Ecological connectivity of the River Murray: Managing ecological outcomes and water quality risks through integrated river management

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Executive summary

In response to degradation of floodplain vegetation (e.g. *Eucalyptus camaldulensis* and *Eucalyptus largiflorens* woodlands) due to reduced inundation extent and frequency throughout the lower River Murray, there has been increasing focus on using existing weirs, and constructing new regulators, to facilitate increases in floodplain inundation. Specifically, this has included the construction of the Chowilla Regulator and associated ancillary structures, with similar projects underway at the Lindsay-Mullaroo, Pike and Katarapko anabranches. The management of riverine and floodplain structures has the potential to both threaten and enhance in-channel processes, such as the life history processes of large bodied native fish, dispersal of propagules such as seeds, eggs and plankton, and promotion of desirable trophic pathways (e.g. energy sources and types of phytoplankton).

Although the dependence of key ecological processes on changes to hydrology (inundation) and, in turn, hydraulics is known (for example Bunn & Arthington 2002; Poff et al. 1997), there is limited quantitative knowledge available to describe the likely environmental responses to specific management scenarios for the lower River Murray. This project has taken an empirical approach (including field, laboratory and modelling components) to investigate the mechanisms relating to how changes in hydraulic conditions alter the transport and transformation of resources (carbon and nutrients), propagules (e.g. eggs, seeds, larvae) and biota within and along the River Murray Channel. This synthesis document provides an overview of the components considered in this project, how the empirical results have been related to underlying hydraulic and hydrological processes understood to be driving the observed changes, and how these relationships have been integrated into the hydrological model to simulate potential ecological responses under different operational scenarios.

Dissolved oxygen dynamics

During periods of elevated water level, due to high flows and/or infrastructure operation, the inundation of soils and accumulated plant and other organic material (e.g. scat) in the riparian zone and on the floodplain results in the mobilisation of water soluble dissolved organic carbon (DOC) and nutrients. The microbial degradation (heterotrophic metabolism) of this DOC can result in low dissolved oxygen (DO) concentrations (hypoxic blackwater events).

The current work has undertaken extensive field sampling and data analysis to enable the sampling data to be used as model inputs. A total of 878 samples of natural organic matter standing load were collected, across Calperum, Chowilla and Katarapko floodplains, and a range of meso-habitats, including black box woodland, cooba woodland, river red gum woodland, mixed tree species woodland, grassland and lignum shrubland. Estimates of litter fall rates were also made by deploying leaf litter traps within woodland habitats on the Chowilla and Calperum floodplains, comprised of 10 black box and 10 red gum sites, and emptied on regular occasions to determine the litter fall rates over time.

The field work has enabled modelling to be undertaken to assess the impact of the variability in organic matter on the floodplain on simulated DO concentrations due to infrastructure operation. In particular, a number of components of the model were parameterised using the litter data: 1) the load of organic matter on the floodplain, 2) the fraction of the total organic matter that is readily available (i.e. the labile component), 3) the rate at which litter accumulates each day and 4) The rate of decay of leaf litter on the floodplain. These values, and the variability in these values, were applied based on vegetation type across the South Australian (SA) floodplain, to enable the range in DO concentrations expected from different flow and inundation scenarios to be estimated. A sensitivity analysis was undertaken for parameters in the model that are not related to leaf litter, which found that water temperature followed by the reaeration rate have by far the biggest impact on the modelled DO levels. This provides useful information to guide operations, where water temperature can have a large impact on the resulting DO conditions, and negative impacts can be reduced through timing operations earlier in the season. The results also provide guidance on where investment in future sampling is likely to be most informative, with reaeration rates the next most influential parameter for the resulting modelled DO concentrations.

Phytoplankton abundance

Traditionally, diatoms comprise a large part of the phytoplankton community found in the River Murray, which are highly digestible and are nutritious for grazers. However, they need turbulence to remain in suspension and can therefore be disadvantaged by reduced velocity. Under the low flow scenarios, large low land rivers can stratify, promoting conditions conducive for cyanobacterial growth. There have been several instances of cyanobacterial algal blooms in the River Murray, for example in 1983, 1991, 2009, 2010 and 2016 (Croome et al. 2011; Murray Darling Basin Authority 2016). Blooms of cyanobacteria can cause problems by reducing the recreational and aesthetic value of river systems (Hallegraeff 1993) with periodic production of toxins which can be problematic for water supply. Cyanobacteria are also less nutritious for consumers in the food web (Ahlgren et al. 1990; Lampert 1987; Müller-Navarra et al. 2004). Cyanobacterial blooms are often associated with increased stratification and residence times, which could be expected to increase when inundation (and hence depth and storage volume) is increased using infrastructure, at lower discharges than would otherwise been required to create the same inundation. Two approaches have been implemented in this work to assess the likelihood of cyanobacterial blooms, a water column mixing criterion and a machine learning approach.

The mixing criterion is an equation that accounts for changes in velocity and water depth when using structures to increase inundation (as well as temperature, light attenuation and solar radiation), and as such provides an indication of any increased risk of stratification due to regulator operations. This project has validated the application of the mixing criterion equation to the River Murray in SA, and implemented the resulting methodology in the Source model to enable operations that increase the risk of persistent stratification to be identified.

The occurrence of persistent stratification provides an indication of increased likelihood of harmful algal blooms, however the two do not always coincide. To address this difference, modelling of cyanobacteria occurrence was also undertaken, to test if models that directly predict cyanobacteria blooms can be developed to inform planning and operations. A dataset covering historical phytoplankton cell counts was collated to train the model, covering the period 1994–2018 for five locations with high quality cell counts was collated and subject to extensive quality assurance and quality control. Variables used to predict the historical cell counts tested included air and water temperature, flow discharge and velocity, salinity, solar radiation, cell counts at the same location one week before and cell counts at the upstream location one week before. The random forest machine learning algorithm was applied using k-fold cross validation to evaluate the model performance. Overall calibration performance was acceptable (typically Nash Sutcliffe Efficiencies greater than 0.7) with the overall seasonal cycle and inter-annual variability of the data was predicted. However, the magnitudes of the cell counts were always underestimated and in validation mode (i.e. when the model was tested on data not used in calibration) the model had a lower predictive capability.

Transport of biological propagules

In regulated rivers, dams and weirs disrupt connectivity and modify hydrodynamics, particularly upstream of regulating structures where lotic systems are converted to lentic impoundments. This project was undertaken to investigate relationships between hydrodynamics and the transport and deposition/retention of propagules at species-specific (e.g. abundance, distribution) and community levels (e.g. species composition and abundance) for plant propagules (seeds), microinvertebrates and fish larvae (Murray cod, *Maccullochella peelii*). For all biotic components, the Chowilla Anabranch during 2017 and 2018 was the focus of the field investigations, given the wide range in hydraulic conditions present in the anabranch, the infrastructure available to control inflows and water levels, and the presence of a population of reproductively mature Murray cod.

Seed bank

The aim of this study was to compare seed deposition with the sediment seed bank in the riparian zones of Chowilla and Monoman creeks (part of the Chowilla Anabranch) under different inundation regimes: a natural flow pulse and a small regulated inundation by the Chowilla Environmental Regulator. Soil samples and sediment traps were deployed to thirteen transects on the banks of Chowilla and Monoman creeks. The

flow pulse in spring 2017 resulted in only two of the 11 elevations where sediment traps were deployed being inundated, which allowed anemochory to also be investigated despite not being a primary aim of the study.

The results indicated that sediment deposition was significantly greater in association with regulated inchannel inundation (in 2018) compared to an in-channel flow pulse (in 2017). However, despite the large differences in sediment deposited, there was no significant difference in seed density between regulated inchannel inundation and an in-channel flow pulse, and sites that were inundated and not. There was also no significant difference in germinable seed composition between the traps inundated in 2018 and traps not inundated (2017). However, there was a significant difference in the germinable seed composition deposited on the traps inundated in 2017 (flow pulse) that was likely due to lower species richness in 2017.

Microinvertebrates

There were two distinct components to this study. The first component aimed to investigate the spatial and temporal changes in generation of littoral and limnetic microcrustaceans as food resources for Murray cod larvae in the Chowilla Anabranch. The results indicate that there was a significant difference in the species composition between trips (i.e. over time) and across sample locations. Post-hoc comparisons revealed that total microcrustacean density was significantly higher in early-November than in late-November and mid-December 2017. Microcrustacean density was also significantly higher in late-October than in late-November 2018 (P=0.0072). The higher microcrustacean densities in mid-late spring in 2017 (late October to early November) were largely due to high densities of *Bosmina meridionalis*, a common cladoceran in the pelagic zone of rivers and lakes including the lower River Murray. This taxon was found to be consumed by Murray cod larvae collected during the same sampling period and highlights the importance of this pelagic riverine species, previously unidentified as a food resource for Murray cod larvae. Even though cyclopoid copepods were not present in high densities during the sampling, Murray cod were selectively feeding on them and made up the highest proportion of organisms found within the stomach content in early November 2017. As in previous studies, this highlights the importance of this littoral food resource for Murray cod larvae.

The second component aimed to investigate the entrainment and downstream transportation of *Trichocerca*, a genus of rotifers that commonly prefer littoral habitats yet are commonly detected within lotic habitats in spring, along the Chowilla Anabranch and the Lock 5 weir pool. The specific aims of this component were to investigate longitudinal changes in the density of *Trichocerca*, and investigate correlations between the density of *Trichocerca* and modelled water level (as an indication of differences in changes in the availability of littoral habitat) and water velocity (as a driver of entrainment).

The results indicated that changes in the density of *Trichocerca* appeared to be associated with both changes in water velocity and water level at different times, potentially driven by both the entrainment and downstream transportation of organisms in late spring. Whether the process association with water velocity or water level was stronger, however, differed under different hydrological conditions. For example, in early-November 2017, when sampling was conducted at the very beginning of an in-channel pulse, neither the best-fit water velocity variable nor water level appeared to be strongly correlated with the density of *Trichocerca*. This suggests that a large amount of variability at this time was being driven by other factors not investigated as part of this study, for example, hatching from the egg-bank, turbulence, food availability and/or water quality. In contrast, in late-November 2017 the combination of water level and velocity explained the majority of variability in the density of *Trichocerca*, suggesting that either the entrainment and the sourcing of organisms from the littoral zone were important under these condition, or the water level and velocity parameters are indicators of other processes driving the density of *Trichocerca*.

Murray cod larvae

In the lower River Murray, the fragmentation of the river by sequential weirs and loss of lotic habitats are considered primary threats to riverine biota, including Murray cod (Mallen-Cooper & Zampatti 2018; Zampatti et al. 2014). Regular recruitment and robust populations of Murray cod are likely supported by the presence of structural (e.g. large wood) and hydraulic habitats that support Murray cod throughout ontogeny, but in particular during early life history processes (i.e. drift and retention in suitable nursery habitats) and survival. In this study, we investigated the retention of Murray cod larvae in littoral habitats of

the Chowilla Anabranch over two spawning seasons, comprising a year of no regulator operation (2017) and a year of operation (2018).

Light trap sampling was conducted at six sites within the Chowilla Anabranch and Slaney Creek over threeweek periods in October–November of 2017 and 2018. At each site microhabitat water depth (m), water velocity (m.s⁻¹), and physical habitat cover were measured. Depth and downstream water velocity were measured at five points, every 0.25 m along a 1 m transect that ran perpendicular to the creek bank and through the position of the light trap. An additional microhabitat hydraulic metric, *velocity difference*, was then calculated for each light trap as site mean cross sectional velocity (from Acoustic Doppler Current Profiler (ADCP) generated data) minus mean microhabitat velocity.

Mixed-effects modelling indicated littoral larval Murray cod abundances were best predicted by microhabitat velocity and velocity difference. Specifically, Murray cod larvae were more likely associated with littoral habitats with mean velocities <0.1 m.s⁻¹, but high velocity difference (e.g. >0.3 m.s⁻¹). This suggests a preference of Murray cod larvae for slackwater habitats within broader fast-flowing reaches. As such, in 2017, Slaney Creek likely represented a generally poor nursery due to a low proportion of slackwater habitats in this fast-flowing creek. In contrast, sites along the length of Chowilla Creek represented suitable nurseries due to a high proportion of microhabitats with low velocity, but high velocity difference. Slaney Creek, however, is an important spawning site for Murray cod and hence constitutes a consistent source of larvae. In contrast, during regulator operation in 2018, reductions in overall flow velocity in Slaney Creek resulted in increased prevalence of low velocity slackwaters and moderate velocity differences, with an associated order of magnitude increase in the abundance of larvae. Conversely, at sites further downstream, whilst slackwaters were prevalent, these were characterised by lower velocity difference, with associated reductions in larval abundance.

Modelling operational scenarios

The results outlined above were used to identify a range of criteria that could be used to assess changes to in-channel ecological connectivity and water quality risks. These criteria were implemented in the Source hydrological model of the SA River Murray (Beh, Montzeri & Gibbs 2019) to enable the assessment of different operational scenarios. To demonstrate the application of these criteria, four were considered, representing low flow (peaking at QSA 14,000 ML.d⁻¹) conditions, two operational scenarios for these low flow conditions, and a high flow scenario (peaking at QSA 60,000 ML.d⁻¹). The metrics considered, based on the results from this work were:

- Defined velocity thresholds to promote important ecological processes:
 - Entrainment and drift of zooplankton (mean cross-sectional velocity, v > 0.2 m.s⁻¹)
 - \circ Creation of suitable retention habitat for Murray cod larvae (v> 0.3 m.s⁻¹)
- Assessment of water quality risks:
 - Information to parameterise the DODOC model to simulate changes in DO and DOC
 - A mixing criterion to assess changes in velocity and depth on the ability to disrupt thermal stratification, and in turn cyanobacteria blooms.
- Other metrics to assess changes of interest:
 - Maximum inundated area over the simulation. This metric provides a representation of the benefits that might be expected, compared to the impacts that might be calculated using the above metrics.
 - Percentage of days a minimum flow requirement was met at each node.

The results suggest that for the scenario based on current operational plans at floodplain sites, only water quality impacts that can be mitigated were expected. An extreme scenario outside of these bounds was required to generate results of concern, however even for this scenario the impacts were generally confined to the anabranches, as opposed to the main River Murray channel. The high flow scenario provides a contrast between the outcomes that can be expected from inundation created by flow and infrastructure operations. This study has demonstrated how the findings from this project can be applied to inform cumulative

operational planning in the River Murray, and that the tools available, namely the Source hydrological model has been expected to provide this additional functionality.

Food web dynamics

The previous sections have outlined studies focused on quantify changes to key in-stream components of the River Murray ecosystem expected to be influenced by the operation of infrastructure. However, the exchange of energy and nutrients is also strongly influenced by the flow regime, and is a primary characteristic controlling ecosystem productivity and community dynamics in riverine systems. The aim of this study was to investigate the flow of basal resources (carbon and nitrogen) during the two contrasting flow scenarios in 2017 and 2018, and improve the understanding of the relative role of different basal energy sources (e.g. phytoplankton, biofilms, terrestrial plant material and sediments/soils) on fuelling primary and secondary productivity. The key focus of this work package was to investigate if managed inundations drive shifts in food web dynamics through changes in basal resource supply and delivery.

Sampling was undertaken at nine sites in November 2017 and November 2018, and for each site water column, biofilms, soils, sediment (benthos), phytoplankton and zooplankton, and crustaceans were sampled. Each sample was analysed for dissolved organic carbon (DOC), total carbon (TC), dissolved organic nitrogen (DON), total nitrogen (TN), nitrate (NO₃⁻), and ammonium (NH₄⁺). Stable isotope analysis was also undertaken to trace patterns of energy flow and food web linkages. Statistical modelling was undertaken to test for statistically significant differences between years, sites and samples for different trophic levels, as well as to identify the main factorial effects and different basal energy sources between trophic levels.

Results indicated that there was significant between year differences for the carbon and nitrogen pools of both the water column and the sediment pools. Higher concentrations of carbon (both total and organic) and nitrogen (total, NO₃ and NH₄) were measured along with lower suspended solids in 2017 than 2018. Isotopic differences were observed between the years for the terrestrial vegetation, as well as the suspended solid load and the benthic sediments, suggesting a terrestrial shift in response to climate in 2017 relative to 2018. Zooplankton were enriched for 15N in 2017 suggesting they were feeding at higher trophic levels in 2017 compared to 2018. The shift in measured C:N ratio suggests mobilisation of a large carbon pool in 2018 relative to 2017. As both the terrestrial and riverine pools of nitrogen decreased in 2018, this suggests an overall ecosystem response to drying conditions since the 2016/17 high flow event, rather than as a result of the managed inundation in 2018. The mobilisation of carbon into the biofilm pool may be a result of either an increase in ambient concentration/change in availability, or a result of the within channel water level rise operation in 2018 promoting carbon mobilisation. Sediment desiccation causes the decline of bacterial activity and biomass and reactivation occurs when rewetting, hence the managed inundation in 2018, is likely to have enhanced sediment C and N mineralisation.

Summary

This report has presented a summary of new research focused on the gap in understanding on changes to inchannel processes from the operation of large infrastructure designed to enhance floodplain inundation. This will enable a more a more holistic evaluation of environmental water delivery and infrastructure operation, extending analysis beyond inundated area to both risks and benefits occurring across the ecosystems. The implementation of the findings from this research into the Source model will enable further integrated assessment of operational scenarios, from the site to the whole of South Australian River Murray scale.

