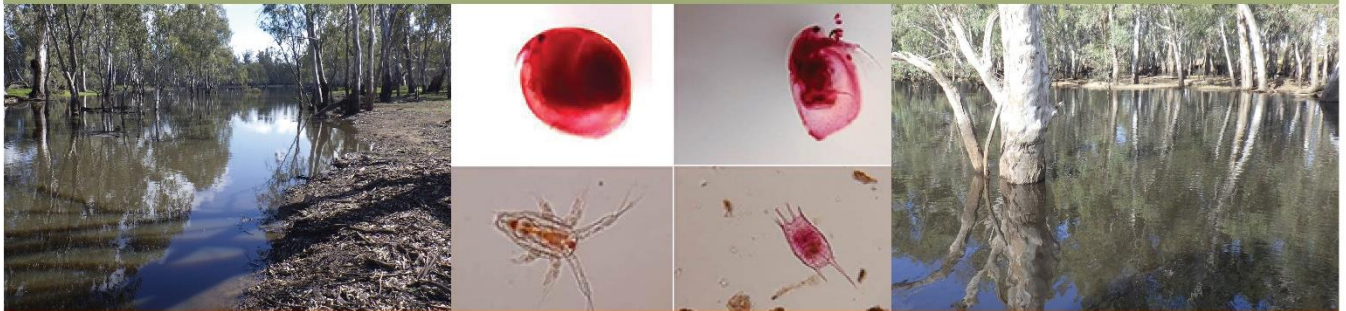
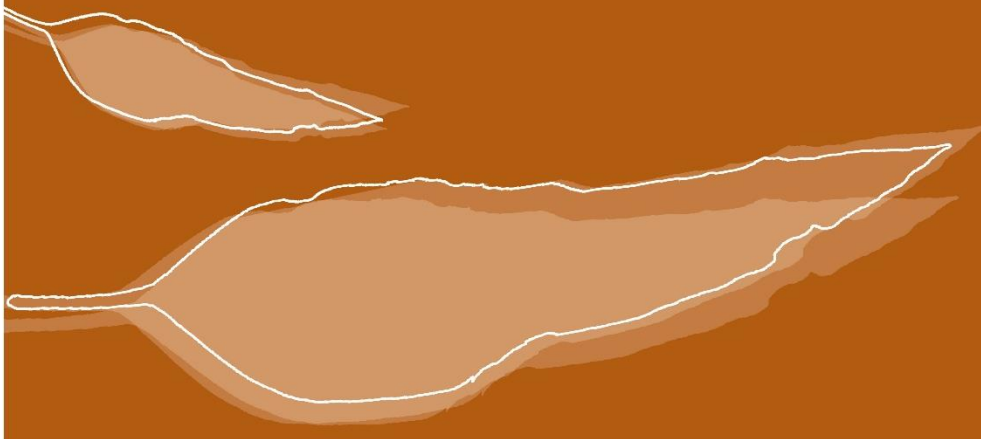




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## Contribution of Koondrook-Perricoota floodplain runoff to the productivity of the Wakool River



## **Contribution of Koondrook-Perricoota floodplain runoff to the productivity of the Wakool River**

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## EXECUTIVE SUMMARY

In late 2016 there was a widespread flood in the southern Murray-Darling Basin associated with record-breaking rainfall in parts of the catchment. Some areas of the floodplain were inundated that had not been flooded for more than 20 years. In the Murray catchment the Murray River flows at Yarrawonga in October were the highest since 1993. Unregulated flows from the Murray River inundated the Barmah Forest, Werai Forest and Koondrook-Perricoota (KP) Forest. In association with the floods there was a hypoxic (low oxygen) blackwater event that extended throughout the Murray River system, including the Edward-Wakool system. Fish kills were reported in many areas with very low dissolved oxygen levels.

This report documents the contribution of KP Forest run-off to the productivity of the Wakool River from late August to late November 2016. It was hypothesised that flows from the Murray River through the KP Forest to the Wakool River would make a significant contribution to instream productivity in the reaches of the mid-Wakool River and ultimately to the lower Wakool River and downstream of the KP Forest. This project was undertaken by Charles Sturt University Institute for Land, Water and Society for the Forestry Corporation of NSW.

Field sampling was undertaken weekly at two sites in each of three reaches of the Wakool River (reach 1 upstream of Thule Creek, reach 2 between Thule Creek and Barbers Creek, and reach 3 downstream of Barbers Creek) and one site in each of Thule Creek and Barbers Creek, outflowing creeks from KP Forest. As the flood event extended over several months, the productivity response to the flood was examined in three periods:

- 1) Return flow period - Three consecutive weeks monitoring at the beginning of the flood event when there were return flows from KP Forest to the Wakool River and flows in the Wakool River remained within the channel (22 August, 29 August, 5 September)
- 2) Flood flow period – Three consecutive weeks monitoring during the peak of the event in the Wakool River when there were overbank flows in the Wakool River and outflows from KP Forest (26 September, 4 October, 10 October)
- 3) Recession flow period - Three consecutive weeks monitoring during the recession of flows when the Wakool River flows had returned to within channel but outflows from KP Forest continued (14, 21, 28 November)

Indicators used to assess the water quality and productivity responses to the flood were:

- Hydrological variables: Median, range and coefficient of variation of daily discharge
- Water quality and carbon variables: Dissolved oxygen (DO) and temperature (logged continuously), dissolved organic carbon (DOC), total phosphorus (TP), bioavailable phosphorus (FRP), total nitrogen (TN), nitrates + nitrites (NO<sub>x</sub>), ammonia (NH<sub>3</sub>) organic carbon absorbance and fluorescence, and Chlorophyll-a
- Spot water quality: DO, temperature, electrical conductivity, pH, turbidity
- Stream metabolism: gross primary production (GPP), ecosystem respiration (ER), and ratio of GPP to ER (P:R ratio)
- Benthic microinvertebrates: abundance and diversity of key microcrustaceans

The flooding of KP Forest and outflows via Thule and Barbers Creeks were important sources of carbon and nutrients to the Wakool River during this flood event. Increasing concentrations of floodplain-derived carbon and nutrients accelerated ecosystem processes. Ecosystem responses during the three stages of the flood event (return flow period, flood flow period, recession period) were considerably different.

Return flow period (22 August to 5 September 2016)

- Thule and Barbers Creeks were important sources of carbon to the Wakool River. During the return flow period. Thule Creek inputs markedly changed the DOC profile and more than doubled the concentration of DOC in the Wakool River.
- Dissolved oxygen concentrations were in the acceptable range at all Wakool River sites
- Although there were detectable increases in nutrient and carbon concentrations, flows from KP Forest during this stage of the flood did not appear to increase rates of metabolism in the Wakool River downstream of Thule Creek and Barbers Creek, possibly due to lower water temperatures at the time
- The abundance of microinvertebrates during the return flow period were generally low

Flood flow period (26 September to 10 October 2016)

- Connection with the floodplain during this period had a strong and rapid influence on DOC concentrations in the Wakool River. During the extensive flooding DOC entered the system from upstream forested areas as well as flooded grazing and cropping land. There were complex and varied sources of the DOC in this system.
- Total phosphorous, total nitrogen, nitrate, nitrite and filterable reactive phosphorous were much lower during the flood flow period than during both the return flow period and recession period.
- An accelerated decline in DO to hypoxia was observed at all sites over a period of a few days in early October. The same trend was present in the dissolved oxygen saturation data, suggesting a rapid increase in oxygen consumption from the water column at this time rather than a decline in oxygen solubility. There was a strong effect of temperature on the onset of hypoxia. The rapid decline in DO on the 6<sup>th</sup> October occurred when there was a rise in water temp of approximately 3 degrees.
- During the flood flow period there was high rates of ER (more than four times that reported for the same reaches in 2015) pushing the system toward being highly heterotrophic. With the onset of flooding chlorophyll-a concentrations reduced across all sites. It is likely that during the peak of the flood chlorophyll-a concentration was diluted by low-chlorophyll water flowing from upstream and DOC may have been attenuated light penetrating the water column suppressing the rates of GPP.
- As predicted, the increased discharge during the flood appeared to dilute the abundance of microinvertebrates in return flows from KP Forest.

Recession flow period (14 to 28 November 2016)

- During the flood recession the discharge in Thule Creek reduced to very low flow, whereas Barbers Creek was still receiving considerable return flows from KP Forest.

The flow in Wakool River reach 1 and 2 had reduced considerably, but Wakool River reach 3 continued to flow strongly, partly due to the return flow from Barbers Creek and also because it is the most downstream reach.

- Inputs of carbon to the Wakool River Reach 2 and Reach 3 from Thule and Barbers Creek that had a strong influence on the water quality and productivity of the Wakool River.
- Total phosphorous, total nitrogen, nitrate, nitrite and filterable reactive phosphorous were much higher during the recession flow period. The ANZECC trigger levels for nitrate, nitrite and ammonia were exceeded at this time.
- During the recession flow period DO concentrations increased in the Wakool River reach 1, reflecting improved water quality entering from the Edward River upstream.
- As expected, during the recession flow period there was increasing GPP, coinciding with increasing chlorophyll-a and falling ER. It is unclear of the mechanism for this – it could be due to change in DOC speciation, potential reduction in light attenuation, an increase in available inorganic nutrients, or increase in temperature.
- The abundance of microinvertebrates did not increase during the flood recession. It should be noted that flows remained higher than baseline discharge in 2015 study and it is likely that there would be significant increase in microinvertebrate productivity at a later stage as flows decreased and temperatures increased in summer. I

In conclusion, the relationship between the various components that influence ecosystem metabolism is complex and the scale and timing of flows is important. Careful planning of timing, magnitude and duration of delivery of environmental water through KP Forest when the Wakool River is not in flood could increase GPP in the mid and lower Wakool River and possibly contribute to the productivity of the Murray River downstream of the confluence with the Wakool River. The magnitude of flows through KP Forest with respect to flows in the Wakool River would need to be optimised and the time of year of water delivery is critical to avoid poor water quality outcomes, due to the strong influence of temperature on river processes.

# 1. INTRODUCTION

## 1.1 Background

The loss of river-floodplain connectivity is one of the factors contributing to the declining health of aquatic ecosystems across the Murray-Darling Basin (Kingsford 2000; Bunn and Arthington 2002). During over-bank flooding, the inundated floodplain creates a mosaic of productive habitat that supports a high diversity and abundance of wetland flora and fauna. During subsequent reconnections, species that have spawned or recruited on the floodplain are able to move back to the river along with a pulse of nutrients and carbon to augment riverine processes (Junk et al. 1989). River regulation and floodplain development have disrupted this connectivity through changes to the volume, timing, and duration of high flow events (Ward et al. 1995; Bunn and Arthington 2002). The subsequent loss of floodplain-derived carbon from the energy budget of adjacent rivers has consequences for the ecology of floodplain-river ecosystems (Baldwin et al. 2016).

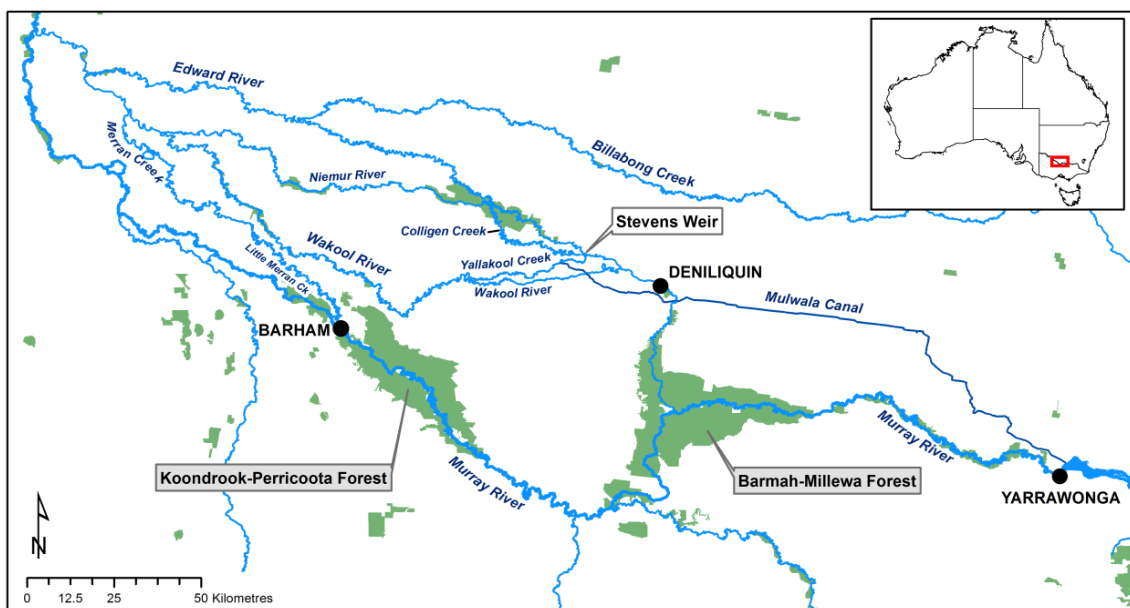
The Murray River system in the southern Murray-Darling Basin includes the main river channel and a number of rivers and streams that intersect across low-lying country in the mid catchment. The Edward-Wakool system (Figure 1.1) is a large anabranch system of the Murray River that carries the majority of water at times of flood. 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 (Rowland 2004). Under regulated conditions flows in the Edward River and tributaries remain within the channel, whereas during high flows there is connectivity between the river channels, floodplains and several large forests including the Barmah-Millewa Forest, Koondrook-Perricoota (KP) Forest and Werai Forest.

The KP Forest (Figure 1.1) is one of the largest forested floodplains in Australia, covering over 32,000 ha. Historically, the KP Forest would have frequently contributed large amounts of floodplain-derived carbon to the Murray River via the Wakool River, although this has been impacted by the regulation of the Murray River. River regulation has resulted in fewer carbon-transporting events; being smaller events (both in terms of flow and area inundated) and shorter events.

New regulators and levees have recently been constructed at the KP Forest under the KP Forest Flood Enhancement Project that was part of the Environmental Works and Measures Program of The Living Murray Program - a joint initiative funded by the New South Wales, Victorian, South Australian, Australian Capital Territory and Commonwealth governments, coordinated by the Murray-Darling Basin Authority. This infrastructure creates opportunities for managed environmental water to be delivered both into and out of the KP Forest. The operating plan for KP Forest outlines the default settings for different regulators (Forests NSW and NSW Office of Water 2012). These include: the Swan Lagoon regulators are kept open

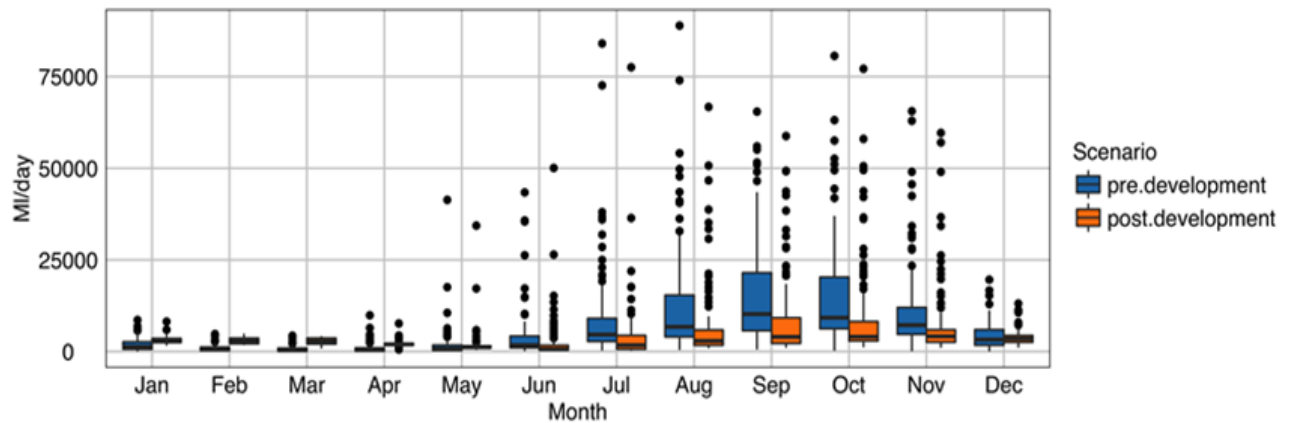


(primary natural inflow point), Thule Creek, Barbers Creek, Runner A, Cow Creek and Calf Creek regulators are open (natural outflow points), and the inlet channel and return channel are shut. The default settings ensure that natural overbank flood events can proceed without obstruction. The infrastructure also creates opportunities for controlled releases of environmental flows to the KP Forest and into the Wakool River via Thule Creek and Barbers Creek. Managed diversions of environmental water through KP Forest only happen when environmental water is made available for that purpose. The operating plan permits the diversion of environmental flows from the Murray River through the KP Forest and into the Wakool River whenever discharge in the Murray River downstream of Torrumbarry Weir is below 18,000 ML/day.

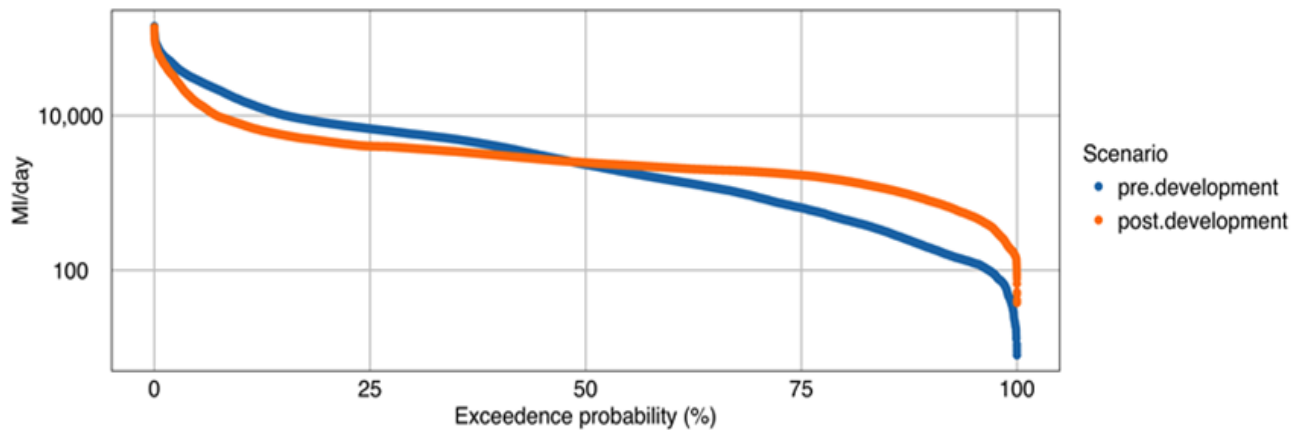


**Figure 1.1:** Map showing the main rivers in the Edward-Wakool system and the location of the Barmah-Millewa forest and Koondrook-Perricoota Forest on the Murray River. (Source: Watts et al. 2013)

Like many rivers of the Murray-Darling Basin, the flow regimes of rivers in the Edward-Wakool system have been significantly altered by river regulation (Green 2001; Hale and SKM 2011). Natural flows in this system are strongly seasonal, with high flows typically occurring from July to November. Analysis of long-term modelled flow data at Deniliquin on the Edward River, showed that flow regulation has been associated with a marked reduction in winter high flows, including both extreme high flow events, but also average daily flows during the winter period (Figure 1.2) (Watts et al. 2015). There is also an elevated frequency of low to median flows and reduced frequency of moderate high flows (Figure 1.3). These flow changes reflect the typical effects of flow-regime reversal observed in systems used to deliver dry-season irrigation flows (Maheshwari et al. 1995).



