitment success of Murray cod* as only 10 individuals were sampled in fish community surveys. Additional efforts targeting young-of-year recruits of large bodied species including Murray cod, golden perch and silver perch are warranted and could include back-pack electrofishing or community-based angling.

Background

One objective of environmental watering option 1 in the Edward-Wakool system (Table 3, Section 3) is to provide recruitment outcomes for native fish. Once reproduction has occurred, the definitive measure of spawning success is the number of individuals that survive to a particular point in time; referred to hereafter as recruitment. For purposes of this report, recruitment was defined as survival to the end of the spring-summer reproductive season. Environmental watering in the Edward-Wakool system during 2012 -2013 aimed to create conditions to facilitate native fish spawning and recruitment. Fish related flow objectives were to: enhance Murray Cod recruitment in Yallakool Creek (19 October - 7 December 2012), provide opportunities for small bodied fish recruitment, and flow specialists (golden perch and silver perch) movement and spawning in Yallakool Creek (1 February – 23 February 2013, and 13 March – 5 April), and to promote small bodied fish recruitment and flow specialist movement and potential spawning in Colligen Creek (13 March – 5 April).

Recruitment in fish that live in flowing water is tightly coupled with historical patterns of the natural flow regime associated with flooding, drought and regular seasonal cycles. Fish fauna of the Murray-Darling Basin evolved in a highly-variable flow system and species have subsequently developed a range of strategies for successful migration, spawning and recruitment (Humphries et al. 1999; Tedesco et al. 2008). It is therefore unlikely that a single flow regime will provide equal benefits for the entire fish community. Given the relatively high reproductive output of fishes, small changes to recruitment success can result in cascading effects on populations (King et al. 2013). Therefore, improvements in managing regulated river flow regimes through environmental watering actions has potential to benefit native fish communities (e.g. Baumgartner et al. 2013). Likewise, the mismanagement of river flow regimes may be detrimental to recruitment of native fishes and their populations.

There is some disagreement in the peer-reviewed literature concerning how river flow regimes and regulation influences the recruitment of Murray-Darling Basin fishes (see Harris and Gehrke 1994; Humphries et al. 1999). Given the erratic rainfall patterns of south-eastern Australia many, if not most, species of fish in the Murray-Darling Basin are able to spawn and recruit under low-flow conditions (see Humphries et al. 1999). Understanding how flooding (Harris and Gehrke 1994) and natural flow regimes (Humphries and Lake 2000) influences the magnitude of recruitment, however, remains speculation and an emerging area of investigation (see King et al. 2013).

A useful mechanism to determine the timing of spawning and recruitment success following environmental flow delivery is to determine the age of individual fish and relate that back to the time of eggs hatching. For older fish, age can be determined to a specific year, but for young fish (up to 200 days old) the birth date can be estimated. Annual age estimation can be used to identify successful recruitment years, which in turn can inform environmental water delivery practices. Daily age estimates provide more accurate estimates of hatch dates and can be used to evaluate the precise environmental and river flow conditions that maximise recruitment (Humphries et al. 2013). Importantly, this information can then help to determine if fish were hatched before, during, or after a period of environmental water delivery.

In this section we assess whether the timing of carp gudgeon recruitment and Murray cod hatch dates were associated with 2012-13 environmental watering actions. The null hypothesis tested was that environmental flows would have no effect on recruitment of carp gudgeon, or the hatch date distribution of Murray cod, and it was therefore consistent with the low-flow recruitment hypothesis (Humphries et al. 1999). Daily and annual age estimates were made on a range of other species sampled in fish community surveys (section 7.5.3) and these were used as a pilot study to establish a proof of concept approach for evaluating fish community recruitment outcomes following environmental watering actions. Statistical analyses could only be carried out on carp gudgeon and Murray cod in relation to 2012-13 environmental watering actions. Murray cod were selected because this species was the target of the October environmental watering action in Yallakool Creek. Carp gudgeon was selected because this was the only species that showed a statistically significant change in larvae following environmental watering actions (Table 24). Other fishes including silver perch, golden perch and several other small-bodied species were not sampled in adequate numbers as recruits to make inferences about 2012-13 environmental watering actions.

Methods

Carp gudgeon and Murray cod

Recruitment was calculated as the number of juveniles or early stage adults sampled throughout the end of the spring-summer reproductive season between January and April 2013. The term 'recruits' therefore refers to young-of-year fish sampled between January and April. Otoliths (sagitta) were extracted from 15 to 20 larvae and juvenile carp gudgeon spanning the full range of lengths sampled from each river between January and April 2013. Fewer than 15 Murray cod larvae were available from most river-month combinations and in these cases all sagittae were extracted and polished.

Otoliths were fixed to a microscope slide with the sulcus facing up using CrystalBond thermoplastic glue. The sagittal plane was polished flat with a grinding wheel fitted with a 15 µm or 6 µm wet polishing pad or with 6 µm, 3 µm and 1 µm dry lapping film. Microincrements were counted from the hatch check out to the anterior edge (Figure 98) using a compound microscope and 20X or 40X objective. In sections where a hatch check was not apparent, microincrement counts started at the mean radius of hatch checks determined from otoliths where a check was discernable. In Murray cod, a hatch check radius of 55 µm was used which was based on validated daily age estimates (Humphries 2005).

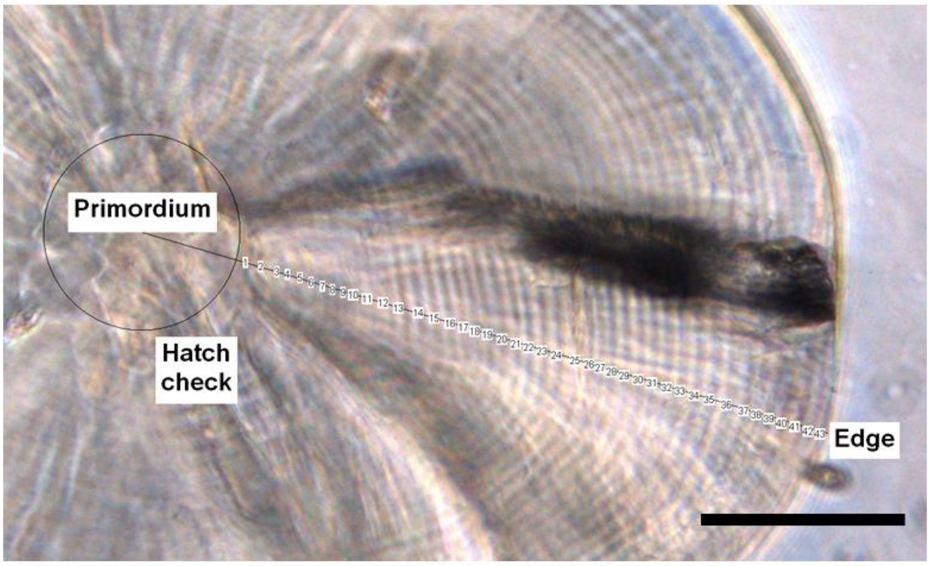


Figure 98. Image of a carp gudgeon (12.0 mm SL) otolith illustrating an estimated 43 daily microincrements after the hatch check. Scale bar represents 100 µm.

Recruitment sampling did not target or sample adults approaching the asymptotic length of species', therefore it was not appropriate to attempt fitting standard fisheries growth curves, such as Gompertz or von Bertalanffy models, to age-length data. A variety of models, including exponential growth, power functions, and polynomial functions, were explored and a three parameter power function provided the best fit to daily age estimates of carp gudgeon, while a simple linear model provided the best fit for Murray cod larvae. Age-length curves for carp gudgeon were pooled across rivers for each month due to the protracted spawning season and wide range of temperatures that could have affected growth. Age-length curves for Murray cod were developed for each river independently, given that the majority of larvae were collected within one month. Estimated daily ages, post-hatch, were rounded to the nearest whole number and were subtracted from the date of capture to determine individual hatch dates of fish. Duration of the spawning season was determined from back-calculated hatch dates of all stages of larvae, while the timing of recruitment was determined from the hatch dates of juveniles surviving to January through April 2013.

A before-after, control and impact, 'repeated BACI' (Underwood 1991), statistical design with multiple controls was used to test the effect of environmental watering actions on recruitment of carp gudgeon and larval hatch dates of Murray cod. The null hypothesis tested was that the mean number of recruits hatched within control rivers was not significantly different to impacted rivers before, during or after environmental watering actions. Means were calculated by the number of recruits hatched per day for the 14 days prior to and after each event and for the duration of each management action.

Statistical analyses were carried out in SigmaPlot (10.0.1). The ability to use hatch dates to evaluate the effect of environmental flows on fish recruitment within rivers relies on assumptions that: 1) selectivity for different age-classes of recruits is comparable among rivers in the BACI design and 2) that changes in the number of recruits within rivers is unrelated to rates immigration or emigration. To test the first assumption we compared the median daily ages of carp gudgeon recruits and Murray cod larvae sampled among rivers using a Kruskal-Wallis One Way ANOVA. To test whether potential increases in recruitment could be attributed to immigration we added the source (Edward River) of impacted rivers to the BACI design. If changes in the number of recruits within the source river was not significantly different to the impacted river during or after environmental flows then the possibility for immigration could not be excluded. Impact rivers received environmental water, while controls were represented by all rivers that did not receive environmental water for each action.
Data were square root transformed to achieve equal variance and normal distribution structure. The mean number of recruits hatched per day were compared among control, impact and source (Edward River) rivers before, during and after environmental watering events using a two-way ANOVA. Post-hoc comparisons were conducted using the Holm-Sidak method which is recommended as more powerful test than Tukey and Bonferroni methods when conducting multiple pairwise comparisons. A Kruskal-Wallis One Way ANOVA on Ranks was used to compare median recruit hatch dates among rivers for the entire year in order to evaluate differences in the seasonal timing of peak recruitment. A Chi-square test was used to evaluate whether a significantly greater proportion of recruit hatch dates occurred during environmental watering actions compared to control rivers.

Fish community recruitment pilot study

Fish suspected to be young of year (0+), based on age-length approximations, were collected from sites during fish community surveys (see section 7.5.3). A sub-sample of otoliths across species and size classes were extracted for daily and annual age analysis. A sample of 30 fish was kept from the focus rivers, including length classes ranging from the smallest to largest estimated young of year fish. All sites occurred within the focus reaches used to monitor other indicators and allow for comparison with larval collection data (section 7.5.1). Additional samples were collected from Werai Forest because this zone may be a source of recruits for the Edward-Wakool river system. The estimated hatch dates of fish younger than 275 days were calculated by subtracting the daily age estimate from the capture date as described in the previous section. Annual age estimates on transverse sections of otoliths were made on older fish by enumerating opaque and translucent annuli (Figure 99).

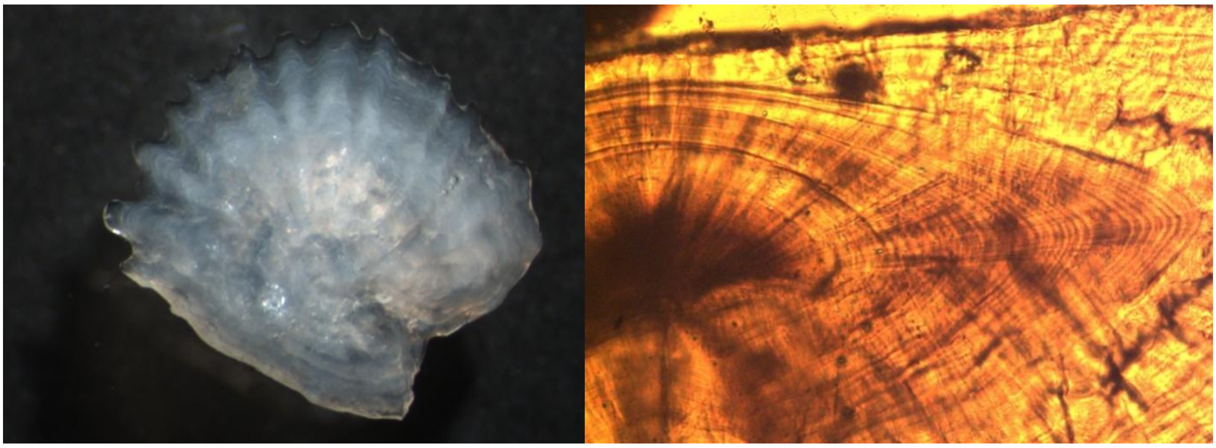


Figure 99. Left; whole juvenile common carp otolith prior to sectioning. Right; magnified view of an otolith daily aged fish, where the rings on the otolith can be counted to get an accurate calculation of the fishes hatch date.

Results and discussion

Carp gudgeon

Daily age-length relationships estimated from readable carp gudgeon otoliths (n=323) were fitted successfully by a power-function for each month between January and April 2013 (Figure 100). An additional 39, or approximately 12% of carp gudgeon otoliths, were considered unreadable due to poor preparation and individual variation in clarity. No significant differences in age-length relationships among rivers for months combined suggested that variation in growth rates could not be attributed to environmental watering actions within impact rivers. Although microincrement banding in otoliths was clearly apparent (see Figure 98) and growth rates were similar to other small-bodied freshwater fish species where ages have been validated (see Tonkin et al. 2008b), it is important to note that the daily periodicity of microincrement formation in carp gudgeon otoliths has not been established.

Power functions provided a statistically significant ($P<0.0001$) fit to age-length relationships of carp gudgeon for all months (Table 25), although there was wide variation in standard error estimates of individual parameters. Standard error estimates associated with parameters y_o and a were consistently high (Table 25) which suggested that model fits were imprecise for the smallest and largest sizes of fish. This variation was likely attributable to relatively few early-stage larvae being sampled in January to April 2013 and the decreasing precision of age estimates with increasing size of fish. Irrespective of the imprecision surrounding large and small fish, 95% confidence bands fitted to power functions (Figure 101) illustrated that age-length models for carp gudgeon provided precise age estimates for fish between lengths of 7 mm SL and 22 mm SL. Hatch dates of carp gugegon recruits (n=20072) sampled between January and April 2013 between the lengths of 8.1 and 25.2 mm SL were back-calculated to compare with the seasonal timing of environmental watering actions and peaks in larval abundance.

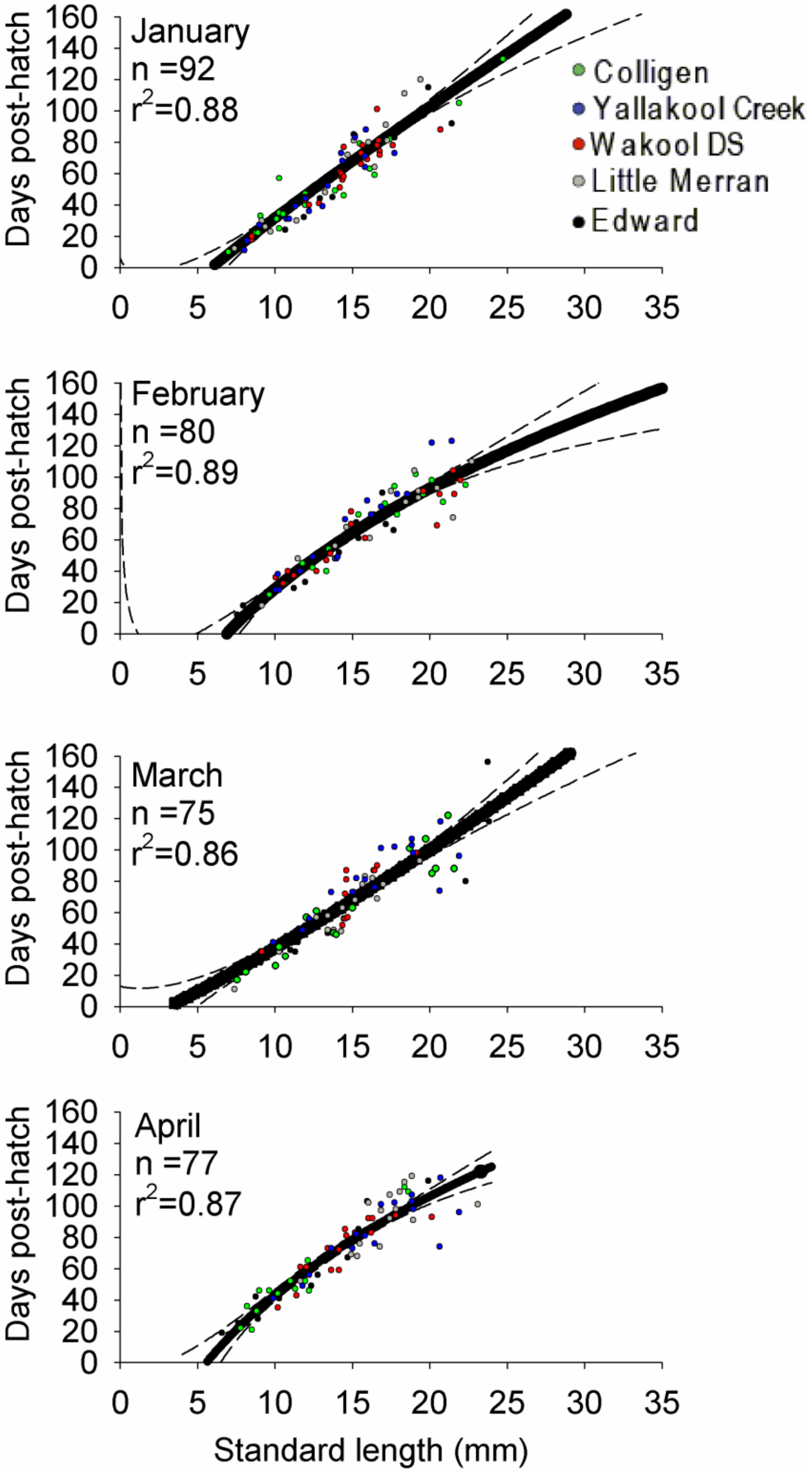


Figure 100. Estimated daily age-length curves for carp gudgeon (n=323) sampled each month in the Edward-Wakool river system between January and April 2013. Dashed lines represent 95% confidence bands.