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1 Introduction

Improving ecological outcomes in the Murray-Darling Basin (MDB) is a priority for the South Australian Government. In addition to natural floods, the primary mechanism for achieving these outcomes is through augmenting regulated and unregulated flows with the delivery of held environmental water. For the lower River Murray channel these flows may improve the condition of ecological communities dependent upon riverine habitat. Due to current constraints on deliverability of flow above certain magnitudes, achieving outcomes on the floodplain is challenging. In response to degradation of floodplain vegetation (e.g. *Eucalyptus camaldulensis* and *Eucalyptus largiflorens* woodlands) due to reduced inundation extent and frequency throughout the lower River Murray, there has been increasing focus on using existing weirs, and constructing new regulators, to facilitate increases in floodplain inundation. Specifically, this has included the construction of the Chowilla Regulator and associated ancillary structures, with similar projects underway at the Lindsay-Mullaroo, Pike and Katarapko anabranches.

The management of riverine and floodplain structures has the potential to both threaten and enhance inchannel processes, such as the life history processes of large bodied native fish, dispersal of propagules such as seeds, eggs and plankton, and promotion of desirable trophic pathways (e.g. energy sources and types of phytoplankton). For instance, actions like weir pool lowering and environmental flow delivery, that promote lotic conditions in altered main channel habitats, have the potential to promote drift of propagules and adult Murray cod habitat and enhance survival of early life stages (Bice et al. 2017). Conversely, actions that promote lentic conditions, like weir pool raising and floodplain regulator operation, have the potential to reduce adult Murray Cod habitat and limit survival of early life stages (Mallen-Cooper et al. 2011). Given these potential risks, there is a need to better understand the relationships between in-channel hydraulics and the ecological functions that are dependent on them.

This is further outlined in Figure 1, where the conceptual relationship between velocity and water level and channel-floodplain connectivity is presented for five different floodplain inundation mechanisms (pumping, weir pool raising, regulated inundation, in-channel flow and overbank flow) at the site scale in the lower River Murray. The provision of environmental flows (or natural flows) results in an increase in water levels with a corresponding increase in flow velocity. In contrast, regulator operation or weir pool raising results in a water level rise decoupled from an increase in flow, and in turn reduce velocity (Figure 1). This increase in inundation for a given flow will alter the riverine hydraulics through the promotion of lentic conditions, and subsequently, the transport of resources, propagules (e.g. eggs, seeds) and biota from the floodplain to and along the river channel – processes that are critical for maintaining riverine-floodplain ecosystems (see Figure 2).

Although the dependence of key ecological processes on changes to hydrology (inundation) and, in turn, hydraulics is known (for example Bunn & Arthington 2002; Poff et al. 1997), there is limited quantitative knowledge available to describe the likely environmental responses to specific management scenarios for the lower River Murray. This project has taken an empirical approach (including field, laboratory and modelling components) to investigate the mechanisms relating to how changes in hydraulic conditions alter the transport and transformation of resources (carbon and nutrients), propagules (e.g. eggs, seeds, larvae) and biota within and along the River Murray Channel. This has previously been identified as a key research need in the lower River Murray (Bice et al. 2014) and internationally (National Research Council 2012). The mechanistic processes selected have direct links to the priority ecosystem functions for the South Australia (SA) River Murray Water Resource Plan Area (DEWNR 2015), including lateral and longitudinal hydrological connectivity, mobilisation and transport of carbon, primary productivity, transport of plant propagules and dispersal of faunal larvae and juveniles.

More specifically, the processes considered in this project were:

- The generation of hypoxic blackwater events through inundation of floodplains at lower flows than would be required without infrastructure,
- The generation of cyanobacterial algal blooms through increasing depth and retention time, and reducing velocity,

- The transport of propagules (seeds, microinvertebrates, Murray Cod larvae) within and along the river channel
- The flow of resources through food web dynamics, to improve the understanding of the relative role of different basal energy sources (e.g. phytoplankton, biofilms, terrestrial plant material and sediments/soils) on fuelling primary and secondary productivity.

This synthesis document provides an overview of the components considered in this project, how the empirical results have been related to underlying hydraulic and hydrological processes understood to be driving the observed changes, and how these relationships have been integrated into the hydrological model to simulate potential ecological outcomes under different operational scenarios.



Figure 1. Relationship between mean water velocity and water level at the site and channel-floodplain (lateral) connectivity for five different floodplain inundation scenarios (pumping, weir pool raising, regulated inundation, inchannel flow and overbank flow) at the site scale in the lower River Murray.



Figure 2. Conceptual representation of in-channel changes when creating inundation using structures. The left image represents normal conditions, with a given water depth (D) and velocity (v). The right image represents increased floodplain inundation due to a downstream structure, for the same flow. The possible changes in-channel include release of dissolved organic carbon (DOC) - resulting in consumption of dissolved oxygen (DO) and lower DO concentrations, reduced velocity, and increased depth - which in combination with the reduced velocity may lead to stratification, a pre-condition to cyanobacterial blooms.

2 Dissolved oxygen dynamics

During periods of elevated water level, due to high flows and/or infrastructure operation, the inundation of soils and accumulated plant and other organic material (e.g. scat) in the riparian zone and on the floodplain results in the mobilisation of water soluble dissolved organic carbon (DOC) and nutrients. Mobilisation of these constituents can provide vital resources to underpin aquatic food webs, however in some cases DOC concentrations can be sufficient to discolour the water and result in challenges for water treatment for potable supply (Simpson & Hayes 1998). The microbial degradation (heterotrophic metabolism) of this DOC can result in low dissolved oxygen (DO) concentrations (hypoxic blackwater events). When pelagic and sediment oxygen demanding processes exceed oxygen re-supply via diffusion across the air-water interface, photosynthesis, or supply of oxygen rich water from inflows, DO concentrations are depleted, and hypoxic (<2 mg $O_2 L^{-1}$) and/or anoxic (~0 mg $O_2 L^{-1}$) conditions become established. Even short periods of exposure to hypoxia can impact on the survival and condition of aquatic biota.

There has been substantial effort in the modelling of floodplain leaf litter dynamics and the resulting DO and DOC, both on the floodplain and the river. The most recent iteration includes the litter accumulation and decay processes developed as part of the Blackwater Risk Assessment Tool (BRAT) (Whitworth & Baldwin 2016) included as a constituent plugin in the Source model, the DODOC plugin (Mosley et al. in prep.). This inclusion in the Source model allows litter accumulation over longer periods, different scales of regulator operations at a floodplain site, as well as cumulative impacts along the river, to be assessed.

The current work has undertaken extensive field sampling, data analysis to enable the sampling data to be used as model inputs, and modelling to assess the impact of the variability in organic matter on the floodplain on simulated DO concentrations due to infrastructure operation. In particular, three components of the model can be parameterised using the litter data:

- The load of organic matter on the floodplain. This can be a combination of the initial standing load at the start of the simulation, and the load that accumulates over time. The model can incorporate a different loads and litter fall rates at different elevations up the floodplain, typically representative of different vegetation types.
- The fraction of the total organic matter that is readily available (i.e. the labile component).
- The rate of decay of leaf litter on the floodplain.

A short summary of the methods and results is provided here, with full details provided in Wallace and Gibbs (in prep.)

2.1 Understanding the amount of organic matter on the floodplains

The two dominant floodplain eucalypts in the lower River Murray; river red gum (*E. camaldulensis*) and black box (*E. largiflorens*) generate a large standing biomass of litter material (e.g. leaves, bark, twigs), that represents a source of allochthonous organic matter to floodplains and wetlands (Francis & Sheldon 2002; Glazebrook & Robertson 1999). The organic loading in the non-wooded basins of large wetlands has been demonstrated to be up to an order of magnitude lower than that at the fringing tree line, and the differences vary between dominant tree species (Wallace et al. 2015). The amount, type, age and inundation history of organic matter on the floodplain inundated will influence the amount and rate of DOC released to the water, and consequent changes in DO in the water column. This component of the project was undertaken to provide necessary site specific data to develop relationships to predict changes in standing organic load (grams of organic material per m²), largely driven by litter fall rates (grams of litter per m² per day), in order to be able to improve the predictive capacity of numerical models to determine the likely range of water quality outcomes associated with infrastructure operation and delivery of environmental water.

2.1.1 STUDY SITES

The study was conducted at the Chowilla Floodplain complex, and the Eckerts-Katarapko Floodplain complex (Figure 3). The Chowilla Floodplain (17,700 ha) is managed as an Icon Site under the Murray-Darling Basin Authority's (MDBA) The Living Murray (TLM) Program and as a game reserve by the Department for Environment and Water. The Calperum Floodplain (12,130 ha) is managed by the Australian Landscape Trust. Both floodplain areas form part of the 30,600 ha Riverland Ramsar Site (DEH 2010). Katarapko Floodplain is located on the Katarapko/Eckert Creeks anabranch system of the lower River Murray located in the Riverland. The floodplain covers 9,000 ha and 38 km of waterways and is situated near the town of Loxton to the southwest of Berri. Infrastructure to create large scale managed inundation has been in operation since 2014 on the Chowilla Floodplain and is currently being built on the Eckerts-Katarapko Floodplain, and as such these sites are of interest for how managed operations may alter DO concentrations.

2.1.2 STANDING LOAD OF ORGANIC MATERIAL

Natural organic matter (NOM) loading was assessed at 111 sites distributed across Calperum (n = 3 sites, 30th July 2018), Chowilla (n = 48 sites, 31st July – 1st August 2018) and Katarapko (n = 60 sites, 13-16 August 2018). The meso-habitats sampled included black box woodland (248 samples), cooba woodland (40 samples), river red gum woodland (311 samples), mixed tree species woodland (119 samples), grassland (88 samples) and lignum shrubland (72 samples), with samples collected from along temporary and permanent creek lines, temporary wetlands, and shedding floodplain, with samples within each meso-habitat type. At each site, 8 replicate samples were collected (total n recorded = 878) *via* an adaptation of the method previously utilised at Chowilla Floodplain (Brookes et al. 2007; Hackbusch 2011; Wallace & Lenon 2010; Wallace 2008; Wallace et al. 2015) and Pike Floodplain (Wallace 2009). Each sample of organic matter was air dried, weighed, and then split into the different components that are expected to release DOC at different rates, leaves, bark, twigs, coarse particulate organic matter (CPOM), scat and understory vegetation.

The total standing load samples can be seen in Figure 4, with the median values (solid lines within the bars) slightly higher than those recorded by Hladyz et al. (2011) of 798 \pm 265 g.m⁻² in the Edwards-Wakool river system (New South Wales).



Figure 3. Map of the lower River Murray floodplain, with location of the locks and floodplains considered in this study. The leaf litter sampling locations, and vegetation present at each location, is shown as the circles.



Figure 4. Total standing load of organic material (g.m⁻²) in each of the six meso-habitats sampled. Boxes enclose the 25th to 75th percentiles, whiskers enclose the 10th to 90th percentiles, outliers are identified by closed circles; dashed line within box plots depicts mean and solid line the median. Outliers with very high loading (>10,000 gm⁻²) are not shown in this plot but are included in the mean, median and percentiles shown.

2.1.3 LITTER FALL RATE

Estimates of litter fall rates were made by deploying leaf litter traps within woodland habitats on the Chowilla and Calperum floodplains, comprised of 10 black box and 10 red gum sites. The leaf litter trap was placed within the drip zone of a tree crown, with traps deployed under three trees at each site. All selected sites have existing tree condition transects where data on crown condition (extent and density) is routinely collected using the standardised TLM method (Souter et al. 2010). Traps were installed in November 2017 and emptied on four occasions to provide an estimate of the total litter fall between visits. For the last two trips, the collected material was also weighed in four fractions that are expected to have different DOC availability (i) leaves that were essentially intact, (ii) bark, (iii) twigs (<2 cm in diameter) and (iv) CPOM.

The data for litter fall rates from the Type 1 traps (Figure 5) demonstrate that the mean rate of litter fall was higher for red gums (1.91 g.m⁻².d⁻¹) than black box (0.44 g.m⁻².d⁻¹) for the summer period, but the rates are similar for the remainder of the year. Median values over the year were 1 and 0.4 g.m⁻².d⁻¹ for red gum and black box, respectively. The data for litter fall rate over the November 2017 to November 2018 period (Figure 6) indicates that there is no clear relationship between tree crown condition and litter fall rates for either red gum or black box.



Date

Figure 5. Litter fall rates (g.m⁻².d⁻¹) (Type 1 traps) for each of the survey periods between November 2017 and November 2018. BB = black box, RRG = river red gum. Symbols show mean, bars are ± 1 Standard Error.



Figure 6. Relationship between litter fall rate (g.m⁻²d⁻¹) November 2017 to November 2018 and tree crown condition (mTCI) [A] = red gum, [B] = black box.

2.2 Parameters for dissolved oxygen modelling

The DODOC model (Mosley et al. in prep.) for eWater Source has been used to model the DO expected for different flow and operational settings in this work. This model represents the most recent developments in the Blackwater Risk Assessment Tool (Whitworth & Baldwin 2016), but has the advantage of using eWater Source to represent complex hydrological scenarios. The model allows for two components of organic matter, one which will release DOC quickly when inundated (readily available), and another component which releases DOC more slowly (non-readily available).

The organic matter sampling results outlined in the previous section have been used to specify model parameters related to the leaf litter, using statistical distributions to account for the substantial variability that was observed (e.g. Figure 4). The parameters that can be estimated in this manner are shaded blue in the list below. A sensitivity analysis was also undertaken to evaluate the remaining model parameters, to understand their relative importance to simulating DO.

To implement the DODOC model, the key model parameters that must be specified are outlined below. Those that have been specified using the field data are shown in blue.

- water temperature (°C),
- reaeration rate, the rate at which oxygen is re-supplied via diffusion across the air-water interface (d⁻¹),
- the fraction of the total litter load that is readily available, with the remainder assumed to be non-readily available (g.g⁻¹),
- total amount of litter that accumulates each day, which is then split into the two components using the above fraction parameter (g.m⁻².d⁻¹), and
- the proportion of the DOC that is consumed each day (d⁻¹).

For each of the readily and non-readily components, there are also parameters for:

- the initial standing load at the start of the simulation (g.m⁻²)
- the proportion of litter that will decay each day when dry (d⁻¹)
- The DOC release rate from litter when wet (d⁻¹), and
- The maximum amount of DOC released for a given litter load (mg.g⁻¹)

2.2.1 ORGANIC MATTER MODEL PARAMETERS

The fraction of the organic matter that is readily available

The Type 1 litter trap data has been used to estimate the fraction of organic matter that is readily available, with the histogram of readily available material sampled for red gum and black box seen as the bars in Figure 7. The current version of the DODOC plugin model (Mosley et al. in prep.) has one fraction to represent the component of the organic matter that is readily available, there is not an option to have different fractions for different vegetation types, litter types, or floodplain elevation (for example). However, the probability distribution was relatively similar between the two samples vegetation types (all data, grey area). A normal distribution was used to represent the pooled litter data to represent the variability in the fraction of readily available leaf litter, which can be seen as the light red probability distribution in Figure 7.



Figure 7. Distribution of the fraction of organic matter that is readily available (leaves).

Decay rate of readily available leaf litter

The DODOC model assumes an exponential decay of litter (which can be different for each of the two components), and a constant daily accumulation of leaf litter. To determine the decay rate, the accumulation rates and standing loads were compared to determine how much of the litter that fell must have decayed. To do this analysis accurately, the time when the standing load was zero is required.

The sampling sites were all inundated during the 2016 high flow event. It is unlikely that all of the organic matter was completely removed from the floodplain during this event, and this is confirmed through analysis of the sampling data. Between the 2016 high flow event and the time of standing load sampling, there was approximately 584 days for matter to accumulate. Considering the median litter fall rate values of 1 and 0.4 g.m⁻².d⁻¹ for red gum and black box, this results in median values of 584 g.m⁻² and 234 g.m⁻² of standing load, before any decay of this material over time. These values are substantially below the median of the standing loads sampled (Figure 4) of 1,517 gm⁻² and 1070 gm⁻² for red gum at Chowilla and Katarapko floodplains, respectively, and 1,221 gm⁻² and 945 gm⁻² for black box at Chowilla and Katarapko floodplains, respectively.

The more readily available material, which is the smaller and lighter component of the organic matter, and breaks down quicker, is more likely to have been reset during the inundation event. The readily available component of the litter has been assumed to be the leaf fraction of the samples. Twigs, bark and CPOM are expected to release DOC at a slower rate compared to leaves. The litter data was also split into understory vegetation and scat components, which may also be more readily available in terms of DOC release rates. However, these components would not be expected to be represented by the litter accumulation from vegetation in the model. Instead, these components of the organic load could be represented in the model as an initial loading on the floodplain.

Based on the readily available (i.e. leaves only) standing loads for red gum and black box, fall rate of readily available material for red gum and black box, and accumulation over 584 days between the last inundation event and the time of sampling, a decay rate of $6e^{-4} d^{-1}$ for readily available leaf litter has been estimated. For non-readily available organic matter, a decay rate an order of magnitude slower has been assumed, $6e^{-5} d^{-1}$, similar to the approach taken by Whitworth and Baldwin (2016).

Initial standing load of organic material

The initial standing load at the point scale was sampled directly, for different locations with different vegetation types (Figure 4). The DODOC model can be configured with different initial standing loads for the two components at different floodplain elevations, but the variability in litter loads within an elevation band must be aggregated. The SA Vegetation layer was used to upscale the standing loads for a given vegetation type to represent the vegetation types across the SA floodplain for different elevation bands. The SA

Vegetation layer was simplified into five categories for this purpose: black box woodland, lignum shrubland, mixed woodland, red gum woodland and shrublands. Other data sources to upscale the point standing load samples to the floodplain scale were considered, for example the extension of a remotely sensed vegetation mapping method developed for Katarapko, however this data was found to be less accurate than the SA Vegetation layer when compared to field data of vegetation type on the Chowilla Floodplain. Remotely sensed greenness data was also considered to represent canopy cover. However, it was found that this data did not change substantially when averaged to the scale of a vegetation type across a floodplain elevation.