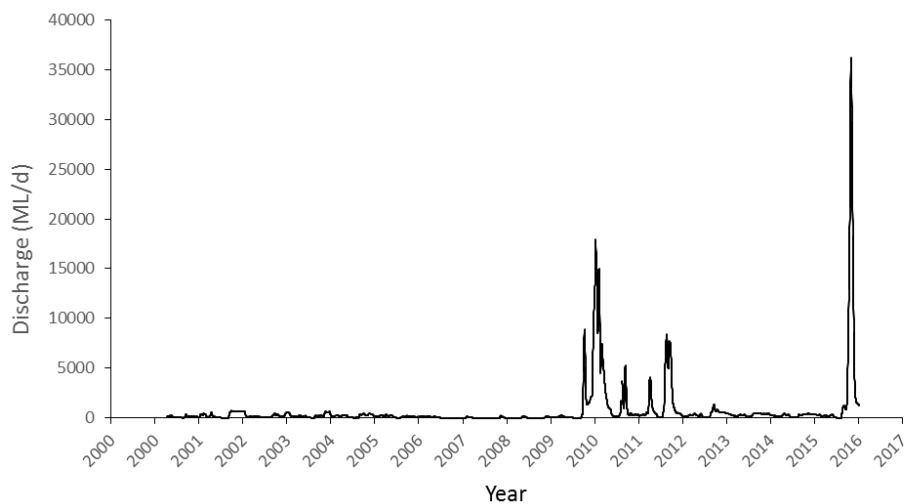
**Figure 1.2:** Boxplots of mean daily flows by month for the Edward River at Deniliquin. Post-development modelled time-series assumes that all current licensed extractions have been in place for the entire record, and that all licenses are active. (Source: Watts et al. 2015).



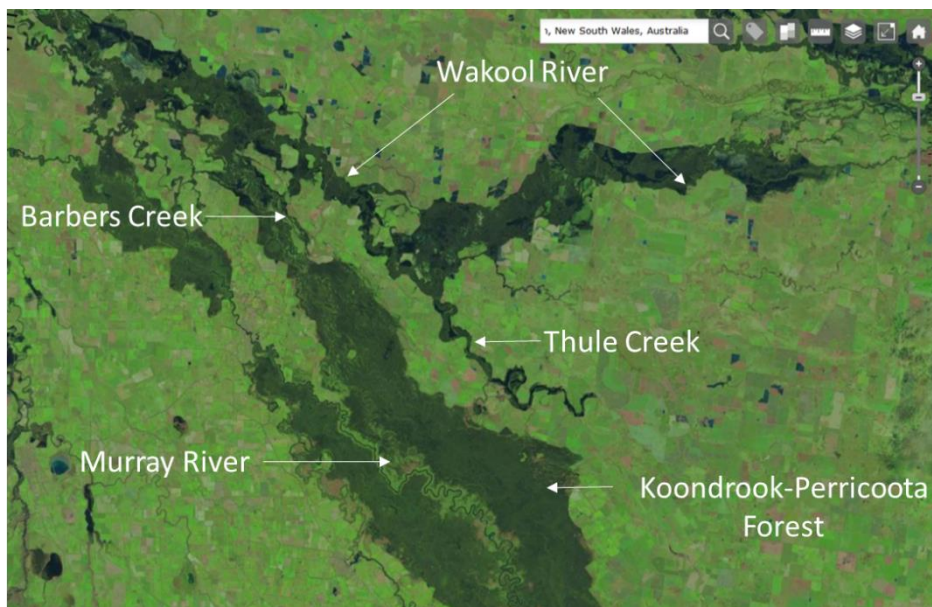
**Figure 1.3:** Annual flow duration curve for the Edward River at Deniliquin. Post-development modelled time-series assumes that all current licensed extractions have been in place for the entire record, and that all licenses are active. (Source: Watts et al. 2015).

From 1998 to 2010 south-eastern Australia experienced a prolonged drought (known as the Millennium drought) and flows in the Murray-Darling Basin were at record low levels (van Dijk 2013; Chiew et al. 2014). During this period the regulators controlling flows from the Edward River into tributary rivers such as Yallakool Creek and the Wakool River were closed. Between February 2006 and September 2010 there were periods of minimal or no flow in the Wakool River (Figure 1.4). At the break of the drought after many years without overbank flows, a sequence of unregulated flow events between September 2010 and April 2011 triggered a hypoxic blackwater event downstream of large river red gum floodplain forests such as the Barmah-Millewa Forest and KP Forest (MDBA 2011; Whitworth et al. 2012). These hypoxic blackwater events resulted in the loss of many thousands of native fish, including large individuals of Murray cod (King et al. 2012; Whitworth et al. 2012; Watts et al. in press).

In late 2016 there was a widespread flood in the southern Murray-Darling Basin associated with record-breaking rainfall in parts of the catchment. Some areas of the floodplain were inundated that had not been flooded for more than 20 years. In the Murray catchment, inputs from the Kiewa and Ovens Rivers were the highest since 2010 and the Murray River flows at Yarrowonga in October were the highest since 1993 (MDBA River Murray Weekly Report, 7th Dec). This resulted in a very large flood event in the Wakool River (Figure 1.4) and unregulated flows from the Murray River inundated the Barmah Forest, Werai Forest and KP Forest (Figure 1.5). In association with the floods there was a hypoxic (low oxygen) blackwater event that extended throughout the Murray River system, including the Edward-Wakool system. Fish kills were reported in many areas with very low dissolved oxygen levels.



**Figure 1.4:** Daily discharge ( $\text{ML}\cdot\text{d}^{-1}$ ) in the Wakool River at Gee Gee Bridge from 1 January 2000 to 30 December 2016, showing period of no flows during the Millennium drought prior to several unregulated flows between 2010 and 2012 and a large unregulated flow in 2016. Daily discharge data was obtained from NSW Office of Water website.



**Figure 1.5:** Landsat image of the inundation of Koondrook-Perricoota Forest and the Wakool River on 25/10/2016 during the flood.

Parts of the floodplain that have not been inundated for many years had built up a substantial amount of leaf litter and soil carbon (Figure 1.6), with leaf fall possibly exacerbated by the record heat in autumn 2016. As river regulation has reduced the number of small to medium overbank flows in winter and early spring, there have been fewer opportunities for carbon to be exported from the floodplain during the cooler months.



**Figure 1.6:** Banks of Barbers Creek (Koondrook-Perricoota Forest) prior to the flood in 2016 showing accumulated leaf litter and bark prior to flooding 13/8/2016 (Photo: Robyn Watts).

Under highly regulated flows the productivity of rivers in the Edward-Wakool system is very low (Watts et al 2013, 2014, 2015, 2016). River operating rules require Commonwealth environmental watering actions in the Wakool River at the confluence of the Wakool River and Yallakool creek to be kept below 600 ML/day, limiting the extent to which low lying parts of the floodplain can be inundated. Consequently, there have been no significant pulses of river productivity recorded during monitoring undertaken in the Wakool River between 2012 and 2016 (Watts et al 2013a, 2013b, 2014, 2015, 2016).

## **1.2 Project aims and hypotheses**

This project examined the contribution of the KP Forest floodplain runoff to the productivity of the Wakool River through spring of 2016 during a widespread flood event. It was hypothesised that flows from the Murray River through the KP Forest to the Wakool River would make a significant contribution to instream productivity in the reaches of the mid-Wakool River and ultimately to the lower Wakool River and downstream of the KP Forest. As the flood event extended over several months, the productivity response to the flood was examined in three periods: 1) Return flow period - early in the event in September 2016 when the return flows were released from KP Forest to the Wakool River but the river was not yet in flood, 2) Flood flow period - during the peak of the event in the Wakool River (late October and early November 2016), and 3) Recession flow period - during the recession of flows (November 2016).

The following hypotheses were examined:

Return flow period (22 August to 5 September 2016):

- 1) Return flows early in the flood event are expected to export carbon and nutrients from the floodplain without causing hypoxia in the Wakool River during periods of low water temperature.
- 2) Return flows early in the flood event are expected to increase rates of metabolism overall, favouring ecosystem respiration (ER) relative to gross primary production (GPP).
- 3) Return flows from the KP Forest to the Wakool River will increase densities and diversity of microinvertebrates in the Wakool River downstream of Thule Creek relative to upstream of Thule Creek, and downstream of Barbers Creek relative to upstream sites.

Flood flow period (26 September to 10 October 2016):

- 1) During flooding, carbon and nutrient concentrations in the Wakool River, Thule and Barbers Creeks are expected to be increased due to widespread contact with the floodplain.
- 2) Flood flows are expected to further increase rates of GPP and ER in the Wakool River at sites downstream of the Barbers and Thule Creek junctions.
- 3) During flooding, densities of microinvertebrates would become similar between sites in the Wakool River due to mixing, and densities would reduce in the creeks due to flushing and dilution of floodwaters.

Recession flow period (14 to 28 November 2016)

- 1) Recession flows are expected to result in gradual decreases in carbon and soluble nutrients as the river becomes restricted to the channel, with continuing inputs until wetlands and forests cease to drain.
- 2) Recession flows are expected to result in a decline in ecosystem metabolism, and a switch from ecosystem respiration (ER) toward gross primary production (GPP).
- 3) During the flood recession, microinvertebrate densities would increase when water levels and discharge fell and during warmer spring temperatures.

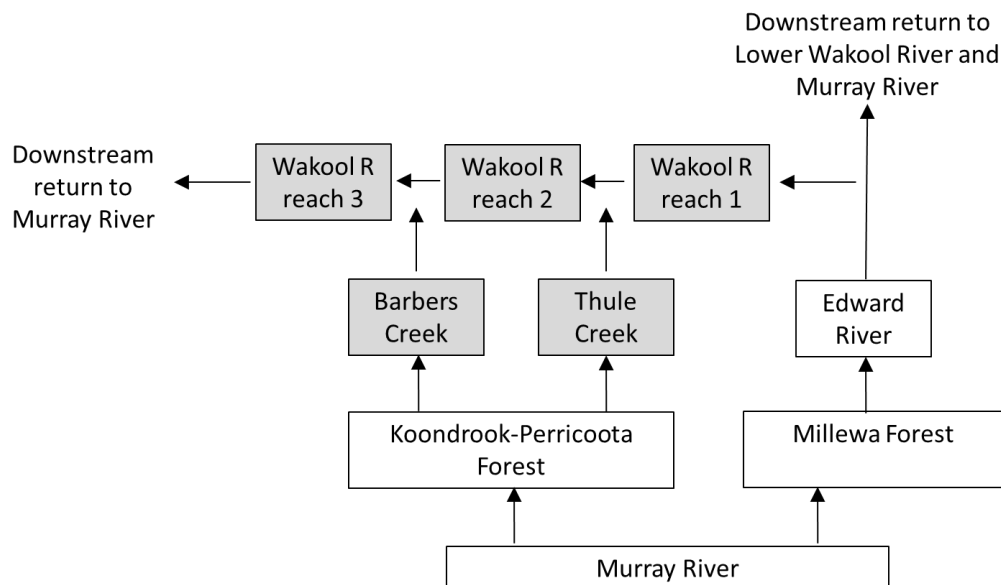
## 2. SITE DESCRIPTION AND STUDY DESIGN

### 2.1 Study design

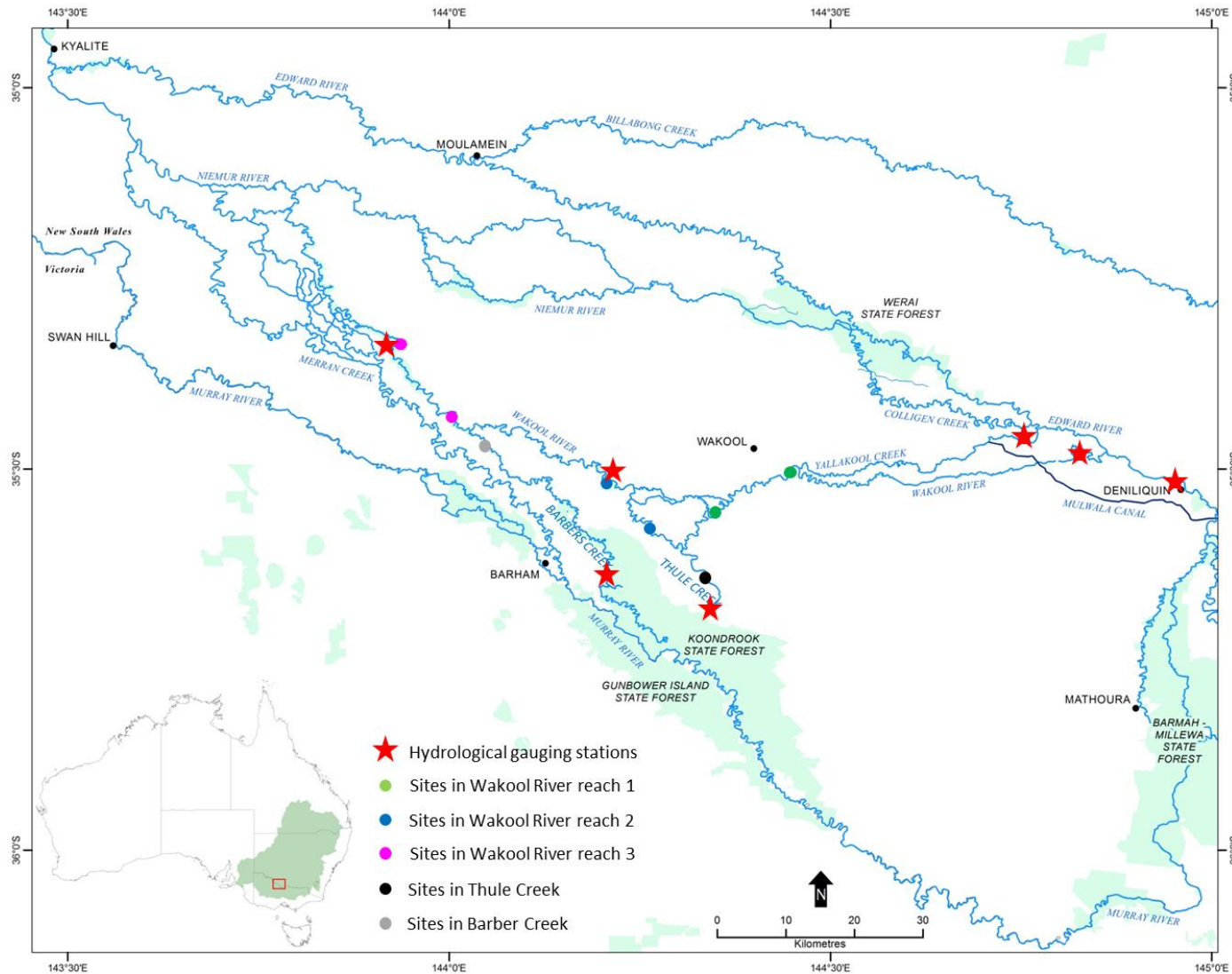
Field sampling was undertaken weekly at two sites in each of three reaches of the Wakool River and one site in each of two inflowing creeks from KP Forest, being Thule Creek and Barbers Creek (Table 2.1). Due to high levels of flooding, not all sites were able to be sampled on all occasions. A schematic diagram (Figure 2.1) and map (Figure 2.2) shows the relationship between the three reaches and two inflowing creeks. Photos of study sites are presented in Figure 2.3.

**Table 2.1.** Monitoring sites for the KP floodplain runoff productivity project

Reach name	Site
Wakool River reach 1, upstream of Thule Creek	LTIM Wakool river zone 3 site 1, zone 3 site 5
Thule Creek	Lower Thule Road bridge, Deniliquin Rd bridge
Wakool River reach 2 between Thule and Barbers Ck	Greenhill Lane, LTIM Wakool R zone 4 site 1
Barbers Creek	Barbers Creek at Woodara
Wakool River reach 3, downstream Barbers Creek	Wakool River at Noorong and Gee Gee bridge



**Figure 2.1.** Schematic diagram of sampling reaches in the Wakool River relative to inflows from Thule Creek and Barbers Creek. River reaches shaded grey were included in this study.



**Figure 2.2.** Location of monitoring sites for the Koondrook-Perricoota Forest run-off project. Wakool River reach 1 is upstream of the confluence with Thule Creek, Wakool River Reach 2 is between Thule Creek and Barbers Creek, and Wakool River Reach 3 is downstream of the confluence with Barbers Creek. Details of hydrological gauges are provided in section 3.



Wakool River reach 1, 1/12/16 (Photo R Cook)



Thule Creek 21/9/16 (Photo R Watts)



Wakool R reach 2, Greenhill L 24/10/16 (Photo J Abell)



Wakool R reach 2 15/11/16 (Photo N McCasker)



Barbers Creek 15/11/16 (Photo N McCasker)



Wakool R reach 3 15/11/16 (Photo N McCasker)

**Figure 2.3:** Photos of study sites for the Koondrook-Perricoota productivity project.



Sampling was undertaken over a period of 16 weeks in 2016, with the sample weeks grouped into three periods for analysis:

- Return flow period: Three consecutive weeks monitoring at the beginning of the flood event when flows in the Wakool River remained within the channel, but there were outflows from KP Forest (22 August, 29 August, 5 September)
- Flood flow period: Three consecutive weeks monitoring when there were overbank flows in the Wakool River and outflows from KP Forest (26 September, 4 October, 10 October)
- Recession flow period: Three consecutive weeks towards the end of the flood event when Wakool River flows had returned to within channel but outflows from KP Forest continued (14, 21, 28 November)

## 2.2 Indicators

Indicators were selected to assess the water quality and productivity responses to the flood and recession flows:

- Hydrological variables
  - Median, range and coefficient of variation of daily discharge
- Water quality and carbon variables
  - Dissolved oxygen/temperature: Logged continuously at each site
  - Dissolved Organic Carbon (DOC)
  - Nutrients (Ammonia ( $\text{NH}_4^+$ ), filtered reactive phosphorus (FRP), dissolved nitrate + nitrite ( $\text{NO}_x$ ), Total Nitrogen (TN) and Total Phosphorus (TP))
  - Absorbance and fluorescence spectroscopy for organic matter characterisation.
  - Chlorophyll-a
  - Spot water quality: Dissolved oxygen, temperature, electrical conductivity, pH, turbidity
- Stream metabolism
  - gross primary production (GPP)
  - ecosystem respiration (ER)
  - ratio of GPP to ER (P:R ratio)
- Benthic microinvertebrates:
  - density of microcrustaceans and small macroinvertebrates

## 3. HYDROLOGY

### 3.1 Methods

Daily discharge data for automated hydrometric gauges were obtained from the New South Wales Office of Water website, and daily discharge data from the Wakool escape were obtained from WaterNSW:

- Wakool River Reach 1 (upstream Thule Creek): The daily discharge was estimated by combining daily discharge data from Yallakool Creek regulator (gauge 409020 Yallakool Creek @ Offtake), the Wakool offtake (409019 Wakool River offtake regulator) and the Wakool escape with an adjustment to account for travel time (4 days) and estimated 20% losses. The estimate of 20% loss in this reach has been previously used for calculations of within channel regulated flows (V. Kelly, WaterNSW pers. comm.) but may not accurately reflect losses in this reach during overbank flows. A slight inaccuracy in the estimate of losses in this reach will not greatly influence the comparison between sampling periods, because the differences in discharge between the three time periods are very large.
- Thule Creek: Daily discharge data obtained from gauge 409109 (Thule @ low Thule Rd)
- Wakool River Reach 2 (Wakool River between Thule Creek and Barbers Creek): Daily discharge data were obtained from gauge 409045 (Wakool @ Wakool-Barham Road).
- Barbers Creek: daily discharge data were obtained from gauge 409111 (Barbers @ Sandy Bridge).
- Wakool River Reach 3 downstream of Barbers Creek: Daily discharge data were obtained from gauge 409062 (Wakool @Gee Gee Bridge)

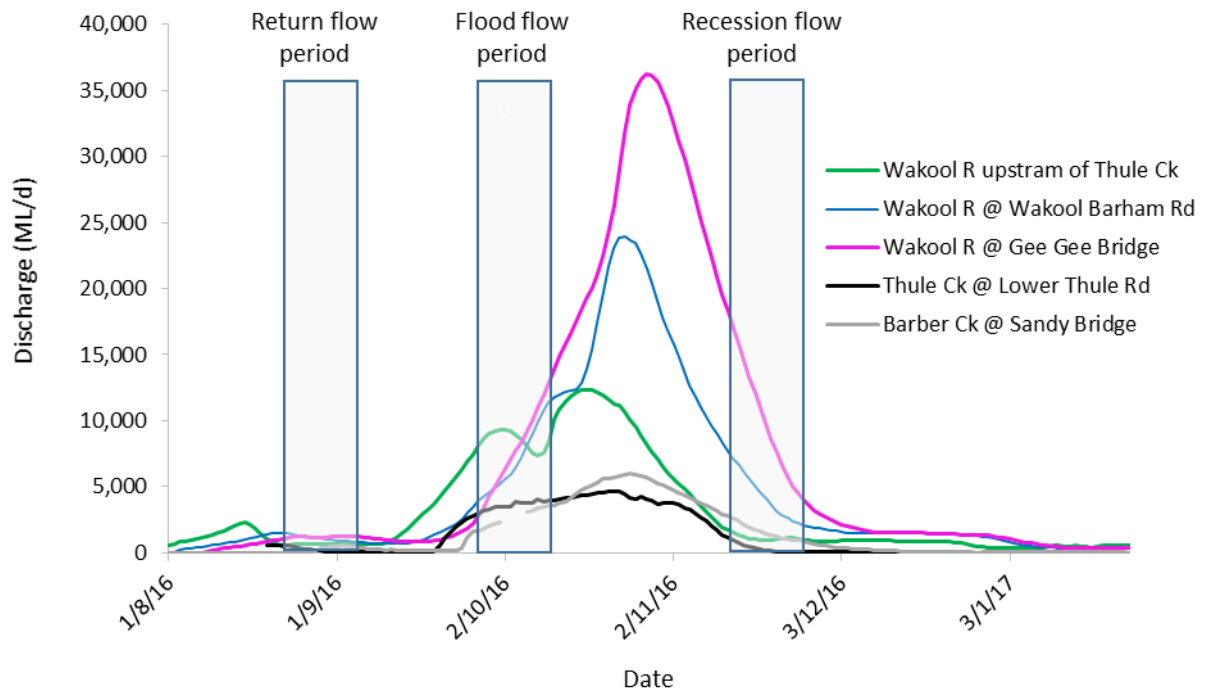
There were a small number of missing daily discharge data from 12<sup>th</sup> to 22<sup>nd</sup> October at the Wakool offtake regulator when a hydrological gauge failed. This was for relatively short periods and data for these dates were estimated based on relationship curves with the Yallakool Creek offtake data.