Table 25. Power function (daily age = $y_0+a \times SL$ ^ ^{b}) parameters fitted to carp gudgeon daily age-standard length (*SL*) data used to back-calculated hatch dates of juvenile recruits sampled between January and April 2013. Parameter estimates ± Standard Error (SE).

Month	*n*	r^2	y_o	*SE*	*a*	*SE*	*b*	*SE*	F	*P*
January	92	0.88	-55.40	30.06	11.35	10.07	0.88	0.24	324.93	<0.0001
February	80	0.89	-212.22	233.03	110.11	180.41	0.34	0.29	296.65	<0.0001
March	75	0.86	-15.02	13.13	3.48	2.60	1.16	0.21	233.66	<0.0001
April	77	0.87	-212.92	252.34	123.32	203.63	0.32	0.29	245.04	<0.0001

Back-calculated hatch dates of carp gudgeon recruits were estimated to fall between 24 August 2012 and 20 March 2013, with peaks occurring in December 2012 or early January 2013 in all rivers (Figure 100). There were small but significant differences (K-W ANOVA Ranks; H=2899; DF = 4; P<0.001) in the median hatch dates of carp gudgeon recruits among rivers. However, environmental watering actions did not significantly shift the timing of recruitment in control rivers compared to impact rivers. The earliest median hatch date (9 December 2012) occurred in Colligen Creek and the latest (1 January 2013) in the Edward river. Hatch dates of recruits generally occurred later in the year compared to the timing of peak larval abundance within respective rivers (see section 7.5.1) and compared to the timing of peak larval abundance in Barmah Millewa Forest (see King et al. 2013) upstream of the Edward-Wakool system. Later season recruitment compared to peak larval abundance of carp gudgeon observed here was a similar mismatch to that observed in Australian smelt by Humphries et al. (2013).

The majority of recruits in Colligen Creek (62%) and 42% of recruits in Yallakool Creek were hatched during environmental watering actions, although these percentages were significantly lower (Chi-square both > 5.89; DF=1; P< 0.05) than the percentages of recruits hatched within control rivers. These results could suggest that the average survival of early life history stages of carp gudgeon in Colligen Creek and Yallakool Creek may have been lower than in control rivers following environmental watering actions. This speculation highlights the importance of managing rivers to provide adequate river flow and ecological conditions following environmental watering actions that are targeted for enhancing fish spawning. Some evidence suggests that carp gudgeon recruitment is maximised by low-flow (Humphries et al. 1999) conditions during summer. It is plausible, therefore, that administering multiple environmental flows after spring-time spawning peaks could be detrimental to survival of carp gudgeon early life-history stages.

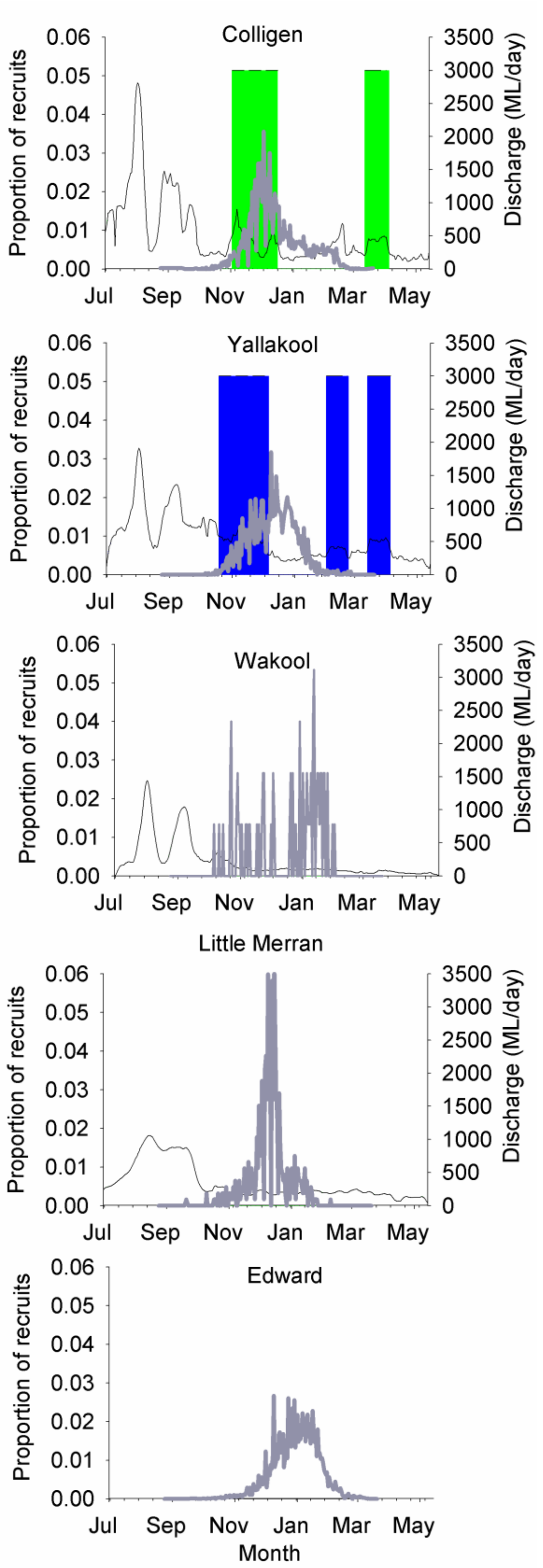
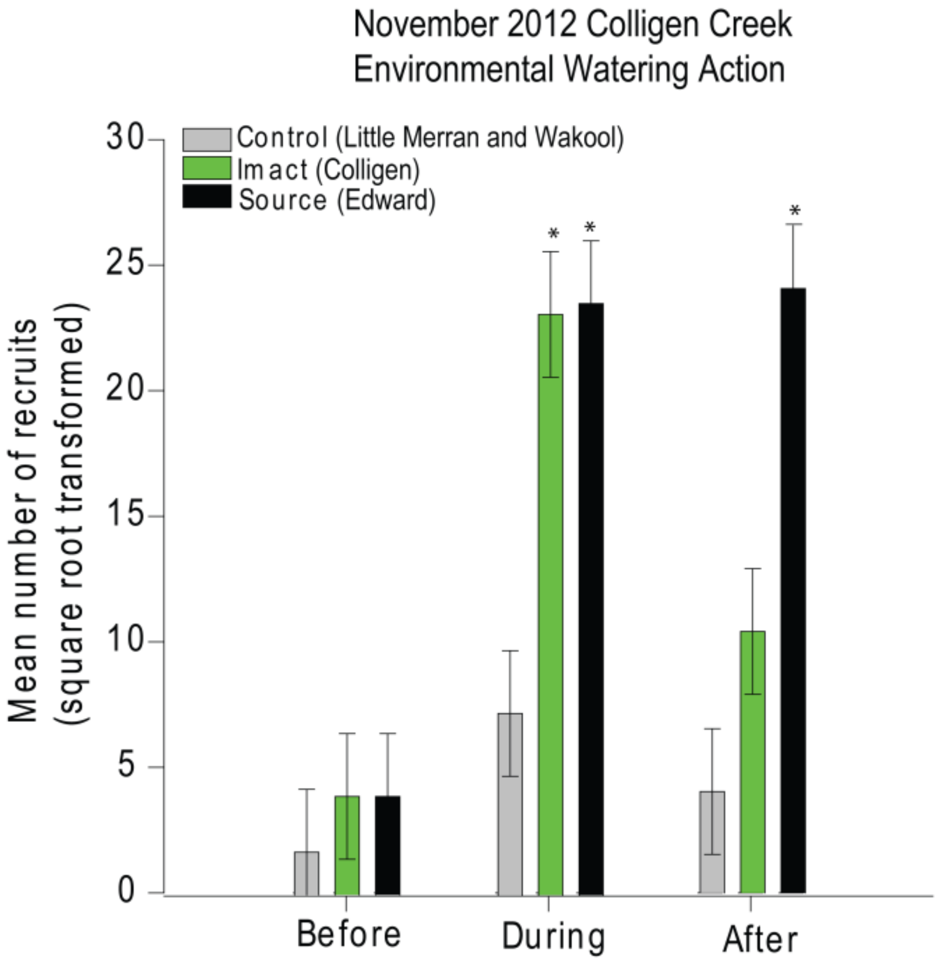


Figure 101. Back-calculated daily hatch dates of carp gudgeon (n=20072) recruits (juveniles and adults) sampled between January and April 2013. Black lines represent daily discharge and grey lines represent the proportion of recruits hatched each day. Green and blue bands represent the timing of environmental watering actions.

Although proportionally fewer carp gudgeon larvae may have survived in the impacted rivers following environmental watering, the mean number of recruit hatch dates generated from the October 2012 Yallakool Creek watering action and the November 2012 Colligen action (TW ANOVA both; F>5.25; P<0.003) were significantly higher (Holm-Sidak both; t > 4.49; P<0.05) compared to control rivers (Figure 102). However, trends in the timing of larval abundance (see section 7.5.1) and hatch dates of recruits within the source river (Edward River) also increased over the same periods of time (Figure 102). This similarity means that it is not possible to determine whether larvae or recruits were generated from within Colligen and Yallakool Creek, or were transported downstream from the Edward River source.

These data lead to two possible conclusions: 1) the magnitude of carp gudgeon recruitment observed in Colligen Creek and Yallakool Creek during 2012-13 was augmented by environmental watering actions, or 2) the number of recruits observed after environmental watering actions in Colligen Creek and Yallakool Creek was attributed to immigration from the Edward River (source). Given that primary productivity and larval fish food availability did not increase in response to environmental watering and the body of empirical evidence suggesting that carp gudgeon are a low-flow recruitment specialist (Humphries et al. 1999), the second conclusion appears to be most probable. In the future it would be useful to investigate how, or if, environmental flows influence downstream dispersal of fish larvae and recruits from the Edward River into distributaries (Colligen Creek, Yallakool Creek, Wakool River) and how this affects native fish populations. Drift net samples did not collect elevated numbers of carp gudgeon larvae or juveniles following environmental watering actions, although sampling closer to the Edward River may help identify changes in the number of individuals entering distributaries.

The observation of environmental watering actions in Colligen and Yallakool Creek during spring resulting in an apparent increase in the early life history stages of carp gudgeon, due either to increased recruitment or downstream dispersal, is a similar result to environmental watering actions in November 2011 within Colligen Creek (Watts et al. 2013). Given that abundances of carp gudgeon larvae and juveniles were lowest in Yallakool Creek compared to all rivers in 2011 (Watts et al. 2013) and that that the total number of recruits increased significantly this year (Section 7.5.1), it appears that increasing river discharge associated with environmental watering actions can increase the number of early life-history stage carp gudgeon sampled in rivers of the Edward-Wakool system.



Source of Variation	*DF*	*SS*	*MS*	*F*	*P*
Control/Impact/Source (CIS)	2	1270.25	635.13	20.29	<0.001
Before/During/After (BDA)	2	1692.24	846.12	27.03	<0.001
CIS x BDA	4	658.91	164.73	5.26	0.002

Figure 102. Two-way ANOVA comparing the mean number of carp gudgeon recruits hatched per day before, during and after the November 2012 Colligen Creek environmental watering action. Asterisks denote significant ($P < 0.05$) differences.

Murray cod

Daily age-length regressions (Figure 103) were developed from readable otoliths of 58 Murray cod larvae, while an additional 13 otoliths were considered unreadable. Due to the narrow range of lengths and small sample sizes of Murray cod larvae, regression fits for the Wakool River and Little Merran Creek were not significant (Table 26). Nonetheless, daily age-length estimates from all rivers fell within range of expected variation based on validated age estimates (Humphries 2005). Aged larvae ranged from 8.6 mm to 12.2 mm SL with a mean length of 9.7 ± 0.71 SD and mean daily age of 11 ± 4 days. No Murray cod recruits were collected using drift nets, light traps or boat tows but the hatch dates of 225 larvae were back-calculated to more closely approximate the timing of spawning in relation to environmental watering actions.

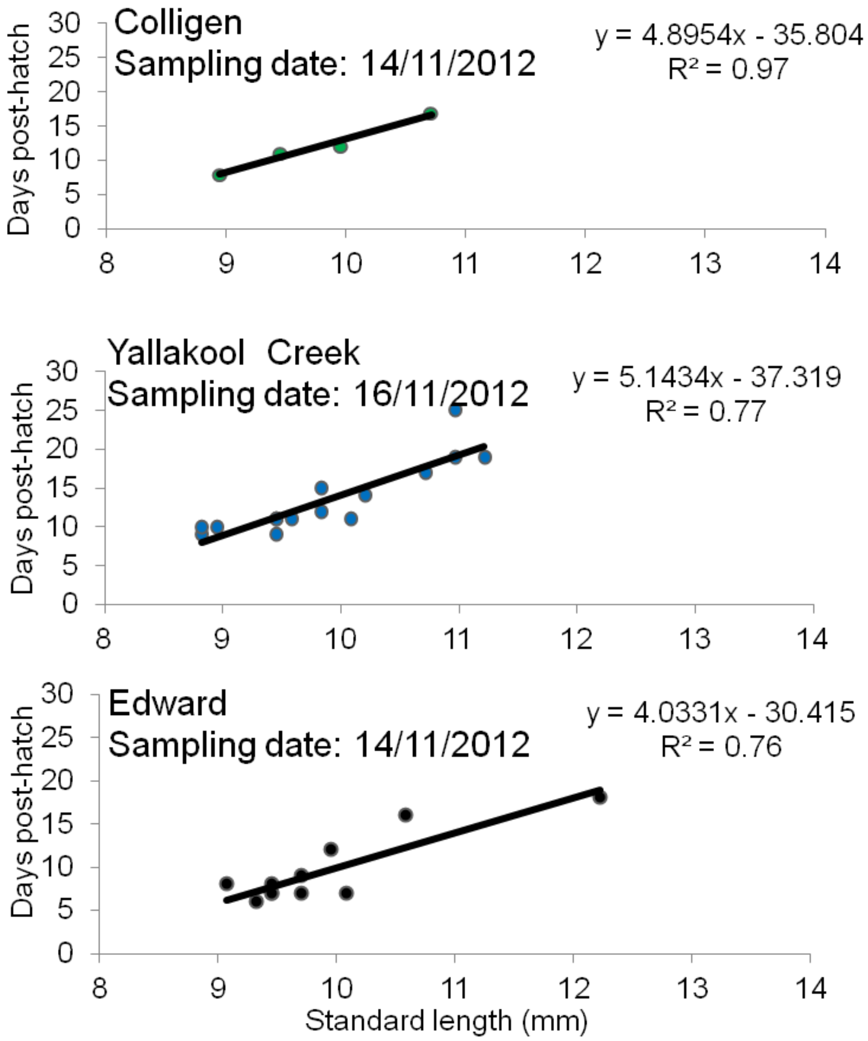


Figure 103. Estimated daily age-length curves for Murray cod sampled in the Edward-Wakool river system in November 2012.

Table 26. Linear regression (daily age = $y_0 + a$ X SL) parameters fitted to Murray cod daily age-standard length (*SL*) data used to back-calculated hatch dates. Parameter estimates ± Standard Error (SE).

Month	*n*	*r2*	*yo*	*SE*	*a*	*SE*	F	*P*
Colligen	4	0.97	-35.80	6.03	4.90	0.62	63.13	0.02
Yallakool	14	0.77	-24.35	6.29	3.75	0.65	33.26	0.0002
Edward	12	0.76	-30.42	7.52	4.03	0.76	28.41	0.0005

Back-calculated larval hatch dates of Murray cod ranged from 16 October to 4 December 2012 (Figure 104) and there were no significant differences in median hatch date among rivers. No Murray cod recruits were sampled using drift nets, light traps or boat tows but the hatch-dates of recruits (n=10) sampled in annual fisheries surveys (see section 7.5.3) fell within the seasonal timing of spawning estimated above.

There was a small peak in Murray cod larvae hatching around 20 October and a larger peak around 4 November 2012 (Figure 104); both near the onset environmental watering actions in Colligen Creek and Yallakool Creek. However, there were no significant changes in mean abundance of Murray cod hatch dates among control or impacted river reaches for either of the environmental watering actions in Yallakool Creek or Colligen Creek. These data corroborate results from fortnightly abundance of larvae (see section 7.5.1) and the peer-reviewed literature (Humphries et al. 1999; Humphries 2005) further suggesting that spawning of Murray cod is unrelated to river discharge. However, it remains uncertain whether environmental flows affected recruitment success of large bodied species in the Edward-Wakool system. It was not possible to evaluate whether environmental watering actions influenced recruitment success of Murray cod given that only 10 individuals were sampled in fish community surveys. Additional efforts targeting young-of-year recruits of large bodied species including Murray cod, golden perch and silver perch are warranted and could include back-pack electrofishing or community-based angling events. More effective sampling of young-of-year large bodied species will be needed to statistically evaluate whether future environmental watering actions influence recruitment success of these species.