Box-Cox distributions were fit to the readily available standing load (i.e. leaves) samples for each vegetation classification, with resulting distributions can be seen in Figure 8. The non-readily available standing load was determined from the readily available standing load, by dividing by the fraction of readily available material.

Litter accumulation rates

Litter accumulation rates are a desirable input to the model when simulating the litter dynamics between inundation events over longer periods of time. Litter fall rates were sampled for red gum and black box, however there is limited information for other vegetation types across the SA floodplain. The sampled standing loads can be converted into accumulation rates for modelling using the same approach used to determine the decay rate above, if the decay rate is assumed to the estimated value of $6e^{-4} d^{-1}$. The derived litter fall rates has been compared to the fall rates determined from the leaf litter traps, and it can be seen that similar distributions have been generated for the red gum and black box vegetation types in Figure 8. These distributions, representing the variability in litter loads on the floodplain, are used in the DO modelling in Section 5.

2.2.2 SENSITIVITY ANALYSIS FOR OTHER DODOC PARAMETERS

A Sobol' sensitivity analysis (Sobol' 2001) using the method of Touati (2016) has been undertaken to assess the impact of the other parameters on the outputs from the DODOC model. The parameter ranges in Table 1 have been adopted, and the modelling scenario considered was an operation of the Pike floodplain regulator with a fill rate of 0.05 m.d⁻¹ to a full inundation height of 16.4 m AHD (Australian Height Datum). The results suggest that water temperature followed by reaeration rate have by far the biggest impact on the modelled DO (Figure 9). This provides useful information to guide operations, where water temperature can have a large impact on the resulting DO conditions, and negative impacts can be reduced through timing operations earlier in the season. The results also provide guidance on where investment in future sampling is likely to be most informative, with reaeration rates the next most influential parameter for the resulting modelled DO concentrations.

Parameter	Minimum	Maximum
DOC decomposition rate (d ⁻¹)	0.02	0.3
DOC release rate – readily available (d ⁻¹)	0.5	0.99
DOC release rate – non-readily available (d-1)	0.05	0.3
Maximum DOC release rate – readily available (mg g ⁻¹)	45	125
Maximum DOC release rate – non-readily available (mg g ⁻¹)	4.5	12.5
Reaeration rate (d ⁻¹)	0.01	0.08
Water temperature (°C)	10	25

Table 1: Bounds of uniform distributions used to testing sensitivity of DODOC parameters.



Figure 8. Standing load of leaves sampled, and Box-Cox transformed normal distributions fit to the standing loads for each vegetation type (left), and generated distributions for leaf accumulation rates, compared to Type 1 litter trap data for red gum and black box (right).



Figure 9. Sobol' sensitivity analysis results for DODOC model parameters not related to leaf litter over the ranges outlined in Table 1.

3 Phytoplankton abundance

Traditionally, diatoms comprise a large part of the phytoplankton community found in the River Murray (Reid & Ogden 2006; Sherman et al. 1998), and while diatoms generally grow quickly, are highly digestible and are nutritious for grazers (Brett & Müller-Navarra 1997; Hampton, Scheuerell & Schindler 2006; Lefèvre et al. 2008), they also need turbulence to remain in suspension (Brett & Müller-Navarra 1997; Litchman & Klausmeier 2008) and can therefore be disadvantaged by reduced velocity. Under the low flow scenario large low land rivers can stratify promoting conditions conducive for cyanobacterial growth. Global warming and increased growing seasons are predicted to contribute to a general increase in phytoplankton growth, however this may exaggerate problems with stratification and undesirable phytoplankton responses, such as cyanobacterial blooms (Sherman et al. 1998; Visser et al. 2005; Webster et al. 2000).

There have been several instances of prolonged algal blooms in the River Murray, for example in 1983, 1991, 2009, 2010 and 2016 (Croome et al. 2011; Murray Darling Basin Authority 2016). Blooms of cyanobacteria can cause problems by reducing the recreational and aesthetic value of river systems (Hallegraeff 1993) with periodic production of toxins which can be problematic for water supply. Cyanobacteria are also less nutritious for consumers in the food web (Ahlgren et al. 1990; Lampert 1987; Müller-Navarra et al. 2004). Cyanobacterial blooms are often associated with increased nutrient supply (Hallegraeff 1993), increased residence times and low flow leading to stratification in rivers (Cloern 1996; Sherman et al. 1998; Webster et al. 2000). In Australian rivers, flow has been found to be a key controlling factor of blooms since it controls stratification (Hoetzel & Croome 1994; Maier, Burch & Bormans 2001; Sherman et al. 1998). Velocities of 0.03 m.s⁻¹ have been shown to be sufficient to suppress persistent thermal stratification and *Anabaena* (recently taxonomically revised to *Dolichospermum*, the dominant genus of cyanobacteria) (Mitrovic et al. 2003). However, much higher velocities are required to resolve an existing bloom, with velocities above 0.3 m.s⁻¹ found to be required to remove an established cyanobacterial bloom (Mitrovic, Hardwick & Dorani 2010).

3.1 Mixing criterion as a measure of stratification

Temperature stratification tends to be a pre-condition for cyanobacteria to bloom and reach problematic concentrations in Australian rivers. The use of hydrologic manipulations to prevent temperature stratification is a demonstrated method of controlling cyanobacteria blooms. Increased flow has been shown to reduce cyanobacteria blooms even in nutrient-rich freshwaters, due to the controlling influence of flow on river stratification and phytoplankton population dynamics (Moritz et al. 2019; Oliver & Ganf 2000; Paerl 2008; Sherman et al. 1998). This has been demonstrated in Australian freshwater systems, for example, with negative correlations between flow and cyanobacterial abundance in the Murrumbidgee River (Jones 1993; Sherman et al. 1998), the lower River Murray (Baker et al. 2000; Bormans et al. 1997), and the Darling River (Mitrovic et al. 2003). The SA River Murray Long Term Watering Plan (DEWNR 2015) has an ecological objective to maintain a diurnally-mixed water column to ensure diverse phytoplankton and avoid negative water quality outcomes, with a target that thermal stratification does not persist for more than 5 days at any time.

3.1.1 MIXING CRITERION APPLICATION IN THE RIVER MURRAY

Walsh et al. (2018) estimated the critical flow required to disrupt thermal stratification in key regions of the Murray-Darling Basin (MDB) and its tributaries using a mixing criterion model (Bormans & Webster 1997). The authors also analysed flow and water column temperature data to compare the critical flow from the model to that observed in the river when stratification (defined as a temperature difference of >0.1°C between the epilimnion and hypolimnion) was and was not present. They found flows in the range of 1,400 – 3,000 ML.d⁻¹ were required to prevent stratification during the summer months within the SA River Murray, depending on the location.

The mixing criterion accounts for changes in velocity and water depth when using structures to increase inundation (as well as temperature, light attenuation and solar radiation), and as such provides an indication

of any increased risk of stratification due to regulator operations. Therefore, this project has implemented the mixing criterion, R, developed by Bormans and Webster (1997) (Equation 3) and used by Walsh et al. (2018), developed by Bormans and Webster (1997), in the Source model to enable operations that increase risk of persistent stratification to be identified.

3.1.2 VALIDATION IN SOUTH AUSTRALIA

Walsh et al. (2018) tested the criterion using high resolution temperature data from thermistor chains deployed between 2007–2009, a period with very low flows (typically less than 2,000 ML.d⁻¹ at Lock 1). Additional sites that have temperature sensors deployed at the top and bottom of the water column over a longer period of time, with a range of flow conditions, have been reviewed to test application of the Mixing Criterion in the Source model. The accuracy required to identify persistent stratification, typically 0.1°C, is often not available from the sensors deployed along the river, with adjustment of $0.3-1^{\circ}$ C made at times by hydrographers to correct the recorded data based on site measurements. These adjustments, and underlying data accuracy, can make identifying smaller persistent stratification events difficult. Reviewing the temperature data available for sites in SA that have temperature sensors at the top and bottom of the water column identified Customs House (A4261022) as the most suitable gauge, with other sites either having short records (<1 year) or involving significant temperature adjustments that introduce trends into the data.

A short period of the Customs House temperature records is presented in Figure 10. On most days, the surface water temperature heats up, and then cools overnight when the water column mixes, indicated by the equivalence of the top and bottom temperatures (e.g. at the end of the record in Figure 10). Over 8–15 November 2018, the recorded top and bottom temperatures do not meet. This could suggest a period of persistent stratification as the top and bottom temperature data, as it is unclear why the bottom temperature would increase over 11-12 November without mixing, for example. To identify stratification events, the minimum temperature difference between the recordings (taken at 5 minute intervals) each day has been used to indicate periods of persistent stratification.

The mixing criterion was calculated for each day based on:

- Air temperature, humidity, wind speed and solar radiation from the Chowilla AWS (A4261167)
- Water level upstream Lock 6 (A4260510) and flow to SA (A4261001)
- The relationship between water level and cross sectional area for the cross section closest to Customs House (A4261022) from the Chowilla MIKE model.
- Velocity derived as flow discharge divided by cross sectional area.

The resulting minimum temperature differences and mixing criterion value can be seen in Figure 11. High values (note reversed y-axis) for the Mixing Criterion, R, align with periods when the water column is expected to be well mixed, based on a small absolute temperature difference. Identifying periods of persistent stratification is more difficult, in part due to artefacts that are expected in the data at this very fine resolution (i.e. differences at a fraction of a degree, as discussed above). For example, the temperature difference is negative (indicating the bottom is warmer than the surface) for most of 2015 and 2016, and the large fluctuations in 2018 are likely due to corrections made to the data as part of the Quality Assurance process. Bormans and Webster (1997) suggested R<35,000 indicated stratified conditions for the Maude weir in the Murrumbidgee River, and derived a similar threshold using generalised numerical modelling. Extended periods with R<35,000 are shaded grey in Figure 11, which tend to align with the higher temperature differences in 2014. However, after this period, limited conclusions can be drawn from the temperature data due to the gaps seen in Figure 10, and unexplained trends in the data. It is difficult to assess the Mixing Criterion metric more quantitatively based on the temperature data. Nonetheless, the criterion is based on a fundamental heat balance (Bormans & Webster 1997), and is expected to provide an indication of when reduced velocity, increased depth, or increased thermal energy will result in an increased risk of persistent stratification.





Figure 10. Sub daily temperature data at Customs House (A4261022), where for a period between 8–15 November 2018 the water column did not reach the same temperature overnight as the sensor reading at the surface.



Figure 11. Comparison between minimum daily temperature difference at Customs House (A4261022), and the calculated mixing criterion. Low values for the mixing criterion (note axis is reversed) indicate higher liklihood of persistent stratification. Extended periods with a mixing criterion values less than 35,000 are shaded grey, and the horizontal dashed grey line represents a temperature difference of 0.1 °C.

3.2 Machine learning for cyanobacteria forecast

The occurrence of persistent stratification provides an indication of increased likelihood of harmful algal blooms, however the two do not always coincide. To address this difference, modelling of cyanobacteria occurrence was also undertaken, to test if models that directly predict cyanobacteria blooms can be developed to inform planning and operations. The prediction of cyanobacteria in rivers and lakes is traditionally done using process based simulation tools. However, when sufficient data are available a data driven approach using machine learning algorithms may also be used to derive forecast and early warning systems (Recknagel et al. 2018). For the River Murray, artificial neural networks were used to simulate data for a specific cyanobacteria species (Maier & Dandy 1997; Maier, Dandy & Burch 1998), where *Anabaena spp.* was simulated over a 7-year period, followed with a one year validation period. A further improvement was achieved using a neurofuzzy model (Maier, Sayed & Lence 2001). Other machine learning models for cyanobacteria forecasting in rivers are described by Hou et al. (2006) for the Darling River using a radial basis neural network model, or Yi et al. (2019) comparing a model-tree and extreme machine learning approach for a Korean river using daily data. The time series used in all these approaches were relatively short with a maximum of 10 years of data. Furthermore, all studies were looking at single locations.

The aim of this study was to use a long-term cyanobacteria record over a 25–year period which spans normal, wet and drought periods in the MDB available from a series of locations along the lower Murray. It is expected that upstream information will be useful to predict algal blooms at a specific station, as biomass can be seeding from upstream as well as result from local growth (Bowling et al. 2013). Algal growth is mainly limited by water temperature, underwater light conditions and nutrient availability. Here nutrient limitation was assumed to be minor, or highly correlated, to the physical drivers. Light conditions for cyanobacteria depend on variation in irradiance and water turbidity and to a large extend on mixing conditions – or stratification. Salinity can also alter growth and composition of cyanobacteria. The physical drivers are partly available for the 25-year modelling period as measured time series or can be derived using models (e.g., flow, water temperature). To simulate cyanobacteria abundance in the lower River Murray a machine learning algorithm was trained with these data. A regression tree ensemble model was used with a set of local predictors that take into account the upstream conditions on a previous time step and local drivers. The final model selection than took into account the sensitivity of model results to a combination of the main drivers. The predictors are described in the next section followed by a brief model description and results.

3.3 Phytoplankton dataset and drivers of growth

3.3.1 PHYTOPLANKTON DATA

A dataset of historical phytoplankton cell counts was collated, subject to extensive quality assurance and quality control, and then aggregated into five functional groups expected to respond differently to different flow and temperature conditions. The change of cyanobacteria biomass, or cell count, at a single location in the river system is determined by its local production as well as advective transport from upstream populations. Therefore cyanobacteria abundance cannot be modelled in isolation per location, but allowed an influence from upstream. Five locations were selected along the lower Murray to account for this upstream dependence: Lock 9, Morgan, Mannum, Murray Bridge and Tailem Bend. Cell count data is available at more locations, however data from other locations at least at times includes only partial counts, where only specific species of interest and the dominant species were counted, and as such represent an incomplete record.

A total of 128,359 records of plankton cell counts are available for the five locations between 1946 and 2018 including data for phytoplankton – cyanophyta, charophyta, chlorophyta, chromista, diatoms, as well as ciliates, protozoa and rotifers. There is greater frequency of records and confidence in the data from 1994 onward. We thus considered only data from this period in our simulation study, i.e. data after 1994 with a total of 87,386 records available, from which 16,335 were classified as cyanobacteria. Five cyanobacteria groups based on life history and morphology plus one general group capturing all unspecified cyanobacteria were originally distinguished. The number of records for the period 1/1/1994 until 18/3/2018

(23 years) is detailed in Table 2. Typically, thousands of records are required for data-driven models to be able to determine relationships between predictors and outputs/response variables, and it can be seen from Table 2 many of the individual groups do not have the number of records to support this kind of modelling approach. As such, a combined group of all cyanobacteria records was used as the output to predict.

The time series of total cyanobacteria cells for the five selected locations is shown in Figure 12. While the upstream locations show several large blooms over the last decade, these did not necessarily travel downstream. Similar but smaller increases in abundance in the downstream locations are more likely a local effect due to similar growth conditions (warm, sunny) as upstream, and less a transport condition. While a substantial effort was undertaken in quality controlling the phytoplankton data, it should be noted the data collection approach introduces uncertainties that cannot be resolved through post-analysis, for example the counting methodology itself, the counting process might have changed over time (e.g. less detailed to reduce costs), and small scale effects may influence the results (water sample taken from a location is not representative of the river channel).

Group ID	Description	Prominent group	Lock 9	Morgan	Mannum	Murray Bridge	Tailem Bend	Total
CHL	Chlorophyta		2751	4003	1356	1213	3401	12724
DIA	Diatoms		3750	4061	2754	2485	2306	15356
CYA-I	Unicellular cyanobacteria	Microcystis, Aphanocapsa	336	256	156	120	170	1038
CYA- IIIb	Filamentous, heterocystous benthic	Oscillatoria, Anabaena	46	92	246	149	34	567
CYA- IIIp	Filamentous, heterocystous pelagic	Dolichospermum, Chrysosporum, Cylindrospermopsis	3386	3823	1787	1156	1260	11412
CYA- IVb	Filamentous, non- heterocystous, non-branching benthic	Phormidium, Romeria	179	213	241	186	450	1269
CYA- IVp	Filamentous, non- heterocystous, non-branching pelagic	Planktolyngbya, Planktothrix, Pseudoanabaena	498	553	294	238	334	1917
CYA	Cyanobacteria not further classified		25	18	42	28	19	132
Total CYA	Sum of all cyanobacteria		10971	13019	6876	5575	7974	44415

Table 2: Plankton grouping and total number of records across all sites in the period 1994-01-01 to 2018-03-18.



Figure 12. Total cyanobacteria cell count for five locations along the lower Murray, a) since 1985, b) zoomed in since 2010.

3.3.2 PREDICTORS OF CYANOBACTERIA GROWTH

To model the dependence of cyanobacteria growth on transport we used simulated flow values or velocities (see Ye et al. 2016) with daily resolution. Upstream conditions in the cyanobacteria forecast model were taken into account by using the previous cyanobacteria cell count from the upstream location.

Along with flow, water temperature is the most important physical factor affecting cyanobacteria growth. Water temperature recordings are not available for most locations before 2009. Based on large scale water temperature simulations in several catchments in the MDB, Joehnk et al. (2019) found that a simple linear regression with air temperature was not sufficient to capture water temperature variations with sufficient accuracy.