Summary statistics (median, range and coefficient of variation) of daily discharge were calculated for the 99 day period from 22<sup>nd</sup> August to 28<sup>th</sup> November for each reach for 2015 and 2016.

Summary statistics (median, range and coefficient of variation) of daily discharge were also for the three time periods in 2016 relating to the return flow period (22 August to 5 September), flood flow period during the middle of the event (26 August to 10 October) and recession flow period occurring late in the event (14 to 28 November). Each of these time periods were 15 days duration.

### 3.2 Results

The daily discharge in the Wakool River in 2015 was regulated with no periods of unregulated flow (Figure 3.1). In contrast, the Wakool River in 2016 received return flows from KP Forest via Thule Creek and Barbers Creek (late August to early September) followed by a large flood including extended periods of unregulated flow through KP Forest via Thule Creek and Barbers Creek followed by a recession (Figure 3.1).



**Figure 3.1:** Hydrograph for Thule Creek, Barbers Creek and the Wakool River reach 1, 2 for the period 1/8 to 1/11 in both 2015 (above) and 2016 (below) with three sample periods shaded (return flow period, flood flow period, recession flow period).

Comparison of discharge summary statistics is provided for the 99 days from 22<sup>nd</sup> August to 28<sup>th</sup> November in 2015 and 2016 (Table 3.1). The results in this table incorporate the entire study period.

In 2016 Thule Creek was not flowing and Barbers Creek was not flowing but retained water within a pool (Table 3.1). The median discharge in all Wakool River reaches in 2016 was at least an order of magnitude higher than in 2015.

There was a low coefficient of variation flow discharge in all reaches in 2015, when there were regulated flows (Table 3.1). In contrast, there was a considerably higher coefficient of variation in all reaches in 2016, reflecting the variability of discharge from low flows to peak flows followed by recession flows. For much of this period in 2016 flows were unregulated.

**Table 3.1:** Summary hydrological statistics for Thule Creek and Barbers Creek and three reaches in the Wakool River in the Edward-Wakool system for 2015 (22/8/15 to 28/11/15) and 2016 (22/8/16 to 28/11/16) (n=99 days each year) cv = coefficient of variation.

	2015 (22/8 to 28/11) (n=99)			2016 (22/8 to 28/11) (n=99)		
	Median	Range	CV	Median	Range	CV
<b>Wakool River reach 1</b>	416.88	293.88	0.20	3019.69	9482.89	0.82
<b>Thule Creek</b>	0.00	2.81	N/A	1649.04	4617.71	0.92
<b>Wakool River reach 2</b>	425.59	264.21	0.18	4635.51	23234.57	0.96
<b>Barbers Creek</b>	46.49	43.34	0.26	1837.44	5960.56	0.88
<b>Wakool River reach 3</b>	362.65	230.72	0.17	7107.00	35372.17	1.00

A comparison of discharge summary statistics among the three time periods in 2016 is presented in in Table 3.2).

In the 2016 return flow period Thule and Barbers Creek started to receive flood water from KP Forest prior to the flows being delivered into the Wakool River through the Wakool and Yallakool regulators. Consequently both Thule Creek and Barbers Creek had considerably higher CV than all three Wakool River reaches in the return flow period.

In the flood flow period all reaches, with the exception of the Wakool River reach 3, experienced their highest median flow. The peak in Wakool River reach 3 occurred at a later time than other reaches, as it is the most downstream reach

In the recession flow period the discharge in Thule Creek had reduced to very low flow, whereas Barbers Creek was still receiving considerable return flows from KP Forest. The flow in Wakool River reach 1 and 2 had reduced considerably, but Wakool River reach 3 continued to flow strongly, partly due to the return flow from Barbers Creek and because it is the most downstream reach.

**Table 3.2:** Summary of hydrological statistics for Thule Creek, Barbers Creek and three reaches in the Wakool River in the Edward-Wakool system in 2016 for three time periods: return flow period (22/8/15 to 5/9/15), flood flow period (26/9/16 to 10/10/16) and recession flow period (14/11/16 to 28/11/16).

	Return flow period (n=15) 22/8/16 to 5/9/16			Flood flow period (n=15) 26/9/16 to 10/10/16			Recession flow period (n=15) 14/11/16 to 28/11/16		
	Median	Range	CV	Median	Range	CV	Median	Range	CV
<b>Wakool R reach 1</b>	500.25	139.23	0.08	6826.94	1559.64	0.09	1187.37	236.87	0.06
<b>Thule Creek</b>	142.16	519.00	0.82	3515.77	1229.36	0.10	106.19	709.86	1.03
<b>Wakool R reach 2</b>	1049.80	693.33	0.21	5996.03	7925.52	0.38	3181.93	4702.69	0.43
<b>Barbers Creek</b>	334.43	541.45	0.83	2666.20	1916.16	0.25	1193.82	1702.95	0.40
<b>Wakool R reach 3</b>	1202.58	183.64	0.05	7107.00	10725.06	0.47	7575.92	12563.88	0.50

## 4. WATER QUALITY AND CARBON RESPONSES TO FLOODPLAIN RUN-OFF FROM KOONDROOK-PERRICOOTA FOREST

### 4.1 Introduction

Water quality parameters can change rapidly in response to changes in environmental conditions and these changes may be expected to be some of the first measurable impacts of reconnection between a river and the floodplain. The nature of these changes will vary depending on the amount of water, timing of the flow and pre-existing conditions in both the river and on the floodplain. Carbon and nutrients will be exchanged between the river, wetlands and previously dry parts of the channel or floodplain (Baldwin 1999; Baldwin and Mitchell 2000), or increased flow may have a dilution effect on these parameters instead. The introduction of additional carbon and nutrients to the water column is likely to stimulate microbial productivity (with flow-on effects to other parts of the food web). 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). Aquatic environments naturally have quite variable dissolved organic matter concentrations and there are no optimal concentrations or trigger values provided for organic matter (ANZECC 2000).

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. 1999). The very large, complex type of organic matter referred to as humic substances (often very dark coloured) 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 (Moran and Zepp 1997; Howitt et al. 2008).

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). The connection between a river and its floodplain has been shown to generate essential carbon stores to sustain the system through drier periods (Baldwin et al. 2013). However, excessive nutrient and organic carbon inputs can result in poor water quality through the development of algal blooms or blackwater events resulting in very low dissolved oxygen concentrations (Howitt et al. 2007; Hladysz et al. 2011). Not all tea-coloured water is expected to result in low dissolved oxygen concentrations- the development of hypoxia depends on the combination of organic matter inputs (amount and type), rates of oxygen consumption and the processes that re-introduce oxygen to the water column. Inputs of large amounts of organic matter and nutrients during hot weather are particularly problematic due to the influence of temperature on the rates of microbial processes and organic matter leaching (Howitt et al. 2007; Whitworth et al. 2014).

This project addressed the following hypotheses:

- 1) Return flows early in the flood event were expected to export carbon and nutrients from the floodplain without causing hypoxia in the Wakool River during periods of low water temperature.
- 2) During flooding carbon and nutrient concentrations in the Wakool River, Thule and Barbers Creeks were expected to be increased due to widespread contact with the floodplain.
- 3) Recession flows were expected to result in gradual decreases in carbon and soluble nutrients as the river becomes restricted to the channel, with continuing inputs until wetlands cease to drain.

## 4.2 Methods

D-opto dissolved oxygen loggers (Zebra Tech) were deployed at each of the six Wakool River sites, monitoring dissolved oxygen (DO) and water temperature continuously at ten minute intervals between late August 2015 to late October 2015 and August to-December 2016. Thule and Barbers Creeks were only monitored between August and December 2016. Flow conditions limited access to a number of these loggers resulting in some gaps in the data.

Water quality parameters (temperature (°C), electrical conductivity (mS/cm), dissolved oxygen (%), pH, and turbidity (NTU)) were measured as spot recordings on each monitoring trip where access to sites was possible.

Water samples were collected from two sites within each reach, and from Barbers and Thule Creeks, and processed according to the methods detailed in Watts et al. (2014) to measure:

- Dissolved Organic Carbon (DOC)
- Nutrients (Ammonia (NH<sub>4</sub><sup>+</sup>), filtered reactive phosphorus (FRP), dissolved nitrate + nitrite (NO<sub>x</sub>), Total Nitrogen (TN) and Total Phosphorus (TP))
- Absorbance and fluorescence spectroscopy for organic matter characterisation.

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. DOC and nutrient samples were frozen and sent to the Water Studies Centre at Monash University for analysis. Carbon characterisation samples were returned to CSU Wagga Wagga and then analysed within a day 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° 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, Baldwin 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 fluorescence contour plot is shown in Figure 4.1. 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.

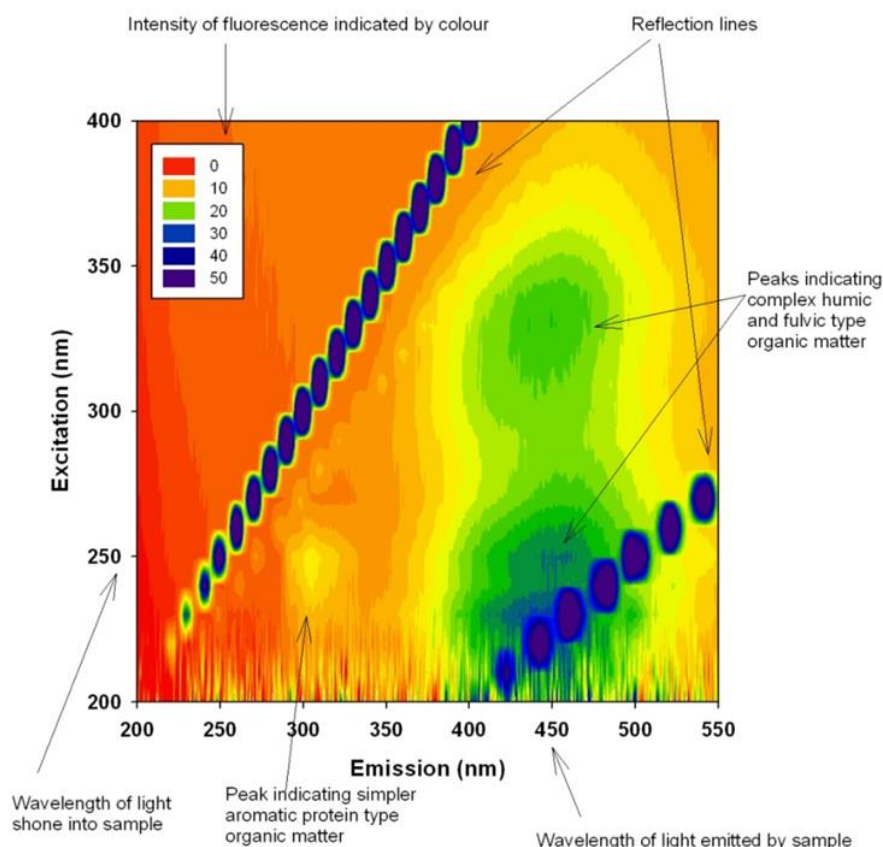


Figure 4.1: Sample excitation emission contour plot indicating key features of the data. (Watts et al. 2013)

The results were assessed against trigger levels from the ANZECC (2000) water quality guidelines. If the concentration of a particular water quality parameter exceeds the trigger level or falls outside of the acceptable range, it is intended that further investigation of the ecosystem is 'triggered' to establish whether the concentrations are causing ecological harm. Systems may vary in their sensitivity to various parameters and therefore exceeding a trigger

level is not an absolute indicator of ecological harm. It is not unexpected that some water quality parameters may exceed the trigger levels during periods of very high flow and this is not necessarily a sign of poor ecosystem health. The ANZECC 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. Given the variable make-up of organic carbon, and the possible range of ecological responses to this, 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.

### 4.3 Results and discussion

#### Temperature

Temperature influences a number of water quality parameters through changes in rates of microbial respiration and chemical reactions, decreases in oxygen solubility at higher water temperatures and increased rates of organic matter leaching from the floodplain. Temperatures at all sites were similar although wider daily fluctuations were recorded in Thule and Barbers Creeks in September 2016 than in the Wakool River reaches (Figure 4.2) and this is likely due to a shallower water depth at these sites. A steep rise in water temperature was observed at all sites over the same period around the 6<sup>th</sup> October (Figure 4.2) coinciding with the time when rapid onset of hypoxia was observed (presented later in Figure 4.7). The 3 to 4 degree increase in water temperature at this time appears to have been sufficient to accelerate respiration beyond the capacity of the system to maintain DO in the water column (microbial respiration is affected by temperature).

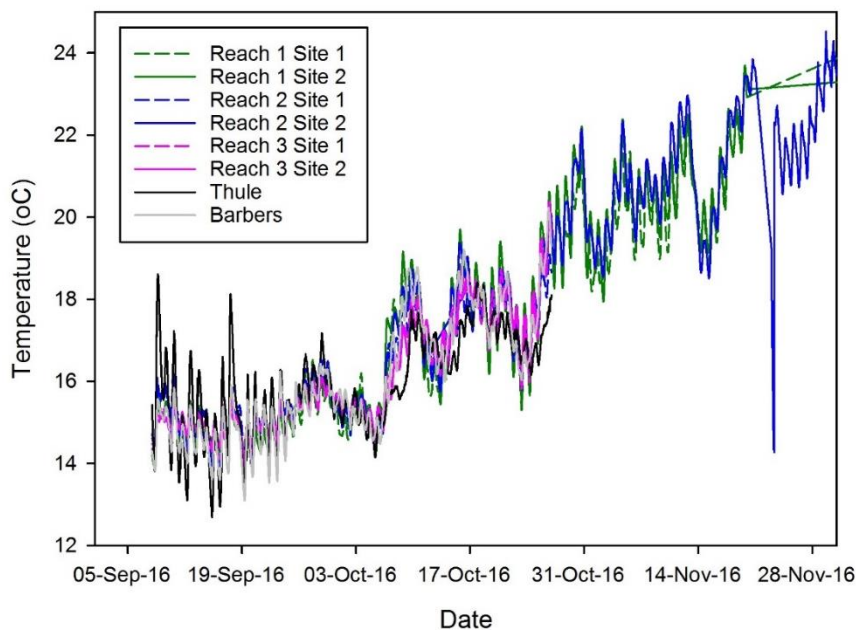


Figure 4.2: Water temperature at all study sites in the Wakool River, Thule Creek, Barbers Creek from 5<sup>th</sup> September to 1<sup>st</sup> December 2016



### Dissolved organic carbon

Connection with the floodplain had a strong and rapid influence on DOC concentrations in the Wakool River (Figure 4.3). It should be noted that during the extensive flooding that occurred during this event, DOC will have entered the system from upstream forested areas (including, but not limited to, Barmah-Millewa Forest), but also from flooded grazing and cropping land and that there are complex and varied sources of the DOC in this system. For almost all sample dates (exception 5/9/2016) the DOC concentrations in Thule Creek and Barbers Creek were higher than all Wakool River reaches (Figure 4.3).

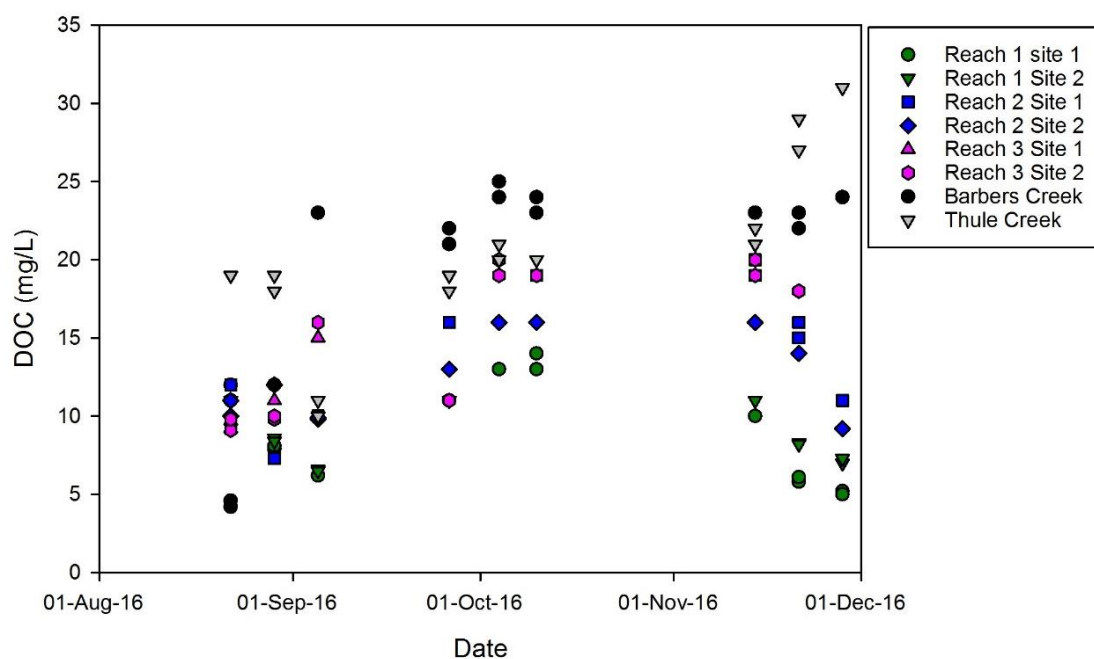


Figure 4.3: Dissolved Organic Carbon concentrations at all study sites in the Wakool River, Thule Creek, Barbers Creek from late August to 1<sup>st</sup> December 2016.