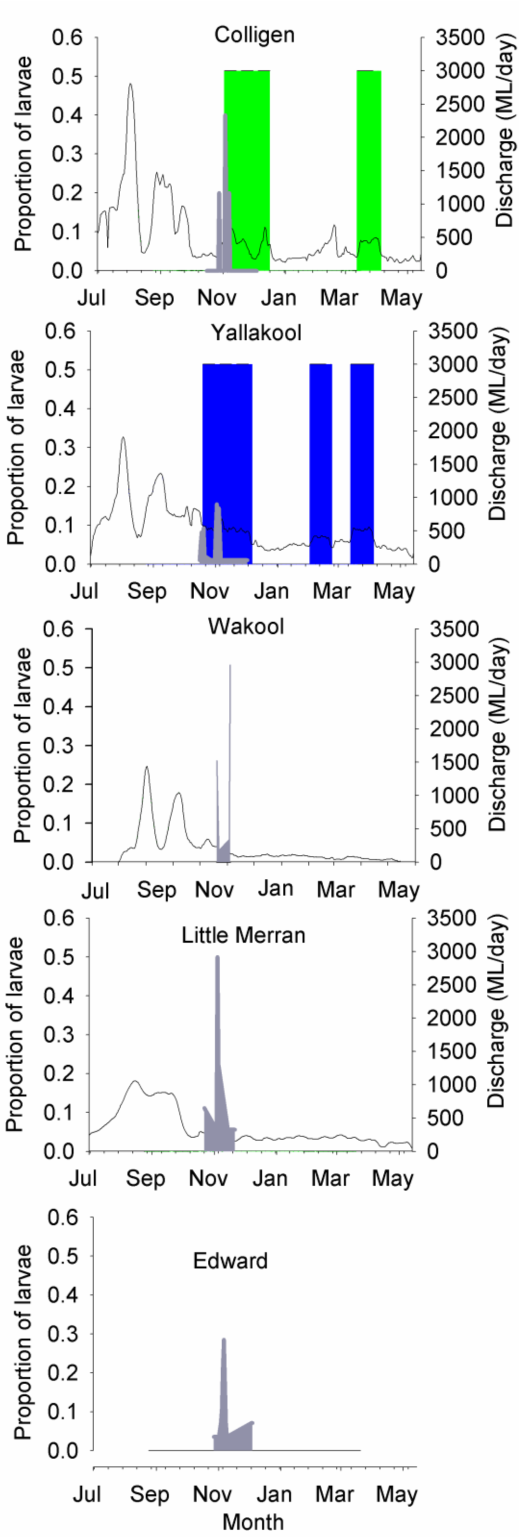


Figure 104. Back-calculated daily hatch dates of Murray cod larvae (n=225) sampled in the Edward-Wakool system in 2012. Black lines represent daily discharge and grey lines represent the proportion of larvae hatched each day. Green and blue bands represent the timing of environmental watering actions

Fish community recruitment pilot study

A total of 391 otoliths from seven species were prepared for age analysis to determine if hatch dates could be compared with the timing of environmental watering actions. Of the aged fish, 100 were estimated to be greater than 1 year old and 14 of these were considered unreliable age estimates. Daily ages of 277 juvenile fish were determined including: 88 Australian smelt, 28 goldfish, 8 carp, 10 Murray cod, 119 carp gudgeon and 24 bony herring. Hatch dates were estimated and plotted for all species (Figure 105-108) but given the small sample size available for each species/month/river combination and the lack of validated age estimates for most species, these data were not used to examine the effects of environmental flows on fish recruitment. These data do, however, illustrate the wide seasonal range of recruit hatch dates among species and how this information could be used to examine the effects of environmental flows. Based on these results, the influence of future environmental watering actions could be evaluated using back-calculated hatch dates across a wide variety of target species and non-native fishes. In order to apply this methodology to evaluate the outcomes of future environmental watering actions, age-length curves need to be established and validated for each target species and these data could then be extended to fish community length data to estimate periods of peak recruitment.

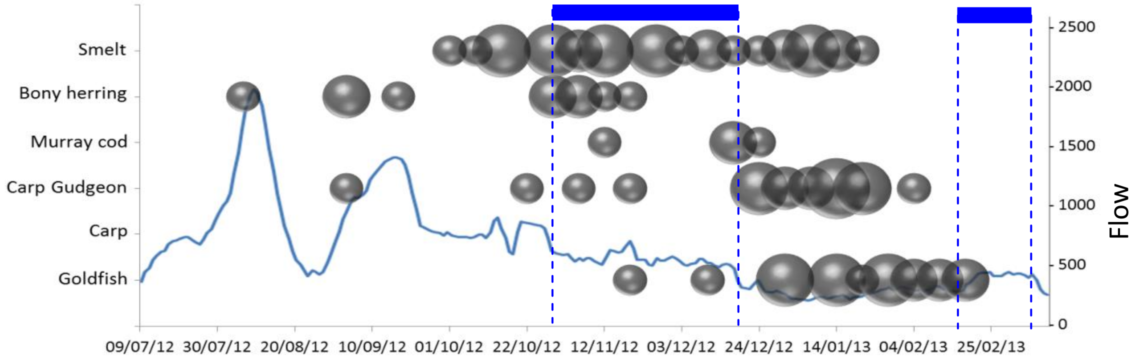


Figure 105. Spawning numbers (bubble sizes) from all sites situated on Yallakool Creek, per species (left axis). Yallakool flow is on right axis. Environmental flow period is highlighted.

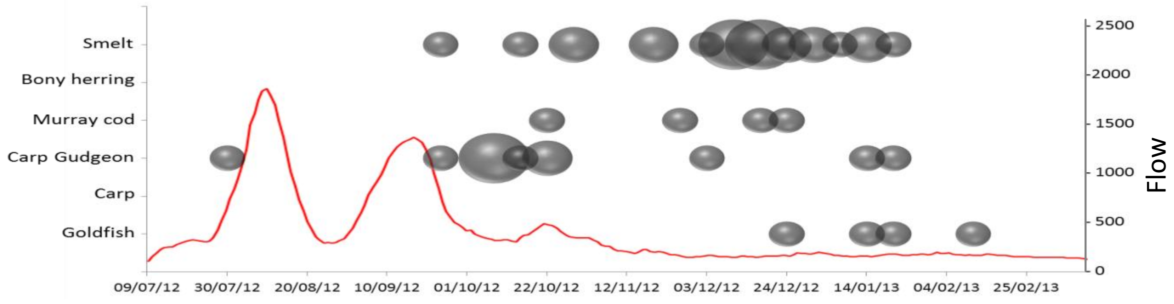


Figure 106. Recruit numbers (bubble sizes) from all sites on the Wakool River (except Wakool Reserve), per species. Wakool River flow is on the right axis. (note; no environmental water delivered to the Wakool)

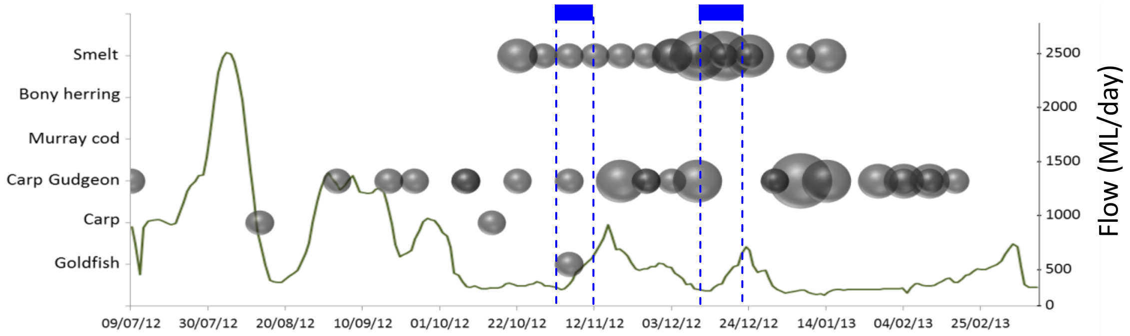


Figure 107. Recruit numbers (bubble sizes) from all sites on Colligen Creek, per species. Colligen Creek flow is on the right axis.

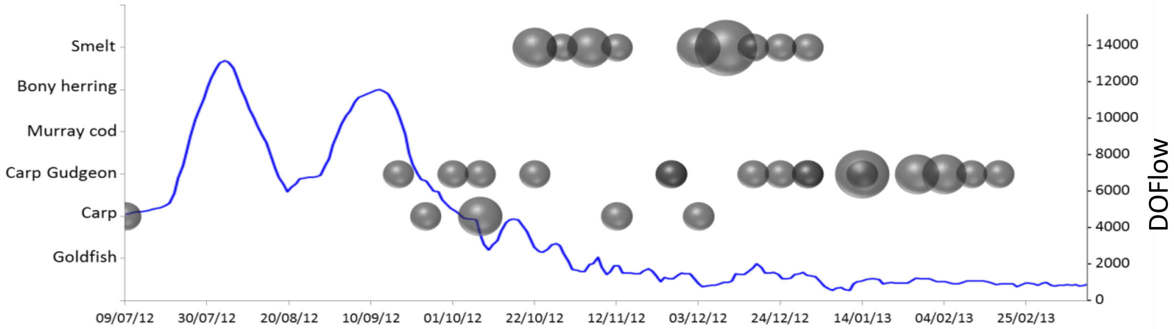


Figure 108. Recruit numbers (bubble sizes) from all sites in the Werai, per species. Edward River flow on the right axis.

7.5.3.Long-term fish community structure (2010-2013)

Key findings

- *The analysis of the fish community from 2010 to 2013 recorded nine of the 21 species thought to occur in the central Murray region of the Murray-Darling Basin prior to European settlement.* Apex predators and flow specialists had higher biomass in channel habitat and flow generalists had higher biomass in wetland habitat.
- *Flooding and subsequent blackwater events in 2010 and 2011 are still having a strong influence on fish community structure.* Blackwater events in 2010 triggered fish kills throughout the system with the exception of the upstream zone where irrigation outfalls were used to deliver environmental water to improve water quality. Murray cod largely disappeared from all but the upstream sections of this system.
- *There has been a small recovery of native fish populations in the system since the blackwater events, however there has been limited recruitment.* Successive years of environmental water delivery targeted at Murray Cod recruitment in the upper zone may have contributed to recovery of the fish community through connection of critical habitat, maintenance of low flow refuges and providing conditions to promote dispersal of individuals to recolonise areas impacted by the blackwater events. *However, the environmental watering in 2012-13 did not trigger widespread recruitment that is necessary for population growth.*
- The lack of evidence for recent recruitment of flow dependent specialists such as golden perch and silver perch suggests that watering strategies have been insufficient to trigger recruitment in these species.

Background

A system-wide monitoring program was established in 2010 to provide baseline information on native fish population status in the Edward-Wakool system and inform environmental water delivery and habitat (e.g. refuge pool) management. The program involved establishing long-term fish monitoring sites that have been sampled consecutively for four years. The overall objective was to determine fish community outcomes from environmental water delivery, including learning from the flow regime delivered within a watering year, and to also examine long term change trajectories.

Unregulated flooding events in the summer of 2010-11 provided information on fish community transition from one largely dependent on drought refuge, to one that responded to a large natural flooding event. This has provided improved understanding of the effect of flow regimes on the fish population, and the benefits of environmental water delivery to the system both during drought, flood (including hypoxic blackwater), and post flood years.

Effective delivery of environmental water requires knowledge and understanding of fish community response to flow. Within the Edward Wakool system a lack of information is precluding effective fisheries management. For example, there is little information on spatial fish community distribution within the system, no data on habitat responses to flow management during drought or high flows, and little data on impacts and recovery from hypoxic blackwater events. The present study sought to gather information on each of these areas to help to progress management outcomes in the short term. The aims of the fish population surveys include:

1. To determine whether peaks in fish abundance and biomass align with years in which environmental watering occurred
2. To determine if environmental water delivery can assist in recovery of populations that were negatively impacted by blackwater events at the end of the milleni um drought in 2009. The release of environmental water is expected to increase the abundance of native fish in blackwater affected zones.
3. To determining if exotic pest fish (e.g. carp) distribution and abundance respond to water delivery actions.

Methods

Water quality parameters (pH, dissolved oxygen, temperature, turbidity) were recorded at each site using a Model U10 Horiba multi-probe water quality meter. Readings were taken at the surface, 0.5 m, 1 m and then at 1 m increments until the bottom was reached. The water quality readings are only short-term indicators but can give a strong appreciation of unseasonal stratification, particularly within deep pool habitat.

Fish were collected from 43 sites within the Edward Wakool system, with sampling occurring annually between 2010 and 2013. Sites were stratified between wetland and channel habitats to determine the role of these different habitat types in supporting fish communities. These habitats were further stratified by position in the system; upper, middle and lower and a Werai zone) to capture any potential differences that may arise over a larger spatial scale (Figure 109). These sites provide data on the distribution, abundance and diversity of juvenile and adult fish in the system.

Fish were collected using a standardised electrofishing (Figure 110),protocol including 10 bait traps (Figure 110) established by the Sustainable Rivers Audit (SRA) for the Murray Darling Basin (Davies et al. 2010). All bait traps were baited with cat food to enhance the potential capture of southern pygmy perch. This was augmented by a netting strategy to capture any cryptic species that may have been present. This involved setting two 15 m long (2 m drop) monofilament multi panel gill nets with single 35, 75, 100 mm mesh panels for two hours (Figure 111). Additionally, two 3 mm dual wing fyke nets and two 25 mm single wing fyke nets were set and retrieved the following day to encompass diurnal periods (Figure 112). At the completion of each electrofishing and netting operation, all fish were identified, counted and measured (maximum of 50 individuals per species per shot). A sub sample of suspected young-of-year fish collected during the course of routine sampling, euthanized and preserved for age recruitment analyses (see section 7.5.2 on fish recruitment).

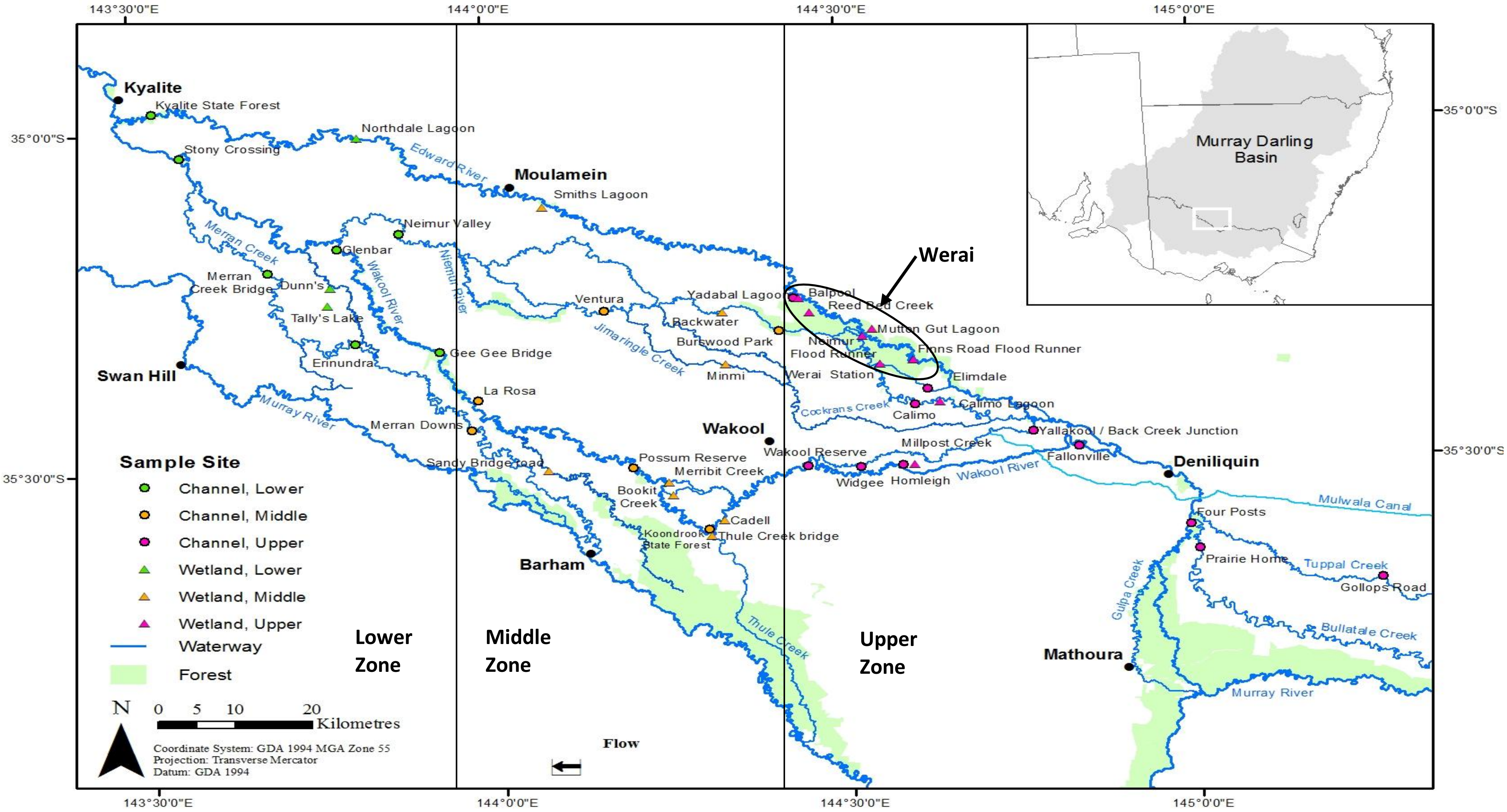


Figure 109. The Edward-Wakool River system highlighting all major rivers and creeks including all zones, and sampling sites delineated by habitat type.

Calculating fish health metrics

Winemiller and Rose (1992) identified a classification system for North American fish and determining likely responses to flows. The work largely identified that fish differ in expected responses to different flow scenarios which are based on specific ecological needs. King et al. (2013) further extrapolated these concepts to the reproductive life history of Murray-Darling fish species. However, neither author extended this work to develop specific hydrographs which link complex ecological processes with flow events. Baumgartner et al. (2013) extended this earlier work by defining environmental water delivery requirements of different groups. Recognising that one flow would not be sufficient to meet the needs of all species, fish with similar trophic and reproductive strategies were grouped, functionally, and hydrographs were developed to guide water delivery planning. Four native fish flow groups and one general alien species group are used; foraging (flow) generalists, apex predators, flow dependant specialists, floodplain (off-channel) specialists and alien species. Rationale for these groupings and nomenclature are presented in Baumgartner et al. (2013).