Stream temperature is dependent on the history of air temperature and flow at the location and upstream. A simplified heat balance equation can be derived using only air temperature and flow rates. The *air2stream* model developed by Toffolon and Piccolroaz (2015) has been used to model stream temperature. The model parameterises the heat balance using eight parameters, and was tested for the lower River Murray using different levels of parameterisations as discussed in Piccolroaz et al. (2016), as well as simple linear regression. Air temperature data were extracted from the SILO database of Australian climate data from 1889 to the present (Jeffrey et al. 2001), and a seven parameter model (Piccolroaz et al. 2016) was selected based on maximising Nash-Sutcliffe-Efficiency, with values of NSE = 0.974, 0.971, 0.973, 0.963, 0.970 for models at Lock 9, Morgan, Mannum, Murray Bridge and Tailem Bend, respectively. As context, NSE = 1 represents perfect predictions, and NSE = 0 represents the model performs only as well as the average of the data. This model outperforms a simple linear relation between air temperature and water temperature with NSE = 0.765, 0.771, 0.691, 0.672, 0.707, for the same locations.

Persistent stratification will promote cyanobacteria growth, while mixing will suppress the growth. This can be described by a simple empirical mixing criterion relating destabilising flow values to stabilising heat supply. For the modelling input, a simplification of the Bormans and Webster (1997) equation outlined in Viney et al. (2007), was adopted, which is based on only flow and air temperature. The formulation needs a site specific threshold value, which is not available *a priori*, and instead the mixing criterion value is used as a potential predictor of stratification likelihood. Several threshold values of the mixing criterion were used to test stratification influence.

High light availability is another factor promoting cyanobacteria growth. Daily solar radiation from the SILO database (Jeffrey et al. 2001) was used as another predictor variable.

Salinity can change phytoplankton composition and growth with a negative correlation between growth and electrical conductivity (EC), which was shown to be one of the drivers in bloom formation in the lower Darling River (Hou et al. 2006). Measured EC was used as a proxy for salinity. Data for EC were not available for all locations over the full period since 1994. Linear interpolation was used to generate a gap free time series, to fill in gaps of a few days. Two of the locations had EC recordings only for periods after 2009. The missing years were extrapolated from nearby stations using a simple correlation factor. The long periods of missing, infilled EC data at the start of the time series (approx. 1994-2009) allows for a characterisation of the influence of salinity on predictability but care should be taken not to over-interpret this influence.

Nutrient levels at Tailem Bend were reported by Biswas and Mosley (2019) to be 0.7 mg.L⁻¹ and 0.1 mg.L⁻¹ for total nitrogen and total phosphorous, respectively. Nutrient values show a strong correlation with river flow. Furthermore it was shown in a previous study that nutrient levels were less important for algal growth than stratification or light penetration in the lower River Murray (Davis & Koop 2006; Maier, Dandy & Burch 1998). Hence, nutrient dependence was not considered further in this analysis.

3.4 Random forest model

3.4.1 MODEL DESCRIPTION

This study applied a generalised version of a machine learning algorithm called "random forest", which is an ensemble of individual regression tree models, termed a Random Tree Ensemble (RTE) (Breiman 2001). The approach has been successfully applied to hydrological data (Zhang et al. 2018) and applied here for the cyanobacteria abundance prediction. The RTE model subsamples the training data set and builds a regression tree for each subsample generating multiple predictions which are then aggregated (Figure 13). The subsampling allows for flexible nonlinear relationships in different regions of the data and it accounts for correlation between predictor variables. For a technical background see Zhang et al. (2018) and Breiman (2001).

The predictive skill of the model is analysed using k-fold cross validation (Hastie, Tibshirani & Friedman 2009). k-fold cross validation splits the data into k groups and uses each group as an independent test dataset and the remaining ones as training set, the procedure is repeated k-times to allow for each group as test data set. The final model skill is then summarised using the held out test evaluations, which provides and independent validation of the model. Depending on data size, k=10 is a commonly used value. In all analyses, small cell counts less than 100 were ignored in the calibration to improve the model.



Figure 13. Regression tree ensemble model framework (after Zhang et al. 2018)

3.5 Results

A regression tree ensemble model was calibrated to each the five stations along the lower River Murray, driven by a series of predictors describing the physical environment:

- Air and water temperature
- Flow discharge and velocity
- Electrical conductivity (salinity)
- Solar radiation

Past information of cyanobacteria abundance for each individual station was included via:

- cell counts at the same location one week before
- cell counts at the next upstream location one week before (except for Lock 9)

Including additional derived quantities:

- simplified mixing criterion representing stratified conditions
- temperature dependent growth rate of cyanobacteria (with a specific set of parameters related to *Chrysosporum ovalisporum*), and
- Week of the year, as a surrogate for seasonal dependence.

Testing of combinations of various predictors found the week of the year, and thus seasonal dependence, together with temperature, velocity and salinity, were the main physical drivers and most important predictors. The prediction improved when further including local and upstream cyanobacteria data from the previous time step.

Solar radiation and the simple mixing criterion for mixing used here did not improve the predictive performance. For the latter, this could be expected as the formulation used a simple relation between temperature and flow, which were included separately in the analysis.

Nash–Sutcliffe model efficiency coefficient (NSE) and root mean square error (RMSE) were used to evaluate model performance and compare results between locations and for calibration and validation results (Table 3). While the calibration achieved high NSE values, the validation, and thus the actual predictive capability, was lower. It is noted that the predictive capability was reduced going from Lock 9 (NSE=0.41) downstream to Tailem Bend where virtual no predictive capability was achieved (NSE=0.05). The simulation results for calibration and validation, together with the observations, are shown in Figure 14. Results are shown for the last four years of the record at Lock 9 in Figure 15, this time using a linear scale and thus focussing on peak growth. It is apparent that in calibration mode can predict the timing of cyanobacteria maxima, albeit underestimating the cell count. In validation mode, it is difficult to detect the peaks in observed cell count from the validation results.

3.6 Discussion

The random forest model used here (regression tree ensemble with bagging) is able to predict cyanobacteria cell counts for river locations with higher abundance, for instance at Lock 9 and Morgan, while the predictive capability decreased going further downstream. Overall calibration performance is acceptable (typically NSE>0.7), but validation shows the model has a lower predictive capability. In general the overall seasonal cycle and interannual variability of the data was predicted (Figure 14), however magnitudes were always underestimated. Compared to previous results achieved for a single location, Morgan, and a short time series of only eight years with a single year validation (Maier, Dandy & Burch 1998) our results are comparable with their predictions using temperature and flow as predictor. Maier, Dandy and Burch (1998) significantly improved predictions using colour as a predictor. This result suggests that other predictors may further improve the model performance.

Table 3: Goodness of fit measures for the random forest models based on the best performing combination of predictors. NSE = Nash–Sutcliffe efficiency, RMSE = root mean square error, Records = number of aggregated cyanobacteria data in data set used for simulation.

Site	Туре	RMSE	NSE	Records
Lock 9	Calibration	0.37	0.78	559
Mannum		0.26	0.73	205
Morgan		0.38	0.74	311
Murray Bridge		0.21	0.76	109
Tailem Bend		0.41	0.67	134
Lock 9	Validation	0.61	0.41	559
Mannum		0.46	0.19	205
Morgan		0.59	0.37	311
Murray Bridge		0.36	0.26	109
Tailem Bend		0.70	0.05	134

The long time series used in this analysis includes higher rainfall as well as drought periods, with changing conditions for cyanobacteria growth, which might not be well captured using the predictors selected. One issue with the low predictability for downstream locations, i.e. Tailem Bend, possibly comes from the general absence of larger cyanobacteria blooms at these locations, and thus too small sample set for predicting events.

The available data for cyanobacteria cell counts have undergone changes over the last 30 years in terms of what and how counts were undertaken. This leads to incompatibilities in the data over time, which may 'confuse' prediction. Another aspect of data uncertainty comes from the method of sampling, which could result in too high cell counts when done in locations with natural, flow driven accumulation. Whether this is the case here is not clear. As only the total cyanobacteria cell counts was used to create the largest dataset possible, changes in cyanobacteria composition over space and time have been ignored. This certainly reduces the predictive capability of any prediction algorithm when large changes in composition occur, and thus cause-effect relationships.

Summarising the application of a random forest model for prediction of cyanobacteria blooms, it was demonstrated that the technology of machine learning is capable of predicting the dataset of cyanobacteria data available for the lower River Murray. However, care has to be taken in the selection of predictor variables, too many might obscure cause-effect relations, and too few can reduce predictive capability. In the case presented here there is expected to be potential to improve upstream (Lock 9 to Morgan) predictive capability by further refining the predictors. It does not seem likely that the type of model used here is able to give good predictions for downstream locations with generally lower cyanobacteria biomass, the trend and seasonality can be matched but the small sample size of bloom events may restrict the ability for a model of this type to be developed.



Figure 14. Calibration and validation results of total cyanobacteria for the best performing prediction model versus observations. Plot is shown in logarithmic scale.


Figure 15. Calibration and validation results of total cyanobacteria for the best performing prediction model versus observations for Lock 9.

4 Transport of biological propagules

The dispersal and transport of biota, laterally between floodplain and main channel habitats and longitudinally within the main channel, is fundamental to the function of floodplain river ecosystems. Dispersal and retention, mediated by the interaction of flow, hydrodynamics and organisms ecology/behaviour, can be a vital life history processes for plants (seed/asexual propagules), microinvertebrates (eggs and adults) and fish (eggs/larvae). For these biota, transport (laterally or longitudinally) may influence the survival of early life-phases, and is critical to the maintenance of populations and communities. Furthermore, for biota that represent important food sources for higher trophic levels (e.g. micro- and macro-invertebrates), transport represents a potential transfer of energy between habitats and may thus influence riverine food webs.

In regulated rivers, dams and weirs disrupt connectivity and modify hydrodynamics, particularly upstream of regulating structures where lotic systems are converted to lentic impoundments. This project was undertaken to investigate relationships between hydrodynamics and the transport and deposition/retention of propagules at species-specific (e.g. abundance, distribution) and community levels (e.g. species composition and abundance) for plant propagules (seeds), microinvertebrates and fish larvae (Murray cod, *Maccullochella peelii*). For all biotic components, the Chowilla Anabranch was the focus of the field investigations, given the wide range in hydraulic conditions present in the anabranch, the infrastructure available to control inflows and water levels, and the presence of a population of reproductively mature Murray cod.

4.1 Sampling sites

This study was undertaken in the Chowilla Anabranch system, approximately 620 km upstream of the River Murray mouth. Chowilla consists of a complex of creeks that bypass Lock and Weir No. 6 on the northern floodplain of the lower River Murray (Figure 16). Due to the head differential created by Lock 6 (~ 3 m), and presence of numerous influent creeks, up to 90% of River Murray flows may be diverted through Chowilla under low flow conditions (i.e. < 10,000 ML.d⁻¹) (Stace and Greenwood 2004). Consequently, Chowilla exhibits permanent lotic habitats in a region dominated by lentic weir pool habitats.

The Chowilla regulator is a ~3.5 m high and 80 m wide weir that consists of 13 concrete piers along a concrete slab and apron, extending the full width of lower Chowilla Creek. The surface water level of the upstream pool is regulated by adding or removing concrete stop logs in bays between the piers (14 bays: 13 x 4.9 m wide and 1 x 2.45 m wide). With no stop logs in place the normal surface water level of Chowilla Creek at entitlement flows of 3,000–7,000 ML.d⁻¹ is 16.25–16.45 m AHD, but the regulator can be used to raise upstream water levels to a maximum of 19.87 m AHD.



Figure 16. Map of the study area including seven sites (black dots) within Chowilla Creek, the four sites (black dots) within the Lock 5 weir pool of the River Murray and the area soil seed bank samples were collected and sediment traps deployed (square). The Chowilla regulator is located just upstream (US) of the Downstream (DS) Regulator site.

4.2 Hydrological and hydraulic conditions during 2017 and 2018 sampling

From June 2017–December 2018, the flow to South Australia (QSA) was characterised by low in-channel flows <18,000 ML.d⁻¹ (Figure 17a). QSA over the general sampling period from October–December ranged 5,170–17,642 ML.d⁻¹ (mean \pm SD = 10,524 \pm 3369 ML.d⁻¹) in 2017, and 5,388–12,060 ML.d⁻¹ in 2018 (mean \pm SD = 7,549 \pm 1,760 ML.d⁻¹). Discharge in Chowilla Creek was slightly greater in 2018, ranging 2,952–5,242 ML.d⁻¹ (mean \pm SD = 4,022 \pm 713 ML.d⁻¹) and 38–68% of QSA, than 2017, when discharge ranged 2,012–5,396 ML.d⁻¹ (mean \pm SD = 3,522 \pm 460 ML.d⁻¹) and 29–49% of QSA. In 2018, elevated discharge in Chowilla Creek was associated with raising of Lock 6 in conjunction with operation of the Chowilla Regulator. From 20 August–10 December 2018, operation of the Chowilla regulator resulted in substantially higher water levels in Chowilla Creek (17.8–18.5 m AHD) than in 2017 (16.4–16.6 m AHD) (Figure 17b).

Operation of the Chowilla Regulator influences hydraulics upstream of the structure, primarily by reducing water velocity (Fredberg & Zampatti 2018; Zampatti, Wilson & Bice 2016). The action of raising water levels with a regulating structure results in a backwater effect that increases channel depth, width and cross-sectional area upstream of the structure. This effect gradually attenuates with increasing distance upstream of the regulator. As such, increased discharge (both absolute and as a proportion of QSA) and water levels in

Chowilla Creek in association with operation of the regulator resulted in substantial differences in the hydraulic characteristics of sites upstream of the regulator in 2017 and 2018. In conjunction with regulator operation in 2018, mean channel widths and cross-sectional area increased by 20–60% and 74–150%, respectively (Figure 18a-b). In association, mean cross-sectional velocities were reduced by 24–51% (Figure 18d). In particular, in Slaney Creek and in Chowilla Creek downstream of Slaney Creek and downstream of Boat Creek, mean cross-sectional velocities were $\geq 0.45 \text{ m.s}^{-1}$ in 2017, but were reduced to 0.25–0.27 m.s⁻¹ in 2018.



Figure 17. a) Discharge (ML.d⁻¹) in the River Murray at the South Australian border (QSA, black line) and in Chowilla Creek (blue line) and b) water level at the Chowilla Regulator from June 2017–December 2018. Grey bars represent timing of Murray cod larvae sampling, blue arrows represent timing of microinvertebrate sampling, while red and green circles represent timing of seed trap deployment and retrieval, respectively.



Figure 18. Mean (± SE) values of a) channel width (m), b) cross-sectional area (m²), c) discharge (m³.s⁻¹), d) crosssectional velocity (m.s⁻¹), e) modified vertical circulation metric (M₃, s⁻¹), f) modified horizontal circulation metric (M₄, s⁻¹), g) Froude Number and h) Reynolds Number at selected sampling sites in 2017 and 2018. Sites are presented as Chowilla flows from east to west (i.e. right to left), with Slaney Creek the most upstream site, and Downstream (DS) Regulator the most downstream site within the system.

4.3 Seed bank

The soil seed bank (reserves of viable seed in and on the soil) is an important component of the vegetation of wetland and floodplain ecosystems (Leck 1989). It provides a mechanism for the regeneration of plant communities after disturbance (Brock et al. 2003), and is of particular importance for arid rivers, where unpredictable flood regimes can result in extended periods of low or no flow and in turn, long periods without inundation of the riparian zone and floodplain (Puckridge et al. 1998; Puckridge, Walker & Costelloe 2000). Compared to the natural flow regime, this is exacerbated by river regulation in the lower River Murray with its largely stable water levels and reduced flooding frequency, duration and magnitude (Maheshwari, Walker & McMahon 1995).

Arid floodplain plant species are adapted to exploit the often short-lived favourable conditions for germination, growth and reproduction that occur after water levels recede following flooding, when there is high soil moisture and a lack of competition (Nicol 2004). In riverine systems, hydrochory is an important mechanism for seed dispersal and has been extensively studied in temperate (e.g. Favre-Bac et al. 2017; Nilsson, Gardfjell & Grelsson 1991) and tropical systems (Esper-Reyes et al. 2018; Lucas et al. 2012; van der Valk et al. 2009). Nevertheless, hydrochory has not been studied to the same degree in arid (e.g. Kehr, Merritt & Stromberg 2014; Sanchez-Salas et al. 2012) or Australian systems (Carthey et al. 2016; Catford & Jansson 2014; Greet, Cousens & Webb 2012, 2013; Johns & Campbell 2011; Murray et al. 2019).

Wind dispersal (anemochory) is also an important mode of seed transport for wetland and floodplain systems (Sarneel et al. 2014; Soons 2006; Zhu et al. 2019) as it provides a mechanism for long-distance dispersal between catchments, and upstream dispersal within catchments not provided by hydrochory (Larsen et al. 2012). It also allows dispersal within fragmented systems such as regulated rivers (Greet, Cousens & Webb 2012; Merritt & Wohl 2006). Most of the studies of seed dispersal in wetland and floodplain systems have focussed on hydrochory and zoochory (in particular the role of waterbirds) (e.g. Raulings et al. 2011) with anemochory more often studied in terrestrial systems (e.g. Beckman, Bullock & Salguero-Gómez 2018; Bullock & Clarke 2000).

The aim of this study was to compare seed deposition with the sediment seed bank in the riparian zones of Chowilla and Monoman creeks (part of the Chowilla Anabranch) under different inundation regimes: a natural flow pulse and regulated inundation by the Chowilla Regulator. The flow pulse in spring 2017 resulted in only two of the 11 elevations where sediment traps were deployed being inundated, which allowed anemochory to also be investigated despite not being a primary aim of the study.