### Carbon Characterisation

Dissolved organic carbon (DOC) analysis degrades all organic matter present in the sample and reports it as equivalent. When considering the influence of carbon inputs into an aquatic ecosystem it is useful to look for markers of changes in the type of carbon present, in addition to the amount. In this work we have used the optical properties (interactions between the sample and light) to provide clues to changes in the mixture of organic materials present.

Scans of the absorbance of ultraviolet and visible light provide an indication of both the amount of DOC (a relationship can be established between absorbance in the UV region and approximate DOC concentrations) and changes to the average size of the molecules. Increasing absorbance in the longer wavelengths suggests the presence of large, complex molecules. Absorbance spectra for representative water samples are provided in Figure 4.4. It should be noted that absorbance graphs for this system would normally not include absorbance values

greater than 1 (frequently less than 0.5), and elevated concentrations of coloured organic matter are present throughout the study period. The first group of three sampling dates captures an increase in organic matter in Barbers Creek as the system transitions from a disconnected pool with a flat scan profile consistent with lower concentrations of aged organic matter, to a flowing system with considerable amounts of fresh organic matter (grey line, equivalent to green and black lines in second and third graphs, respectively). During the recession flow period a 'hump' in the scan around 260 nm and a steep increase near 200 nm is not present in the Zone 1 samples, suggesting the Wakool River organic matter from upstream is reverting to a more usual 'aged' organic matter profile

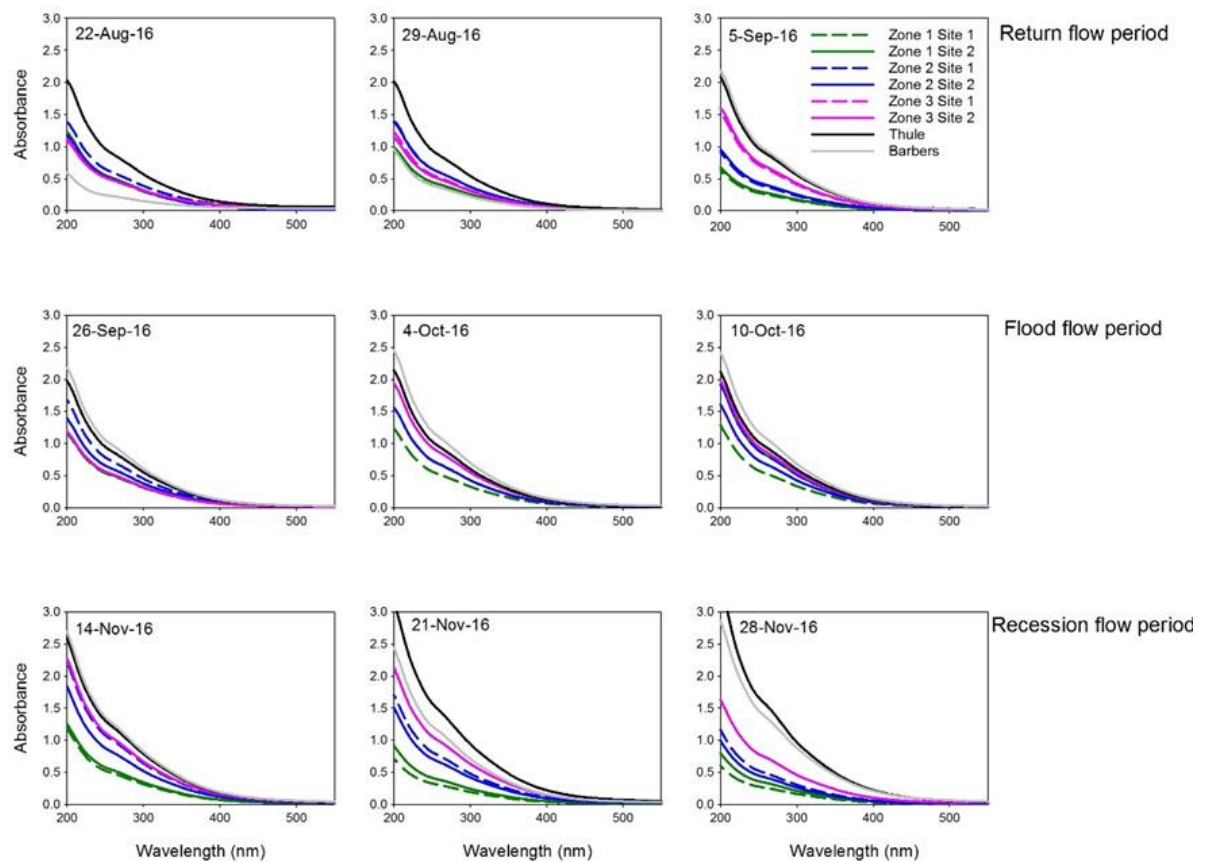


Figure 4.4: Absorbance spectra for all available sites over the study period.

Fluorescence spectroscopy detects organic matter that is composed of large, complex molecules that are capable of absorbing light and re-emitting it at a longer wavelength. Not all organic matter is fluorescent, but changes in the intensity or wavelength of a fluorescence signal can be a sensitive marker of changes to average molecular size, type and amount, and provides clues to different sources of organic matter or different impacts on the ecosystem. The results are presented and discussed for the three periods: return flow period, flood flow period, and recession period.

Return flow period:

Fluorescence plots for 22<sup>nd</sup> August (Figure 4.5a) during the return flow period show that downstream of Thule Creek there is a clear input of carbon from KP Forest (compare plots for Wakool River Reach 1 with Reach 2). There is no evidence that Barbers Creek was receiving flows through KP Forest at this stage. The fluorescence plot for Reach 1 suggests there is upstream DOC inputs from the floodplain or perhaps aged carbon from a wetland. Thule Creek inputs alter the type as well as the amount of DOC. There was decreased carbon fluorescence on 29<sup>th</sup> August compared to the previous week at all sites (Figure 4.5b) and evidence of continued inputs from Thule Creek. There was substantial input of fluorescent DOC from both Thule and Barbers Creek on 5<sup>th</sup> September 2016 (Figure 4.5c). Further analysis is required to see if the type is different (plots are off-scale but kept to the normal scale for ease of comparison with previous years). On the 5<sup>th</sup> September there is clear evidence of floodplain carbon also entering from upstream of Wakool River reach 1.

Flood flow period:

By the 26<sup>th</sup> September all sites show clear input of floodplain carbon at this point in the flood event. The Wakool River reach 2 Site 1 is more impacted by Thule Creek than reach 2 site 2 (Figure 4.6a). This has been noted in some of the other parameters and it may be due to additional water entering the Wakool River via the creek network (e.g. Merrabit Creek and Bookit Creek) that enters the Wakool River between these two sites, thus diluting the inputs from Thule Creek. By the 4<sup>th</sup> October the fluorescence intensity has increased at all sites as the flood continues to cover previously dry floodplain (Figure 4.6b). By the 10<sup>th</sup> October Reach 1 site 1 and Reach 2 Site 2 show signs of some aging of the fluorescent carbon from upstream, while new inputs are received from KP Forest and are evident in the Wakool River reach 2 Site 1 and Reach 3 (Figure 4.6c).

Recession flow period:

During the recession flow period on the 14<sup>th</sup> November there was extensive ongoing inputs of carbon to the Wakool River from both Thule and Barbers Creek (Figure 4.7a). However, as the recession continued by the 21<sup>st</sup> November the decreasing fluorescence intensity is apparent in the Wakool River upstream sites but additional DOC was still entering Reach 2 and Reach 3 from Thule and Barbers Creek (Figure 4.7b). The inputs of carbon from KP Forest during this recession period will be more evident and have more influence as the discharge in the Wakool River recedes (Figure 3.1)

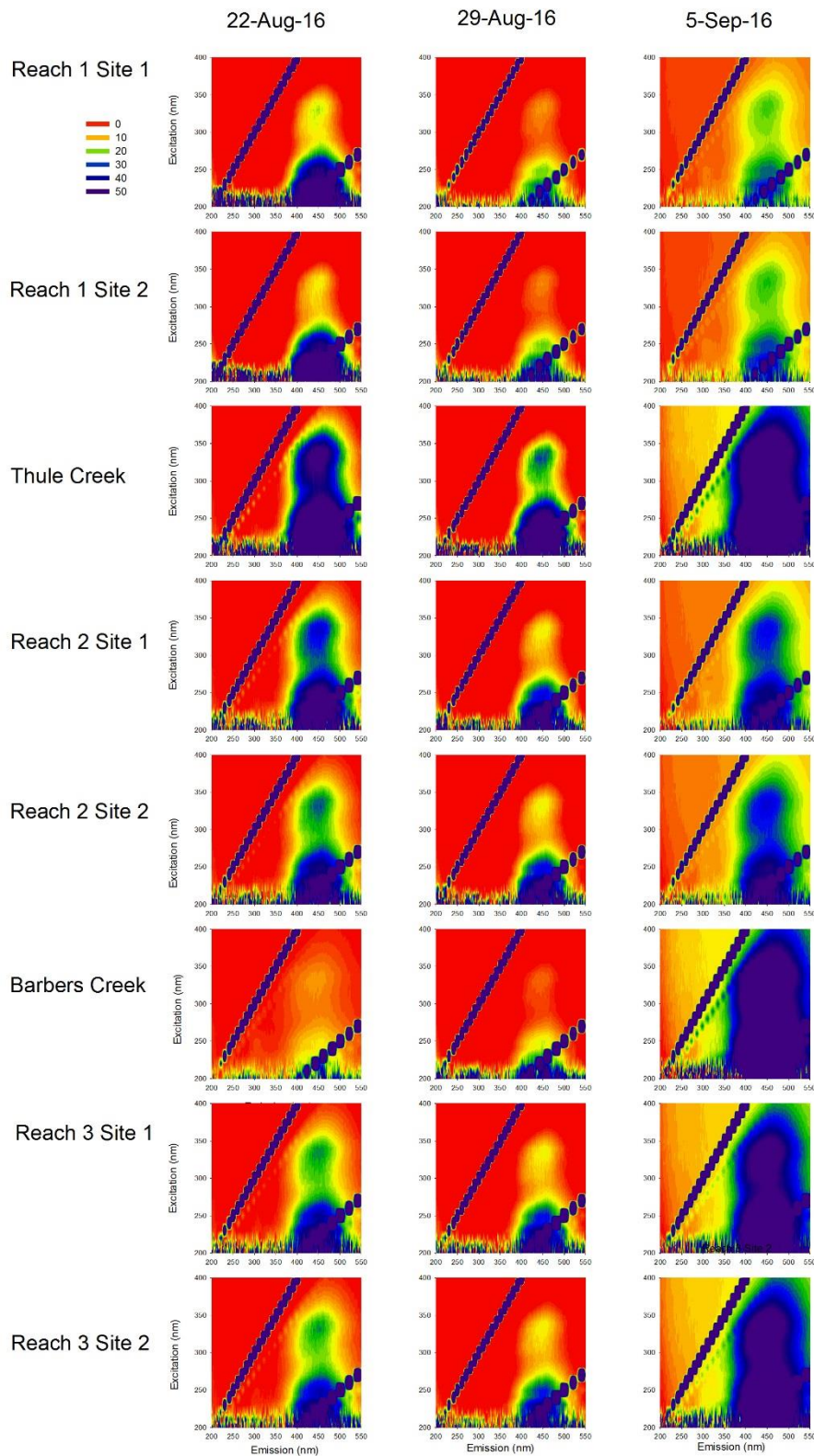


Figure 4.5: Representative fluorescence plots for water samples collected from the Edward-Wakool system during the return flow period on a) 21<sup>st</sup> August, b) 29<sup>th</sup> August and c) 5<sup>th</sup> September 2016



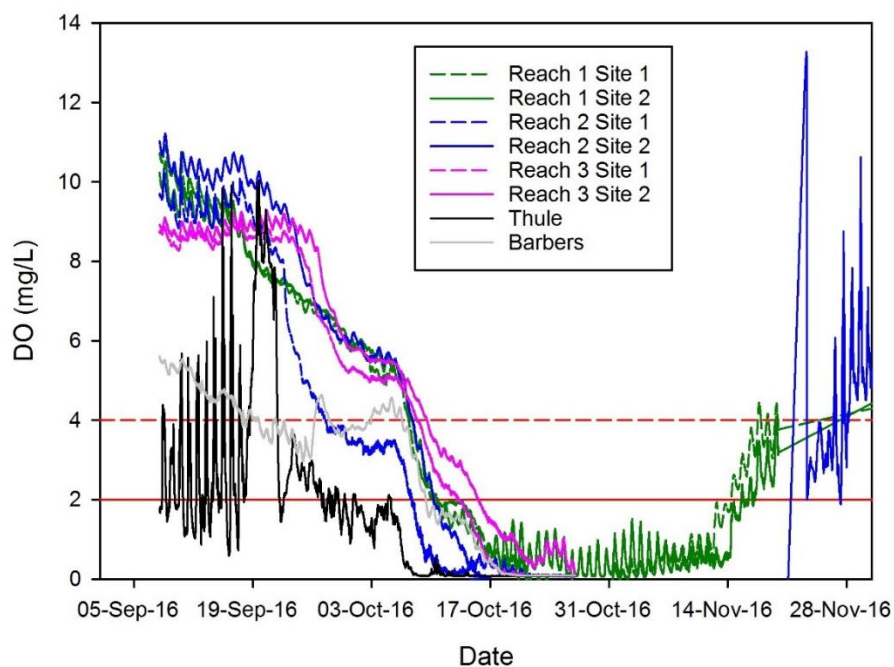


### Dissolved Oxygen

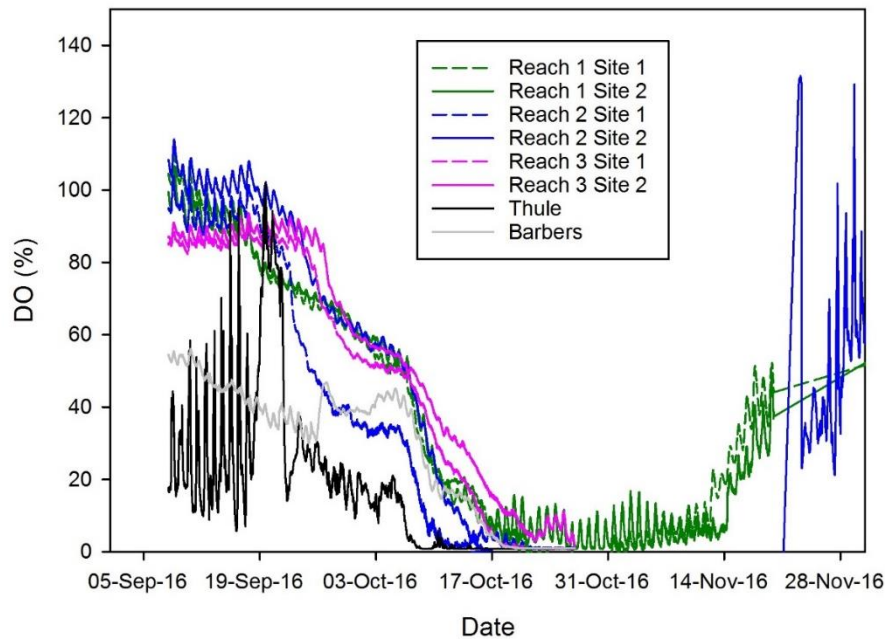
During this study dissolved oxygen in the water column became a critically important water quality parameter as the extensive flooding that continued into December resulted in a hypoxic blackwater event. In figures 4.8 and 4.9 the horizontal lines indicate the thresholds of 4 mg/L where fish populations are likely to show signs of stress, and 2 mg/L where fish deaths may occur. The response of the fish population to hypoxia will reflect the period of time they are exposed to adverse water quality, the severity of the hypoxia, availability of localised refuges and differences in susceptibility of different fish species and of individuals within a particular species.

Dissolved oxygen concentrations were in the acceptable range at all Wakool River sites through to late September 2016 (Figure 4.8). Much lower concentrations were present in Thule and Barbers Creek systems at this time, likely associated with much higher DOC concentrations and low flow rates. An accelerated decline in DO to hypoxia is observed at all sites over a period of a few days in early October (especially the 6<sup>th</sup> to 8<sup>th</sup> October). Comparison with the dissolved oxygen saturation data (Figure 4.9) shows the same trend is present. This suggests a rapid increase in oxygen consumption from the water column at this time, rather than a decline in oxygen solubility.

The decline in DO at all sites around the 6<sup>th</sup> October occurred when there was a rise in water temp of approximately 3 degrees (Figure 4.2) suggesting the strong role of temperature in hypoxia. The earlier fresh in August had very different outcome when the water temperature was lower with increases in DOC not resulting in hypoxia in the Wakool River.



**Figure 4.8:** Dissolved oxygen (mg/L) at all study sites in the Wakool River, Thule Creek and Barbers Creek from September to 1<sup>st</sup> December 2016. Horizontal lines indicate the 4 mg/L and 2 mg/L concentrations where stress of fish populations and the onset of fish kills are likely.



**Figure 4.9:** Dissolved oxygen saturation (%) at all study sites in the Wakool River, Thule Creek and Barbers Creek from September to 1<sup>st</sup> December 2016.

#### *Spot water quality measurements*

Dissolved oxygen was also measured with a hand-held meter at each site one each sample date when water samples were collected. While these measurements do not provide the same high resolution indication of changes with time, the data set presented below covers a wider time period and gives a clear indication of the extent of hypoxia at each site at the time of sampling. Figure 4.10 shows that during the return flow period, the dissolved oxygen concentrations in all three reaches start out similar but by early September Reach 2 site 1 and Reach 3 are showing small reductions in DO relative to Reach 1. Reach 2 Site 1 appears to be more impacted by water quality from Thule Creek than Reach 2 Site 2, which may have received additional dilution flows from the Merrabit C and Bookit Creek network in this area. During the high flow period in October the DO is similar at all sites and this corresponds with the decline into hypoxic conditions noted above.

During the recession flow period in late November increasing DO concentrations are evident in Reach 1, reflecting improved water quality entering the reach from upstream. The slower improvement in DO in the Wakool River reach 2 and 3 are the result of the later receipt of this water further downstream in the system, combined with ongoing inputs of low DO water from Barbers and Thule Creeks. All spot DO measurements in the Wakool River during the period September-October 2015 were in the range 5.2-8.5 mg/L.