Fish community data was summarised to compare results initially to three main SRA Indicators (these are fully explained in Robinson 2012). The SRA derived Indicators calculated were; Expectedness (provides a comparison of existing catch composition with historical fish distributions), Nativeness (combination of abundance and biomass describing the proportion of the community comprised of native fish), and Recruitment (provides a proportion of the entire native fish population that is recruiting within a zone). Recruitment was further divided; recruiting taxa (proportion of native species present recruiting), and recruiting sites (proportion of sites where recruitment occurs). These Indicators produce a score that is related to Reference conditions, and receive a condition rating (Extremely Poor (0-20), Very Poor (21-40), poor (41-60), Moderate (61-80), Good (81-100).

Due to natural flow attenuation, hydrology of the Edward-Wakool system varies in an upstream to downstream direction. Flows in the lower end of the system are consequently lower and it was hypothesised that this may have contributed to some flow-related fish community structuring. It was also hypothesised that fish communities differ at local scales between channel and wetland habitat. Both of these factors were likely to influence spatial structure in fish communities, which may vary on an annual scale depending on catchment conditions. To account for these factors, fish community structure was analysed using PERMANOVA (PRIMER, with region (upper, middle and lower), habitat type (wetland or channel) and year (2010, 2011, 2012 or 2013) as fixed factors. Tests were performed using 999 Monte Carlo randomisations to calculate approximate probabilities.

Figure 110. (2a left) Boat electrofishing used to sample juvenile and adult fish communities. (2b right) Bait traps baited with canned cat food are set to sample small fish not efficiently sampled during routine electrofishing.

Figure 111. Monofilament multi panel gill nets 15 metre long (2 metre drop) with single 35, 75, 100 mm mesh panels are set for 2 hours at survey sites.

Figure 112. Large mesh single wing and small mesh dual wing fyke nets are set and retrieved the following day to encompass diurnal periods.

Results and discussion

Hydrology and water quality

For nine years prior to August 2010 the Edward-Wakool system experienced a period of severe drought with extended low flows, and extreme low flows between 2007 and 2009. In August 2010 system-wide flooding occurred with widespread inundation and overbank flows (Figure 113). Lasting several weeks, flow exceeded bankfull capacity in September 2010 in the upper Edward, the upper Wakool River, Yallakool Creek, Merran Creek and the Upper Colligen-Niemur system. A follow up unregulated high flow occurred in November-December 2010 which resulted in hypoxic blackwater fish kill (particularly in Murray cod). Fish kills were observed throughout the system but were most significant in the middle and lower zones, including Werai zone. Unregulated high flow events have been occurring in most of the system each spring since the initial flooding in August 2010, but no further fish kills have been associated with these events.

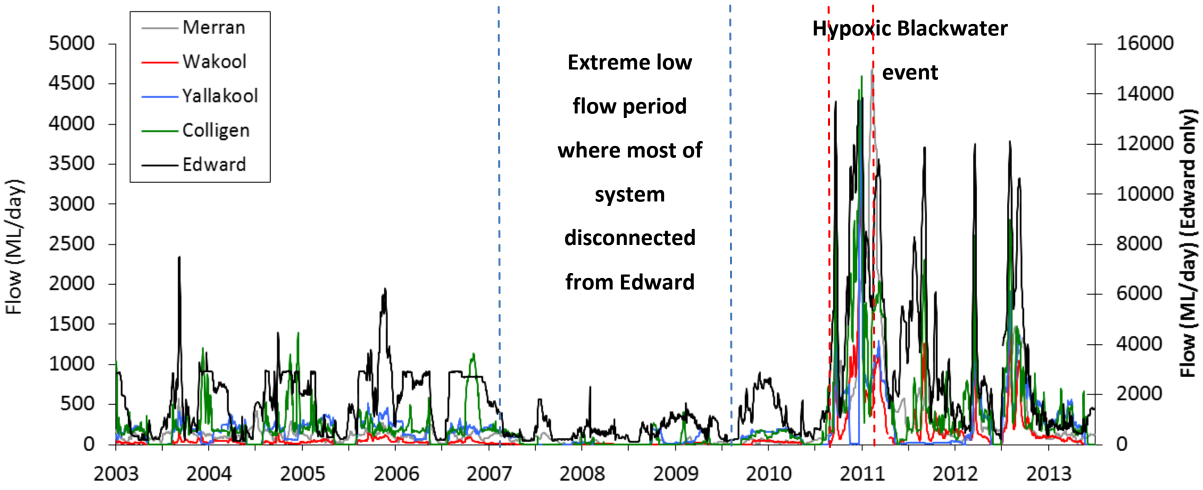


Figure 113.. Hydrograph for the Edward River (right axis) and Wakool, Yallakool, Colligen-Niemur-Merran (left axis) between 2003 and 2013. The drought period extends from 2003 to August 2010, with extreme low flow period between 2007 to 2009. In August 2010 flooding was experienced throughout the system. In December 2010 follow up overbank flooding occurred and hypoxic blackwater fish kills occurred in the entire system.

Surface (top 20 cm) water quality readings were consistent among sites. However, during sampling in 2010, water quality deteriorated within the lower zone at certain sites (example given at Stoney Crossing, Figure 114). Dissolved oxygen, pH, salinity and temperature were significantly different in the lower water column because of stratification during low flows and disconnection during the drought. Flooding caused complete mixing of the water column and water quality at these affected sites in the lower zone returned to safe levels for fish in 2011, and has persisted since.

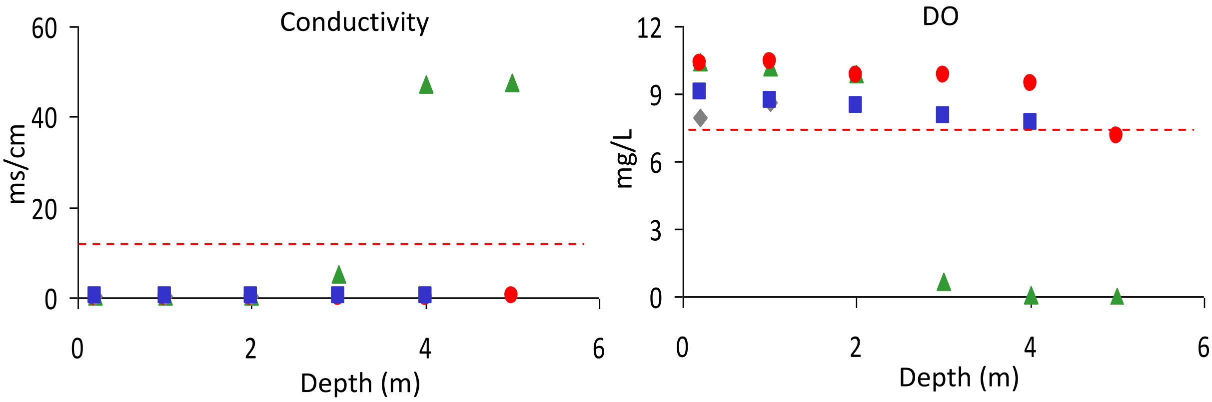


Figure 109. Electrical conductivity, and dissolved oxygen water quality parameters at each metre of water at Stony Crossing (lower zone site). in 2010 (green triangle), 2011 (red circle), 2012 (grey diamond), and 2013 (blue square). Figure depicts the change in water quality with depth and demonstrates stratification despite these readings being taken in July 2010, and water quality after significant mixing in subsequent sampling years (2011 and 2013). Red line denotes levels that are considered harmful to native fish.

Fish results

There were 18,909 fish collected in total from 2010 to 2013; 6,392 in 2010, 5,618 in 2011, 2,186 in 2012, and 4,713 in 2013 (Table 27, Table 28). Nine native species and five alien species were collected in total over the sampling period (Table 27). Individuals from three of the four native flow guilds were represented (Figure 115), as no floodplain specialists were sampled in any year. Flow generalists dominated the total and relative abundances in all zones and habitat types in 2010, but following flooding in 2010-11 the alien group dominated total and relative abundance for many of the zones (Table 27). The alien group (especially carp and goldfish) dominated total and relative biomass in all zones and habitat types in all years. Substantial increases in total and relative biomass occurred in this group after the 2010-11 flooding, but abundance declined in subsequent years (Table 27). Nine of the 21 species thought to occur in the central Murray region of the Murray-Darling Basin prior to European settlement were recorded during this annual survey (Table 29), and one additional species, freshwater blackfish, has been collected as larvae (section 7.5.2).

Table 27. Total number of fish caught in each year of sampling and the mean (and standard error in parenthesis) number of fish per site per year.

	2010				2011				2012				2013			
Species	Channel		Wetland		Channel		Wetland		Channel		Wetland		Channel		Wetland	
Apex Predators																
Murray cod	**101**	(14±4)	**14**	(7±7)	**40**	(6±2)	**0**	(0±0)	**47**	(6±3)	**0**	(0±0)	**70**	(4±1)	**8**	(8±0)
Flow dependent specialists																
Silver perch	**2**	(2±0)	**4**	(2±2)	**2**	(2±0)	**1**	(1±1)	**4**	(1±0)	**0**	(0±0)	**8**	(1±0)	**2**	(1±0)
Golden perch	**36**	(5±2)	**24**	(12±12)	**33**	(5±2)	**4**	(2±1)	**38**	(5±2)	**4**	(2±1)	**55**	(3±1)	**8**	(2±1)
Flow Generalists																
Australian Smelt	**850**	(121±43)	**15**	(8±8)	**1021**	(146±71)	**388**	(194±188)	**427**	(53±34)	**137**	(69±58)	**410**	(17±5)	**152**	(19±11)
Bony Herring	**32**	(5±2)	**67**	(34±34)	**30**	(4±3)	**4**	(2±2)	**44**	(6±4)	**3**	(2±1)	**98**	(8±2)	**11**	(4±1)
Carp Gudgeon	**647**	(92±53)	**939**	(470±165)	**231**	(33±11)	**254**	(127±23)	**128**	(16±4)	**374**	(187±182)	**1813**	(121±116)	**685**	(57±25)
Flat-headed gudgeon	**0**	(0±0)	**3**	(2±2)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)	**16**	(5±2)
Murray Rainbowfish	**865**	(124±49)	**142**	(71±15)	**530**	(76±25)	**149**	(75±70)	**21**	(3±1)	**20**	(10±8)	**11**	(2±1)	**8**	(3±1)
Un-specked hardyhead	**1210**	(173±79)	**314**	(157±126)	**285**	(41±24)	**118**	(59±55)	**2**	(0±0)	**1**	(1±1)	**13**	(7±4)	**6**	(2±1)
Yabby	**2**	(0±0)	**5**	(3±1)	**59**	(8±6)	**1**	(1±1)	**8**	(1±1)	**1**	(1±1)	**1**	(1±0)	**0**	(0±0)
Alien Species																
Carp	**300**	(43±18)	**653**	(327±247)	**1414**	(202±100)	**143**	(72±22)	**574**	(72±16)	**264**	(132±17)	**733**	(28±6)	**454**	(28±2)
Gambusia	**27**	(4±1)	**101**	(51±45)	**48**	(7±4)	**4**	(2±0)	**1**	(1±0)	**0**	(0±0)	**1**	(1±0)	**7**	(2±1)
Goldfish	**106**	(15±5)	**41**	(21±14)	**872**	(125±85)	**22**	(11±9)	**113**	(14±7)	**18**	(9±6)	**137**	(10±3)	**79**	(7±2)
Oriental weatherloach	**0**	(0±0)	**0**	(0±0)	**3**	(3±0)	**0**	(0±0)	**1**	(1±0)	**0**	(0±0)	**0**	(0±0)	**2**	(2±0)
Redfin perch	**7**	(1±1)	**0**	(0±0)	**2**	(2±0)	**0**	(0±0)	**3**	(3±0)	**0**	(0±0)	**1**	(1±1)	**2**	(2±0)

Table 28. Total number of fish caught 2013 and the mean (and standard error in parenthesis) number of fish per site.

2013 Summary	Upper				Middle				Lower				Werai			
Species	Channel		Wetland		Channel		Wetland		Channel		Wetland		Channel		Wetland	
Apex Predators																
Murray cod	**50**	(7±2)	**0**	(0±0)	**10**	(3±2)	**8**	(8±0)	**8**	(8±0)	**0**	(0±0)	**2**	(1±0)	**0**	(0±0)
Flow dependent specialists																
Siver perch	**6**	(2±0)	**0**	(0±0)	**10**	(1±0)	**2**	(1±0)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)
Golden perch	**25**	(4±1)	**0**	(0±0)	**2**	(3±1)	**7**	(2±1)	**18**	(4±1)	**1**	(1±0)	**2**	(2±0)	**0**	(0±0)
Flow Generalists																
Australian Smelt	**228**	(23±9)	**103**	(34±31)	**102**	(20±14)	**38**	(13±2)	**38**	(6±2)	**1**	(1±0)	**42**	(14±8)	**10**	(10±0)
Bony Herring	**46**	(22±11)	**0**	(0±0)	**8**	(3±1)	**11**	(4±1)	**38**	(4±2)	**0**	(0±0)	**6**	(6±0)	**0**	(0±0)
Carp Gudgeon	**1801**	(200±193)	**193**	(97±1)	**2**	(1±0)	**79**	(16±9)	**8**	(4±3)	**100**	(33±26)	**2**	(1±0)	**313**	(157±150)
Flat-headed gudgeon	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)	**1**	(1±0)	**0**	(0±0)	**9**	(9±0)	**0**	(0±0)	**6**	(6±0)
Murray-Darling Rainbowfish	**9**	(2±1)	**2**	(2±0)	**1**	(1±0)	**6**	(3±1)	**1**	(1±0)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)
Un-specked hardyhead	**10**	(10±0)	**3**	(3±0)	**0**	(0±0)	**3**	(2±1)	**0**	(0±0)	**0**	(0±0)	**3**	(3±0)	**0**	(0±0)
Alien Species																
Carp	**434**	(43±13)	**198**	(50±16)	**124**	(21±6)	**227**	(32±15)	**86**	(12±2)	**24**	(8±4)	**89**	(29±10)	**5**	(3±1)
Gambusia	**1**	(1±0)	**1**	(1±0)	**0**	(0±0)	**1**	(1±0)	**0**	(0±0)	**1**	(1±0)	**0**	(0±0)	**4**	(4±0)
Goldfish	**110**	(18±6)	**18**	(9±1)	**3**	(2±1)	**51**	(9±4)	**23**	(5±2)	**10**	(4±1)	**1**	(1±0)	**0**	(0±0)
Redfin Perch	**2**	(2±0)	**0**	(0±0)	**0**	(0±0)	**2**	(2±0)	**1**	(1±0)	**0**	(0±0)	**0**	(0±0)	**0**	(0±0)

Un – specked hardyhead Carp gudgeon Flat-headed gudgeon

Redfin perch Australian smelt Golden perch

Goldfish Oriental weatherloach Bony Bream

Figure 115. Some of fish species caught during fish community sampling in the Edward-Wakool. (Images of Murray cod, silver perch and common carp appear elsewhere in this document).

Table 29. PERCH list (pre-European) of expected native species for the central Murray region of the Murray-Darling Basin

Common Name	**Species Name**	**Found in present study**			
		2010	2011	2012	2013
Flow Generalists					
Freshwater catfish	*Tandanus tandanus*	N	N	N	N
Bony herring	*Nematalosa erebi*	Y	Y	Y	Y
Freshwater blackfish	*Gadopsis marmoratus*	N	N	N	N
Shortheaded lamprey	*Mordacia mordax*	N	N	N	N
Macquarie perch	*Macquaria australasica*	N	N	N	N
Murray-Darling rainbowfish	*Melanotaenia fluviatilis*	Y	Y	Y	Y
Un Specked hardyhead	*Craterocephalus stercusmuscarum*	Y	Y	Y	Y
Murray hardyhead	*Craterocephalus fluviatilis*	N	N	N	N
Australian smelt	*Retropinna semoni*	Y	Y	Y	Y
Carp gudgeon	*Hypseleotris* spp	Y	Y	Y	Y
Flatheaded gudgeon	*Phylipnodon grandiceps*	Y	N	N	Y
Dwarf Flatheadedgudgeon	*Philypnodon macrostomus*	N	N	N	N
Apex Predators					
Murray cod	*Maccullochella peelii*	Y	Y	Y	Y
Trout cod	*Maccullochella macquariensis*	N	N	N	N
Flow Dependent Specialists					
Golden perch	*Macquaria ambigua*	Y	Y	Y	Y
Silver perch	*Bidyanus bidyanus*	Y	Y	Y	Y
Floodplain (or Off Channel) specialists					
Southern pygmy perch	*Nannoperca australis*	N	N	N	N
Southern Purple Spotted gudgeon	*Mogurnda adspersa*	N	N	N	N
Flatheaded galaxias	*Galaxias rostratus*	N	N	N	N
Olive perchlet	*Ambassisagissizi*	N	N	N	N
Mountain galaxias	*Galaxias olidus*	N	N	N	N

Sustainable Rivers Audit (SRA) Indicators

Expectedness was above 70% (moderate condition rating) in all zones in 2010, but decreased in subsequent years of sampling. Each zone (except Werai zone) had fallen below 50% (poor condition rating) by 2013 (Table 30). Although many expected species were present, the moderate rating for 2010 is largely because floodplain specialists remain undetected. Expectedness in the Werai zone increased each sampling year (Table 30) indicating that successive inundation events may be providing a benefit to the wetlands.

Nativeness was above 61% (moderate condition rating) for the upper and lower zones in 2010 but below 50% (poor condition rating) for the middle zone. Nativeness subsequently decreased in every year of sampling in all zones (Table 30). The Werai zone was below 20% (extremely poor condition) in 2011, but improved in 2012 to a 'poor' rating before decreasing in 2013 to below 41% (very poor condition rating). As nativeness is a combination of abundance and biomass, an increase in carp is the primary influence in all zones except Werai . Targeted delivery of water which benefits native fish abundance and biomass or targets removal of carp could be utilised to improve the nativeness rating.

Recruitment (proportion of taxa) remained above 61% (moderate rating) in all zones, in all years, (except for the middle zone in 2011 and Werai 2013). However recruitment (proportion of sites) which was above 61% (moderate) in all zones, had decreased across all zones by 2013. This indicates that most native species present were recruiting across the system as a whole, particularly as the drought broke. Post flooding, most recruitment has been dominated by alien species which spawned after over bank flows in 2011. Following this flood event there has been little recruitment in other species.