4.3.1 METHODS

Thirteen transects extending from normal pool level to 2 m above normal pool level were established on the banks of Chowilla and Monoman creeks within 4 km upstream of the Chowilla Regulator (Figure 16) in reaches with gently sloping banks. In November 2017, soil samples to a depth of 5 cm at 20 cm vertical intervals from normal pool level to 2 m above normal pool level were taken (with a spade) along each transect (these samples will be herein referred to as seed bank samples). In addition, 40 x 40 cm squares of artificial turf were pegged at the same elevations (avoiding the holes left by sediment sampling) along each transect in 2017 (prior to the in-channel pulse) and 2018 (prior to regulator operation) and retrieved when water levels returned to normal pool level (Figure 17). The germinable seed bank was assessed using the seedling emergence technique (sensu Brown 1992; Gross 1990). After 16 weeks, numbers of germinants and species were collated and the seed bank samples converted to germinants.m-2 using the approach of Nicol, Ganf and Pelton (2003). Further details on the methods and analysis is available in Nicol and Frahn (in prep).

4.3.2 RESULTS AND DISCUSSION

Whether seed was transported by water (hydrochory) or wind (anemochory), the artificial turf sediment traps were effective at capturing sediment and in turn seed transported to the banks of creeks in the Chowilla Anabranch system. The sediment traps were also easy to locate, with only two traps lost (>99% of traps

recovered) over the study period. The most sediment was deposited on the traps inundated in 2018 (regulated inundation) when average flow velocities were typically 60% of those measured in 2017 (Section 4.2) and the least sediment was deposited on traps that were not inundated (Figure 19). In several instances during spring 2018 (regulated inundation), over 8 kg (air dried) sediment was deposited on sediment traps 0.16 m² in area, thus equating to ~50 kg.m⁻² of sediment deposited.



Figure 19. Sediment deposited on artificial turf sediment traps inundated in 2017 (flow pulse) and 2018 (regulated inundation) and non-inundated traps (2017) (error bars ±1 S.E.).

Despite the large differences in sediment deposited on the traps there was no significant difference in seed density between traps inundated in 2017, 2018 and not inundated (Figure 20). Seed densities from the sediment traps showed that seed input to the seed bank from hydrochory, anemochory and seed rain is significant, with inputs ranging from 8% (seed traps inundated in 2017) to 17% (seed traps not inundated in 2017) of the soil seed pool.

There was no significant difference in germinable seed composition between the traps inundated in 2018 and traps not inundated (2017). There was; however, a significant difference in the germinable seed composition deposited on the traps inundated in 2017 (flow pulse) that was likely due to lower species richness in 2017 (Figure 21).



Figure 20. Germinable seed density of the soil seed bank (2017), and for the sediment traps inundated in 2017 (flow pulse) and 2018 (regulated inundation) and non-inundated sediment traps (2017) (error bars ±1 S.E.).



Figure 21. Total germinant species richness of the soil seed bank (2017), and for the sediment traps inundated in 2017 (flow pulse) and 2018 (regulated inundation) and non-inundated sediment traps (2017).

This study demonstrates there is significant seed input into the soil seed bank from hydrochory, anemochory and seed rain. Seed deposition by water only occurs when there are changes in water level, with the deposition of seed entrained in the water column on the banks or floodplain as water levels recede. In contrast, anemochory would be less sporadic, and assuming a constant rate of deposition, 14 seeds.m⁻² are deposited on the banks of Chowilla and Monoman creeks each day. There is no way, however, of knowing whether any seed deposited on the traps is derived from seed rain (primary dispersal) or redistribution of dormant seed already in the soil seed bank (secondary dispersal). Whilst not all the seed deposited may be increasing the total soil seed pool, the number and species richness of viable seeds trapped shows that the seed bank is spatially and temporally dynamic. Furthermore, the higher species richness recorded for the sediment traps not inundated and inundated in 2018 compared to the soil seed bank suggest that seed input is important in maintaining diversity in the seed bank and in turn resilience of the plant community.

Most species were recorded across multiple sampling events, which suggests that they have evolved multiple dispersal strategies (sensu Zhu et al. 2019). Whilst ten species were recorded exclusively on sediment traps that were inundated, they were present in low numbers and it cannot be concluded that hydrochory is their primary means of dispersal. All of these species are widespread throughout Australia, including in soil seed banks in the Murray-Darling and Lake Eyre basins; hence, are not reliant on hydrochory. The dominant tree species (*E. camaldulensis*) was present across all sampling events but was a significant indicator of the sediment traps inundated in spring 2018 and was observed in inundated and non-inundated sediment traps in 2017. This species was uncommon in the soil seed bank, as it has a predominantly aerial seed bank (Jensen, Walker & Paton 2008) and times seed release to coincide with high river flows (Jensen, Walker & Paton 2008; Pettit & Froend 2001a, 2001b; Pettit, Froend & Davies 2001). In the lower River Murray, high flows typically occur in spring and early summer (Maheshwari, Walker & McMahon 1995; Walker 1986) coinciding with the deployment of sediment traps in this study.

In arid floodplain systems, where favourable conditions for germination growth and survival can be sporadic and often of short duration, multiple dispersal strategies may be important for survival (Nicol et al. 2018; Puckridge et al. 1998; Puckridge, Walker & Costelloe 2000). Most species recorded in this study require exposed sediment with high soil moisture for germination (Nicol 2004; Nicol & Frahn 2019; Nicol & Ganf 2000; Nicol, Ganf & Pelton 2003). Dispersal by hydrochory will result in seeds having a higher chance of being deposited in an area that fulfils the requirements for germination and in turn growth and survival to maturity to replenish the seed bank. In contrast, anemochory is a riskier strategy with a higher chance of seeds being deposited in areas that are not immediately suitable for germination. Whilst hydrochory is a less riskier strategy in the short-term, dispersal is limited spatially to areas that are subjected to inundation, is generally unidirectional (typically downstream dispersal) and does not disperse between catchments (Larsen et al. 2012). Therefore, long-distance dispersal, dispersal upstream and between catchments is reliant anemochory or zoochory.

Increased sediment deposition without a corresponding increase in seed numbers during regulated inundation may "dilute" the active seed bank (top 5 cm of soil). Most species recorded in this study are small seeded species that lack the carbohydrate reserves to emerge from deep in the soil profile (Cunningham et al. 1992). Whilst this may not result in seed mortality (e.g. Leck & Brock 2000; O'Donnell, Fryirs & Leishman 2015; Thompson et al. 1998; Tsuyuzaki & Goto 2001; van der Valk & Davis 1979), buried seeds are effectively removed from the seed bank and do not contribute to the vegetation.

4.4 Microinvertebrates

Within river-floodplain food webs, microinvertebrates, including rotifers, cladocerans and copepods, provide a critical link to higher order consumers by feeding on bacteria, phytoplankton and organic material (e.g. May, Bailey-Watts & Kirika 2001; Work & Havens 2003) while they themselves are consumed by fish, birds, amphibians and macroinvertebrates (Arumugam & Geddes 1996; Lancaster & Robertson 1995). Riverine ecosystems are characterised by spatial variability in microinvertebrates assemblages. At the simplest level, microinvertebrate assemblages can be classified as either littoral, limnetic, lotic or benthic. In littoral and limnetic environments, water residence time (WRT) has a strong positive relationship with microinvertebrate density and biomass (e.g. Baranyi et al. 2002; Basu & Pick 1996; Obertegger et al. 2007). High microinvertebrate density is favoured by increased WRT largely because microinvertebrates cannot reproduce, maintain their position, or, depending on the species, survive within fast flowing water (e.g. Richardson 1992; Sluss, Cobbs & Thorp 2008). Therefore, most species depend on still or slow flowing water environments to maintain their populations. Consequently, longer WRTs favour microinvertebrate development within off-channel sites including the littoral and limnetic habitats of floodplain lakes, billabongs and wetlands. Within in-channel sites, microinvertebrate community development occurs predominantly in weir pools, littoral, and limnetic backwater habitats (e.g. Baranyi et al. 2002; Reckendorfer et al. 1999). Organisms can then be transferred between habitats by hydrological mixing and exchange, or due to the entrainment of organisms from limnetic or littoral into lotic habitats (Figure 22). As microinvertebrates generally cannot reproduce in lotic habitats use as creeks and river channels, the density and assemblage structure of the community within these habitats is primarily dependent on the characteristics of source communities (e.g. littoral or limnetic habitats) and the ability of different species to survive/cope within flowing water after transferal from slower flowing habitats.



Figure 22. Schematic diagram illustrating the primary habitats and movement of microinvertebrates.

There were two distinct components to this study. Each component was investigated across the spring/summer period of 2017, a year of no regulator operation, and 2018, a year of regulator operation.

The first component aimed to investigate the generation of littoral and limnetic microcrustaceans as food resources for Murray cod larvae in the Chowilla Anabranch. The specific aims of this component were to:

- 1. Investigate spatial and temporal changes in the littoral microcrustacean community, and
- 2. Relate these changes to discharge and water level (as indicators of changes in the availability of littoral habitat), and season.

The second component aimed to investigate the entrainment and downstream transportation of *Trichocerca*, a genus of rotifers that commonly prefer littoral habitats yet are commonly detected within lotic habitats in spring, along the Chowilla Anabranch and the Lock 5 weir pool. The specific aims of this component were to:

1. Investigate longitudinal changes in the density of *Trichocerca*, and

2. Investigate correlations between the density of *Trichocerca* and modelled water level (as an indication of differences in the availability of littoral habitat) and water velocity (as a driver of entrainment).

4.4.1 METHODS

Each of the components had a different sampling design, summarised as follows. Further details of the methods and analysis are outlined in Furst (In prep.).

4.4.1.1 Generation of littoral and limnetic microcrustaceans as food resources for Murray cod

Three sites along the Chowilla Anabranch were sampled, one downstream of Slaney, Pipeclay and Boat Creek. Samples were taken to provide a general comparison with Murray cod stomach content. Between three and four replicates were taken from each site close to the creek bank. Each sample consisted of three 4 L quantitative samples collected using a Haney trap and combined to make a single composite sample. The total volume of each composite sample was concentrated to approximately 50 mL by filtering through a 30 μ m net. Concentrated samples were then transferred to a 200 mL PET jar and preserved with 70% ethanol. The number of microcrustaceans within each subsample were counted, and microcrustaceans identified to the highest level possible from the gross morphology (predominantly order level for copepods and family level for cladocerans). The average number of microcrustaceans were calculated and expressed as numbers of individuals per litre (ind.L⁻¹).

4.4.1.2 Entrainment and downstream transportation of rotifers

During each trip, five sites along the Chowilla Anabranch (downstream of Pipeclay, downstream of Boat, upstream of Monoman, downstream of Monoman and downstream of the Regulator) and four sites within the Lock 5 weir pool (Below Lock 6, Lock 5 Site 1, Lock 5 Site 2 and Lock 5 Site 3) were sampled. Chowilla Creek was sampled due to the wide variety of average water velocities in which a parcel of water experiences as it moves through the anabranch. Sites within the Lock 5 weir pool were sampled to investigate whether trends detected in the Chowilla Anabranch also occur within the main river channel. At each site three replicate samples were taken in the middle of the channel approximately 200 m apart. Each sample was generated using a 4 L Haney trap, collecting a discrete sample from the top, middle and bottom of the water column, and transferring to a pre-rinsed 20 L drum to produce a 12 L sample. The total volume of each composite sample was concentrated to approximately 50 mL by filtering through a 30 μ m net. Concentrated samples were then transferred to a 200 mL PET jar and preserved with 70% ethanol. Quantitative samples were inverted three times and a 1 mL sub-sample transferred into a pyrex gridded Sedgewick-Rafter cell. The entire sub-sample was counted, and microinvertebrates identified using a Leica compound microscope. The average number of organisms from the genus *Trichocerca* were calculated and expressed as numbers of individuals per litre (ind.L⁻¹).

Longitudinal changes in the density of *Trichocerca* along both the Chowilla Anabranch and the main river channel were investigated. Water level and water velocity are two hydrological variables that can be modelled using the Chowilla MIKE FLOOD model and therefore correlations between different variations of these variables and the density of *Trichocerca* explored. These variations included: (1) the average at that site (or the nearest model point) (2) the average within a set distance upstream and (3) the proportion of the reach upstream above 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 m.s⁻¹. The different sections of the reach investigated included between 500 and 5000 m at 500 m intervals. *Trichocerca* are a seasonal taxa and temporal changes in their productivity likely to be driven by a wide range of factors including water temperature and food availability. The required sampling frequency required to explore temporal relationships was not achievable during this study, therefore sampling trips were analysed separately. The velocity variable and the product of the best fit velocity variable and water level which resulted in the best fit relationship with the density of taxa from the genera of Trichocerca for each trip in which *Trichocerca* were the dominant taxa are presented and discussed below.

4.4.2 RESULTS

4.4.2.1 Generation of littoral and limnetic microcrustaceans as food resources for Murray cod

Previous studies have identified that microcrustaceans including cladocerans from the families Macrothricidae, Chydoridae, Moinidae and Daphniidae, as well as cyclopoid copepods, are preferred prey for larvae and juvenile Murray cod (e.g. Ingram & De Silva 2007; Kaminskas & Humphries 2009). In general, microcrustaceans occur in high density in slow/non-flowing littoral and limnetic habitats and low density in lotic habitats due to their inability to survive within fast flowing water. Microcrustacean community dynamics are influenced by the availability of food resources, predation, water quality, geomorphology, habitat and hydraulics. Within the lower River Murray, two of the key drivers of community development are likely to be habitat availability and season (Obertegger et al. 2007; Shiel, Walker & Williams 1982).

A PERMANOVA on microcrustacean density indicated a significant difference between trips and sites (*P*=0.0001 and *P*=0.03, respectively) (Figure 23). Post-hoc comparisons revealed that total microcrustacean density was significantly higher in early-November than in late-November (P=0.0001) and mid-December 2017 (P=0.0002). Microcrustacean density was also significantly higher in late-November than in late-November 2018 (P=0.0072).



Figure 23. Differences in the average density of microcrustaceans (ind.L⁻¹) at three different sites within the Chowilla Anabranch, namely downstream of Slaney (DS Slaney), downstream of Pipeclay (DS Pipeclay) and downstream of Boat Creek (DS Boat) in 2017 and 2018.



Figure 24. Composition of sum of all prey items found in the stomach of Murray cod larvae collected in early November 2017 (n=25) and late October 2018 (n=14).

The higher microcrustacean densities in early-November 2017 were largely due to high densities of Bosmina meridionalis, a common cladoceran in the pelagic zone of rivers and lakes including the lower River Murray (Figure 23). This taxon was found to be consumed by Murray cod larvae collected during the same sampling period and highlights the importance of this pelagic riverine species, previously unidentified as a food resource for Murray cod larvae (Figure 24). During this study, Murray cod larvae were only collected in conjunction with the sampling trips conducted in early-November 2017 and late-October 2018. Interestingly, even though cyclopoid copepods were not present in high densities during these times, Murray cod were selectively feeding on them and made up the highest proportion of organisms found within the stomach content in early-November 2017 (Figure 23 and Figure 24). As in previous studies, this highlights the importance of this littoral food resource for Murray cod larvae. Few of the littoral microcrustaceans previously found to be important food resources for Murray cod larvae were present at high densities at this time, most likely due the absence of adequate littoral habitat. Littoral taxa previously identified as important food resources, such as cyclopoid copepods and cladocerans from the families Chydoridae and Macrothricidae, started to develop in mid-December 2017 when discharge and water level were the highest for the study and most likely due to the creation of adequate habitat (Figure 23). Many of the species from these families characteristically peak in spring (Shiel, Walker & Williams 1982), suggesting that similar hydrological conditions in spring may have produced a greater response from these organisms.

4.4.2.2 Entrainment and downstream transportation of rotifers from the genus *Trichocerca*

Invertebrates such as rotifers, have a limited ability to synthesise long-chain polyunsaturated fatty acids and must obtain them from the food they consume (Guo et al 2017). *Trichocerca pusilla*, often the most abundant species of *Trichocerca* during spring, are thought to feed primarily on *Aulacoseira*, a high quality diatom (May, Bailey-Watts & Kirika 2001). Therefore, *Trichocerca pusilla* are potentially a high-quality food resource for higher trophic organisms. When *Trichocera* species are residing in the littoral (their preferred habitat), they are producing eggs and contributing to the egg-bank that will ensure future communities. If their populations within the littoral zone are not also seeding channel habitats during an in-channel pulse, they will perish as the hydrograph recedes and their habitat vanishes. In these instances, the benefit they provide to higher trophic organisms are likely to be relatively short lived. However, if *Trichocera* species are entrained and transported downstream, the water is likely to become increasingly enriched with these organisms, providing a food resource that builds longitudinally, maximising the benefits to the aquatic food web downstream. Indeed, two recent studies have found this group to occur at higher densities at greater discharges, increase in density longitudinally, and decrease in density when longitudinal connectivity is disrupted and water velocities are reduced (Furst, Aldridge, Bice, et al. 2017; Furst, Aldridge, Ye, et al. 2017).

For the purpose of this study and analysis, a number of assumptions have been made. These include that: (1) spatial differences in the availability of organisms to source lotic habitat are associated with the availability of adjacent littoral habitat, (2) the availability of adjacent littoral habitat is positively associated with water level, (3) once washed into a lotic environment organisms require water velocities and/or turbulence above a certain level to remain within suspension and continue to be transported downstream and (4) once entrained, reproduction is significantly limited. It was expected that longitudinal differences in the density of *Trichocerca* would be positively associated with differences in water level (due to increased availability of organisms within the adjacent littoral habitat) and water velocity (due to enhanced entrainment and downstream transportation).