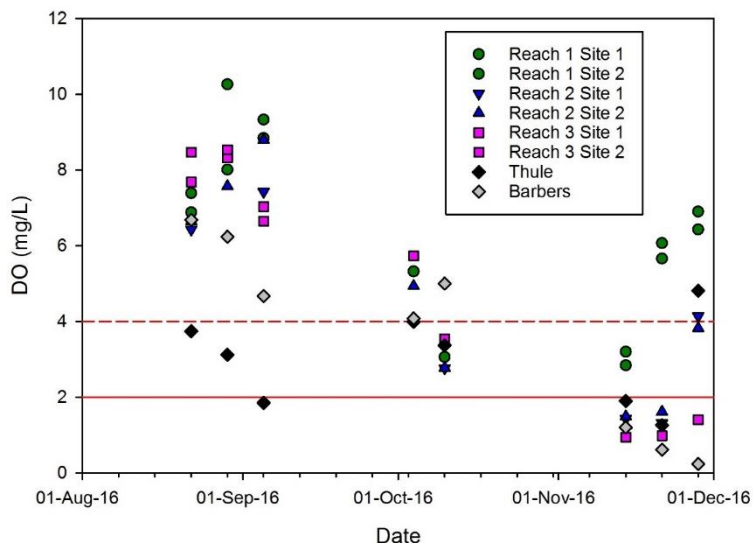


Figure 4.10: Spot Dissolved Oxygen measurements (mg/L) at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

The pH of the water during the return flow and flood flow periods was highly variable and frequently fell below the lower ANZECC (2000) trigger guideline of 6.5 (Figure 4.11). As pH is affected by both respiration and photosynthesis (altering the amount of dissolved carbon dioxide), plus the input of acidic molecules from the floodplain, the variation and tendency towards acidic conditions is not unexpected under these flow conditions. Many dissolved organic carbon molecules will be slightly acidic and will contribute to lowering of pH during blackwater events. During the recession flow period the variability was reduced and the pH was in the normal range. The high pH in Thule Creek may indicate increased algal activity at that time. All pH measurements in Sept-Oct 2015 were in the range 6.3-7.6.

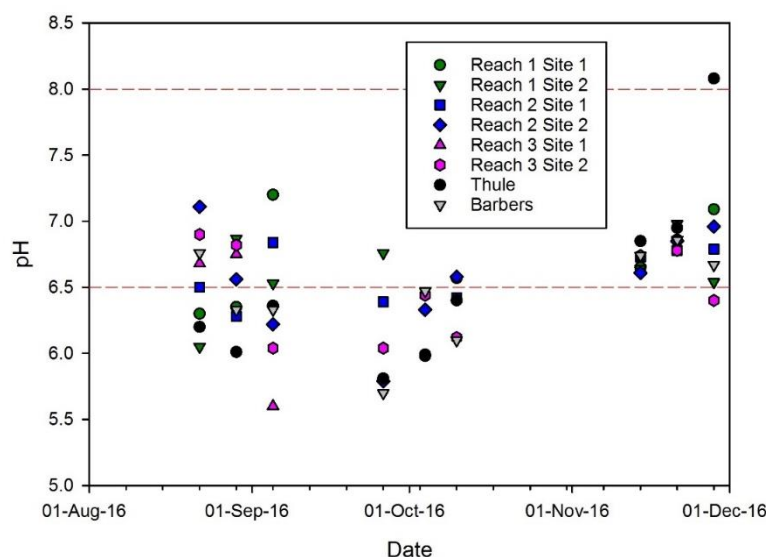


Figure 4.11: pH measurements at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.. Dotted red lines indicate the upper and lower ANZECC (2000) trigger levels.

Electrical conductivity (EC)(Figure 4.12) increased during the flow event, but remained within the lower end of the range expected for lowland rivers (0.125-2.2 mS/cm) (ANZECC 2000). Conductivity is higher in Thule Creek and Barbers Creek and during the recession flows in particular. This is seen to have a measureable effect on Reach 2 and Reach 3. Spot EC results in 2015 were in the range 0.02-0.134 mS/cm.

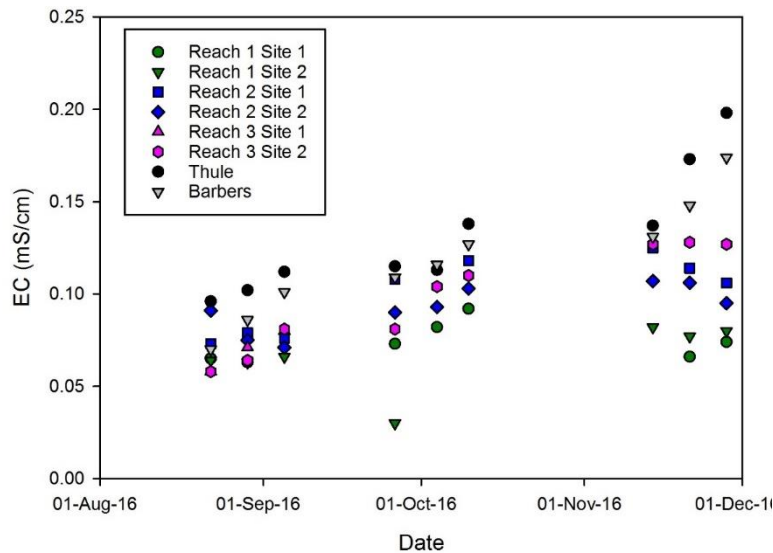


Figure 4.12: Electrical Conductivity (mS/cm) at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

Turbidity of rivers is known to decrease during blackwater events (the water appears dark coloured but not cloudy). Very low turbidity values were recorded during the Return flow period and the peak flows, with the turbidity gradually returning to normal or slightly elevated levels during the recession flow. The range for Sept-Oct 2015 was 43 to 100, whereas in 2016 values both considerably lower and considerably higher were recorded (Figure 4.13).

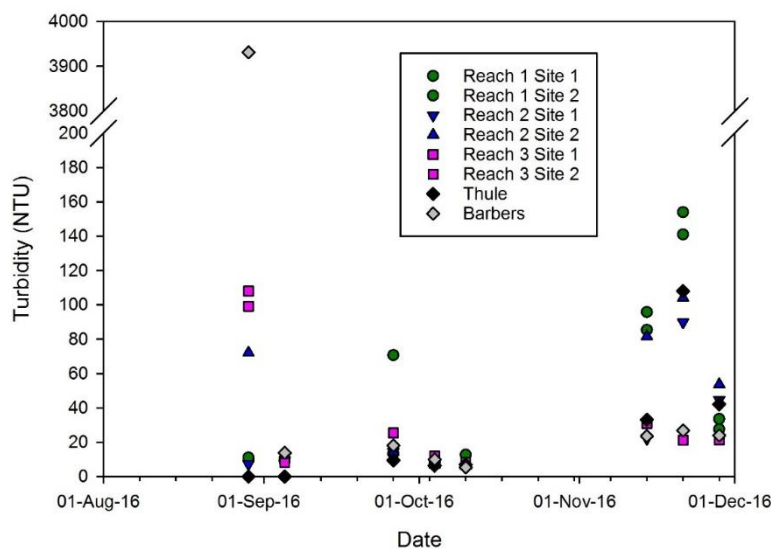


Figure 4.13: Turbidity (NTU) at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016. Note, extremely high reading for Barbers Creek-in Sep needs to be checked as is most likely an error but may be a sign of disturbed sediment or algae.

### Nutrients

Nutrients (nitrogen and phosphorus) were measured in their total forms (TN, TP) and also in the soluble/bioavailable forms: ammonia ( $\text{NH}_3$ ), nitrate + nitrite ( $\text{NO}_x$ ) and filterable reactive phosphorus (FRP). Total Nitrogen concentrations were considerably elevated throughout the study (Figure 4.14). In this system TN is typically close to the ANZECC (2000) trigger level of 0.5 mg/L. However, the concentrations observed during the flood were generally less than during the peak of the 2016 algal bloom (around 4 mg/L, with some measurements over 6 mg/L). The highest concentrations were recorded when floodwater was draining back into the river channel and during this time the inputs from Thule and Barbers Creeks are more evident.

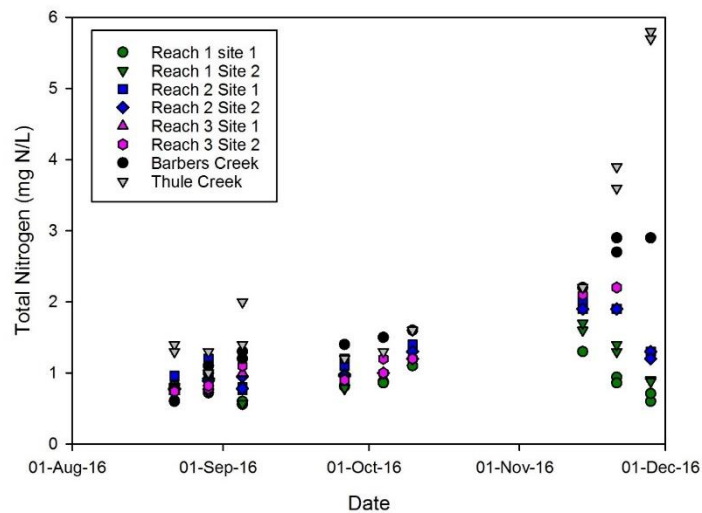


Figure 4.14: Total Nitrogen concentrations at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

Ammonia concentrations well above the ANZECC (2000) trigger level of 0.02 mg/L were observed during this study, most notably during the recession flow period (Figure 4.15). Microbial production of ammonia from organic nitrogen will occur with prolonged hypoxia, especially in anoxic sediments. The results reported here exceed the maximum observed during the 2016 algal bloom (0.4 mg/L after bloom collapse in the canal). Ammonia is a particularly bioavailable form of nitrogen and may stimulate growth of vegetation or algae.

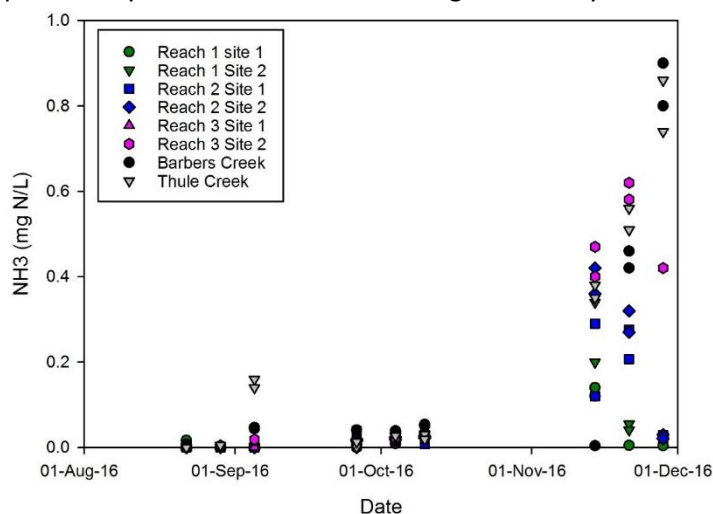
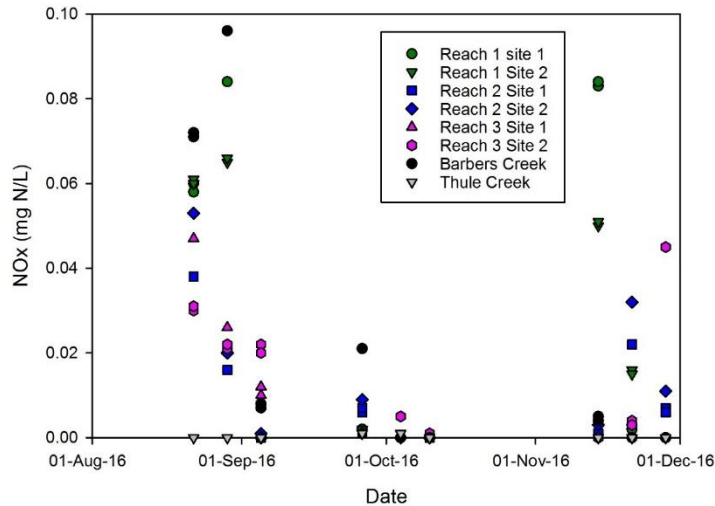


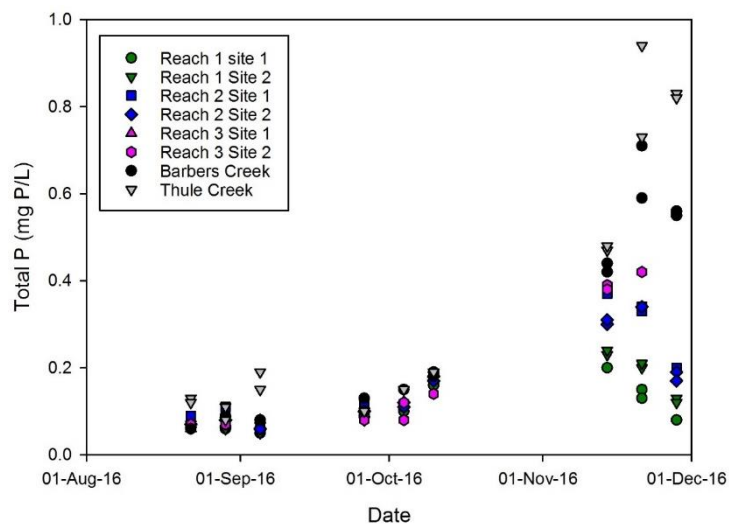
Figure 4.15: Ammonia concentrations at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

On the first two sampling dates in August a pulse of nitrate and nitrite (the technique measures these as a single parameter) was moving down the Wakool River from upstream (Figure 4.16). Barbers Creek was initially a disconnected pool and had high  $\text{NO}_x$ , but nutrients decreased with flushing when the flows commenced. Note that nitrate and nitrite concentrations are highly soluble but also bioavailable and concentrations will reflect the balance of flushing from the floodplain, dilution with large volumes of water and uptake by organisms. Compare concentrations with total N (Figure 4.14) – these soluble forms make up a small fraction of the total with the remainder bound to particles, contained within living cells or in the form of dissolved organic nitrogen. The ANZECC (2000) trigger level is 0.04 mg/L, which was exceeded at both the beginning and end of this event.



**Figure 4.16:** Nitrate and nitrite concentrations at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

A strong flood influence was observed on total phosphorous (TP) in November 2016, with a declining trend present in the Wakool River in the recession period when the water quality improves from the Edward River but inputs were considerable from KP Forest. The highest recorded value in 2015 was 0.2 mg/L. Total phosphorous in this system is normally around the 0.05 mg/L (trigger level) and values during the recession flow period were well above this trigger level (Figure 4.17).



**Figure 4.17:** Total Phosphorous at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

Bioavailable phosphorus (FRP) is notably usually very low in this system (normally less than 0.005 mg/L) relative to the ANZECC (2000) trigger level of 0.02 mg/L. The concentrations reported in Figure 4.18 are considerably elevated compared to baseline levels, especially during the recession flow period (Figure 4.18). KP Forest was a source of FRP, especially in November 2016.

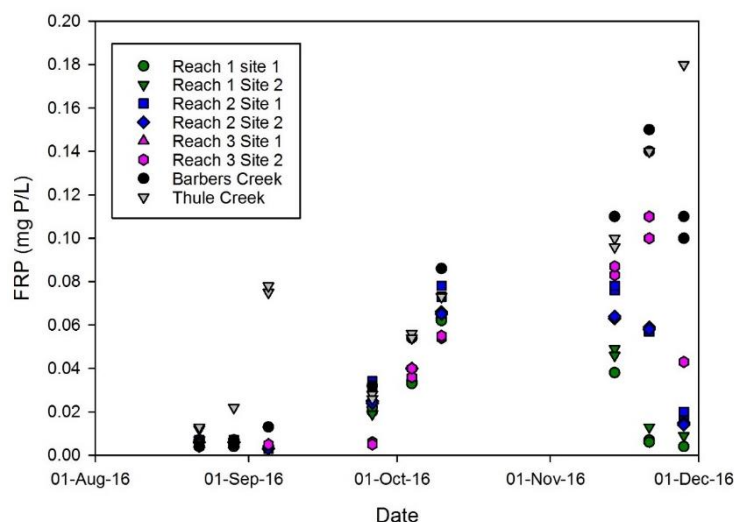


Figure 4.18: Filterable Reactive Phosphorous at all study sites in the Wakool River, Thule Creek and Barbers Creek from late August to 1<sup>st</sup> December 2016.

#### Water quality in context - Summary of key points:

##### Return flow period (22 August to 5 September 2016)

- Thule and Barbers Creeks were both important sources of carbon to the Wakool River. During the return flow period Thule Creek inputs markedly changed the DOC profile in the Wakool River.

##### Flood flow period (26 September to 10 October 2016)

- There was a strong effect of temperature for the onset of hypoxia around the 6<sup>th</sup> October during the flood flow period.
- The fluorescence data suggest that Murrabit and Bookit Creeks were important sources of dilution water in the Wakool River between Greenhill Lane (reach 2 site 1) and the Wakool Barham Rd bridge (reach 2 site 2) during the flood flow period.

##### Recession flow period (14 to 28 November 2016)

- Ammonia production increased in the recession flow period (14-28 November 2016) with prolonged hypoxia (anoxic sediment is a source of ammonia).
- Total phosphorous (TP), total nitrogen (TN), nitrate, nitrite and filterable reactive phosphorous were much higher during the recession flow period when discharge in the Wakool River was decreasing and floodwater from KP was continuing to drain back into the river channel from KP Forest. This may be due to particulate matter draining back into the river from the floodplain, in addition to soluble inputs from wetlands.

## 5. STREAM METABOLISM RESPONSES TO FLOODPLAIN RUN-OFF FROM KOONDROOK-PERRICOOTA FOREST

### 5.1 Introduction

All organisms use carbon-based energy to support basic metabolic processes. In floodplain river ecosystems this carbon is provided in the form of living plant biomass, produced during photosynthesis by in-stream aquatic plants and algae or terrestrial vegetation on the adjacent floodplain. Carbon is subsequently consumed when organisms respire, including when plants consume their own energy reserves or when heterotrophic organisms consume plant biomass. The twin in-stream processes of gross primary production (GP) and ecosystem respiration (ER) are collectively referred to as 'stream metabolism', and they form the basis of all food available in a river ecosystem, with net primary production in systems that are dominated by algal production and net respiration in systems where other out of channel or upstream sources of carbon dominate (Young et al. 2008; Figure 5.1).

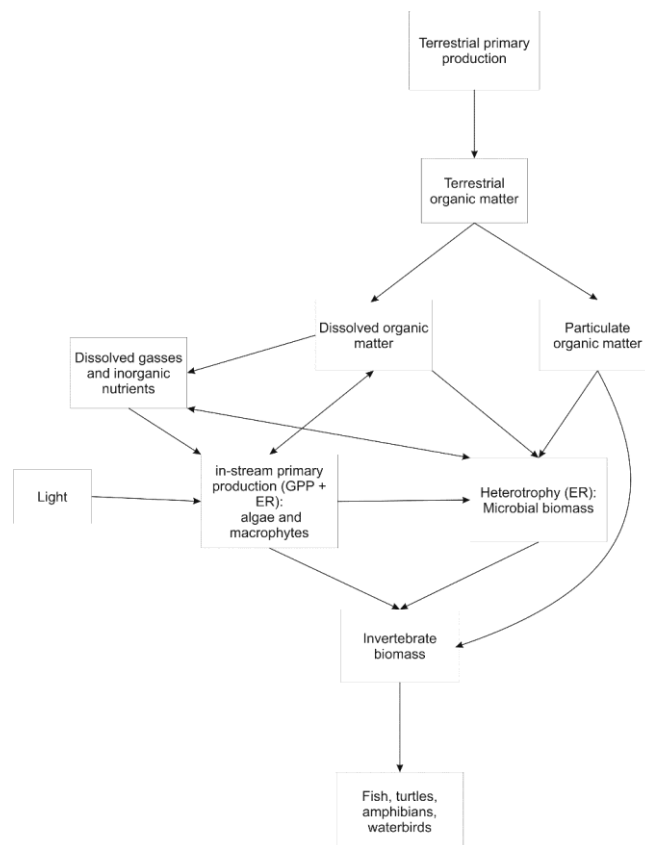
Rates of stream metabolism vary with temperature, as well as the availability of light, nutrients and carbon (Young et al. 2008). As the master variable controlling these drivers (Poff and Zimmerman 2010), flow plays a key role in determining rates of metabolism. In-channel flows create pulses of nutrients while influencing a range of physical changes associated with water depth and habitat inundation (see Watts et al. 2009). However, floodplain rivers are unique in that they are characterised by alternating periods of increased resource availability that is linked to flooding (Junk et al. 1989). The timing, frequency and scale of flooding, and the potential boom in productivity, is an important aspect of the annual hydrological cycle to which many floodplain-river species are adapted.