Table 30. SRA indicators (Expectedness, Nativeness and two recruitment metrics) for all zones and all years

Location	year	OE_metric	Nativeness	Recruitment (Propotion of taxa)	Recruitment (Proportion of sites)
Upper	2010	74	67	67	64
Upper	2011	67	48	63	56
Upper	2012	58	36	63	52
Upper	2013	48	38	78	50
Middle	2010	76	48	78	71
Middle	2011	53	24	63	53
Middle	2012	57	38	75	63
Middle	2013	47	45	78	44
Lower	2010	80	62	78	73
Lower	2011	63	28	57	57
Lower	2012	56	52	75	66
Lower	2013	48	40	75	58
Werai	2011	23	18	67	65
Werai	2012	37	41	67	49
Werai	2013	35	30	57	48

Fish community structure - Abundance

Based on fish abundance data, fish communities significantly differed among zones (PERMANOVA: Pseudo F = 4.47, $P < 0.001$), habitat type (Pseudo F = 13.24; $P < 0.001$) and years (PERMANOVA: Pseudo F = 8.38; $P < 0.001$). Zone based differences arose largely from differing relative higher abundances of Australian smelt and carp gudgeons from middle sections than upper and lower. Fish community observations in 2011 and 2012 were largely influenced by increases in the abundance of carp gudgeon and Australian smelt from upper wetlands and from higher carp abundances from middle and lower channel habitat. Australian smelt and bony herring were generally more commonly captured in channel habitat. Carp gudgeon, Un-specked hardyhead and Murray-Darling rainbowfish were more commonly collected from wetland habitat.

Ordination using Principal Components Analysis (Figure 116) identified clear groupings based on sampling year, which were largely centred around natural flooding in 2011. For flow generalists carp gudgeon, unspecked hardyhead and Murray-Darling rainbowfish were far more abundant prior to flooding than in subsequent years. Carp gudgeon abundance declined substantially after flooding in 2011 but increased in 2012 and again in 2013.

Carp were the most abundant species in all years except 2010 but peaked following flooding in 2011 (Figure 117). Goldfish abundance also peaked following flooding in 2011 but have declined in each subsequent year. Other alien species contributed little to observed annual differences.

Apex predators were abundant throughout the system in 2010 but declined following hypoxic blackwater events in subsequent years. The group has partly recovered post-flooding but are still in low abundance (Figure 118).

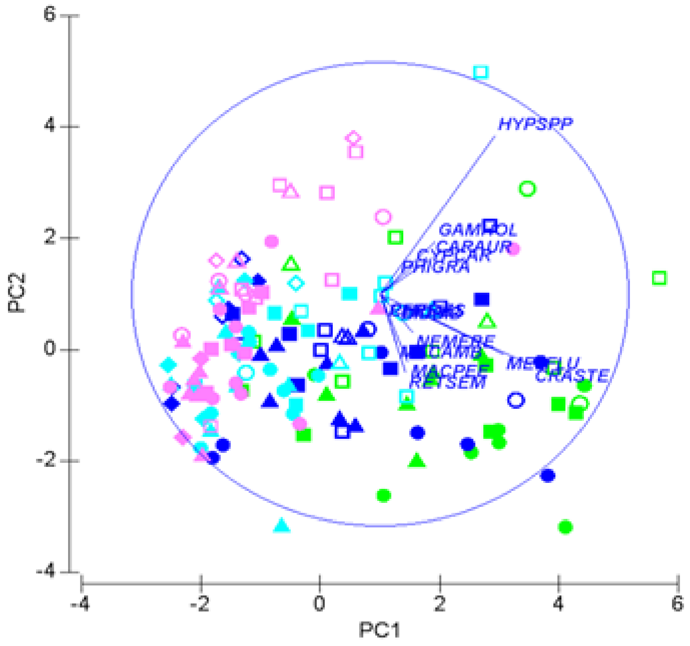


Figure 116. Principal components ordination of fish collection sites in the Edward/Wakool system. Groupings are based on similarity following conversion of standardised abundance to Bray-Curtis similarities measures after pooling electrofishing and netting data and applying a fourth root transformation. Sites are stratified into lower (triangle), middle (square) and upper (circle) geographic regions. Channel sites are solid and wetland sites are hollow. Colour depicts the year of sampling in either 2010 (green), 2011 (dark blue), 2012 (light blue) and 2013 (pink). The overlayed vector plot identifies species contributing to group separation. A longer line suggested a stronger contribution to separation in ordinal space. Species codes refer to carp (cypcar), goldfish (caraur), carp gudgeon (hypspp), yabby (chedes), gambusia (gamhol), Murray Rainbowfish (melflu), un-specked hardyhead (craste), Murray cod (macpee), Australian smelt (retsem), golden perch (macamb), flatheaded gudgeon (phigra) and redfin perch (Perflu).

Fish community structure - Biomass

Fish communities, based on fish biomass data, significantly differed among zones (PERMANOVA: Pseudo F = 6.89, P < 0.001), habitat type (Pseudo F = 12.43; P < 0.001) and years (PERMANOVA: Pseudo F = 6.33; P < 0.001) (Figure 117).

By abundance, there were more small-bodied flow generalist species (Figure 118) than any other across all zones. By biomass, however, carp and goldfish dominated catches in each zone but more were collected from upper zones (Figure 119). Apex predators (Figure 120) and flow specialists (Figure 121) contributed more to overall catches from the lower zone than upper or middle.

Differences among habitat types were largely driven by golden perch and Murray cod. These species were far more abundant in channel habitat than wetland suggesting that in channel flow delivery would be much more efficient at maintaining habitat quality and for stimulating movement. Carp biomass was generally higher within channel but goldfish biomass was greater from wetland habitat.

Significant differences in biomass among years were largely due to an increase in carp and goldfish following flooding in 2011 (Figure 118). In addition, Murray cod, Murray-Darling rainbowfish, Australian smelt and Golden perch were in substantially greater abundance prior to the widespread hypoxic blackwater event. Murray cod were not collected in 2011 and few in 2012 (Figure 119). Many larger fish were collected in 2013 demonstrating that the species is recovering.

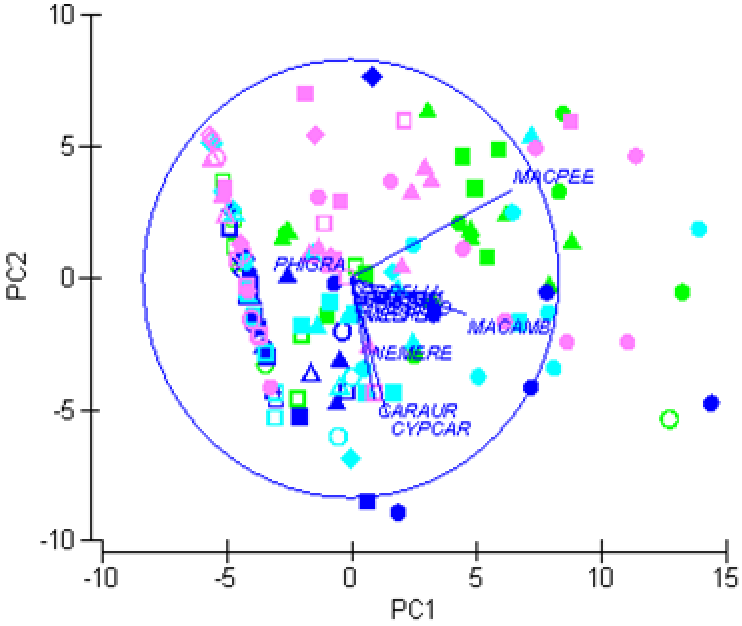


Figure 117. Principal components ordination of biomass of fish collection sites in the Edward/Wakool system. Groupings are based on similarity following conversion of standardised biomass to Bray-Curtis similarities measures after pooling electrofishing and netting data and applying a fourth root transformation. Sites are stratified into lower (triangle), middle (square) and upper (circle) geographic regions. Channel sites are solid and wetland sites are hollow. Colour depicts the year of sampling in either 2010 (green), 2011 (dark blue), 2012 (light blue) and 2013 (pink). The overlayed vector plot identifies species contributing to group separation. A longer line suggested a stronger contribution to separation in ordinal space. Species codes refer to carp (cypcar), goldfish (caraur), carp gudgeon (hypspp), yabby (chedes), gambusia (gamhol), Murray Rainbowfish (melflu), un-specked hardyhead (craste), Murray cod (macpee), Australian smelt (retsem), golden perch (macamb), flatheaded gudgeon (phigra) and redfin perch (Perflu).

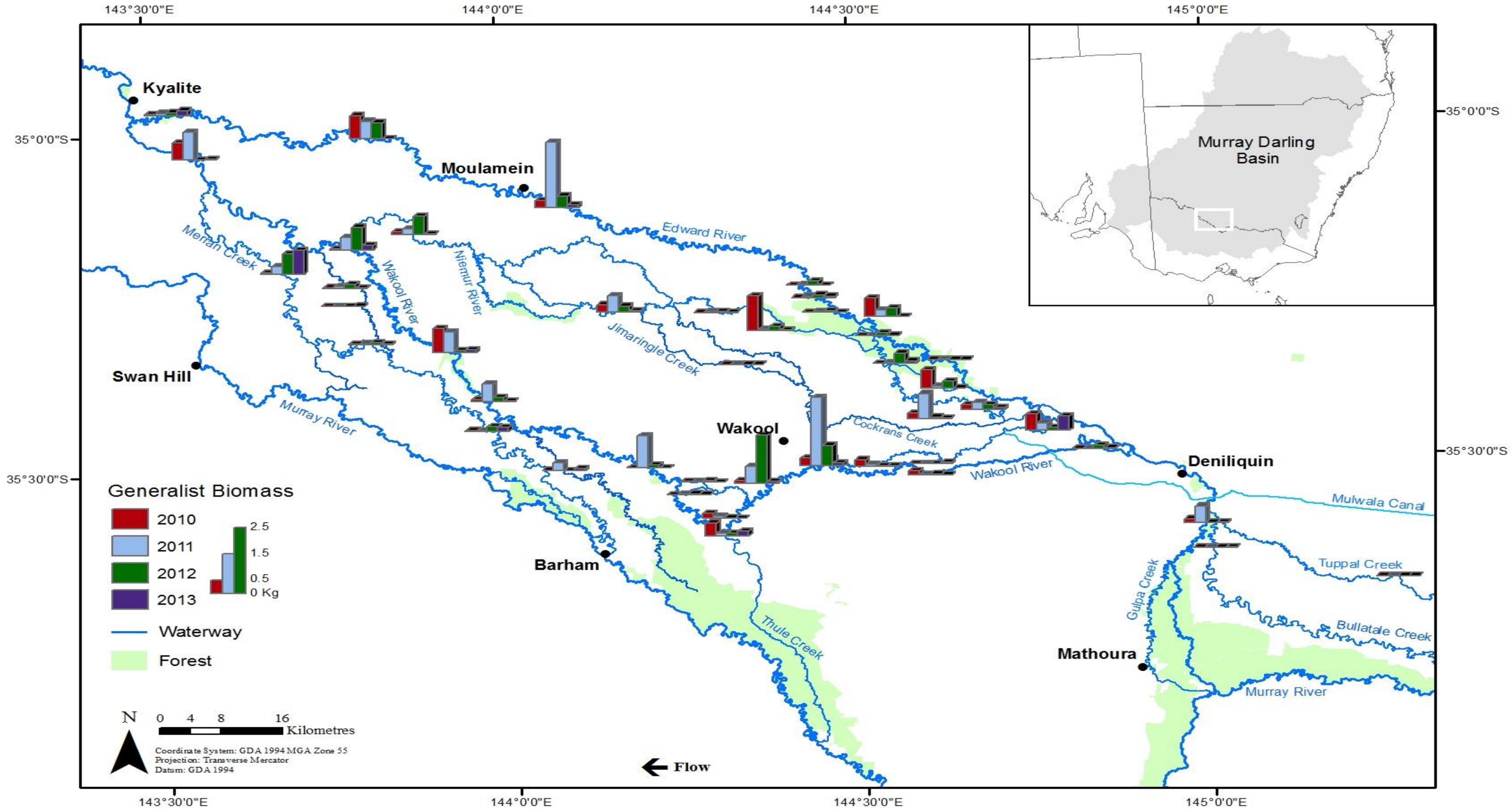


Figure 118. Total biomass of flow generalist species (biomass pooled among species) caught at each sampling site in the Edward-Wakool river system (2010-2013).

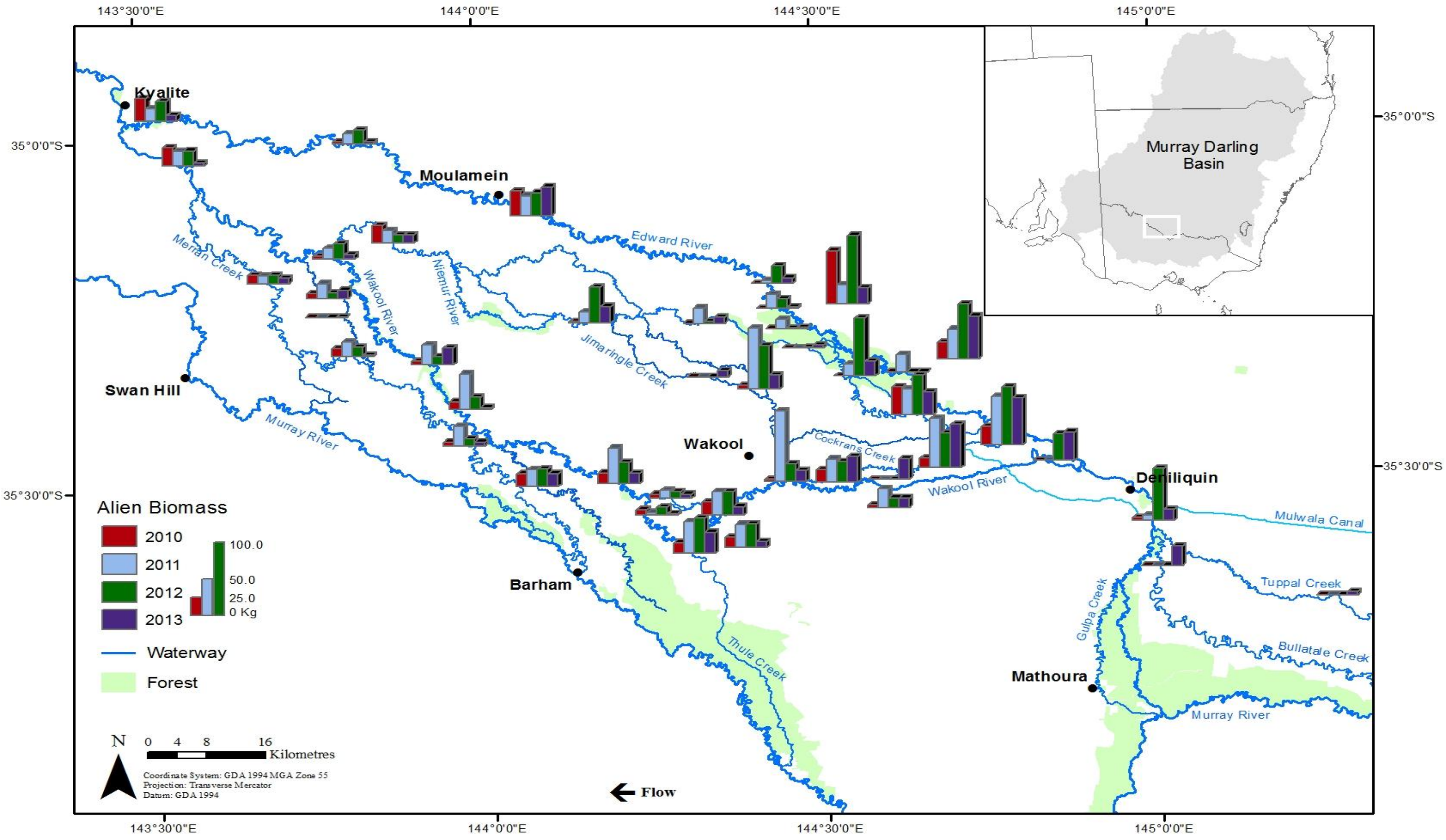


Figure 119. Total biomass of alien species (biomass pooled among species) caught at each sampling site in the Edward-Wakool river system (2010-2013).