Taxa from the typically littoral genus *Trichocerca*, dominated the microinvertebrate community (> 50% of the total density) in the Chowilla Anabranch and the River Murray during the sampling trips conducted in early-November 2017, late-November 2017 and late-November 2018. Changes in the density of *Trichocerca* appeared to be associated with both changes in water velocity and water level at different times, potentially driven by both the entrainment and downstream transportation of organisms in late spring. Whether the association with water velocity or water level was stronger, however, differed under different hydrological conditions.

In early-November 2017, sampling was conducted at the very beginning of an in-channel pulse. During this trip, when analysed independently, neither the best-fit water velocity variable nor water level appeared to be strongly correlated with the density of *Trichocerca* (R^2 =0.37 and 0.026, respectively) (Figure 25a). The product of water level and the best-fit water velocity variable also demonstrated a relatively weak correlation (R^2 =0.41) suggesting that the model could not explain nearly 60% of the variability in the density of *Trichocerca*. This suggests that a large amount of variability at this time was being driven by other factors not investigated as part of this study, for example, hatching from the egg-bank, turbulence, food availability and/or water quality.

In late-November 2017, sampling was conducted on the rising limb of the in-channel pulse. As discharge increases, dry sediments bordering the channel become inundated and the area of slow flowing habitat grows as water level rises. The egg-bank within these sediments are likely to contain considerable quantities of littoral organisms as these areas are likely to have been slow flowing littoral habitat during previous inchannel rises. Therefore, the slightly stronger correlation between the density of Trichocerca and water level $(R^2=0.55)$, rather than with the best-fit water velocity variable $(R^2=0.37)$ during this sampling trip may have been due to the influence of hatching from the egg-bank and increases in productivity in the adjacent littoral habitats (Figure 25b). Despite water level being a slightly better predictor than the best-fit water velocity variable when investigated independently, the product of the two variables demonstrated a strong correlation with Trichocerca density (R²=0.75). This suggests that the combination of the two variables, explained the majority of variability in the density of *Trichocerca*. It is possible that both the entrainment and the sourcing of organisms from the littoral zone were important under these conditions. However, water level and water velocity are correlated and therefore the strong correlation with the product of the two variables may indicate that the relationship is non-linear, or these variables represent attributes of an overarching driver not investigated. For example, one of the assumptions of this analysis was that once entrained within flowing water, organisms are unable to reproduce due to the limitations that water velocity are thought to exert on rotifer reproduction (e.g. Czerniawski & Sługocki 2017). However, this assumption may not hold true, and changes in their densities may have been driven by a change in instream primary production in relation to increases in water level and water velocity, driving productivity in the Trichocerca community in-channel.

In late-November 2018, sampling was conducted during a period of very low discharge following a small inchannel regulator operation. During such low discharge, little to no littoral zone is likely to be engaged within the area of interest and therefore increases in density due to entrainment are unlikely. This was supported by a very weak correlation between the density of *Trichocerca* and water level (R^2 =0.23) (Figure 25 c). Despite little entrainment occurring within the study area, downstream transportation of the organisms already within the system appeared to be important with the best-fit water velocity variable (> 0.15 m.s⁻¹) having a strong correlation with the density of *Trichocerca* (R^2 =0.67). Again, the product of water level and the bestfit water velocity variable was also strongly correlated (R²=0.71), however only slightly more than velocity alone.

Organisms from the genus *Trichocerca* are commonly the most dominant genus of rotifers within the lower River Murray at times in spring. Due to the limitations of traditional gut content analysis, the higher trophic levels that consume *Trichocerca* in the lower River Murray have not been identified however a number of international studies have found them to be important food resources for shrimp (e.g. Grossnickle 2001; Haskell & Stanford 2006) and fish (e.g. Dev & Rahmatullah 1998; McCullough & Stanley 1981; Sampson, Chick & Pegg 2009; Van Den Avyle & Wilson 1980). This study provides some indication that engaging the littoral zone through increases in water level and maintaining average cross-sectional velocities above 0.15-0.2 m.s⁻¹ are beneficial to the entrainment and downstream transportation of littoral rotifers from the genus *Trichocerca*. However, there are a number of factors that need further exploration including the inconsistency in the best-fit water velocity variable across sampling trips (e.g. 0.15 versus 0.2 m.s⁻¹), the large degree of unexplained variability, and the significance of covariance between water level and water velocity. Improving our understanding of the relationships between hydrology and hydrodynamics and therefore our ability to protect and restore these relationships is likely to have positive outcomes for the aquatic food web.



Figure 25. Relationships between *Trichocerca* density and the best fit cross-sectional water velocity variable, water level and the product of the two within the Chowilla Anabranch and the Lock 5 weir pool in (a) early November 2017, (b) late November 2017 and (c) late November 2018. Blue line represents the 95% confidence band and the red line represents 95% prediction band. Prop. = "proportion of the".

4.5 Murray cod larvae

The early life stages of fishes (eggs, larvae and juveniles) are subject to high levels of mortality, and survival through this vulnerable period can influence cohort strength and population abundance (Houde 1997). Critical to survival is the coincidence of larvae with favourable physico-chemical conditions (e.g. temperature) and food, and mitigation of predation and competition (Cushing 1990; Keckeis et al. 1997; Pavlov 1994). Dispersal from spawning habitats and retention in nursery habitats, where nutrients and prey are concentrated, are likely fundamental processes that dictate survival (i.e. Fundamental Triad Hypothesis; Bakun 1998).

In riverine ecosystems, the dispersal of fish larvae commonly occurs as downstream drift, whilst nurseries, either off-channel or within the main channel, are associated with hydraulic features (e.g. low water velocities) where prey are concentrated (Humphries et al. 2019). The spatio-temporal distribution of these habitat patches (Price et al. 2012), as well as the processes of dispersal and retention of larvae, are a function

of the interaction among river morphology, hydrodynamics and fish behaviour (Lechner, Keckeis & Humphries 2016; Lechner, Keckeis, Schludermann, Humphries, et al. 2014; Lechner, Keckeis, Schludermann, Loisl, et al. 2014). Anthropogenic changes to river morphology (e.g. channelisation) and hydrodynamics (e.g. flow regulation) may therefore influence dispersal and retention, and subsequent survival of early life stages, with potential population level effects (Humphries et al. 2019).

Murray cod is a large (total length >1.4 m) and long lived (~48 years) apex predator of riverine ecosystems in the MDB (Ebner 2006). Murray cod have undergone substantial declines in abundance in association with the anthropogenic modification of river flow and habitats, barriers to movement and over-harvesting (Ye et al. 2018). Murray cod spawn annually in October–December, irrespective of hydrology, but coincident with water temperatures ≥15°C (Humphries 2005). Eggs are laid at a 'nest' site and larvae subsequently disperse from the nest, and may drift downstream, before settling in littoral nursery habitats (Koehn & Harrington 2005). Murray cod larvae have considerable swimming ability (Kopf, Humphries & Watts 2014), suggesting drift is acti-passive (Pavlov 1994), and entry and exit from drift may relate to behavioural choices that enable individuals to assess littoral nursery habitat prior to settlement (Lechner, Keckeis & Humphries 2016).

In the lower River Murray, the fragmentation of the river by sequential weirs and loss of lotic habitats are considered primary threats to riverine biota, including Murray cod (Mallen-Cooper & Zampatti 2018; Zampatti et al. 2014). Whilst Murray cod are still found throughout the lower River Murray, they are most prominent in a few locations where lotic habitats remain (Zampatti et al. 2014). These include large flowing anabranch systems (e.g. Lindsay-Mullaroo, Chowilla), and the tailwaters and upper weir pools of specific main channel reaches (e.g. Lock 3–4). Regular recruitment and robust populations of Murray cod in these lotic regions are likely supported by the presence of structural (e.g. large wood) and hydraulic habitats that support Murray cod throughout ontogeny, but in particular during early life history processes (i.e. drift and retention in suitable nursery habitats) and survival.

In this study, we investigated the retention of Murray cod larvae in littoral habitats of the Chowilla Anabranch over two spawning seasons, comprising a year of no regulator operation (2017) and a year of operation (2018). Specifically, we aimed to:

- Quantify spatio-temporal variability in the abundance of Murray cod larvae in the littoral zone (retention);
- Investigate environmental correlates (e.g. water velocity) associated with variability in larval retention, and quantify micro-scale nursery habitat preferences of larvae; and
- Develop ecohydraulic relationships and conceptual models of Murray cod early life history to support future management

4.5.1 METHODS

In 2017 and 2018, sampling was conducted at six sites within the Chowilla Anabranch over three-week periods in October–November. The most upstream site was within Slaney Creek, while the remaining sites were located in Chowilla Creek at approximately 4 km intervals beginning 0.5 km downstream of the Slaney Creek Junction and ending at a site 0.5 km downstream of the Chowilla Regulator. Previous tracking of radio-tagged Murray cod at Chowilla indicates that the majority (typically >80%) of tagged male fish reside within Slaney Creek during the spawning season, suggesting the majority of larvae in the Chowilla system are sourced from this creek.

In each year, littoral habitats at each site were sampled over 3–6 nights with a suite of six quatre-foil light traps (225 x 225 x 225 mm; Floyd, Courtenay & Hoyt 1984), clad with 5 mm mesh, and lit with a yellow cyalume stick (Cyalume Technologies Inc., West Springfield, MA, USA) inside the trap. Light traps were set at dusk, adjacent to physical habitat (e.g. macrophytes and woody debris), and retrieved the following morning. Samples were preserved in 95% ethanol and returned to the laboratory for processing.

In the immediate vicinity of every light trap set, microhabitat water depth (m) and velocity (m.s⁻¹), and physical habitat cover were measured. Depth and downstream water velocity were measured at five points, every 0.25 m along a 1 m transect that ran perpendicular to the creek bank and through the position of the

light trap. An additional microhabitat hydraulic metric, *velocity difference*, was then calculated for each light trap as site mean cross sectional velocity (from ADCP generated data) minus mean microhabitat velocity. Physical habitat cover was assessed by placing a 1×1 m quadrat over the positon of the light trap and determining the number of 0.1 x 0.1 m cells comprised of submerged vegetation, emergent vegetation, coarse woody debris (>20 cm diameter), fine woody debris (<20 cm diameter), rock or open water. Further details of the methods and analysis is available in Bice and Zampatti (in prep.).

4.5.2 SPATIO-TEMPORAL VARIABILITY IN MICROHABITAT HYDRAULICS AND THE ABUNDANCE OF MURRAY COD LARVAE IN THE LITTORAL ZONE

In 2017, larval Murray cod abundances were lowest in Slaney Creek and highest in Chowilla Creek immediately downstream of Slaney Creek. Abundances among the four sites further downstream in Chowilla Creek were relatively consistent (Figure 26a). Conversely, in 2018, larvae were sampled in greatest abundance in Slaney Creek, and there was a general trend of declining abundance with distance downstream in Chowilla Creek, with the lowest abundance downstream of the regulator (Figure 26a).

Spatio-temporal change in larval Murray cod abundance was associated with concomitant change in microhabitat hydraulic characteristics (Figure 26b-d). In association with regulator operation in 2018: 1) microhabitat depth increased, particularly at sites in Chowilla Creek, with the exception of the site downstream of the regulator (Figure 26b); 2) median values and, in some cases, the distribution of velocity difference decreased (Figure 26c); and 3) microhabitat water velocities decreased substantially in Slaney Creek, and to a lesser degree at downstream of Boat Creek, but were generally consistent across years at other sites (Figure 26d).

A number of factors, and correlation between factors, were considered prior to modelling Murray cod abundance. Mean site velocity and velocity gradient were highly correlated, and because of this autocorrelation one factor was redundant as an effect in the model. Velocity difference was adopted as 1) it captures the requirement for presence of slackwater in the literal zone, which mean cross sectional velocity does not, and 2) it showed lower correlation with microhabitat velocity than mean cross sectional velocity, suggesting velocity difference provided more new information. While velocity difference provided a stronger predictor for Murray cod larvae abundance, this metric requires information at a much finer scale than mean cross sectional velocity, and is harder to represent with current hydraulic modelling tools. As such, the correlation with mean cross sectional velocity is exploited for the assessment of operational scenarios in Section 5.

Mixed-effects modelling indicated littoral larval Murray cod abundances were best predicted by microhabitat velocity and velocity difference (Figure 27 and Figure 28). Specifically, Murray cod larvae were more likely associated with littoral habitats with mean velocities <0.1 m.s⁻¹, but high velocity difference (e.g. >0.3 m.s⁻¹) (Figure 26). This suggests a preference of Murray cod larvae for slackwater habitats within broader fast-flowing reaches. As such, in 2017, Slaney Creek likely represented a generally poor nursery due to a low proportion of slackwater habitats in this fast-flowing creek, and in contrast, sites along the length of Chowilla Creek represented suitable nurseries due to a high proportion of microhabitats with low velocity, but high velocity difference. Slaney Creek, however, is an important spawning site for Murray cod and hence constitutes a consistent source of larvae. In contrast, during regulator operation in 2018, reductions in overall flow velocity in Slaney Creek resulted in increased prevalence of low velocity slackwaters and moderate velocity differences, with an associated order of magnitude increase in the abundance of larvae. Conversely, at sites further downstream, whilst slackwaters were prevalent, these were characterised by lower velocity difference, with associated reductions in larval abundance.



Figure 26. a) Mean catch per unit effort, CPUE (larvae.hr⁻¹ ± SE), of Murray cod larvae, b) mean (± SE) microhabitat depth (m), c) density plot of velocity difference (m.s⁻¹), and d) mean (± SE) (black line and boxes) microhabitat velocity (m.s⁻¹) at six sites sampled with light traps in 2017 (light blue) and 2018 (purple). Standard errors represent a negative binomial structure. Dots on figures b and d represent observed values, DS=downstream and US=upstream.



Figure 27. Predicted fixed effects of a) microhabitat velocity and b) velocity difference across all sites in 2017 and 2018. These are predicted while holding all other variables constant to determine relative effects of each individual variable on Murray cod larvae catch per unit effort, CPUE.



Figure 28. Mean catch per unit effort, CPUE (larvae.hr⁻¹), \pm SE of Murray cod larvae from littoral microhabitats with varying velocity difference (0.02 m.s⁻¹ increments). Data from both years are pooled and all microhabitats with a mean velocity >0.1 m.s⁻¹ were excluded, to highlight the influence of velocity difference alone.

The results of the current study suggest that operation of the Chowilla Regulator alters meso- and microscale hydraulics, in turn influencing nursery habitat quality through the prevalence and nature of littoral slackwaters, and subsequently, affects Murray cod larvae distribution. In 2017, when the regulator was not in operation, larval Murray cod drift was likely supported throughout the creek system and settlement occurred throughout Chowilla Creek where suitable littoral nursery habitats were present (Figure 29a). Conversely, during operation in 2018, settlement occurred predominantly in Slaney Creek and in Chowilla Creek immediately downstream of Slaney Creek, and then declined with distance downstream (Figure 29b).

The drift and dispersion of fish larvae in riverine environments is commonly thought to occur for the purpose of: 1) transport to favourable nursery habitats (e.g. hydraulics, temperature), where food is concentrated, and energy uptake maximised (Humphries et al. 2019); whilst 2) reducing concentrations of con-specifics, and associated risks of predation, cannibalism, and competition for food and space (Copp et al. 2002; Lechner, Keckeis & Humphries 2016). Changes in the spatial distribution of favourable nursery habitats in Chowilla, during regulator operation, may be counter to these requirements. Restriction of such habitats to Slaney Creek may have resulted in aggregation of larvae in Slaney Creek and the upper reaches of Chowilla Creek. Density dependent factors (e.g. competition and predation) that effect the survival of early life stages are believed critical in determining recruitment and cohort strength (Economou 1991). As such, while overall abundances of Murray cod larvae did not vary among years, aggregation, as opposed to dispersion, may result in poor survival and subsequent recruitment to young-of-year. This notion, however, requires subsequent investigations of the age structure and abundance of Murray cod in the Chowilla system.

4.6 Summary

The transport of biological propagules is fundamental to the function of terrestrial and aquatic ecosystems. In this study, we have employed an empirical approach to identify water velocity thresholds and seasonal timing associated with key dispersal process of riverine and floodplain flora and fauna. This information can be used to inform operational and environmental water delivery planning and river flow management. This quantitative information can be summarised as:

- Sediment deposition was significantly greater in association with regulated in-channel inundation than during an in-channel flow pulse.
- Despite the large differences in sediment deposited, there was no significant difference in seed density between regulated in-channel inundation and an in-channel flow pulse. Mechanisms for seed input to the seed bank include from hydrochory, anemochory, zoochory and seed rain.
- Flow delivery or regulator operation should be timed to coincide with seed fall of eucalypts to maximise chances of recruitment.
- Murray cod larvae were associated with littoral habitats with mean velocities <0.1 m.s⁻¹, but high velocity difference (>0.3 m.s⁻¹). This suggests a preference of Murray cod larvae for slackwater habitats within broader fast-flowing reaches.
- Velocities > 0.2 m.s⁻¹ are expected to entrain and transport rotifers.





Figure 29. Generalised conceptual model of Murray cod larvae drift and retention during a) typical conditions and b) during regulator operation. Regulator operation is associated with increased water levels, declines in mean cross-sectional water velocities, and changes in the spatial distribution of favourable larval nursery habitat.