For many regulated floodplain rivers the loss of forested floodplain habitat and reduced frequency and duration of overbank flooding has altered the pattern of carbon and nutrients supplied during flooding (Robertson et al. 1999). This means that food webs in regulated floodplain rivers are often increasingly supported by in-stream primary production, rather than respiration (e.g. Robertson et al. 1999; Vink et al. 2005), with potential flow on effects to the type and number of species that a river can support (Marcarelli et al. 2011). How river food webs are affected by changes to the amount and source of carbon in regulated systems is still being debated (e.g. Baldwin et al 2016). Holistic approaches to environmental water management continue to recognise the importance of floodplain-river linkages and the potential importance of increased carbon subsidies during and after flooding. Solving the problem of diminished or altered productivity pathways for regulated systems is a complex task for water managers. Delivering environmental flows to achieve large-scale overbank inundation events is limited by third party impacts, policy constraints, limited water availability and floodplain development (Wolfenden et al. 2017).

'Return flows' (Wolfenden et al. 2017) describe the movement of water diverted onto floodplains back to the main source. In some regulated systems, small-scale return flows can be used to create one-way hydrological connections between river channels and adjacent river habitats, mimicking aspects of natural connectivity in an otherwise highly-constrained environment (Wolfenden et al. 2017). The degree that return flows can influence riverine processes depends on the relative scale, timing, and duration of managed events.

The KP Forest is a large wetland complex that is densely populated by river red gum (*Eucalyptus camaldulensis*). The scale of engineering works, coupled with the size of the forested system, creates an opportunity to mimic rates of ecosystem processes in receiving stream water by managing wetland connection events to the Wakool River. In this section we examined these metabolism responses to floodplain water diverted from the Murray River to the Wakool River via KP Forest. Specifically, we sought to test whether

- 1) Return flows increase rates of metabolism (GPP and ER) in the Wakool River at sites downstream of the Barbers and Thule Creek junctions. Specifically, return flows are expected to increase rates of ER relative to GPP.
- 2) Flood flows are expected to further increase rates of GPP and ER in the Wakool River at sites downstream of the Barbers and Thule Creek junctions.
- 3) Recession flows were associated with a decline in ecosystem metabolism, and a switch from ER toward GPP.



**Figure 5.1:** A simplified conceptual model showing the relationships between components of ecosystem metabolism, their drivers and outputs.

## 5.2 Methods

### *Chlorophyll-a*

At each site on each sample occasion, a known volume of water (approximately 250mL) was filtered through a glass-fibre filter paper. The filter paper was immediately frozen for transport to the laboratory. The concentration of chlorophyll-a ( $\mu\text{g L}^{-1}$ ) was later determined using a Spectrosonic 20 Genesys spectrophotometer after extraction in a mixture of methanol and magnesium carbonate (after Ryder 2004).

### *Ecosystem metabolism*

Ecosystem metabolism was measured using the single-station open-system method first described by Odum (1956) and calculated using the Bayesian model fitting method described by Grace et al. (2015) modified after Song et al. (2016). D-opto dissolved oxygen loggers (Zebra Tech) were deployed at each of the six sites, monitoring dissolved oxygen (DO) and water temperature continuously at ten minute intervals between late August 2015-late October 2015 and August 2016-December 2016. Light (photosynthetically active radiation, PAR) and barometric pressure were also measured at 10 minute intervals using an Odyssey PAR meter and U20 barometric pressure sensor (HOBO). Metabolism was not monitored in Barbers or Thule Creeks in 2015 as the creeks were dry. To expand the time series, additional oxygen data were obtained from the NSW DPI website (<http://waterinfo.nsw.gov.au/>) for the Deniliquin town gauge and the Gee Gee bridge gauge.

Rates of gross primary production (GPP;  $\text{mgO}_2\text{L}^{-1}\text{d}^{-1}$ ) and ecosystem respiration (ER;  $\text{mgO}_2\text{L}^{-1}\text{d}^{-1}$ ) were calculated using the BASE package (BASE version 3.3.3; Grace et al. 2015) in the R environment (*R Core Team 2012*). Data were excluded from further analysis if  $R^2 < 0.75$  or the coefficient of variation of GPP was  $> 50$ . Calculating metabolism requires a daily rise and fall in dissolved oxygen, with rates of change used to estimate rates of primary production, respiration and reaeration. Because of continuously falling and/or consistently low dissolved oxygen concentrations across all sites (section 4), rates of metabolism could not be calculated for most days in 2016.

### *Data analysis*

To test for differences in chlorophyll-a concentration among flow periods and reaches data were analysed using a two-way permutational analysis of variance (PERMANOVA; Anderson et al. 2008). Flow period and Reach (Table 5.1) were treated as fixed factors with  $n=2$  sites in each reach as the error term. The mean chlorophyll-a concentration, rather than individual sample times, in each flow period was used for the analysis (i.e. the average of three sample occasions for each site). Some missing site/time combinations meant there were no degrees of freedom for some tests, particularly in Reach 3. Resemblance matrices were calculated using a Euclidian distance measure. Prior to analysis, all main effects were tested for significant dispersals using the PERMDISP procedure and  $\log_{10}$  transformed if necessary. Post-hoc tests



were used to further isolate significant terms, using Monte-Carlo tests where numbers of unique permutations were low. All data analyses were performed using Primer 6 with PERMANOVA (Primer-E Ltd.).

**Table 5.1:** PERMANOVA design factors and number of levels used in the analysis of return flows metabolism data.

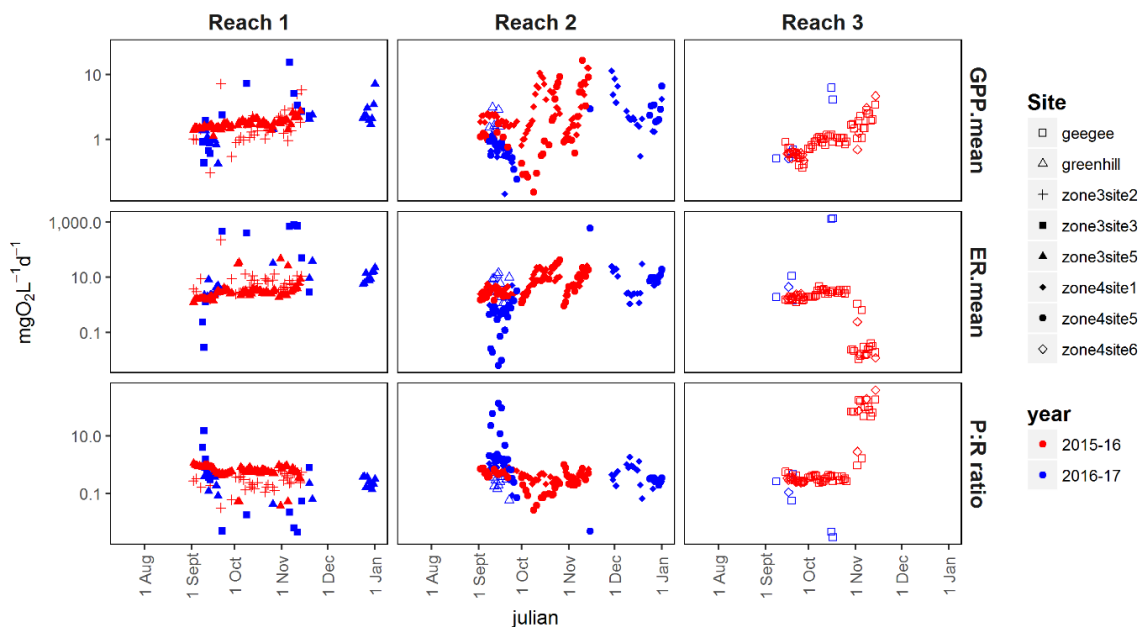
<b>Factor</b>	<b>Levels</b>
Zone	4 (Inflows, Reach 1, Reach 2, Reach 3)
Flow Period	4 (2015 - no return, 2016 return, 2016 flood, 2016 flood recession)
Error term (site)	2 sites in each reach

### 5.3 Results

#### *Metabolism*

Overall, metabolism in the Wakool River was heterotrophic across both years, with ER exceeding GPP by about 2:1. Metabolism was net heterotrophic (P:R <1) for most of 2015, but began with phase of net autotrophy during August 2015 (Figure 5.). There also appeared to be inherent differences among reaches, with Reaches 2 and 3 downstream of KP Forest inflows tending to have slightly higher rates of metabolism than reach 1 (Table 5.2,

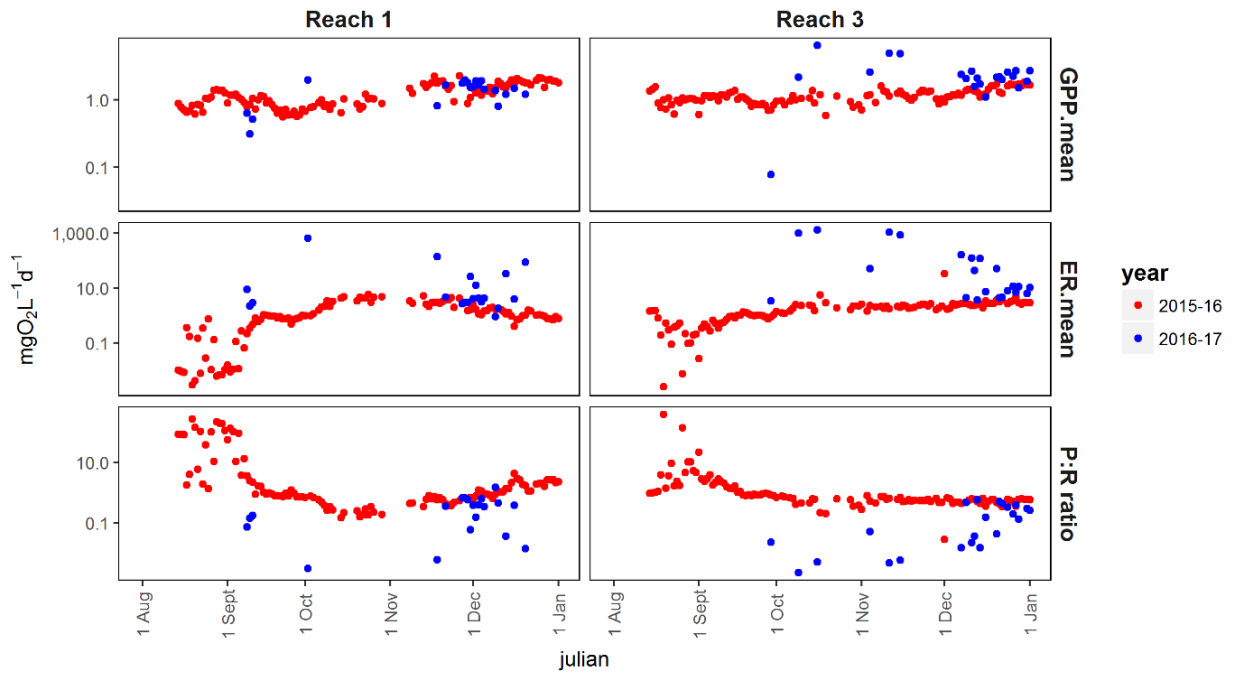
Table 5.3). Where data were able to be calculated, rates of metabolism were typically lower in September 2016 than 2015 (Figure 5.2), toward lower GPP. With the onset of flooding and the flood recession, there is some evidence that ER, in particular, increased and shifted the system toward net heterotrophy (Figure 5.2, Figure 5.3).



**Figure 5.2:** Gross primary production (GPP), ecosystem respiration (ER) and the ratio of GPP and ER (P:R ratio) for all metabolism results calculated for the eight sites included in this study. Note the y-axis is  $\log_{10}$ .

**Table 5.2:** Median (min-max) of gross primary production (GPP), ecosystem respiration (ER) and the ratio of GPP to ER (P:R ratio) for all metabolism data calculated for 2015 and 2016. Data treat all results from the two sites in each reach as a single dataset. See Figure 5.1.

Reach	Water Year	GPP	ER	P:R	<i>n</i>
1 – Wakool River U/S Thule	2015	1.66 (0.30-7.20)	3.22 (1.23-226.51)	0.53 (0.03-1.12)	100
2 – Wakool River D/S Thule		1.89 (0.15-16.62)	4.82 (0.90-41.81)	0.41 (0.02-1.01)	102
3 – Wakool River D/S Barbers		0.91 (0.36-4.70)	1.89 (0.01-4.72)	0.37 (0.23-387.87)	65
1 – Wakool River U/S Thule	2016	2.71 (0.41-15.62)	5.70 (0.02-784.39)	0.43 (0.005-15.03)	61
2 – Wakool River D/S Thule		1.62 (0.14-27.22)	3.25 (0.006-587.87)	0.56 (0.005-134.64)	91
3 – Wakool River D/S Barbers		0.65 (0.50-6.31)	7.80 (1.44-1353.19)	0.08 (0.003-0.48)	6



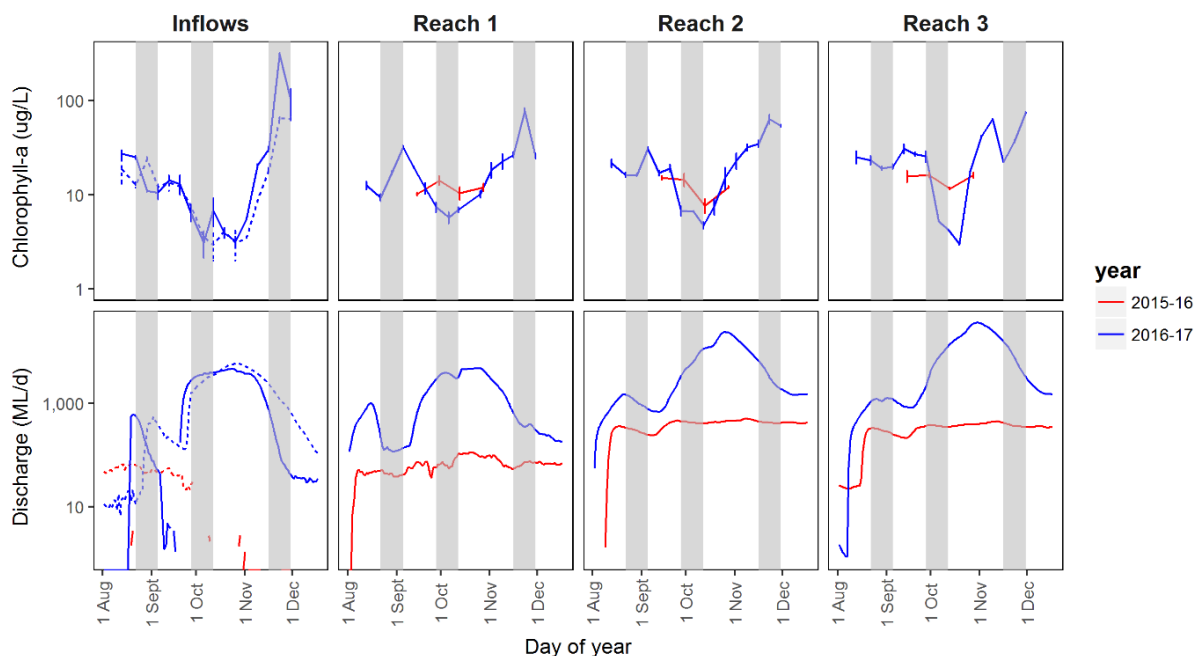
**Figure 5.3:** Gross primary production (GPP), ecosystem respiration (ER) and the ratio of GPP and ER (P:R ratio) for all metabolism results calculated for the gauge site at Deniliquin and Gee Gee bridge. Note the y-axis is log<sub>10</sub>.

**Table 5.3:** Median (min-max) of gross primary production (GPP), ecosystem respiration (ER) and the ratio of GPP to ER (P:R ratio) for all metabolism data calculated for 2015 and 2016. Data are collected continuously at the Deniliquin (Reach 1) and Wakool River (at Gee Gee, Reach 3).

Reach	Water Year	GPP	ER	P:R	n
1 – Wakool River @ Deniliquin	2015	1.46 (0.57-2.99)	1.09 (0.003-5.83)	1.35 (0.15-275.85)	160
3 – Wakool River downstream junction with Barbers Creek		1.20 (0.03-2.79)	2.19 (0.002-32.74)	0.63 (0.002-395.82)	155
1 – Wakool River @ Deniliquin	2016	1.92 (0.31-3.66)	4.00 (0.91-656.54)	0.46 (0.003-1.507)	34
3 – Wakool River downstream junction with Barbers Creek		2.59 (0.78-6.47)	11.32 (3.44-1301.18)	0.26 (0.002-0.58)	40

### Chlorophyll-a

Chlorophyll-a concentrations varied consistently among the different flow periods across all sites (pseudo-F=44.325, p(permutation)=0.001) but did not differ among reaches within flow periods (pseudo-F=0.55146, p(permutation)=0.762). Overall, median chlorophyll-a concentrations were 18.8 ( $\pm 1.54$ )  $\mu\text{g L}^{-1}$  during the return flow period and reduced to 6.37 ( $\pm 0.60$ )  $\mu\text{g L}^{-1}$  during the flood, rising again to 65.0 ( $\pm 11.00$ )  $\mu\text{g L}^{-1}$  during the recession (Figure 5.4). The median reference concentration for 2015 was 11.98 ( $\pm 0.31$ )  $\mu\text{g L}^{-1}$ .



**Figure 5.4:** Concentration of chlorophyll-a and stream discharge at the four study reaches during 2015 and 2016. This graph shows all chlorophyll-a data available, however the analysis was limited to the 2015 data (red) and the three flow periods (shaded). Data are shown on a  $\log_{10}$  scale.

## 5.4 Discussion

This study sought evidence that increasing in channel concentrations of carbon and nutrients with return flows would lead to a detectable increase in rates of both GPP and ER and a subsequent increase in chlorophyll-a. Although there were detectable increases in nutrient and carbon concentrations (Section 4), we were unable to directly test whether return flows increased rates of metabolism in reaches downstream of the KP Forest return flow delivery points. Rather, potential patterns in the data were masked by a combination of widespread flooding upstream of the return flows release point and prolonged hypoxic conditions that prevented a complete dataset from being collected.

It is important to consider that the inability to measure metabolism in this study is at least partly caused by a temporary increase in rates of ER attributed to labile carbon flushing from floodplain habitats (Hladyz et al. 2011). It isn't known what rates of metabolism typify the Wakool River in an undisturbed state, although based on the presence of large-bodied fish it is expected that annual flooding regulated carbon accumulation in floodplain areas, lessening the impact of floods on both oxygen levels and rates of production.

Wolfenden et al. (2017) found only a minor increase in concentrations of nutrients and no detectable increase in ecosystem metabolism when return flows were delivered from the lower Murrumbidgee Floodplain to the Murrumbidgee River. They attributed the lack of response to a relatively small return flow volume (<10% of total river flows downstream of the return flow point), coupled with low concentrations of nutrients (relative to the receiving water) in the adjacent wetland that had received flushing flows in the previous year. Nielsen et al. (2016) also found that large-scale return flows were needed to elicit functional responses in receiving waters, with Cook et al. (2015) finding a two-fold increase in DOC (from ~3-4 mg L<sup>-1</sup> to ~8-14) associated with an increase in DOC linked to an increase in rates of GPP and ER (respectively) when flood flows returned large volumes of floodplain water to the Murray River in 2010. Return flows to the Wakool River system have the potential to deliver smaller loadings of floodplain DOC, more closely mimicking natural delivery patterns while helping mitigate future hypoxic events by reducing leaf litter accumulation.