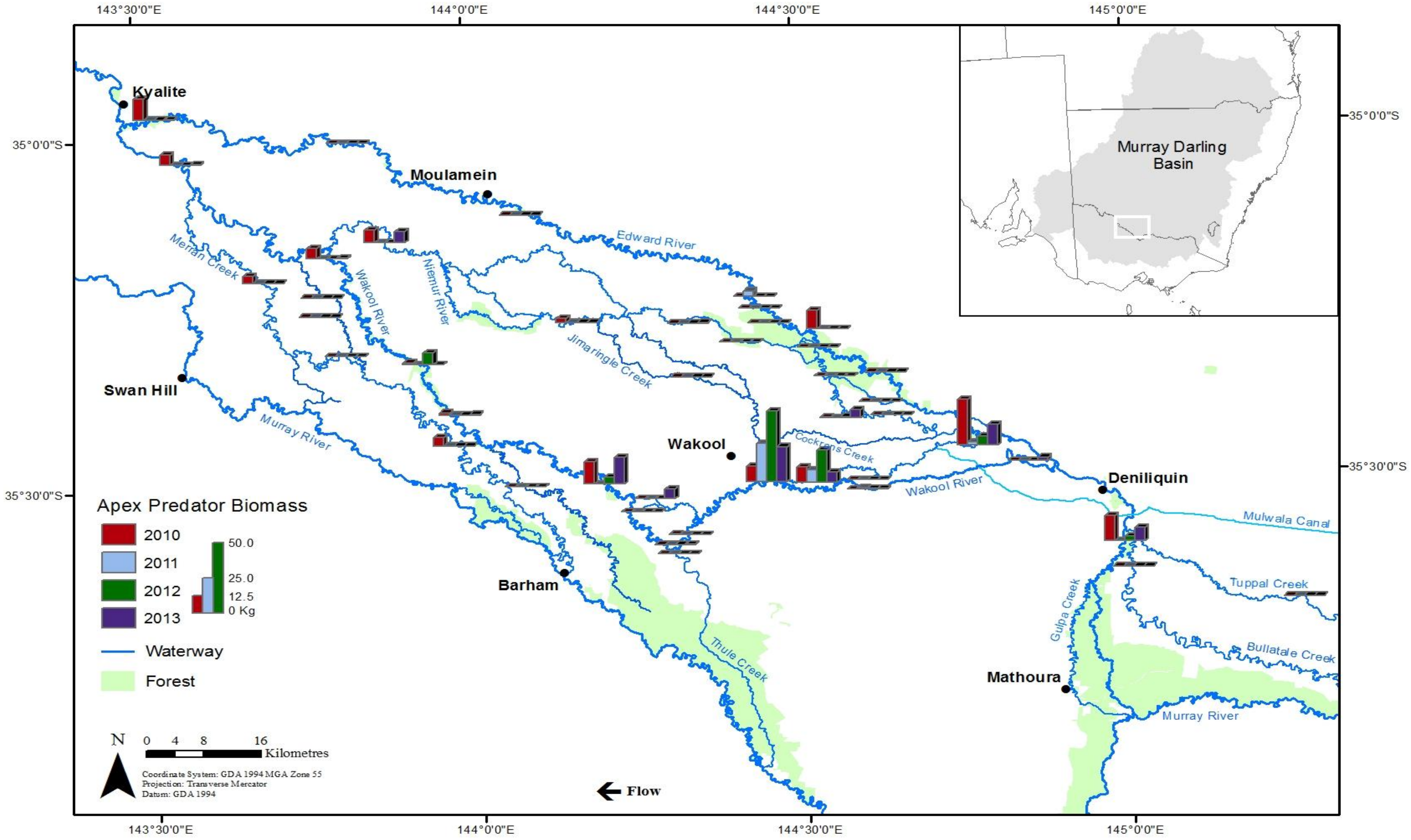


Figure 120. Total biomass of apex predators (Murray cod) caught at each sampling site in the Edward-Wakool river system (2010-2013).

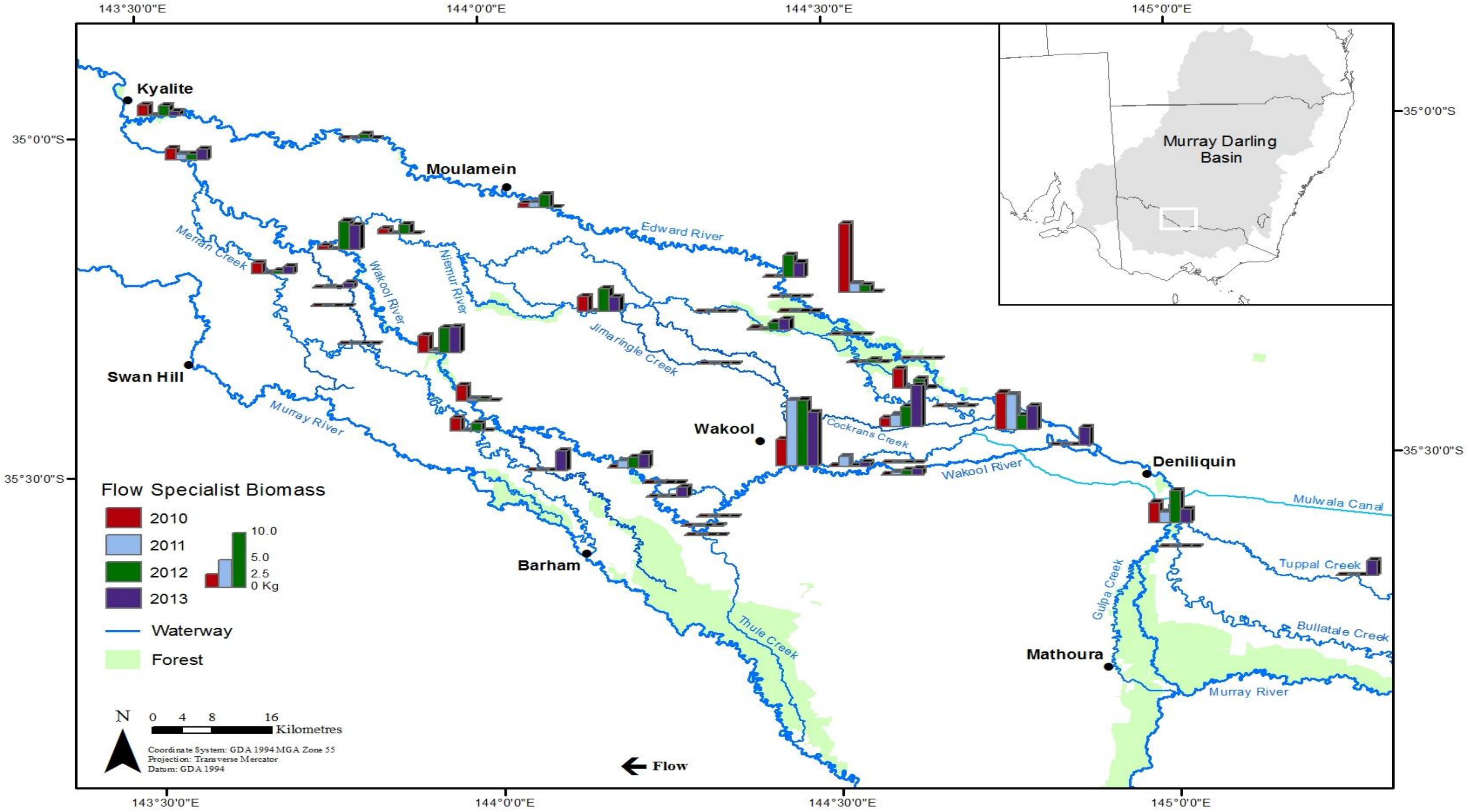


Figure 121. Total biomass of flow specialist species (biomass pooled among species) caught at each sampling site in the Edward-Wakool river system (2010-2013).

Discussion

Environmental flow delivery and water quality management during drought

There was stronger native fish recruitment and lower alien species recruitment during drought conditions. Native fish recruitment during drought was dominated by small bodied generalist species which are likely to be able to persist under such conditions. During drought, a main issue impacting on fish persistence is water quality (Closs and Lake 1996). Reduced water quality was experienced at sites in the lower zone, likely impacting native fish populations, which were least abundant in this zone at that time. Environmental water delivery to target poor water quality should be a priority during low flow periods. Watkins et al. (2010) suggested maintenance of continuous flows in warmer months to avoid stratification in waterholes <3 m in depth. If insufficient water is available (e.g. severe drought) to allow this to occur, then environmental water should be targeted at avoiding mixing with stratified layers, but allowing the upper layers of the water column to maintain fish populations.

There was high biomass of apex predators and flow specialists in channel habitat and high abundance of flow generalists in wetland habitat. During drought, flow should be delivered to maintain source populations, including those which utilise both channel and wetland sites. Specific refuge holes in channel (e.g. Wakool Reserve) and wetlands or complexes (e.g. Werai zone) should be targeted during drought periods to maintain refuges and allow source populations to persist until larger-scale targeted flows can be delivered or natural high unregulated flows return to allow for dispersal. In-channel flows should be provided to promote a productivity boost to the system, and allow movement and spawning opportunities for in-channel spawners, such as apex predators and flow dependant specialists. Wetland watering should be targeted to areas with good native populations (source populations maintained during drought), preferably with low numbers of aliens, with consideration given to water quality.

Influence of flooding on fish community structure

Flooding, and subsequent blackwater events had the greatest influence on fish community structure. Firstly, following the blackwater events the apex predators, such as Murray cod, largely disappeared from all but the upper zone, where in 2010 and 2011 irrigation offtakes were used improve water quality. Murray cod were largely absent from lower zones following flooding, but the species is slowly beginning to recolonise the hypoxic blackwater-affected areas. A targeted re-stocking program has sought to re-introduce Murray cod and golden perch to blackwater affected areas. Each fish was marked with Calcein, to determine the overall contribution of stocking to population recovery. However, only a single stocked fish has been recovered from the release sites, demonstrating that recolonisation is from natural recruitment. Delivery of environmental water during the Murray cod spawning season (October – December) could be a mechanism to facilitate natural recovery by providing opportunities for downstream larval drift.

Secondly, flooding triggered the proliferation and widespread recruitment of carp and goldfish throughout the system. Both species are known to respond to high flows and demonstrated strong recruitment responses. Alien species initially spiked with strong flood recruitment, however this increase in abundance and biomass did not persist and has consistently reduced each subsequent year. It is likely that the flood created conditions favourable for larval survival in these alien species (Brown et al. 2005). In addition, carp and goldfish have known tolerances to hypoxic conditions and an ability to perform anaerobic metabolism during periods of stress (Balashov and Recoubratsky 2011) and these physiological characteristics would have helped these species persist through the hypoxic blackwater conditions.

Environmental watering outcomes for specific flow guilds

Flow generalists

Flow generalists were in highest abundance following the low flow period of the drought in all habitat types and zones. This may indicate a possible preference for low flow recruitment (Balcombe and Closs 2000), or an ability to respond quickly to small increases in flow that occurred during the drought (Gilligan et al. 2009). This highlights the importance of having low flow periods within channel. Flow generalists also increased in the Werai wetlands each year indicating that the inundation events in this wetland system may increase the ability of flow generalists to proliferate in floodplain wetlands. Therefore, establishment of some semi-permanent wetlands in the Werai zone may be warranted, and may also help to encourage other species (other than aliens) to utilise these habitats as habitat conditions improve.

Apex Predators (Murray cod)

Murray Cod are known to spawn irrespective of flow conditions (Humphries 2005) and peaked in abundance and biomass during drought conditions. The susceptibility to hypoxic blackwater water was obvious, but it remains unknown whether the species would have benefited from the flood, and subsequent wetland inundation, if water quality did not deteriorate. Successive years of environmental water delivery targeted at Murray Cod recruitment in the upper zone may have contributed to species persistence. Ongoing spawning and recruitment of populations in the upper zone will be critical to support population expansion back into the lower and middle zones. Flow delivery for apex predators should therefore aim to support maintenance of nest sites, connection of critical habitat, support low flow refuges (such as the Wakool reserve) and also promote opportunities for larval and adult dispersal.

Flow dependant specialists

Flow dependant specialists were widespread throughout the system (mainly in channel habitats) and maintained populations, even following the system-wide hypoxic blackwater event. However, little recruitment has been observed in this group.These species are thought to have flexible life history strategies, and it has been hypothesised that they spawn only in years where conditions favour post-larval recruitment (Mallen-Cooper and Stuart 2006). It is likely that conditions were not suitable during the flooding and subsequent hypoxic blackwater event. The lack of evidence for recent recruitment suggests that watering strategies have, so far, been insufficient to satisfy this life history need. To date, juveniles have been collected only from wetland habitats in 2010. Maintenance of refuges will be required to provide benefits to this group whilst specific flow requirements for this gorup are further investigated and refined.

Floodplain specialists

Due to the complete absence of floodplain specialists, environmental water delivery for this group should be focused on restoring the habitat for this group within a number of target wetlands (e.g. Werai zone). Once adequate habitat is established, the group could be re-introduced through a conservation stocking program and these wetlands maintained through environmental water delivery (Baumgartner et al. 2013). The Werai zone shows great potential for such a targeted program as it is already able to support high abundances of some small-bodied flow generalist fish after several flow event years, and historically would have contained permanent and semi-permanent wetlands holding floodplain specialists. The re-establishment of floodplain specialists should form a part of the future research needs within the system.

Aliens

The alien group (mostly carp and goldfish) dominated biomass throughout the entire system in all years, zones and habitat types. Biomass and abundance peaked after flooding, and a major challenge for environmental watering is to avoid increases in alien fish populations, but this should not be to the detriment of environmental watering for native fish species. Even though there was prolific carp recruitment in 2011, subsequent re-wetting of wetlands including Werai zone did not result in the same recruitment success in this species (section 7.5.2). In 2012-13 carp were restricted to mostly short home range movements with few individuals moving large distances (section 7.5.1), however, during flooding the carp population moved significant distances and system wide recruitment resulted. The lack of substantial movement or recruitment during periods of targeted environmental watering is encouraging from a carp management perspective. It demonstrated that outside of large flood events, there was observed declines in overall biomass of aliens. Longer term confirmation of these trends during future watering events will provide management options to minimise further expansion of carp throughout the system.

General Conclusion

Annual sampling of fish populations within the Edward-Wakool has already contributed to the management of environmental water. In addition it has provided information on community changes in response to changes from drought to flooding and hypoxic blackwater events of 2010-11. The data demonstrate a consistent and system wide reduction in carp biomass since flooding. It is also helping to understand recolonisation pathways for Murray cod following hypoxic blackwater. The annual fish sampling program will help guide long term environmental flow delivery strategies and help to improve overall ecosystem health in the Edward-Wakool system, whilst also assessing the incremental value in annual water deliveries for fish recruitment and abundance.

8. SYNTHESIS

8.1. Objective 1: Support habitat requirements of native aquatic species, maintain health of existing extent of riparian, floodplain and wetland native vegetation communities

Summary of main findings

The Yallakool Creek environmental watering from 19 October 2012 to 7 December 2012 was held in the range of approximately 360 ML/day to 683 ML/day during this period with a median discharge of 526 ML/day. This objective of this event was to maintain inundation of habitat for Murray cod nests and maintain the flow until cod eggs could hatch and drift downstream. In Yallakool Creek a modelled discharge scenario of 560 ML/day resulted in a 22% increase in wetted benthic surface area relative to the base flow 170 ML/day scenario. There was a significant increase in submerged aquatic vegetation during this event, however the increase was short lived, because when the water levels were reduced at the end of the environmental watering event the submerged aquatic vegetation was fully exposed and vegetation was desiccated.

The Colligen Creek environmental watering in November and December 2012 consisted of two freshes to promote golden perch and silver perch spawning. The first fresh reached a peak of approximately 903 ML/day and the second fresh reached a peak of approximately 655 ML/day. In Colligen Creek a modelled environmental flow of 800 ML/day resulted in a 14% increase in wetted benthic surface area relative to the base flow 200 ML/day scenario. These events did not result in any noticeable change in submerged aquatic vegetation, which is not unexpected as the objective of the environmental watering was for short duration of peaks to encourage movement and spawning of golden perch and silver perch.

The Yallakool Creek environmental watering action in February 2013 commenced on 1 February 2013 and finished on 23 February 2013. The fresh increased over 3 days to a peak of 430 ML/d on 13 February 2013. This event was aimed to provide opportunities for small bodied fish to breed. A discharge of 430 ML/day in Yallakool Creek has not been modelled, but is between the modelled median discharge (271 ML/day) and environmental watering modelled discharge (560 ML/day). Based on this, the wetted benthic surface area on the peak of the environmental watering is estimated to have increased between 9.5% and 22% relative to the base flow 170 ML/day scenario. These events did not result in any reportable change in submerged aquatic vegetation.

A watering action in Yallakool and Colligen Creeks commenced on 13 March 2013 and ceased on 5 April 2013. In Yallakool Creek there was a one day duration peak of 563 ML/d and in Colligen Creek a one day duration peak of 499 ML/d. The objective of these watering actions was to test whether a spawning response could be achieved in small bodied fish from a 30 cm rise in water levels in autumn. A secondary objective was to test whether or not a small water level rise would result in the movement of medium/large bodied fish during autumn. The peak of the environmental watering in Yallakool Creek is estimated to have resulted in a short-lived increase in wetted benthic surface area of 22% relative to the base flow 170 ML/day scenario. A discharge of 499 ML/day in Yallakool Creek was not modelled but is between the modelled median discharge (314 ML/day) and estimated half bank discharge (800 ML/day). Based on this, the wetted benthic surface area on the peak of the environmental watering is estimated to have increased between 3 % and 14 % relative to the base flow 200 ML/day scenario. There was no reportable change in submerged aquatic vegetation associated with this action.

The modelled wetted benthic surface area during the maximum daily discharge of the unregulated flows in Colligen Creek (2808 ML/day on 2/8/2012) and Yallakool Creek (1913 ML/day on 2/8/2012) was considerably higher than that during the base flow or environmental watering scenarios in these rivers. On the peak of this unregulated flow event the modelled wetted benthic surface area relative to the base flow scenario increased by 47.8% in Colligen Creek and 58.9% in Yallakool Creek. During these events there would have been a considerable increase in inundated riverbank vegetation. The modelled bankfull scenarios produced even larger increase in wetted benthic surface area than the unregulated flows.

Conclusion

In 2012 the CEWO determined that mid-Murray region was considered to be in a moderate to-wet resource availability scenario and the catchment condition was considered to be wet (CEWO 2012). Under that scenario the ecological management objectives were expected to be in the moderate to wet range. The objective of the moderate range is to ‘maintain ecological health and resilience to improve the health and resilience of aquatic ecosystems’ and the objective for the wet range is to ‘Improve the health and resilience of aquatic ecosystems’ (CEWO 2012). The environmental watering action in Yallakool Creek from October to December 2012 was of sufficient duration that it achieved the objective of maintaining inundation of habitat for Murray cod nests and maintaining the flow until cod eggs could hatch and drift downstream. The duration of inundation provided the opportunity for submerged vegetation to significantly increase in area and there was a visible increase in the activity of macroinvertebrates and other organisms in this newly grown vegetation. However, the benefits of this increase in submerged aquatic vegetation were short lived, because when the water levels were reduced at the end of the environmental watering event the aquatic vegetation was fully exposed and submerged vegetation was desiccated.