5 Modelling operational scenarios

The results outlined previously have identified a range of relationships that were used to assess changes to in-channel ecological connectivity and water quality risks. In particular:

- Defining velocity thresholds to promote important ecological processes:
 - Entrainment and drift of nutritious zooplankton (mean cross sectional velocity, v > 0.2 m.s⁻¹)
 - Creation of suitable retention habitat for Murray cod larvae (v > 0.3 m.s⁻¹, used as a surrogate for velocity difference, see Section 4.5.2)
- Assessment of water quality risks:
 - o Information to parameterise the DODOC model to simulate changes in DO and DOC
 - A mixing criterion to assess changes in velocity and depth on the ability to disrupt thermal stratification, and in turn cyanobacteria blooms.

These criteria have been implemented in the Source hydrological model of the SA River Murray (Beh, Montzeri & Gibbs 2019) to enable the assessment of different operational scenarios. This functionality is demonstrated in this section.

5.1 Implementation of criteria

5.1.1 VELOCITY

A large repository of hydraulic model results have been developed for each weir pool over the recent years, from the MIKE FLOOD models available (DHI 2014; McCullough, Montazeri & Esprey 2017). This repository provides a range of metrics summarising the results from steady state conditions for different upstream discharge into the weir pool, and the operating height of the weir. Metrics of the proportion of the reach with a velocity greater than 0.2 m.s⁻¹ and 0.3 m.s⁻¹ for the each case of flow and operating height have been compiled into lookup matrices. In this case, the higher spatial resolution velocity results from the hydraulic model have been summarised to average velocities every 100 m, representing cross section average velocities used to derive the relationships in this work. These matrices can then be interpolated each day in the Source hydrological model, for a given flow and weir pool height, the resulting velocity metric can be derived by the model.

Results are presented for the locations where this information is available, weir pools 1-5. To some extent, the velocity requirements in floodplain sites will be considered in much more detail in the operating plans for each site, and if the operating rules are followed, velocity will be maintained. Nonetheless, similar lookup tables could be developed for other sites if the hydraulic model information is compiled.

5.1.2 DISSOLVED OXYGEN

DO concentration were modelled throughout the Source model using the DODOC plugin (Mosley et al. in prep.). 500 separate simulations were undertaken, with samples drawn from the statistical distributions of standing load leaf litter distributions for each vegetation type presented in Figure 8 were simulated to represent the variability in litter load, and ultimately DO concentration, expected. Each leaf litter sample was applied to each node in the model using the weighted average based on the vegetation areas for each node. For the operational scenarios considered in this section, leaf litter was assumed to not decay or accumulate, as such the standing load applied was maintained on the floodplain until inundated. A monthly pattern of water temperature in the Lock 5 weir pool (A4260703) was used for water temperature, and other parameters for the DODOC model were as outlined in Mosley et al. (in prep.).

5.1.3 MIXING CRITERION

The mixing criterion equation from Bormans and Webster (1997) was also implemented in the DODOC plugin, to enable this equation to be easily calculated within the Source model. The inputs to the model were:

- Monthly patterns of air temperature, humidity, wind speed and solar radiation derived from the Chowilla AWS (A4261167)
- The same water temperature pattern as used for the DODOC modelling
- The median velocity in the weir pool was implemented in the model in the same way as the proportion of the reach with a velocity greater than 0.2 m.s⁻¹ and 0.3 m.s⁻¹ to provide an input to the Mixing Criterion equation. As such, results for this metric are also currently calculated at weir pools 1 5
- Water depth as modelled by Source for each weir pool

5.2 Modelled scenarios

Four scenarios have been considered, which were informed by the integrated operations planning underway within the South Australian Department for Environment and Water. The scenarios are summarised as:

- 1. No operations (No Ops). The QSA inflow at the SA border were based on a previous annual operating plan developed by the MDBA for planning purposes (75th percentile for 2017/18).
- 2. All operations (All Ops). Multiple operations across the lower River Murray, at Chowilla, Pike and Katarapko floodplains with associate weir pool raisings at Locks 4, 5 and 6, as well as weir pool lowering and raising at Lock 2. These operations were based on operation plans that exist or are under development for each site. Additional environmental water was added to the QSA inflow for this scenario on top of that used for Scenario 1, to account for filling and losses from the operations.
- 3. Extreme operations (Extreme Ops). This scenario undertakes operations that are outside the bounds of the operating plans. It should be stressed that this scenario is not planned to be undertaken, but used to demonstrate the metrics under higher risk operations. Inflows for this scenario were the same as the All Ops scenario.
- 4. A high flow event (High Flow). Inflows were adopted based on observed flows during 1978, peaking to 60,000 ML.d⁻¹ at the SA border, creating a similar area of inundation to Extreme Ops scenario.

Table 4 presents a summary of the regulator management for the All Ops and Extreme Ops scenario, and Figure 30 the inflow at the SA border. The model was run for a 6-month period from 1 July to 31 December. Climate data were based on monthly average pattern based on the observed climate over 1970-2019 and diversions were assumed based on full utilisation of consumptive entitlements, applied over the year using the historical pattern of use.

Site	Maximum water level (m AHD)			Period of operation			
	Normal level	All Ops.	Extreme Ops.	Start raise	Full extent	Start drawdown	End
Lock 6	19.25	19.8	19.9	29-Aug	08-Sep	03-Nov	13-Nov
Chowilla	16.45	19.45	19.87	17-Aug	16-Sep	03-Nov	31-Dec
Lock 5	16.3	16.8	16.8	02-Jul	21-Jul	19-Oct	07-Nov
Pike	14.55	15.6	16.4	22-Jul	01-Sep	30-Sep	20-Oct
Lock 4	13.2	13.5	13.8	17-Jul	01-Aug	07-Dec	24-Dec
Katarapko	10	12.9	13.5	17-Jul	05-Sep	04-Oct	24-Dec
Lock 2 lowering	6.1	6.02	6.02	7-Jul	11-Jul	27-Jul	1-Aug
Lock 2 raising	6.1	6.6	6.6	5-Aug	31-Aug	28-Sep	23-Oct

Table 4: Regulator operating heights and timing for all operations and extreme operations scenarios.



Figure 30. Assumed flow to South Australia (QSA) for the scenarios considered.

5.3 Assessment criteria

The criteria identified through this work to represent the processes considered are calculated every day over the model simulation. There are numerous possibilities to summarise these criteria into metrics, which are expected to evolve over time. To summarise the outputs into a metric for each location and scenario, the following metrics have been used in this work:

- Average proportion of the reach with velocity exceeding 0.2 m.s⁻¹ and 0.3 m.s⁻¹ over the simulation period. The average value is presented, with the shading based on the percentage change from the No Ops scenario.
- Percentage of days mixing criterion exceeded the R>35,000 threshold in each weir pool, representing a low likelihood of stratification.
- Percentage of days DO exceeded 6 mg.L⁻¹, at each node each weir pool and within the anabranch for each floodplain site.

Along with these metrics based on the results from this work, additional metrics have been calculated to assess the scenarios:

- Maximum inundated area over the simulation. This metric provides a representation of the benefits that might be expected, compared to the impacts that might be calculated using the above metrics.
- Percentage of days a minimum flow requirement was met. The minimum flow requirement was assumed to be 1,000 ML.d⁻¹ at Locks 4, 5, 6 and Chowilla regulator, 100 ML.d⁻¹ at Pike, Mundic and Katarapko, and 2,500 ML.d⁻¹ at Locks 1, 2 and 3.

5.4 Results and discussion

The results for the metrics outlined above are presented in Figures 31- 35, followed by time series plots in Figures 37 - 41.

5.4.1 VELOCITY

The velocity metrics indicate that small reductions in the proportion of the reach with velocities expected to support drift and entrainment of propagules from the All Ops scenario compared to No Ops. From Figure 37 and Figure 38, this can typically be seen to occur in September when structures were raised to the maximum level. The additional flow included in the All Ops scenario to provide for filling and loss requirements (Figure 30) was not sufficient to offset the increase in cross sectional area due to the operation, resulting in a reduction in fast flowing reach length in Weir Pools 2, 4 and 5 when Locks 2, 4, 5 structures were raised. In contrast, as Locks 1 and 3 structures were not raised, Weir Pools 1 and 3 generally have improved velocities throughout the simulation, with some small reductions due to travel times through the system.

Additional inflows were not included to offset the additional water requirements for the Extreme Ops scenario, and the resulting reductions in velocity can be seen in Figures 31, 37 and 38. Conversely, the High flow scenario results in 100% of all reaches exceeding 0.3 ms⁻¹ for a large proportion of the simulation. The metrics in Figure 31 and 32 showing the average reach length exceeding 0.2 ms⁻¹ and 0.3 ms⁻¹, respectively, over the simulation period, where from Figure 38 it can be seen the increase in the percentage of the reach with higher velocities much greater than this over the simulation.

5.4.2 WATER QUALITY

The percentage of days with DO greater than 6 mg.L⁻¹ metrics indicate that the No Ops and High Flow scenarios maintain DO concentrations above this threshold irrespective of the leaf litter loads. This is not surprising for the No Ops scenario, as there is very little overbank inundation occurring to inundate the litter.

The High Flow scenario does generate overbank inundation (percentage increase in inundated area compare to normal pool level in Figure 35), however the additional dilution from the high flows is sufficient to maintain high DO concentrations.

Only the most extreme litter loads resulted in a small number of days with DO less than 6 mg.L⁻¹ at the Pike floodplain for the All Ops scenario. From Figure 39 the DO concentrations can be seen to be highly skewed, with a large difference between the median DO concentration and the 97.5th percentile DO concentration (as the bottom of the shaded area), given by the large difference between these two lines compared to the distance to the 2.5th percentile (upper bound of the shaded area).

In contrast, the Extreme Ops scenario results in low DO concentrations at the Pike Floodplain, with the median representing hypoxic conditions (2 mg.L⁻¹), and no oxygen at all for an extended period in the 97.5th percentile case. It should be reiterated that this is a hypothetical scenario to test the metrics and not intended to be undertaken in reality. Even with these very low DO concentrations within the floodplain, at the next location downstream in the main River Murray channel, the DO concentrations have returned to higher concentrations (Lock 4 in Figure 40).

The mixing criterion results can be seen in Figure 41, with the increase in air temperature and solar radiation resulting in reduced values over time as the season unfolds from July to December. The increase in water depth and reduction in velocity due to the weir pool raisings at Locks 2, 4 and 5 in the all Ops and Extreme Ops scenarios can be seen to reduce the value when these operations are undertaken. However, the value is not reduce to more than the normal values later in the year when conditions are warmer, and are generally not below the 35,000 threshold identified in Section 3.1.2. This result highlights the importance of timing operations earlier in the season to reduce the risk of cyanobacteria blooms, before higher air temperatures and solar radiation increase the thermal energy over the hotter months.

5.5 Summary

This section has demonstrated how the findings from this research can be applied to inform cumulative operational planning in the River Murray. The Source hydrological model is the main tool for assessing these operations, and additional functionality has been added to this model to allow metrics representing transport of propagules and water quality risks of hypoxic blackwater and algal blooms to be quantified.

The results suggest that current operational plans at floodplain sites are not expected to create water quality risks that cannot be mitigated (All Ops). An extreme scenario outside of these bounds was required to generate results of concern, however even for this scenario the impacts were generally confined to the floodplains with the main River Murray channel providing enough dilution and travel time to prevent impacts accumulating between sites (for dissolved oxygen in particular).

The high flow scenario also provides a contrast between the outcomes that can be expected from inundation created by flow and infrastructure operations. While there was variation between sites along the river, similar increases in inundation area were simulated between the operational and high flow scenarios. However, the velocity, and to a lesser extent water quality, results were significantly different, supporting the conceptualisation of the relationship between velocity and channel-floodplain connectivity for different floodplain inundation mechanisms, outlined in Figure 1.



Figure 31. Average proportion of the reach exceeding 0.2 m.s⁻¹ over the simulation period, with the shading representing the change from the No Ops scenario.



Average proportion of reach exceeding 0.3 $\mathrm{m.s}^{-1}$

Figure 32. Average proportion of the reach exceeding 0.3 m.s⁻¹ over the simulation period, with the shading representing the change from the No Ops scenario. Note that maximum value on the colour scale is 100%, however some changes exceed this amount.



Figure 33. Percentage of days the mixing criterion was greater than 35,500, with the shading representing the change from the No Ops scenario. Note that maximum value on the colour scale is 100%, however some changes exceed this amount.







Figure 35. Summary metric for the percentage of days the minimum flow requirement was met at each location, with the shading representing the change from the No Ops scenario.



Figure 36. Maximum inundated area (hectares) for each reach, with the shading representing the change from the No Ops scenario. Note that maximum value on the colour scale is 100%, however some changes exceed this amount.



Figure 37. Time series of the percentage of the reach exceeding 0.2 m.s⁻¹ for scenario in each weir pool.



Figure 38. Time series of the percentage of the reach exceeding 0.3 m.s⁻¹ for scenario in each weir pool.



Figure 39. Dissolved oxygen (DO) concentrations for locations off the main River Murray channel for each scenario. The line is the median DO from the range in standing litter loads, and the shaded area the 95th percentile range.



Figure 40. Dissolved oxygen (DO) concentrations for locations within the River Murray channel for each scenario. The line is the median DO from the range in standing litter loads, and the shaded area the 95th percentile range.


Figure 41. Mixing criterion for locations within the River Murray channel for each scenario.

6 Food web dynamics

The previous sections have outlined studies focused on quantify changes to key in-stream components of the River Murray ecosystem expected to be influenced by the operation of infrastructure intended to increase the extent of floodplain inundation. However, the exchange of energy and nutrients is also strongly influenced by the flow regime, and is a primary characteristic controlling ecosystem productivity and community dynamics in riverine systems. In developed catchments, management of flow and energy needs to be based on a balance between the needs of agriculture, society, and ecology. Achieving this will require underlying knowledge of the food web and carbon pathways that deliver an appropriate load of carbon and nutrients at times of the year that enable trophic uptake of those resources. By examining the linkages between consumers and resources within a community, we can provide advice to underpin management decisions that support ecosystem function, while balancing the needs of agriculture and society.

The aim of this study was to investigate the flow of basal resources (carbon and nitrogen) during two contrasting flow scenarios in a heavily regulated reach of the lower River Murray. It is intended that the knowledge generated will contribute to an improved understanding of site-specific ecosystem responses to managed inundations, and subsequently help plan and implement management actions that make substantial positive contributions to increasing the carrying capacity of the local and/or reach scale ecosystem.

This work package was developed in order to be able to improve our understanding of the relative role of different basal energy sources (e.g. phytoplankton, biofilms, terrestrial plant material and sediments/soils) on fuelling primary and secondary productivity. The data generated contributes to reducing knowledge gaps around difference between managed inundations, base flows and unregulated floods. The key focus of this work package was to investigate if managed inundations drive shifts in food web dynamics through changes in basal resource supply and delivery.

The floodplain system in the lower River Murray was sampled in spring in two consecutive years to characterise the food web. The first sampling event occurred during spring 2017, a period of regulated low flow. The second sampling period occurred in spring 2018, a period where a managed inundation using constructed infrastructure was conducted to raise water levels and deliver environmental water to approximately 2,000 ha of floodplain (see Section 4.2). Concentration and natural abundances of stable carbon and nitrogen isotopes (δ 13C, δ 15N) were measured in a range of consumers to provide integrated measures of assimilation and partitioning of the relative contributions of dominant basal carbon sources. We differentiate between allochthonous and autochthonous inputs into food web of the riverine system.

6.1 Understanding basal resource dynamics

Throughout the Murray-Darling Basin there is an increasing reliance of managed delivery of environmental water through a combination of managed releases from water storages and the use of constructed infrastructure to target ecological outcomes at specific riverine and floodplain ecological assets. Whilst using infrastructure to deliver environmental water to targeted locations has been demonstrated to be an effective mechanism to maintain some ecological functions during periods of low water availability, there are a number of key differences in potential outcomes between 'Natural' (unregulated flooding) and 'Artificial' (managed) inundation of floodplains and wetlands. These differences include hydraulics (velocity, turbulence [mixing energy], dilution [daily exchange] and retention time within the managed area), and changes to lateral and longitudinal connectivity. These differences provide pathways for ecological and biogeochemical responses to differ markedly between natural and managed inundations (Wallace et al. 2011; Welti et al. 2012) and between different locations and similar scale managed events at the same location.

The two dominant floodplain eucalypts in the lower River Murray; river red gum (*E. camaldulensis*) and black box (*E. largiflorens*) generate a large standing biomass of litter material (e.g. leaves, bark, twigs, understory vegetation) that represents a large source of allochthonous organic matter to floodplains and wetlands (Francis & Sheldon 2002; Glazebrook & Robertson 1999). Therefore, unregulated flooding and managed inundations will "wet-up" large standing stocks of natural organic material (leaves, bark, twig, course

particulate material, understory vegetation) and trigger the rapid release (within hours) of water soluble compounds from soils (Banach et al. 2009; Kobayashi et al. 2008; Scholz et al. 2002; Wilson et al. 2010) and plant material (Brookes et al. 2007; Watkins et al. 2010; Whitworth, Baldwin & Kerr 2012). The carbon and nutrients released into the water column can be incorporated into microbial and algal biomass and cycled through the food web via multiple pathways, including via micro- and macro-invertebrates to higher trophic level organisms (e.g. birds and fish).

It has been demonstrated that during low flows, the River Murray is energy constrained with net production close to zero (Oliver & Lorenz 2007; Oliver & Lorenz 2013; Oliver & Merrick 2006; Wallace 2019; Wallace & Furst 2016). Recent studies have demonstrated that open water productivity in the river channel increases in response to inputs of allochthonous carbon associated with unregulated floods and managed floodplain inundation (Cook et al. 2015; Oliver & Lorenz 2013; Wallace 2019; Wallace & Furst 2016). Therefore, developing an understanding of (i) which sources of basal energy are effectively taken up through the foodweb, and (ii) differences in responses between low flow, unregulated floods and managed inundations is a key part of maximising desirable outcomes, and avoiding perverse outcomes such as hypoxic blackwater events (Hladyz et al. 2011; Whitworth, Baldwin & Kerr 2012).