### *Return flows*

During the return flow period in 2016, inflows from Thule Creek and Barbers Creek more than doubled the concentration of DOC in the Wakool River from approximately 5 mg/L in Reach 1 to more than 10 mg/L in reaches 2 and 3 (see section 4). These concentrations are commensurate with similar increases in DOC reported by Cook et al. (2015), and might be expected to have also increased rates of metabolism (specifically ER). There is little to suggest any difference in GPP or ER between the two study years in early September that might be consistent with increased metabolism in 2016. Though we note that there is insufficient data to properly test the relationship. Chlorophyll data was not collected in late August/early September 2015.

### *Flood flows*

With the onset of flooding during early October 2016, chlorophyll-a concentrations fell from ~18 to ~6 ug/L across all sites. Chlorophyll-a is a measure of the approximate density of water-column phytoplankton, and might increase after sustained increased primary production. In turn, chlorophyll-a is sometimes used as a surrogate for food availability when studying food webs at lower trophic levels (Nielsen et al 2016). It is likely that during the peak of the flood chlorophyll-a concentration was diluted by low-chlorophyll water flowing from upstream. Dissolved organic carbon can also attenuate light penetrating the water column (e.g. Bukaveckas and Robbins-Forbes 2000, Rose et al. 2014) so the rates of GPP and subsequent chlorophyll-a concentration may have been suppressed in the early stages of the blackwater event. The interaction between DOC concentration, changes in DOC speciation across time and subsequent rates of primary production in Australian systems requires further study.

Hypoxic conditions meant little data could be calculated for the period between September and early December 2016 for any of the studied reaches. However, the few data points for which metabolism that could be calculated indicate high rates of ER more than 4 times that reported for the same reaches in 2015, pushing the system toward being highly heterotrophic. It isn't known whether the BASE program is capable of calculating metabolism for datasets with extremely low dissolved oxygen. For this reason, we limited results to meet strict quality criteria, and so the findings presented here are relatively conservative. The results are also broadly consistent with accelerated rates of ER in response to increased availability of nutrients and carbon, supporting the overarching hypothesis.

### *Recession flows*

During the recession flow period GPP appeared to increase coinciding with a fall in ER and a return to P:R ~1. Chlorophyll-a concentrations also increased significantly, reaching a median of 65ug/L, approaching chlorophyll-a concentrations seen in nearby wetland systems (Wassens et al. 2015) and exceeding chlorophyll-a data for a similar period during the previous year. These findings are consistent with the prediction that during the recession of flows when discharge in the Wakool River is receding, there would be a decline in ecosystem metabolism, and a switch from ER toward GPP. It also supports the prediction that the natural flood supported increased ecosystem production by increasing the availability of resources.

## **6. MICROINVERTEBRATE RESPONSES TO FLOODPLAIN RUN-OFF FROM KOONDROOK-PERRICOOTA FOREST**

### **6.1 Introduction**

Return flows from inundated floodplains can deliver floodplain-derived inputs of nutrients and organic matter to rivers (Wassens et al. 2015). These floodplain nutrients may then fuel secondary productivity in riverine microinvertebrate communities, boosting abundances. Further, the return flows may also transport floodplain taxa of microinvertebrates to rivers. Connections between the floodplain and river are considered important for recruitment of larval fish by stimulating primary and secondary production (Balcombe et al. 2007).

Microinvertebrates play a key role in floodplain river food webs, as prey to a wide range of fauna including fish (King 2004) and as important consumers of algae, bacteria and biofilms. Microinvertebrates are the critical link between stream metabolism and larval fish survival and recruitment (King 2004). As fish are gape limited, the availability of microinvertebrate prey in each size class at different times in the larval fish development is a critical factor influencing growth and survival. Density of microinvertebrates is also considered important for larval success, with densities between 100 and 1000/L reported for marine fish and densities within this range noted in hatching experiments and aquaculture for freshwater species (King 2004). They respond strongly to flow pulses and inundation, mediated by antecedent conditions and season (Jenkins et al. 2003; Jenkins et al. 2007).

In this project we aimed to study the ecological effect of the return of water to the Wakool River via two floodplain creeks, Thule Creek and Barbers Creek following inundation of the KP Forest. We predicted;

1. Return flows from the KP forest to the Wakool River will increase densities and diversity of microinvertebrates in the Wakool River downstream of Thule Creek relative to upstream of Thule Creek, and downstream of Barbers Creek relative to upstream.
2. During flooding, densities of microinvertebrates would become similar between sites in the Wakool River due to mixing and densities would reduce due to flushing and dilution of floodwaters.
3. During the flood recession, microinvertebrate densities would increase when water levels and discharge fell and during warmer spring temperatures.

## 6.2 Methods

### *Study design*

We sampled 18 sites, stratified across five longitudinal reach categories US (Upstream, Reach 1), Inflow from Thule Creek, MS (Midstream, Reach 2), Inflow from Barbers Creek, DS (Downstream, Reach 3)), ranging from the sites furthest upstream (US), to those furthest downstream (DS). Sites were re-visited during different flow periods on 1 to 9 occasions. Trips from mid-August to early September were during the return flow period when creeks were returning water to the Wakool River, trips during October were during flood flow period and trips in November were during the flood recession period when flows were receding in the Wakool River, but outflows from KP Forest were continuing (see section 2). To quantify differences in flow across space and over time, we measured flow discharges (ML/day) for each flow period (see section 3).

### *Sample collection and laboratory processing*

Composite benthic cores (50 mm diameter x 120 mm long, 250 mL volume) were collected at each site at the same time as water quality monitoring (see section 4). Five cores were collected from haphazard locations within each site with replicates spaced at least 20 m apart. The corer was placed onto the sediment surface, the top then sealed with a plastic cap and the water overlaying the sediment was extracted with the aid of a hardened rubber trowel. The aim was to sample the microinvertebrates immediately on or above the benthic sediment rather than to sample the sediment, which makes sample processing difficult. The contents of the corer were emptied into a 4 litre bucket and allowed to settle for at least one hour. Once settled, the supernatant was poured through a 63  $\mu\text{m}$  sieve to retain microcrustaceans, larger rotifers and macroinvertebrates. The retained sample was washed into a sample jar and stored in ethanol (70% w/v) with rose Bengal until time of enumeration.

In the laboratory, benthic microinvertebrate samples were poured into a Bogorov tray and enumerated with the aid of a dissecting microscope (Leica M125) at a magnification of 32x to 80x. Due to high densities and dense organic matter in many samples we sub-sampled all samples by dividing Bogorov trays into 44 cells (1.5 x 1.3 cm) and counting and measuring individuals in every fourth cell (25 per cent of sample processed). Rose Bengal stain was used in the field to highlight individuals in samples with excessive sediment present. Specimens were identified with relevant guides to species where possible (Williams 1980; Smirnov et al. 1983; Shiel et al. 1995; Shiel 1995).

### *Analysis*

We examined the community level response of microinvertebrate communities to changing flow conditions using multivariate negative binomial generalized linear models (Wang et al. 2012). We selected the negative binomial family to account for overdispersion in the data (Warton et al. 2016). Nine taxa that were only recorded in one site were excluded from this

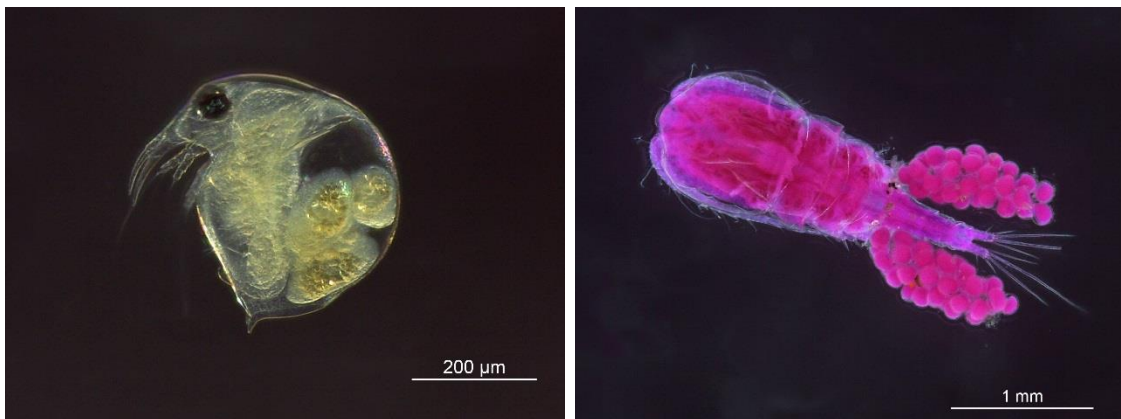


model. This left us with 73 multivariate abundance measurements of 37 species at 18 sites. We included longitudinal category (river reach), flow discharge and site as predictor variables in the model. For longitudinal category, we set the furthest upstream category (US) as the intercept. Site was used as a predictor variable to account for the variability in sampling effort between sites (1-9 visits). We fit a separate model with the same predictor variables, but grouping the 37 taxa to 11 broader taxa groups.

The significance of each variable was tested using the likelihood ratio test (LRT) both across all taxa/group and for each taxa/group specifically. Taxa/group-specific P values were estimated using resampling and were conservative, as they were adjusted for multiple comparisons (Wang et al. 2012). All analyses were undertaken in *R* v 3.3.1 (R Development Core Team 2016) with the *mvabund* v 3.11.9 package for the multivariate glms (Wang et al. 2012).

### 6.3 Results

Overall we sampled 46 taxa from 11 broader taxa groups. Nine of these taxa were recorded only in one site. The key taxa groups included chydorid, macrothricid and bosminid cladocerans (Figure 6.1), cyclopoid copepods (Figure 6.1) and naupli.

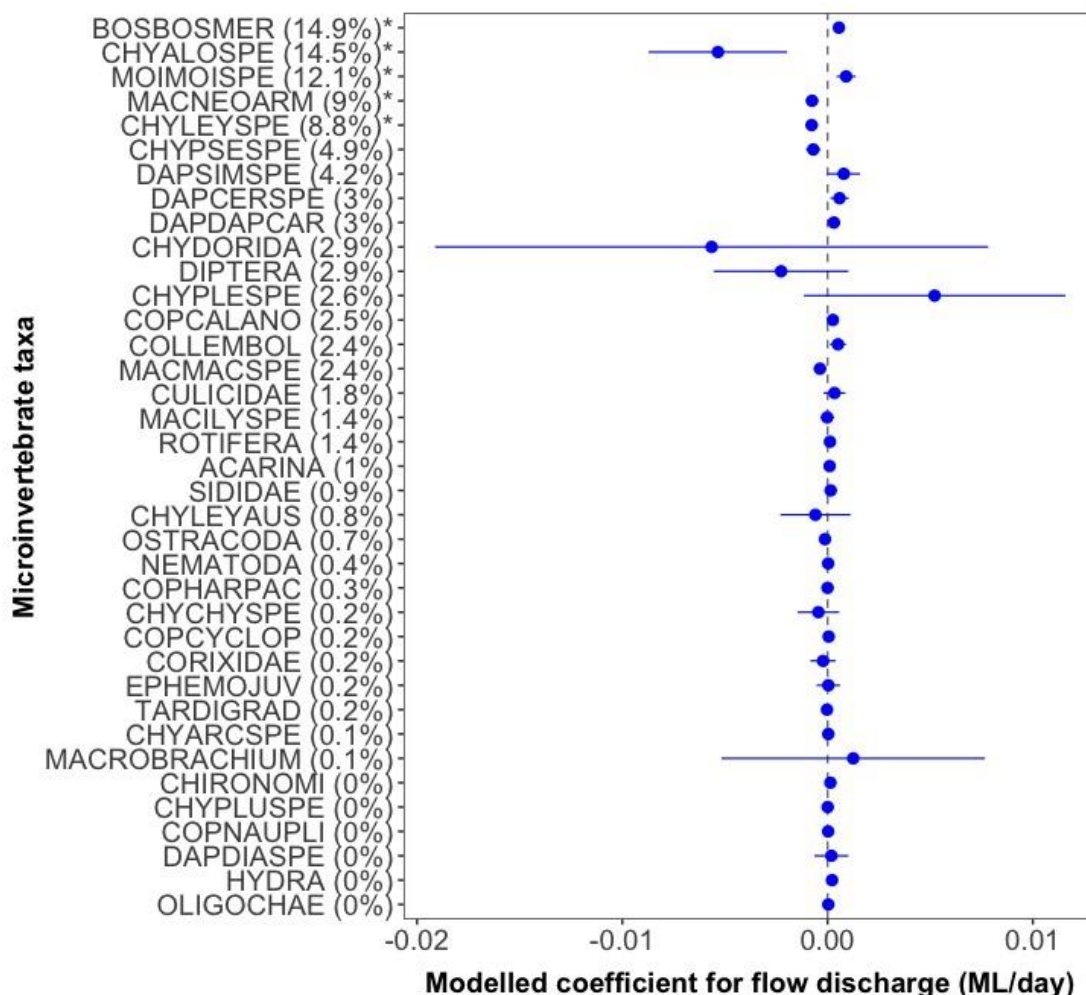


**Figure 6.1:** Bosminia cladoceran (left) and Cyclopoid copepod stained with rose Bengal (right). (Photos Caire Sives)

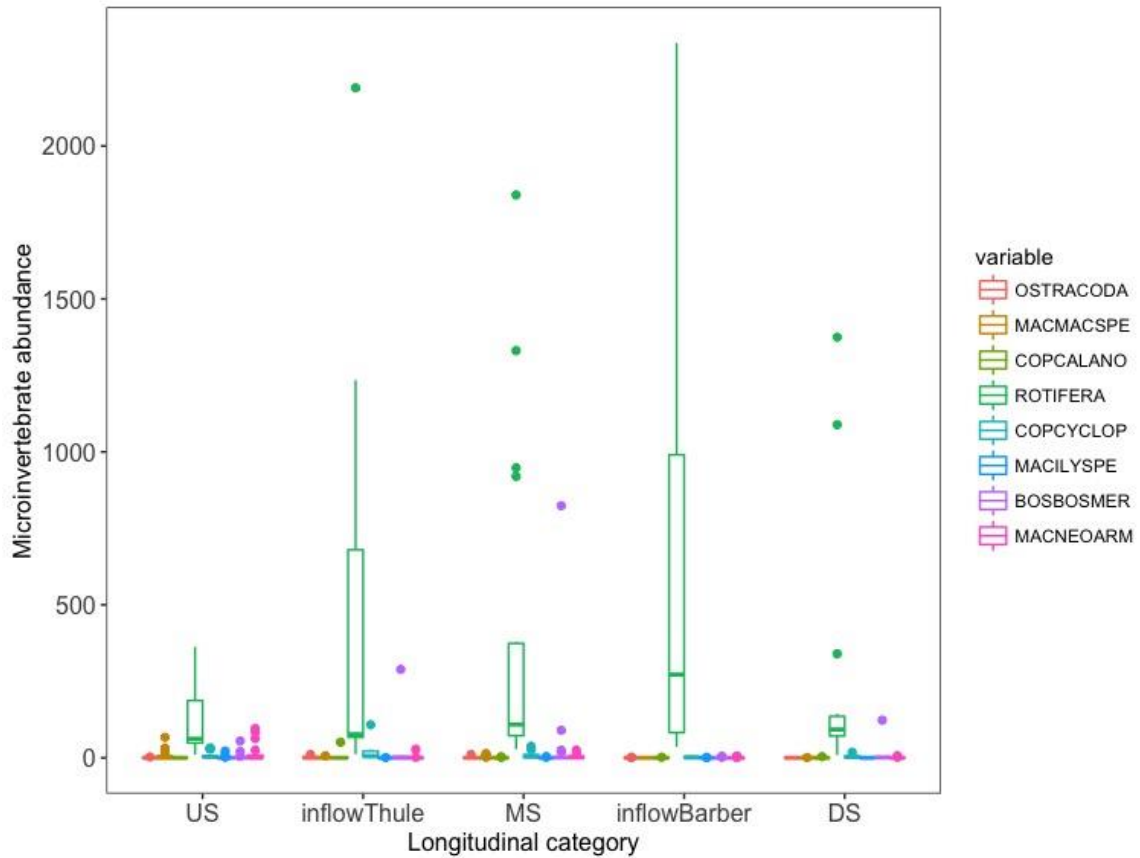
There was a significant effect of flow discharge (LRT = 191.2,  $P = 0.001$ ), longitudinal location (LRT = 252.9,  $P = 0.048$ ) and site (LRT = 530.5,  $P = 0.004$ ) on microinvertebrate taxa abundance.

At the taxa level, the abundance of five taxa were significantly associated with flow discharge, accounting for 59% of the variability in community composition with flow discharge (Figure 6.2). Two of these taxa were positively associated with flow discharge (*Bosmina meridionalis* BOSBOSMER:  $P = 0.001$ , 14.9 %; *Moina* sp. MOIMPISPE:  $P = 0.001$ , 12.1 %) while three were negatively associated with flow discharge (*Alonella* sp. CHYALOSPE:  $P = 0.001$ , 14.5 %; *Neothrix* sp. MACNEOARM:  $P = 0.004$ , 9.0 %; *Leydigia* sp. CHYLEYSPE:  $P = 0.005$ , 8.8 %) (Figure 6.2).

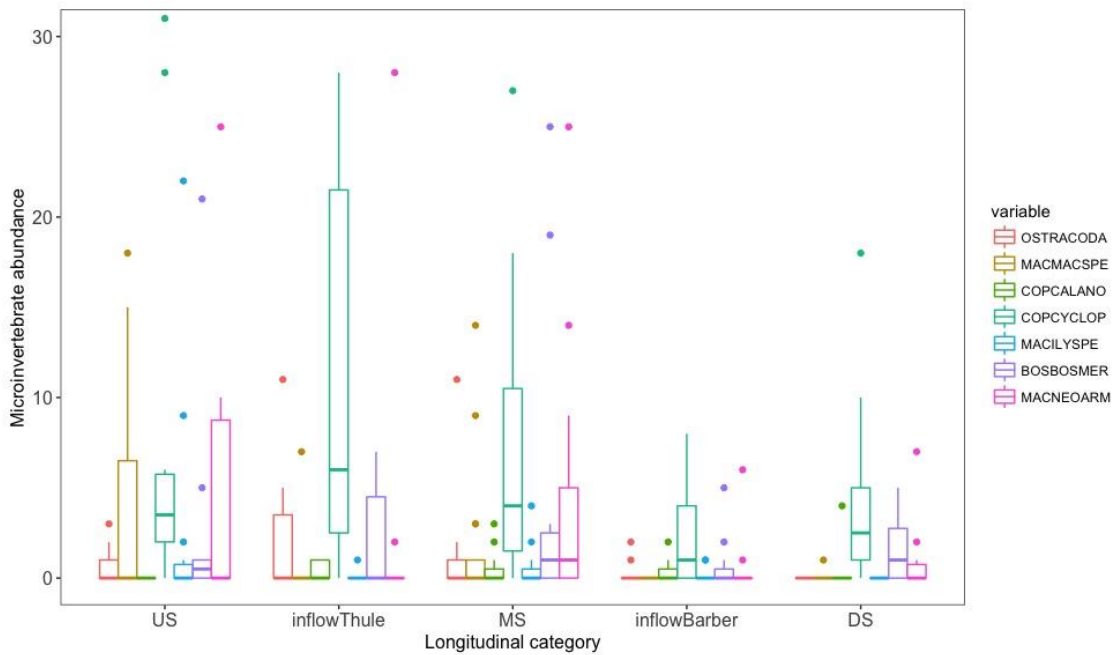
No significant taxa-level relationships were found for longitudinal category (river reach) and only one taxa was found to differ significantly between sites (Copepod nauplii COPAUPLI:  $P = 0.012$ ). Box plots of the results including rotifers (Figure 6.3) and without rotifers (Figure 6.4) indicate the lack of consistent relationship among longitudinal sites for key taxa groups. Nevertheless, there are some patterns of interest in the data. Abundances of Cylopoid copepods (COPCYCLOP), *Neothrix* sp. (MACNEOARM) and *Bosmina meridionalis* (BOSBOSMER) appear to have slightly increased in the Wakool River reach downstream of Thule Creek (reach 2), merging key taxa from the Wakool River reach 1 (upstream) and inflow from Thule Creek (Figure 6.4). Patterns of abundances and variability at in inflow Barbers and downstream Wakool River appear similar (Figure 6.4).