The environmental watering actions in Yallakool Creek in February 2013 and in Yallakool Creek and Colligen Creek in March 2013 were targeted to provide opportunities for small bodied fish to breed. It is unlikely that the short duration of the peak of these events would have been sufficient to create suitable breeding and nursery habitat for small bodied fishes. However, these events would have wetted the river banks and this may have made some contribution to sustaining riverbank plants that would provide important habitat during any subsequent higher flows.

An important finding is that the relationship between discharge and wetted benthic surface area in these rivers is not linear. For example, an increase from 200 ML/day to 800 ML/day in Colligen Creek resulted in a modelled 14% increase in wetted benthic area, whereas an increase from 170 ML/day to 800 ML/day in Yallakool Creek resulted in a modelled 30% increase in wetted benthic area. It is likely that that the effect of environmental watering on the habitat of native aquatic species is strongly influenced by the geomorphology of the rivers in the Edward-Wakool system.

From the data presented in this report it can be concluded that the 2012-13 environmental watering in the Edward-Wakool system contributed to maintaining the habitat of native aquatic species. However, there is no evidence that the environmental watering improved the habitat requirements of native aquatic species, other than a short-term improvement in submerged aquatic habitat in Yallakool Creek from October to December 2012.

Suggestions for future monitoring and assessment

The modelling of in-channel habitat inundation area provides critical information to allow integration of results across a range of indicators. Inundation modelling for additional flow scenarios and over a longer river distance would facilitate better planning for the magnitude and duration of environmental watering events. It would also assist in the interpretation of monitoring results and enable better links among parameters indicators. Extending the monitoring of habitat parameters to additional sites across the catchment would facilitate assessment of longer term condition trend in this system.

8.2. Objective 2: Support ecosystem functions that relate to mobilisation, transport and dispersal of biotic and abiotic material

The overarching conclusion from the monitoring of water chemistry (nutrients, organic carbon) and stream metabolism is that throughout the entire period, encompassing base flow and freshes, water quality posed no threat at all to these aquatic ecosystems. At no stage did the dissolved oxygen concentration fall below 4 mg O_2/L, which may threaten viability of invertebrates and fish communities. There were no ‘blackwater events’, which usually arise from water from inundated floodplains, devoid of oxygen, flowing back into the stream channels, and this is more commonly a problem when combined with high water temperatures. The hydrograph peaks throughout 2012-13 were insufficient for such inundation to occur. The following synthesis must be read with a large caveat: “But the water did not get onto the floodplain”. The possible outcomes of floodplain inundation are discussed at the end of this section.

With the exception of NO_x on one occasion, bioavailable nutrient concentrations, ammonia, filterable reactive phosphorus (FRP) and NO_x (nitrate plus nitrite) remained below the ANZECC Trigger concentrations of 20, 20 and 40 µg/L respectively. The organic carbon concentrations in this system can be subject to large fluctuations especially during periods of high flow, however the normal baseline concentrations observed during the study period are towards the lower end of those normally observed in lakes and rivers- generally in the range 2-10 mg/L (Morel and Hering 1993). The ANZECC (2000) water quality guidelines do not provide trigger levels for total organic carbon and dissolved organic carbon, reflecting the fact that there is great diversity in the ‘normal’ concentrations of organic carbon between ecosystems and also that there can be considerable variation in the chemical and biological reactivity of the mixture of organic compounds making up the DOC and TOC at a particular site.

The 2012-13 monitoring data show that there were no large nutrient and organic carbon additions, at least of sufficient duration to be detected by the fortnightly sampling. Some addition of organic carbon occurred during the unregulated flow in August 2012, although much of this may have occurred upstream of the study sites as the water quality in the study rivers was similar to that of the source water from the Edward River. Larger increases in Little Merran Creek and the addition of some bioavailable nutrients in this river may have been due to a combination of different water source and greater area of inundated riverbank in this system. This highlights the value of connection with the floodplain for the mobilisation of carbon and nutrients in these lowland river systems. One of the anticipated ecological benefits of freshes is the delivery of bioavailable materials (nutrients, carbon) to stimulate plant growth and hence enhance the food supply to higher level organisms (notably fish). Elevated nutrient and organic carbon concentrations generally originate from the floodplain and benches in the stream channel above the usual water level can also be repositories of organic C and nutrients. However, there were no large fow events or overbank flows and hence no large increases in nutrient and organic carbon were recorded during 2012-13.

Rates of gross primary production (GPP) and ecosystem respiration (ER) in these streams were typical of lowland streams with good water quality. These rates were constrained by some of the key driving variables. GPP rates were controlled by a combination of very low nutrient concentrations and strong light attenuation limiting the biomass of primary producers. This turbidity-induced light attenuation means that any stream reach deeper than about 1.0 m (Little Merran Creek) to 1.5 m (Colligen Creek) will not have sufficient light reaching the sediment to sustain biofilm growth.

The key point from all of the water quality measurements in 2012-13, is that the nutrient and organic carbon concentrations and rates of primary production and organic matter processing were sufficient to provide the energy base to support the the recruitment of small bodied fish and apex predators such as Murray Cod. However, the question whether fish populations would increase further with higher concentrations and rates can only be determined by further monitoring after flow events that bring higher nutrient and organic carbon concentrations from the floodplain to the river channel.

In the absence of freshes that inundated the floodplain, the effect of such flows on water quality in the study streams remains a matter of conjecture. It is highly likely that water flowing back to the stream channel from the floodplain would be much higher in nutrients and organic carbon than exist in the stream during in-channel flows. Decisions regarding the timing of any proposed large freshes should take into account the prospect for creating 'blackwater events'. The severity of blackwater events would be highly impacted by the timing of flow through the dependence of a number of the key processes on water temperature (Howitt et al. 2007). High flows or overbank flows during periods of low water temperature are less likely to result in hypoxic blackwater events due to slower rates of organic carbon leaching and microbial metabolism, coupled with higher oxygen solubility, however, consideration also has to be given to the period of time the water will be sitting on the floodplain.

8.3. Objective 3: Support breeding and recruitment of frogs, turtles, invertebrates

This objective was assessed through monitoring frogs, shrimp and zooplankton. Turtles were not assessed as part of this monitoring and evaluation program in 2012-13.

Summary of main findings

The watering actions in Colligen and Yallakool Creeks during the 2012 – 2013 sampling period had no effect on the abundance of zooplankton, including individual size classes of zooplankton, nor did it appear to stimulate reproduction. Zooplankton abundance was instead highly seasonal, affected by factors unrelated to flow, such as temperature. It is possible that the magnitude of the watering actions were insufficient to inundate habitat and stimulate productivity thereby increasing abundance and taxonomic diversity of zooplankton. Connectivity and upstream sources may also play a role in zooplankton abundance, as suggested by the similarity in zooplankton abundance of Colligen Creek and Yallakool Creek to the Edward River.

The abundance of shrimp during 2012 – 2013 was not significantly different across the four rivers. The timing of shrimp spawning also did not seem to be influenced by environmental watering. In fact, Colligen Creek and Yallakool Creek had less shrimp overall compared to the control rivers, perhaps as a result of increased flows reducing the size and availability of slackwaters crucial to larval development and juvenile recruitment. In addition, sharp increases in flow may result in higher larvae mortality. The magnitude of flow provided by the watering actions in Colligen Creek and Yallakool Creek may have decreased the size and availability of slackwaters. Larger flows reaching near bankfull levels are likely to inundate benches and create a greater area of slackwaters, resulting in a general increase in shrimp habitat availability.

There was little response of frogs to watering actions in Colligen and Yallakool Creeks. In Colligen Creek frog calling increased when flows were sufficiently high to inundate vegetation. However, no response to environmental watering was evident in Yallakool Creek. The lack of frog response in Colligen and Yallakool Creeks to the environmental freshes (and the other focus reaches which did not receive freshes) may be due to the unprecedented hot weather and low rainfall conditions experienced in the region at this time. Furthermore, riverbank cover was low in each focus reach, which potentially reduced suitable habitat available for frogs and may also explain the lack of a response to watering actions.

Conclusions

The watering actions in Colligen and Yallakool Creeks during 2012–2013 had little influence on the abundance of zooplankton, shrimp and frogs. In addition, few changes occurred in the 'control' rivers at the time of the watering actions, suggesting the objective of 'maintaining ecological health and resilience' in the mid-Murray region under the moderate resource availability scenario was not achieved. Under the experimental design used here, we would expect to see a decline in condition (in this case abundances of zooplankton, shrimp and frogs) in rivers not receiving environmental water compared to those receiving environmental water, if ecological health and resilience had been maintained by the environmental watering. The suggestion that slackwater habitats for shrimp were possibly reduced by these watering actions needs to be considered when future watering actions are planned.

The objective to 'Improve the health and resilience of aquatic ecosystems' under the wet resource availability scenario may be expected to translate to higher abundances and evidence of breeding among zooplankton, shrimp and frogs in rivers receiving environmental water compared to control rivers. This was not observed during 2012-2013, therefore this objective was not achieved. The primary reason for this appears to be the lack of inundation of habitat areas (slackwaters and inundated vegetation) during watering actions. Freshes remained in-channel and did not reach sufficient heights to introduce nutrients and stimulate zooplankton production, nor inundate vegetation and large slackwaters to create habitat for frogs and shrimp respectively. More work is needed to model habitat inundation under a range of flow scenarios to assist the planning of future watering actions.

8.4. Objective 4: Support ecosystem functions that relate to longitudinal connectivity and lateral connectivity to maintain populations

Longitudinal and lateral connectivity is important for supporting ecosystem functions and is critical for the long-term survival of populations of fish and other aquatic organisms. The Edward-Wakool system recedes to a series of pools during low flow conditions, reconnecting when flows resume. During dry periods, off channel wetlands are regularly disconnected from main channel habitats but become connected during higher flow events. The flow regime in the Edward-Wakool system has been significantly altered by river regulation (Green 2001; Watkins et al. 2010), with changes to the timing and volume of flows. Environmental watering actions can help to increase longitudinal and lateral connectivity within this system. When connectivity is restored to aquatic ecosystems fish and other organisms can move between habitats to feed, reproduce, colonise new habitats, and avoid unfavourable conditions. Longitudnal and lateral connectivity is also vital for healthy ecosystem functions by facilitating the transport of nutrients and carbon and thus supporting primary production that underpins food webs.

Summary of main findings

The environmental watering actions in 2012-13 did not result in a significant increase in lateral connection or inundation of riverbank habitat and backwaters (objective 1). Consequently, during the 2012-13 environmental watering actions there were no significant input of nutrient and organic carbon, or detectable change in ecosystem metabolism (objective 2) or zooplankton abundance (objective 3). This suggests that the environmental watering actions did not produce significant ecological changes that would be expected if there was increased lateral connectivity and inundation of backwater habitats. There was evidence of some increased lateral connection during the unregulated flows in August 2012, and there was an increase in nutrients and carbon observed at this time. This highlights the value of lateral connection with the floodplain for the mobilisation of carbon and nutrients in these lowland river systems, The rates of primary production appeared to be sufficient to provide the energy base to maintain the fish population in this system in the absence of significant increased lateral connections. However, the question whether fish populations would increase further with higher primary production rates remains, and can be answered only by further monitoring of flow events that result in significant lateral connection.

The environmental watering actions in the Edward-Wakool system in 2012-13 had an effect on the longitudinal movements of four large-bodied fish species that were monitored in the Wakool River, Yallakool Creek and the Edward River using acoustic telemetry. However, these longitudinal movements did not result in detectible differences in spawning or recruitment based on our sampling of larvae and juveniles. Movement of fish in Colligen Creek could not be assessed due to the absence of an acoustic array and tagged fish in this system.

All four tagged fish species responded quickly to changes in flow, displaying increased activity with increasing temperature and flow during spring and early summer. This period of increased activity corresponded with spawning periods for these species. Fish displayed high site fidelity, and movement was linked to the hydrograph. Although all tagged species displayed increased activity in response to environmental delivery, this increased activity did not result in displacement movements, but may represent survival related behaviour, such as an increased feeding response.

Of the fish that moved in response to delivery of environmental water, all species, except Murray cod displayed a preference to move into the Yallakool Creek habitat and not into the Wakool River control reach. This could be a direct response to the environmental watering actions and change in longitudinal connectivity in this system or could be influenced by other factors or constraints affecting fish movement. In contrast, Murray cod made displacing movements during spring and early summer predominantly into the Wakool River. This pattern of movement was consistent with spawning related behaviour, with cod moving upstream prior to a sedentary period, suggestive of nesting, before returning down river. Back calculated spawning dates for young of year Murray cod recruits collected later in the year aligns with this period.

The results confirm that the Wakool Reserve is an important refuge pool in the region and is used by all fish species during periods of low flow. The flow regimes delivered in 2012-13 were sufficient for fish to leave the pool and access new habitat, and also to subsequently return when flows ceased. However, movement patterns in 2012-13 differed from previous years. A strong movement response similar to that observed during flooding in 2010 was not observed in 2012-13. Both the displacement distance, and the number of fish undertaking these movements was reduced in 2012-13. Knowledge of fish migration patterns, home range, spawning related activity and responses to environmental watering can provide information for improving the management of environmental watering actions and management of native and alien fish and aquatic habitat.

8.5. Objective 5: Support breeding and recruitment of native fish

We use a multiple lines of evidence approach (Downes *et al.* 2002), to assess whether the environmental watering actions delivered from October 2012 to April 2013 to rivers in the Edward-Wakool system achieved the CEWO objectives of supporting the breeding and recruitment of native fish. Specific aims of the watering actions included eliciting a spawning response from golden and silver perch, enhancing the nursery and dispersal conditions of Murray Cod larvae, and promoting spawning and recruitment of small-bodied fish. We investigated if the watering actions led to *a)* spawning related movement of flow specialists such as golden and silver perch, *b)* increases in the abundance of eggs and larvae of the fish species during or immediately after water actions, and *c)* an increase in the number of 0+ recruits of fish species in rivers that received the watering actions.

Edward-Wakool fish assemblage

Ten species of native fish have been identified in the Edward-Wakool river system since 2010. Using the presence of larvae and juveniles, we detected that eight of these species spawned, and spawning was independent of most environmental watering actions (Table 33). Overall, the hydrological conditions were very similar to 2011-2012, where the majority of fish species also spawned (Watts et al. 2013). While it is encouraging to note that most of the native fish species populations in the Edward-Wakool system appear self-sustaining, it is important to keep in mind that the diversity of composition of the community is no doubt greatly reduced as a result of river regulation, and therefore, it may well only be the species that have life histories amenable to persisting in highly regulated systems, that are currently present in the area.

Worthy targets of restoration should aim to consider the promotion of environmental (including hydraulic) conditions previously present within the system, as well as to increase locally extant populations. While historical records for the Edwards-Wakool river system are elusive, it is likely to have supported a similar fish community to that found upstream in the Barmah-Millewa area of the Murray River, and downstream in the Koondrook-Pericoota region. For example, King et al. (2009) cites that the Barmah-Millewa region is home to 18 native fish species, nearly twice the number currently found in the Edward-Wakool system to date. Species presumably once present Edward Wakool region may no longer be present because changes in hydrological conditions as a result of many years of river regulation leaving them unable to persist. However, they should not be forgotten when setting restoration targets and goals for what environmental watering actions might be able to achieve. As asserted by King et al. (2009a), the regulation and reduced variability in flows as a result of regulation will inevitably lead to a reduced fish fauna that reflecting a more homogenous subset of life history strategies and flow requirements. It is vital therefore that planning for environmental watering actions encompassing the diversity of environmental conditions required by different fish species, as well as other key taxonomic groups.

Table 33. Multiple of lines of evidence results to assess the effect of 2012-2013 environmental watering actions on the breeding and recruitment of native fish present in the Edward Wakool system. Yellow – no response, green – significant response, white – response not assessable. A= adults, L/J = larvae/juveniles.

Flow guild	**Species**	**Collected in 2012-13**		**Response to environmental watering actions**		
		A	L/J	Fish spawning movement	Fish larvae & eggs	Fish recruitment
Native species ***Mode 1***	Murray cod	✓	✓	no	no	no
	River blackfish		✓	not targeted	low abundance not assessable	low abundance not assessable
Native species ***Mode 2***	golden perch	✓		no	no	low abundance not assessable
	silver perch	✓	✓	no	no	low abundance not assessable
Native species ***Mode 3***	Australian smelt	✓	✓	not targeted	no	no
	carp gudgeon	✓	✓	not targeted	yes	yes
	flathead gudgeon	✓	✓	not targeted	no	low abundance not assessable
	Murray river rainbowfish	✓	✓	not targeted	low abundance not assessable	low abundance not assessable
	unspecked hardyhead	✓	✓	not targeted	low abundance not assessable	low abundance not assessable
	bony herring	✓		not targeted	not collected	low abundance not assessable
Alien species	carp	✓	✓	no	no	no
	goldfish	✓	✓	not targeted	not collected	no
	gambusia	✓	✓	not targeted	low abundance not assessable	low abundance not assessable
	redfin	✓		not targeted	not collected	low abundance not assessable
	oriental weatherloach		✓	not targeted	low abundance not assessable	not collected

Golden and silver perch

Using multiple lines of evidence, we conclude that environmental watering actions did not result in a spawning outcome for golden and silver perch. No large-scale increase in upstream displacement movements (pre-spawning migration) by golden perch were detected, (though there was an increase in the proportion of tagged fish moving from the refuge pool into Yallakool Creek during all three environmental watering events). Movement in Colligen Creek could not be assessed due to the absence of an acoustic array. It is not known if any of these movements were spawning related behaviour because most adult golden perch migrated downstream outside the larval monitoring zone. No eggs or larvae of silver or golden perch were found during or immediately after the watering actions, and no young-of-year were collected from any of the focus rivers or community sampling sites. These findings repeat those reported in 2011-2012, and possible reasons for the environmental watering actions failing to result in a golden or silver perch spawning event are discussed below.