**Figure 6.2:** Mean coefficient estimates ( $\pm$  95 % CI) for multivariate generalized linear models relating flow discharge (ML/day) to 37 microinvertebrate taxa. The percentages that each taxa contributes to the overall community relationship with flow discharge are given in parentheses next to the taxa name. Where confidence intervals cross the zero line, there is no evidence for a relationship between that taxa and flow discharge. \*Significant ( $P < 0.05$ ) taxa-specific relationships adjusted for multiple testing.

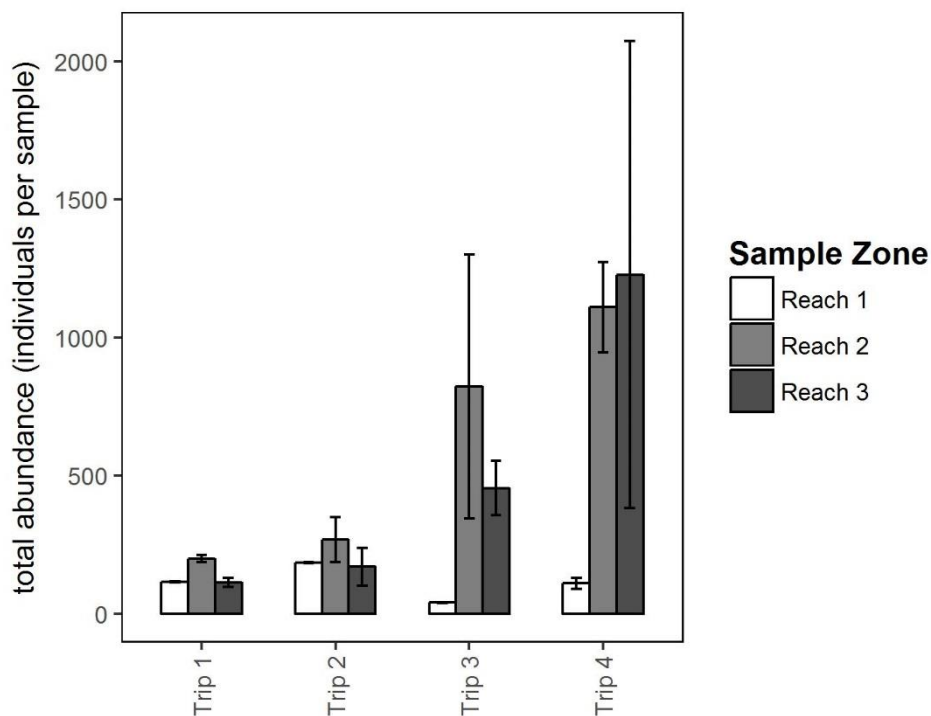


**Figure 6.3:** Box plots of the abundance of key taxonomic groups including rotifers versus longitudinal locations US (upstream of Thule), inflow Thule (Thule Creek), MS (midstream between Thule and Barbers Creek), inflow Barbers (Barbers Creek) and DS (downstream of Barbers and Thule Creeks).



**Figure 6.4:** Box plots of number of key taxonomic groups excluding rotifers versus longitudinal locations US (upstream of Thule), inflow Thule (Thule Creek), MS (midstream between Thule and Barbers Creek), inflow Barbers (Barbers Creek) and DS (downstream of Barbers and Thule Creeks).

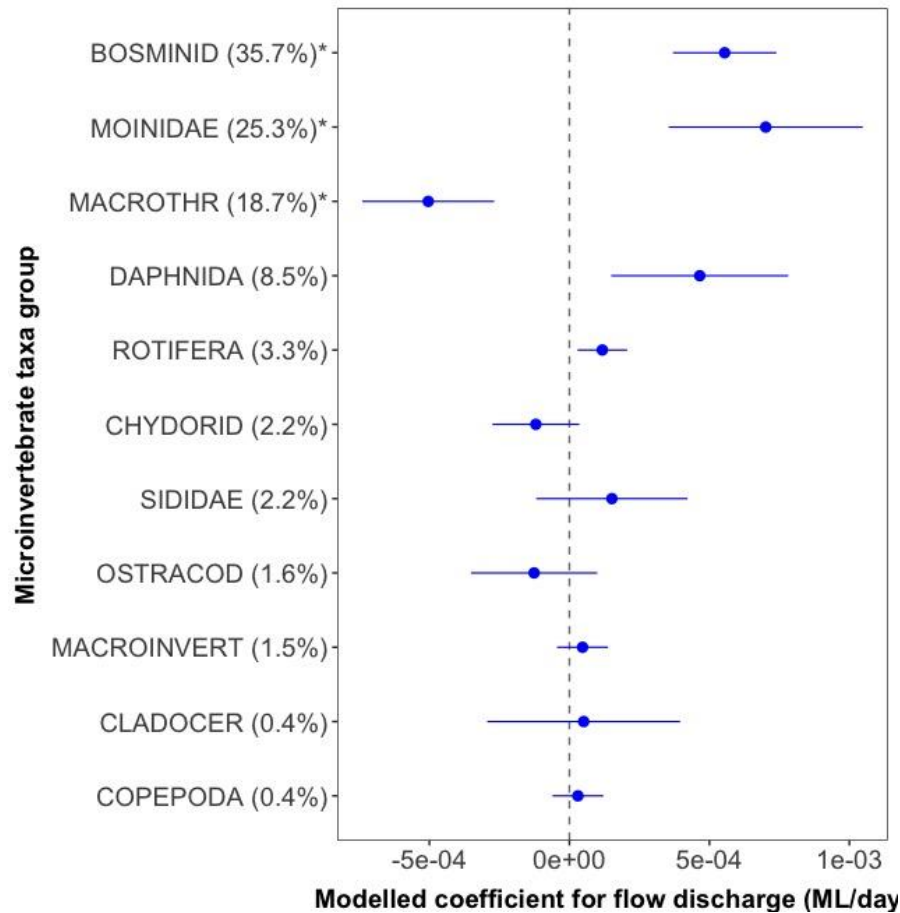
Abundances of microinvertebrates (excluding rotifers) in the current study were generally lower than abundances recorded in 2015-16 when they reached more than 500 and 1000 (in five cores with volume 1.178 ~ density/L) in the warmer weather in late October (Figure 6.5). In particular abundances increased in the mid-stream and downstream reaches in 2015-16. In the current study abundances across time were generally less than 100 with some notable abundances above 200 primarily in late August and early September before flooding [Thule Creek 252 (29/8), 467 (21/11); MS 239 (29/8), 232 and 254 (5/9) and 1115 (10/10); US 220 and 283 (29/8) and 343 (5/9)]. The highest abundance of 1115 is a MS site occurred during flooding, comprised primarily of *Bosmina mediodonalis* and a mixture of other cladocerans and copepods.



**Figure 6.5:** Box plots of total abundance of microinvertebrates excluding rotifers for each of four sampling trips in 2015-16 (13 September to 29 October 2016). Reach 1 relates to US 2016-17, Reach 2 relates to MS in 2016-17 and Reach 3 relates to DS in 2016-17.

There was a significant effect of flow discharge (LRT = 80.0, P = 0.001), longitudinal location (river reach) (LRT = 98.8, P = 0.026) and site (LRT = 219.5, P = 0.003) on abundance of microinvertebrate taxa groups. At the group level, the abundance of three groups were significantly associated with flow discharge, accounting for 80% of the variability in community composition with flow discharge (Figure 6.5). Two of these groups were positively associated with flow discharge (Bosminidae cladocerans BOSMINID: P = 0.001, 35.7 %; moinidae

cladocerans MOINIDAE:  $P = 0.001$ , 25.3 %) while one was negatively associated with flow discharge (Macrothricidae MACROTHR:  $P = 0.006$ , 18.7 %) (Figure 6.5). No significant taxa-level relationships were found for longitudinal category (river reach) and two taxa were found to differ significantly between sites (Chydoridae CHYDORID:  $P = 0.043$ ; Copepods COPEPODA:  $P = 0.010$ ).



**Figure 6.5:** Mean coefficient estimates ( $\pm$  95 % CI) for multivariate generalized linear models relating flow discharge (ML/day) to 11 microinvertebrate taxa groups. The percentages that each taxa group contributes to the overall community relationship with flow discharge are given in parentheses next to the taxa group name. Where confidence intervals cross the zero line, there is no evidence for a relationship between that taxa group and flow discharge. \*Significant ( $P < 0.05$ ) group-specific relationships adjusted for multiple testing.

## 6.4 Discussion

The microinvertebrate assemblage sampled in 2016-17 was diverse, with 46 taxa representing 11 broad taxa groups identified. Abundances were however low, generally less than 100 in the five cores sampled at each site, compared to abundances from 2015-16 that reached more than 500 in the midstream and downstream reaches in late October 2015 as temperatures rose. As predicted, flooding appears to have led to a dilution in abundances in 2016-17 with numbers not recovering even during the flood recession when flows still remained higher than baseline. It is likely that there would have been significant bursts in microinvertebrate productivity as flows returned within channels and temperatures were high in summer. In addition, the benefits of flooding are expected to be seen with productivity increases in subsequent years.

In 2016-17 it was not possible to test our critical hypothesis about the effects of return flows along Thule and Barber's Creeks due to flooding. Nevertheless abundances of Cyclopoid copepods (COPCYCLOP), *Neothrix* sp. (MACNEOARM) and *Bosmina meriodonalis* (BOSBOSMER) appear to have slightly increased in the Wakool River reach downstream of Thule Creek (reach 2), combining key taxa from the Wakool River reach 1 (upstream) and inflow from Thule Creek (see Figure 6.4). A pattern of higher abundances in the midstream reach as well as the downstream reach was also observed in 2015-16. It is likely that inflows from Thule Creek and Barbers Creek boost diversity and productivity in downstream reaches over long time periods, but this response remains to be tested in a year when return flows are possible.

There was a significant effect of flow discharge on the abundance of individual microinvertebrate taxa and on abundance of taxa groups. At the taxa level, the abundance of two taxa were positively associated with flow discharge and three were negatively associated with flow discharge. The abundance of three taxa groups were significantly associated with flow discharge, accounting for 80% of the variability in community composition with flow discharge. Two groups of cladocera (commonly known as water fleas) were positively associated with flow discharge; the riverine taxa Bosminidae and floodplain taxa group Moinidae both increased in abundance, while the Macrothricid cladoceran taxa group was negatively associated with flow discharge. Macrothricidae cladocerans are a key prey item for larval fish like Murray cod (King 2004) being largish and fleshy, and it is interesting to see they would be less available during floods. During low flow years in the Murrumbidgee catchment, Macrothricids are prevalent in late spring when fish larvae are most abundant (Wassens et al 2017).

In summary, flooding in 2016-17 overpowered the response of microinvertebrates to return flows from KP Forest. However, some patterns in the results suggest that the inflows from Thule Creek increased the abundances of some taxa in the Wakool River reach 2 (downstream of Thule Creek). In contrast, the inflow from Barbers Creek did not appear to affect the patterns of abundances of microinvertebrates in the Wakool River reach 3 (downstream of Barbers Creek). This pattern was similar to that in 2015-16 when the highest abundances were recorded in the midstream and downstream reaches.

## 7. SUMMARY AND CONCLUSION

In spring of 2016 there was a widespread flood in the southern Murray-Darling Basin and some areas of the floodplain were inundated that had not been flooded for more than 20 years.

During the flood, water flowed from the Murray River through KP Forest and into the Wakool River via Thule and Barbers Creeks. At the peak of the flood the Wakool River experienced very high discharge, with overbank flows and connections to the floodplain. During the recession of the flood Thule Creek and Barbers Creek continued flowing into the Wakool River.

In this study we examined the contribution of the KP Forest floodplain runoff to the productivity of the Wakool River. We found that the flooding of KP Forest and outflows via Thule and Barbers Creeks were important sources of carbon and nutrients to the Wakool River during this flood event and that increasing concentrations of floodplain-derived carbon and nutrients accelerates ecosystem processes. In addition, the study demonstrates that the scale and timing of flows is important and that the relationship between the various components that influence ecosystem metabolism is complex.

Ecosystem responses during the three stages of the flood event (return flow period, flood flow period, recession period) were considerably different.

### *Return flow period (22 August – 5 September 2016)*

During the return flow period Thule and Barbers Creek started to receive flood water from KP Forest and there were outflows from these creeks to the Wakool River. These return flows from KP Forest occurred prior to the flood flows coming down the Wakool River via the Edward River system.

During the return flow period inflows from Thule Creek and Barbers Creek more than doubled the concentration of DOC in the Wakool River. Early in the event there was input of carbon from KP Forest via Thule Creek but not Barbers Creek, as it commenced flowing later than Thule Creek. However, by 25<sup>th</sup> September there was substantial input of DOC to the Wakool River from both Thule and Barbers Creek. On almost all sample occasions the DOC concentrations in Thule Creek and Barbers Creek were higher than all Wakool River reaches. In addition the ANZECC trigger levels for nitrate and nitrite was exceeded at the beginning of this event. During the return flow period chlorophyll-a concentrations (a measure of the approximate density of water-column phytoplankton) were higher than baseline 2015 levels. Most importantly, dissolved oxygen concentrations were in the acceptable range at all Wakool River sites during this period.

Although there were detectable increases in nutrient and carbon concentrations, there was limited evidence that flows from KP Forest during this early stages of the flood increased rates of metabolism in the Wakool River downstream of Thule Creek and Barbers Creek. The abundance of microinvertebrates during the return flow period was generally low in late August and early September.

*Flood flow period (26 September to 10 October)*

In the flood flow period most of the study reaches experienced their highest median flow of the three study periods. The exception to this was the Wakool River reach 3 (the most downstream reach) that was still increasing in flow at this time.

Connection with the floodplain during this period had a strong and rapid influence on DOC concentrations in the Wakool River. On the 26<sup>th</sup> September there was evidence of clear input of floodplain carbon at all sites. During the extensive flooding that occurred during this event, DOC will have entered the system from upstream forested areas but also from flooded grazing and cropping land. The results demonstrated that there were complex and varied sources of the DOC in this system. By the 4<sup>th</sup> October the fluorescence intensity had increased at all sites as the flood continued to cover previously dry floodplain.

Total phosphorous (TP), total nitrogen (TN), nitrate, nitrite and filterable reactive phosphorous were much lower during the flood flow period than during both the return flow period and recession period.

An accelerated decline in DO to hypoxia was observed at all sites over a period of a few days in early October. The same trend was present in the dissolved oxygen saturation data, suggesting a rapid increase in oxygen consumption from the water column at this time rather than a decline in oxygen solubility. There was a strong effect of temperature for the onset of hypoxia. The decline in DO at all sites around the 6<sup>th</sup> October occurred when there was a rise in water temp of approximately 3 degrees suggesting the strong role of temperature in hypoxia. The earlier fresh in August had very different outcome when the water temperature was lower with increases in DOC not resulting in hypoxia in the Wakool River.

The hypoxic conditions during the flood flow period meant that only a small amount of data could be used to estimate GPP and ER. However, the few data points for which metabolism could be calculated indicate high rates of ER more than four times that reported for the same reaches in 2015, pushing the system toward being highly heterotrophic. The results are broadly consistent with accelerated rates of ER in response to increased availability of nutrients and carbon, supporting the overarching hypothesis. With the onset of flooding chlorophyll-a concentrations reduced across all sites. It is likely that during the peak of the flood chlorophyll-a concentration was diluted by low-chlorophyll water flowing from upstream and DOC may have attenuated light penetrating the water column suppressing the rates of GPP.

As predicted, in general the increased discharge during the flood appeared to dilute the response of microinvertebrates to return flows from KP Forest, but the patterns of abundance suggest that inflows from Thule Creek increased the abundances of some taxa in the Wakool River reach 2 (downstream of Thule Creek). Inflows from Barbers Creek did not appear to affect the abundances of microinvertebrates in the Wakool River reach 3 (downstream of Barbers Creek).



*Recession flow period (14 to 28 November 2016)*

In the recession flow period the discharge in Thule Creek had reduced to very low flow, whereas Barbers Creek was still receiving considerable return flows from KP Forest. The flow in Wakool River reach 1 and 2 had reduced considerably, but Wakool River reach 3 continued to flow strongly, partly due to the return flow from Barbers Creek and because it is the most downstream reach.

During the recession flow period there was inputs of carbon to the Wakool River Reach 2 and Reach 3 from Thule and Barbers Creek. This had a strong influence on the water quality and productivity of the Wakool River as the upstream discharge in the Wakool River was receding. Total phosphorous (TP), total nitrogen (TN), nitrate, nitrite and filterable reactive phosphorous were much higher during the recession flow period when discharge in the Wakool River was decreasing and floodwater from KP was continuing to drain back into the river channel from KP Forest. The ANZECC trigger levels for nitrate and nitrite was exceeded at this time. Ammonia production was well above the ANZECC trigger level, during the recession flow period (November 2016) (anoxic sediment is a source of ammonia). Ammonia is a particularly bioavailable form of nitrogen and may stimulate growth of vegetation or algae.

During the recession flow period DO concentrations increased in the Wakool River reach 1, reflecting improved water quality entering from the Edward River upstream. The slower improvement in DO in the Wakool River reach 2 and 3 were the result of the later receipt of this water combined with ongoing inputs of low DO water from Barbers and Thule Creeks

As expected, during the recession flow period there was increasing GPP, coinciding with increasing chlorophyll-a and falling ER. All are consistent with the prediction that during the recession of flows when discharge in the Wakool River is receding, there would be a decline in ecosystem metabolism, and a switch from ER toward GPP. It is unclear of the mechanism for this – it could be due to change in DOC speciation, potential reduction in light attenuation, an increase in available inorganic nutrients, or increase in temperature.

The abundance of microinvertebrates did not recover during the flood recession when flows still remained higher than baseline discharge in 2015 study. It is likely that there would be significant increase in microinvertebrate productivity at a later stage as flows returned within channels and temperatures were high in summer. In addition, the benefits of flooding are expected to be seen with productivity increases in subsequent years.

*Conclusion*

In conclusion, the flood event in 2016 was extensive, inundating parts of the floodplain that had not been connected for over 20 years. The combination of the extensive flooding plus a sharp rise in water temperature in early October resulted in a widespread hypoxic blackwater event. This influenced the degree to which KP Forest could influence the productivity of the Wakool River during the flood. However, during the recession of the flood, when discharge in the Wakool River was receding but outflows from KP Forest continued, there was a decline in

ecosystem metabolism and a switch from ER toward increased in GPP, coinciding with increasing chlorophyll-a and increased abundance of some microinvertebrates in the Wakool River.

This study has demonstrated that managed return flows from KP Forest to the Wakool River can lead to increased productivity downstream and could support higher ecosystem production in the Wakool River. There is a risk of hypoxic blackwater during a managed return flow from KP, however return flows from KP could be managed in conjunction with flows in the Wakool River to minimise the risk of hypoxic blackwater. Careful planning of timing, magnitude and duration of delivery of environmental water through KP Forest when the Wakool River is not in flood, has the potential to have a positive outcome by increasing GPP in the mid and lower Wakool River and possibly contributing to the Murray River downstream of the confluence with the Wakool River. The size of flows through KP Forest with respect to flows in the Wakool River would need to be optimised and the time of year of water delivery is critical to minimise the risk of poor water quality outcomes and hypoxia due to the strong influence of temperature on river processes.

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