Golden and silver perch are considered to be flow recruitment specialists because they are found to spawn on flow pulses that occur either within-channel or overbank (Mallen-Cooper and Stuart 2003; Roberts et al. 2008; King et al. 2009; Zampatti and Leigh 2013). The seasonal timing, duration and magnitude of these rises are three important factors thought to determine the degree of spawning and consequent recruitment for these species (Mallen-Cooper and Stuart 2003; King et al. 2009; Zampatti and Leigh 2011). Even though temperatures were adequate for golden and silver perch to spawn (>18°C), the 2012-2013 environmental watering in the Edward-Wakool system did not result in spawning relating movements. Mallen-Cooper and Stuart (2003) found that strong year-classes of golden perch were found in the mid-Murray when variability of within channel flows in late spring and summer was greatest, with water levels increasing one to two metres. Further, golden perch were found to spawn and recruit on flows where discharge exceeded 70% of bankfull conditions. King et al. (2009b) monitored the spawning and recruitment of golden and silver perch in the Murray River in the Barmah-Millewa from 2003-2006 and found that low levels of spawning could occurr for both species during within channel high flows, but that a large increase in spawning intensity occurred as a result of an overbank flood in 2005. The spawning responses to the within channel flows occurred when discharge was at near bank full levels (King et al. 2009b). In the lower Murray, South Australia, Zampatti and Leigh (2013) found strong recruitment of golden perch followed spring-summer spawning when water temperatures would have exceeded 20°C and magnitude of peak flow in which fish larvae were found occurred on a pulse with discharge levels 5 times greater than at the start of the pulse, and took three months to reach is peak. In contrast, environmental water delivered to the Colligen in November 2012 peaked at 903 ML/day, equating to only 22% of the discharge needed to reach bankfull, and took 6 days to reach its peak. While additional work is required to identify the most appropriate flows needed by golden and silver perch spawn and recruit in significant numbers in this region, it is likely that the magnitude and rate at which the pulse of environmental water is ramped up will need to be much greater than what has been delivered to date. Also the timing of flows needs to be closely considered.

Murray cod

There was no evidence that the nursery and larval dispersal conditions of Murray cod were enhanced as a result of the November 2012 Yallakool watering action. The watering action was provided to maintain the water level heights moving through Yallakool Creek during the Murray cod breeding season. Murray cod did not utilise Yallakool Creek habitat in response to environmental water delivery, however increased activity within the Yallakool - Wakool confluence refuge pool was detected. Increased activity may be indicative of a feeding response to flow delivery. Murray cod larvae and young-of-year recruits were found in Yallakool Creek, but not significantly more than the rivers that did not receive environmental watering actions.

The findings from this study support the large body of literature that now exists which consistently conclude that Murray Cod spawn at peak times in November-December, and regardless of flow conditions (Rowland 1983; Humphries et al. 2002; Gilligan and Schiller 2003; King et al. 2003; Humphries 2005; Koehn and Harrington 2005; King et al. 2009b). While flow conditions may not influence Murray cod spawning, speculation exists that recruitment of Murray cod can be greater in times of high flows, however evidence for this remains inconclusive (Rowland 1998; Ye et al. 2000, King et al. 2009b). For increased recruitment to occur as a result of environmental flows, a significant increase in habitat and food resources must be provided with the delivered flows, at a level substantial enough to lead to increased survivorship and growth of larvae and juveniles (King et al. 2009b). We hypothesised that watering actions delivered in the Yallakool in November 2012 would maximise recruitment by inundating more nursery habitats. This hypothesis could not be supported, as the number of 0+ recruits sampled in the Yallakool at the end of the study period was not significantly different than in other rivers that did not receive any environmental water. The lack of enhanced recruitment in Murray Cod in the Yallakool could be due to two possible reasons – firstly, inundation modelling showed that the Yallakool flows lead to only a small (22%) increase in wetted benthic surface area, suggesting that nursery and habitat areas were not increased significantly, and secondly, there was no significant increase food resources (zooplankton) for larval and juvenile cod as a result of the watering actions. We recommend that future watering actions targeted at enhancing native fish populations must take into consideration the importance of promoting in-stream productivity in order to provide a food rich environment essential for larval and juvenile growth, survival and ultimate recruitment.

Small bodied fish

There was little response from small-bodied fish to the environmental water actions. However, the spawning and recruitment of carp gudgeon, one of the five small bodied fish species found in the Edward-Wakool system, benefited from the early season environmental watering actions that took place in the 2012-2013 watering year. There were greater numbers of larvae found after the first November environmental water action in Colligen Creek compared to the other rivers. The number of recruits was also significantly higher in Yallakool Creek and Colligen Creek immediately after the November environmental watering actions compared to rivers that did not receive environmental water. However, the number of recruits in the Edward River, the source of the environmental water, was equally as high as those found in the Yallakool and Colligen rivers during this time. Therefore, it is not possible to conclude if the increase in numbers of recruits in the Yallakool and Colligen is due to increased recruitment occurring within these rivers themselves, or is due to the more recruits moving into these systems from the Edward River. Carp gudgeon are commonly found to be one of the most numerically abundant species across the Murray Darling Basin, and its prevalence throughout the system suggest this species flourishes under regulated conditions (Humphries et al. 1999).

Larvae of small-bodied, seasonal spanners like flathead gudgeon, unspecked hardy head and Murray River rainbow fish were present in the Edward-Wakool region, but in very low numbers. While this could be due to sampling bias, studies by Meredith et al. (2002) have found these species can be collected in much higher numbers with quatrefoil light traps, and therefore, it may be that the low numbers of larvae collected for these species, is an indication that these species currently have relatively low population sizes in the Edward-Wakool system. Fish species that occur in low populations may do so because they persist in non-ideal current hydrological and habitat conditions, and understanding their ecology is vital if their populations are to be encouraged to flourish with environmental flows. For example, macrophytes and submerged littoral vegetation are important habitat spawning substrate for numerous native species, including unspecked hardyhead and Murray river rainbowfish (Llewellyn 1970). The use of environmental flows to promote the establishment and persistence of macrophytes in the Edward-Wakool river system, is therefore a worthy restoration goal, and likely to have significant and positive impact on the size of numerous resident native fish populations.

Role of flow for enhancing fish recruitment

Understanding the causal mechanisms which underlie recruitment strength is critical for effective conservation of fish (Fuiman and Werner 2002). One of the dominant fish recruitment hypotheses relevant to freshwater fish is the "Match/Mismatch" hypothesis; which proposes that fish recruitment will be high if the peak of the prey availability temporally matches the peak in larval production, while a mismatch will lead to poor recruitment (Cushing 1990). Zooplankton is the major food item for larval fish and alignment between zooplankton abundance and larval fish abundance may be an important determinant of recruitment strength (Cushing 1990; King 2003; Humphries et al. 2013). The use of environmental watering actions to promote strong zooplankton production, during periods when larval production is high, may be key to enhancing strong recruitment levels of fish populations in the Edward Wakool. Comparisons of recruitment levels across years, and across rivers, under different magnitudes and flow durations, will provide a greater understanding of how zooplankton densities shape the survival and subsequent recruitment of native fish in the area, and the role of flow in shaping these recruitment events.

8.6. Conclusion

In summary, in 2012-13 there were a small number of significant responses to the October to December 2012 in-channel environmental watering, however some of the expected benefits of environmental watering were not observed. This is consistent with the findings from the 2011-12 environmental watering in this system (Watts et al. 2013). In contrast, there were almost no significant responses to the February, March and April environmental watering events in Colligen Creek and Yallakool Creek. The environmental watering in the Edward-Wakool system in 2012-13 achieved the objective of maintaining the ecological health and resilience of the ecosystem but the timing, magnitude and duration of watering events were not sufficient to make a significant improvement in the the health and resilience of the aquatic ecosystem in the Edward-Wakool system.

9. ENVIRONMENTAL WATERING RECOMMENDATIONS

The possibility of a wet to moderate resource availability scenario meant that ecological objectives for environmental water use in the Edward-Wakool system in 2012-13 were expressed in terms of *improving* ecological outcomes (CEWO 2012). However, above average temperatures and below average rainfall in 2012-13 meant that environmental water largely contributed to *maintaining* ecological outcomes, consistent with a moderate resource availability scenario.

The monitoring and evaluation of environmental watering in the Edward-Wakool system in the 2012-13 showed that the objective of improving ecological outcomes was generally not achieved, however, in some cases there is evidence that the environmental water contributed to maintaining ecological outcomes.

Recommendations relating to the timing of environmental watering

1. *To achieve the objective of 'improving the health and resilience of aquatic ecosystems', environmental watering actions under water use option 1 should be targeted during spring and early summer.* This is the time of year when the greatest benefit for spawning and recruitment of most aquatic species can be realised. An additional benefit of undertaking environmental watering in spring and early summer is that it is less likely to trigger water quality issues than environmental watering undertaken in late summer or early autumn when water temperatures are higher and the concentration of dissolved oxygen is lower. Furthermore, delivery of environmental water in spring or early summer may be more straightforward to implement given existing operational constraints, as it can be difficult for river operators to meet all license holders water needs in the Edward-Wakool system during late summer and early autumn during periods of high consumptive demand.

2. *Environmental watering actions under water use option 2 (Edward-Wakool system refuge habitat) can be implemented at any time of the year to avoid damage to key assets and provide refuge during hypoxic blackwater events.* If there is a high likelihood of a blackwater event, the ecosystem can benefit from environmental watering to provide refuges that have a higher concentration of dissolved oxygen. This option was implemented in 2011-12.

3. *Decisions involving the timing of environmental watering should consider the water temperature at the proposed time of of the environmental watering actions because it will strongly influence the success of fish spawning, the risk of hypoxic blackwater events and rate of ecosystem productivity.*

Recommendations relating to the magnitude of environmental watering

4. *To achieve the objective of 'improving the health and resilience of aquatic ecosystems' under water use option 1, the magnitude of environmental watering freshes should be larger than environmental flow actions delivered in 2012-13.* In contrast to the small or absent responses to environmental watering in Yallakool Creek and Colligen Creek in 2012-13, the larger magnitude unregulated flow events in August and September 2012 inundated a significantly larger area of riverbank and triggered an increase in river productivity. Better ecological outcomes could be achieved through delivery of environmental freshes of sufficient magnitude to inundate low lying benches and backwaters and create shallow water habitat and slackwaters and inundate riverbank vegetation. *Additional modelling of inchannel inundation should be undertaken during the planning of environmental watering actions to assist with optimisation of flow magnitude to help achieve watering objectives and maximise the creation of shallow inundated areas.* It is possible that for a given volume of environmental water, a better ecological outcome could have been achieved in 2012-13 by delivery of fewer larger freshes rather than several smaller freshes, however this hypothesis needs to be tested in an adaptive management context.

5. *Smaller magnitude freshes (such as those delivered in 2012-13) can be delivered to achieve the ecological objective of 'avoid damage', or to 'provide capacity for recovery or maintain health'.* When smaller magnitude watering actions are being planned and implemented it is important that realistic watering objectives are set for each watering action.

6. *A comprehensive community engagement program will be required to facilitate the delivery of larger environmental freshes to the Edward-Wakool system.* The in-channel inundation modelling, mentioned in recommendation 4, will enable scenarios to be presented to stakeholders prior to implementation, which will help identify and minimise risks and serve to inform and engage stakeholders in the planning process.

Recommendations relating to the duration of environmental watering

7. *The duration and shape of the hydrograph of environmental watering events should be carefully planned to avoid rapid rates of recession to minimise stranding of aquatic biota and desiccation of newly established submerged plants*. For example, future watering actions could include a longer, gradual recession to ensure a portion of newly established submerged habitat remains inundated and has the opportunity to increase in area following the flow recession. This will also ensure that organisms utilising the inundated shallow areas have sufficient time to return to in-channel habitats and avoid stranding.

8. *There is a need to outline multiple objectives when setting the duration of environmental watering events.* For example, the objective of the environmental watering event in Yallakool Creek from October to December 2012 was targeted for Murray cod but was also of sufficient duration that it resulted in a significant increase in aquatic vegetation that was not observed during the shorter duration environmental watering events in Colligen Creek or Yallakool Creek later in the year.

General recommendations for environmental watering

10. *The quality of the source water should be carefully considered prior to each environmental watering action as it will influence the outcome of environmental watering*. The quality of source water for environmental watering actions in the Edward-Wakool system (e.g. the Edward River or Mulwala canal) can vary considerably. For example, on occasions when there is considerable overbank flooding in the Murray catchment, the water in the Edward River or the Mulwala canal may carry high dissolved carbon loads (Watts et al 2013).

11. *Decisions around the timing of environmental watering should consider the antecedent hydrological conditions because they can strongly influence the success of subsequent environmental watering actions.*

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12. APPENDICES

Appendix 1. Statistical results for 2 way Analysis of Variance (ANOVA) to examine differences across rivers.

				Factor								
				River			Time			River x Time		
Parameter	*Response variable*	*Size class*	*Transformation*	df	F-test	P value	df	F-test	P value	df	F-test	P value
Total zooplankton	Density (animals/L)	Total	Log 10	4	1.208	Non sig	17	13.445	< 0.001	66	1.076	Non sig
Total zooplankton	Density (animals/L)	53–106 µm	Log 10	4	14.232	< 0.001	17	29.942	< 0.001	66	3.840	< 0.001
Total zooplankton	Density (animals/L)	106–200 µm	Log 10	4	0.817	Non sig	17	43.838	< 0.001	66	4.396	< 0.001
Total zooplankton	Density (animals/L)	200–500 µm	Log 10	4	31.566	< 0.001	17	55.805	< 0.001	66	3.874	< 0.001
Rotifers	Density (animals/L)	Total	Log 10	4	3.714	< 0.01	17	14.477	< 0.001	66	1.759	< 0.001
Cladocera	Density (animals/L)	Total	Log 10*	4	54.743	< 0.001	17	4.284	< 0.001	66	3.051	< 0.001
Copepods	Density (animals/L)	Total	Log 10	4	3.518	< 0.01	17	18.918	< 0.001	66	2.781	< 0.001
Total ovigerous zooplankton	%	Total	Arcsine	4	9.079	< 0.001	16	50.63	< 0.001	63	2.732	< 0.001
Ovigerous rotifers	%	Total	Arcsine	4	3.918	< 0.01	16	36.475	< 0.001	63	2.033	< 0.001
Ovigerous cladocera	%	Total	Arcsine*	4	0.269	Non sig	16	4.201	< 0.001	63	0.659	Non sig
Ovigerous copepods	%	Total	Arcsine*	4	5.186	< 0.001	16	1.674	< 0.05	63	0.959	Non sig

Appendix 2. Statistical results for 2 way mixed-effects Analysis of Variance (ANOVA). A significant interaction between the two fixed factors: Period (before, during, after) and CI (control rivers, impact rivers) indicates that the mean abundance of zooplankton within Impact Rivers was significantly different to changes that occurred over the same period of time within the Control Rivers. Significant interactions are in bold.

Environmental flow	Parameter	Main effect	d.f	F-test	p-value
Nov 2012 – Colligen Creek fresh #1					
	Total zooplankton	Period (B-D-A)	2,54	0.380	0.686
		CI (C-I)	1,54	0.0405	0.841
		Period*CI	2,54	1.7151	0.189
	53 – 106 µm	Period (B-D-A)	2,54	0.453	0.638
		CI (C-I)	1,54	0.0236	0.879
		Period*CI	2,54	1.1573	0.322
	106 - 200 µm	Period (B-D-A)	2,54	0.1326	0.876
		CI (C-I)	1,54	0.0044	0.947
		Period*CI	2,54	2.1156	0.130
	200 - 500 µm	Period (B-D-A)	2,54	0.2757	0.760
		CI (C-I)	1,54	0.2581	0.614
		Period*CI	2,54	1.189	0.312
	Total ovigerous zoops	Period (B-D-A)	2,54	5.369	0.007
		CI (C-I)	1,54	0.3549	0.554
		Period*CI	2,54	0.655	0.524
	Ovigerous rotifers	Period (B-D-A)	2,54	7.329	0.002
		CI (C-I)	1,54	2.205	0.143
		Period*CI	2,54	0.275	0.761
	Ovigerous cladocera	Period (B-D-A)	2,54	0.0848	0.919
		CI (C-I)	1,54	0.958	0.332
		Period*CI	2,54	0.9172	0.406
	Ovigerous copepods	Period (B-D-A)	2,54	0.0677	0.935
		CI (C-I)	1,54	0.461	0.500
		Period*CI	2,54	0.4174	0.661
Nov 2012 – Colligen Creek fresh #2					
	Total zooplankton	Period (B-D-A)	2,38	21.263	<0.0001
		CI (C-I)	1,1,	1.141	0.479
		Period*CI	2,38	1.947	0.157
	53 – 106 µm	Period (B-D-A)	2,38	9.753	0.004
		CI (C-I)	1,1,	0.263	0.696
		Period*CI	2,38	1.947	0.157
	106 - 200 µm	Period (B-D-A)	2,38	20.321	<.0001
		CI (C-I)	1,1,	9.457	0.2002
		Period*CI	2,38	6.715	0.0032
	200 - 500 µm	Period (B-D-A)	2,38	60.4503	<.0001
		CI (C-I)	1,1,	19.719	0.141
		Period*CI	2,38	10.844	0.0002
	Total ovigerous zoops	Period (B-D-A)	2,38	34.383	<0.0001
		CI (C-I)	1,1,	1.328	0.455
		Period*CI	2,38	2.221	0.1224
	Ovigerous rotifers	Period (B-D-A)	2,38	23.054	<0.0001
		CI (C-I)	1,1,	1.520	0.4338
		Period*CI	2,38	4.334	0.0202
	Ovigerous cladocera	Period (B-D-A)	2,38	13.473	<0.0001
		CI (C-I)	1,1,	1.683	0.418
		Period*CI	2,38	0.0668	0.9355
	Ovigerous copepods	Period (B-D-A)	2,38	1.2815	0.2893
		CI (C-I)	1,1,	2.626	0.352
		Period*CI	2,38	0.165	0.848