6.2 Methods

6.2.1 FIELD SAMPLING

Field sampling occurred in November 2017 and November 2018. All sites were sampled within 48 hours in the same sampling period in order to minimise variation in seasonal and environmental variability.

A range of sites, representing the range and diversity of meso-habitats present within the study area were selected to include three main channel river sites, three anabranch creek sites, and three wetland sites (Table 5, Figure 42).

Lake Merretti was sampled in 2017. However, as part of the long-term wetland management plan, the inlet regulator was closed following that sampling and the lake was subsequently dry, and not sampled during the 2018 sampling period. Merretti swamp (a shallow depression adjacent to Lake Merretti, that requires flows >100,000 ML.d⁻¹ to generate a lateral connection and subsequent fill) was dry in the 2017 sampling period, but environmental water was pumped to the site and sampled during the 2018 sampling period.

Site name	Site type	Description	Year sampled
Upstream Lock 6	Main	Main channel upstream Lock 6: between lock 6 and Custom's house	2017, 2018
Downstream Chowilla	Main	Main channel lock 5 tail water, 1.9 km downstream Chowilla Creek return	2017, 2018
Upstream Lock 5	Main	Main channel –weir pool upstream lock 5, 40km downstream Chowilla	2017, 2018
Chowilla Creek	Creek	Moderate creek downstream of faster flowing creeks	2017, 2018
Ral Ral Creek	Creek	Slow flow anabranch creek, mid-pool	2017, 2018
Monomon Creek	Creek	Slow flow anabranch creek	2017, 2018
Coppermine Wetland	Wetland	Ephemeral wetland, non-regulated, flows during inundations	2017, 2018
Lake Merretti	Wetland	Structured, regulated wetland	2017 only
Merretti Swamp	Wetland	Meretti Swamp. Almost dry.	2018 only

Table 5: Site descriptions of food web sampling locations.



Figure 42. Map of food web sampling sites.

At each site the water column, biofilms, soils, sediment (benthos), phytoplankton and zooplankton, and crustaceans were sampled (Table 6). Adjacent to each aquatic site, terrestrial sampling occurred in order to follow the organic matter movement and decomposition.

Water samples: At each river and creek sampling site, a boat was used to provide sampling access. Composite samples were generated by using a 4 L Haney trap and transferring a "grab" from the top, middle and bottom of the water column respectively, to a pre-rinsed and muffled 2 L glass bottle that was filled for subsequent processing and analysis. Due to the shallow (<1 m) and confined nature of the channel at the wetlands, grab samples were collected directly into the 2 L sample bottles. At each sampling time, three grab samples were collected from three spatially separated (>50 m) locations.

Phytoplankton and zooplankton samples: River and creek samples were collected by towing a 37 μ m net at slow speed behind the boat. Wetland samples were collected by a "walked" tow as the wetlands were too shallow to launch the boat. The total volume was not measured or estimated. The net sample was concentrated and transferred to a pre-rinsed bottle for subsequent processing.

Crustaceans: Decapod crustaceans (*Cherax destructor* (yabbies) and *Macrobrachium australiense* (shrimp)) were sampled using traps baited with beef bones. Soak time was <1 hour, with the traps deployed whilst the other sampling was occurring.

Sediments: Benthic sediments were sampled to a depth of 0-10 cm using an 80 mm internal diameter sediment corer. The upper 5 cm (i.e. 0-5 cm depth) of the sample was transferred into 200 mL PET container.

Each sample contains any benthic biofilm growing at the sediment-water interface as well as the underlying benthos. Samples were homogenised upon return to the lab.

Biofilms: Biofilm material was collected by scraping shallow submerged woody material directly into 50 mL centrifuge tubes.

Soil: Soil samples (0-5 cm depth), plant material (leaf litter, bark) and scat samples were taken at the littoral edge of the water body (Littoral Edge), midway between the littoral edge and high flood water mark (Midway), and above any apparent flood markings (Above).

Vegetation: Specific vegetation and organic matter (i.e. Red gum (*Eucalyptus camaldulensis*); Black Box (*Eucalyptus largiflorens*) and Macropus rufus/Macropus giganteus scat (Scat) were sampled at the same time. The vegetation sampling in 2017 was used to inform the 2018 sampling plan. Therefore, the sample number increased from 12 in 2017 to 43 in 2018 to include a more comprehensive sampling regime, which included bark, both fresh leaf and degraded leaf litter at all sites.

All collected samples were stored in insulated containers in the dark, on ice and returned to the laboratory for processing and analysis.

Upon return to the laboratory, each sample was filtered through 0.45 μ m Millipore filter (GF/F). All filters were muffled and weighed prior to being used. The filtrate (water samples) were analysed for dissolved organic carbon (DOC), total carbon (TC), dissolved organic nitrogen (DON), total nitrogen (TN), nitrate (NO₃⁻), and ammonium (NH₄⁺). Analysis of DOC, TC, DON, TN was undertaken using by high temp combustion (Thermalox TOC/TN, Analytical Sciences Ltd. Tewkesbury, UK), and NO₃⁻, and NH₄⁺ using standard colorimetric methods (APHA 1998). The filters were oven dried at 60°C and then reweighed to calculate total suspended solids (TSS) (ASTM 1999).

6.2.2 STABLE ISOTOPE MEASUREMENTS

Stable isotope analysis is a widely used technique for tracing patterns of energy flow and food web linkages in ecosystem. Natural abundances of carbon and nitrogen stable isotopes (δ^{13} C and δ^{15} N) provide integrated measures of assimilation and partitioning of the relative contributions of basal carbon sources to food webs. Biological fractionation of δ^{13} C is generally negligible (< 1%) and estimates the energy source whereas fraction of δ^{15} N indicates the trophic position of a consumer and shifts at 2-4‰ per trophic level. Together, δ^{13} C and δ^{15} N can also indicate a food source as a viable energy source through one or more trophic transfers (Perdue and Koprivnjak 2007; Pingram et al. 2014).

All solid samples were freeze dried and then ground to a fine powder before being measured by elemental analyser coupled to an isotope ratio mass spectrometry (IRMS) (Delta V Thermo) for both C and N concentration (mg), CN ratios (weight based) and isotopic (δ^{13} C and δ^{15} N) values.Delta values were calculated by measuring the relative different between the isotope ratio of the sample (Rsp) and the reference material (Rst) by the following equation, where R is the concentration ratio of heavy to light isotope:

 $\delta(\text{sample}) = [(\text{Rsp/Rst})-1] \times 103$

6.2.3 STATISTICAL MODELLING

Normality of data and homogeneity of the variance was tested using Shapiro-Wilk tests and Bartlett's test before further statistical analysis ($\alpha = 0.05$ in all cases). One Way Analysis of Variance followed by a Tukey's multiple comparison were used to assess differences in δ^{13} C and δ^{15} N values and C:N ratios among treatments for all data combined and for individual sampling years, 2017 and 2018. A Generalized Least Square model was used in cases when there was no homogeneity of the variance. To test the main factorial effects, we grouped both consumers (Zooplankton, Crustaceans, Scat) and basal sources (soil, sediment, TSS, biofilms, vegetation). All statistical analyses were done in R with a criterion of p < 0.01.

The relative contribution of different basal energy sources (soil, sediment, TSS, biofilms, vegetation) to consumer zooplankton and crustaceans, based on the δ^{13} C and δ^{15} N values, was assessed using Bayesian

mixing models of Stable Isotopes in R (MixSIAR package v3.1; (Stock et al. 2018)). In MixSIAR, model fitting is hierarchical and uses the Markov Chain Monte Carlo method that produces iterations of plausible values of source contributions to consumer diets. Each model was the product of 500,000 iterations with the first 50,000 discarded, and we report results as the 95% confidence intervals. We employed widely-used values for trophic discrimination factors as outlined by Post (2002). In our models we used discrimination values of $1\% \pm 0.5\%$ for δ^{13} C and $3.5\% \pm 0.5\%$ for δ^{15} N values.

6.3 Results

6.3.1 WATER MEASUREMENTS

In 2017, significantly higher values of TSS and lower C:N ratios were observed than in 2018 across all sites (Table 7). Wetland sites had significantly higher values of TC, TOC, TN and NH_4^+ than main channel or creek sites in both 2017 and 2018. Due to the low sample size at each site we were unable to statistically test for within year site differences, however visually site 3 (Coppermine Wetland) had higher values of TC, TOC, TN, and NH_4^+ than all other sites (Figure 43). For all water samples combined (both 2017 and 2018) there the only significant spatial (site or within river location) difference was observed for C:N. Year was a significant factor for both TSS and C:N in the water column (Table 8).

6.3.2 ISOTOPIC COMPOSITION OF BASAL ENERGY RESOURCES

Sample sizes and mean δ^{13} C and δ^{15} N values and C:N ratios of each basal source sampled in 2017 and 2018 are reported in Table 8. Isotope values and C:N ratios were significantly different between the five types of basal resources sampled (p <0.001, Table 9, Figure 44). Values of δ^{13} C were less negative in biofilm and more negative in TSS while δ^{15} N values were lowest in soil and highest in sediment and vegetation. C:N ratios were highly variable between sample types years ranging between mean values of 0.62 to 40.8 (for vegetation samples in 2017 and 2018, respectively). The values measured in 2017 were unusually low, mostly likely due to the variation in the type of vegetation measured in 2017 relative to 2018.

Isotope values and C:N ratios of all pooled basal samples were significantly different between the two sampling years (p <0.001, Table 10). Yearly differences were also observed for all TSS isotope variables and in δ^{13} C values of Sediment and Vegetation, and C:N ratios of soil, vegetation, sediment and biofilm (Table 11, Figure 45. Biplots of basal resources sampled in 2017 and 2018 at sites that were classified by their association with (A) the main channel of the river, (B) Creeks - low or mid-flowing creeks adjustment to the main channel, and (C) wetlands. Note that the three sites within each group are not considered replicates, they are different representations of that habitat type. Symbols represent the same samples as above.). In 2018, all sampling groups were observed to converge around a common basal resource. Less negative δ^{13} C values, higher δ^{15} N values and higher C:N ratios were consistently observed in 2018 than 2017 (Figure 46), except for C:N ratios of TSS which were lower in 2018 than 2017.

There were some significant spatial (site) effects on basal δ^{13} C and δ^{15} N values in 2017 but not 2018 (p<0.01 Table 10). δ^{13} C values and C:N ratios of basal samples were most different at site 4 (Lake Merretti), while δ^{15} N values of basal samples at Site 5 (Chowilla Creek) were significantly different to many other sites (Tukey p < 0.007; Figure 46). The soil C:N pool is varied across the sampling sites in 2017 but becomes consistent across the field sites in 2018 (Figure 46C). Similarly, the C:N ratio increases in 2018 across all measured vegetation pools (Figure 46E). Statistically significant differences were found between basal δ^{15} N values at sites sampled in 2017 that were grouped as 'Creeks' to those in the main channel and wetland sites and between wetland and main channel sites sampled in 2018. No statistical differences in basal δ^{13} C values and C:N ratios were found between site types.

The change in C:N ratio observed in 2018 relative to 2017 is driven by overall ecosystem changes to the nitrogen pool, rather than the carbon pool (Figure 47). Both riverine (sediment, biofilm) and terrestrial (soil, vegetation) components have a decrease in the %N in 2018 relative to 2017.

Table 6: Mean (±SD) of water properties measured at the different sites in 2017 and 2018. Site type include: Main – main channel, Wet – wetlands, or Creek – low or mid-flowing creeks adjustment to the main channel. Water properties are total carbon (TC), total organic carbon (TOC), total nitrogen (TN), ammonia (NH₄), carbon to nitrogen ratio (C:N) and total suspended solids (TSS).

	Туре	n	TC (r	ng/L)	TOC (r	ng/L)	TN (m	TN (mg/l)		NH4 (mg/L)		io	TSS (mg)		TSS (g	TSS (g/L)	
2017		21	35.19	±38.91	12.94	±18.25	0.95	±1.28	0.011	±0.02	43.09	±6.03	6.55	±1.67	19.87	±10.80	
			(15.65	-132.33)	(4.54	-57.65)	(0.36	-4.04)	(0.00	-0.06)	(31.26	-52.43)	(4.27	-10.58)	(8.54	-42.32)	
Upstream Lock 6	Main	3	16.2	±0.08	4.9	±0.30	0.38	±0.02	-0.001	±0.00	42.96	±2.09	4.48	±0.28	8.95	±0.55	
Downstream Lock 6	Main	3	18.01	±0.28	5.25	±0.32	0.38	±0.00	0	±0.00	47.15	±1.22	7.7	±2.68	29.69	±16.86	
Coppermine Wetland	Wet	3	127.33	±4.53	56.37	±1.23	3.99	±0.04	0.047	±0.02	31.89	±0.79	7.48	±1.72	21.84	±6.09	
Lake Merretti	Wet	3	31.7	±0.38	9.55	±0.29	0.77	±0.01	0.022	±0.01	40.92	±0.54	6.77	±1.04	33.86	±5.18	
Chowilla Creek	Creek	3	17.15	±1.34	4.69	±0.19	0.38	±0.01	0.009	±0.00	45.51	±3.69	6.19	±1.38	12.39	±2.76	
Ral Ral Creek	Creek	3	17.13	±1.08	4.96	±0.26	0.41	±0.01	0	±0.00	42.12	±3.81	7.3	±1.72	20.56	±2.02	
Upstream Lock 5	Main	3	18.84	±0.08	4.89	±0.10	0.37	±0.01	0	±0.00	51.06	±1.72	5.91	±0.59	11.82	±1.18	
2018		24	24.18	±27.30	6.19	±5.96	0.73	±0.65	0.130	±0.27	33.22	±11.22	11.60	±4.08	48.76	±43.71	
			(9.26 -	93.87)	(1.99	-19.90)	(0.29	-1.94)	(0.001	-0.95)	(15.38	-57.70)	(5.8	-22.9)	(7.73	–171.59)	
Upstream Lock 6	Main	3	11.82	±2.27	3.93	±1.52	0.39	±0.01	0.029	±0.01	30.25	±5.22	8.28	±1.58	36.03	±0.84	
Downstream Lock 6	Main	3	12.07	±3.07	2.99	±0.29	0.39	±0.03	0.016	±0.01	31.16	±9.56	10.89	±3.24	46.09	±10.05	
Coppermine Wetland	Wet	3	30.25	±2.17	11.40	±1.01	1.74	±0.13	0.816	±0.14	17.41	±1.77	19.01	±3.90	152.81	±31.16	
Chowilla Creek	Creek	3	10.10	±0.30	2.87	±0.09	0.38	±0.04	0.015	±0.00	26.59	±2.72	9.90	±0.86	57.22	±11.12	
Ral Ral Creek	Creek	3	10.86	±0.23	3.39	±1.26	0.32	±0.03	0.027	±0.01	34.28	±2.94	12.98	±1.83	34.72	±12.32	
Monomon Creek	Creek	3	10.81	±0.42	2.38	±0.24	0.34	±0.07	0.020	±0.01	33.05	±7.33	14.57	±0.77	29.15	±1.54	
Upstream Lock 5	Main	3	14.72	±4.73	2.93	±1.52	0.34	±0.03	0.102	±0.13	44.27	±15.69	10.07	±1.71	20.15	±3.42	
Merretti Swamp	Wet	3	92.77	±0.96	19.64	±0.23	1.90	±0.05	0.015	±0.02	48.76	±1.70	7.11	±1.24	13.91	±9.07	



Figure 43. Water properties (TC – total carbon, TOC – total organic carbon, TN – total nitrogen, NH₄ – Nitrate, CN ratio – ratios of % carbon and % nitrogen, and TSS – total suspended solids) measured (n=3) at different sampling sties in (red) 2017 and (black) 2018. Sites are grouped by site type: Main – main channel of the river, Creeks - low or mid-flowing creeks adjustment to the main channel, and wetlands. Note that the three sites within each group are not considered replicates, they are different representations of that habitat type.

	df	тс	тос	ΤΝ	NH4	C:N	TSS (as mg)	TSS (as g/L)
Year	1	0.273	0.0937	0.444	0.0521	0.000824	3.80E-06	0.00517
Site	8	0.537	0.579	0.679	0.356	0.0119	0.815	0.0848
Site type	2	2.02E-08	2.86E-06	2.05E-09	0.00937	0.361	0.11	0.0643

Table 7: Summary of p-value ANOVA results for water properties. Bold represents significant effects (p < 0.001) while those in italics are of a lesser significance (p < 0.05).



Figure 44. Biplots of carbon and nitrogen isotope (δ 13C and δ 15N) values showing differences in basal resources and consumers sampled at all sites in 2017 and 2018.



Figure 45. Biplots of basal resources sampled in 2017 and 2018 at sites that were classified by their association with (A) the main channel of the river, (B) Creeks - low or mid-flowing creeks adjustment to the main channel, and (C) wetlands. Note that the three sites within each group are not considered replicates, they are different representations of that habitat type. Symbols represent the same samples as above.

	n	$\delta^{ t 13}$ C values (‰)		$\delta^{ extsf{15}}$ N valu	es (‰)	С	C:N ratio			
2017	167	-27.88	±3.99	3.13	±5.14	4.62	±10.66			
Soil	51	-27.12	±3.98	0.40	±6.97	3.70	±5.56			
TSS	20	-30.01	±0.68	1.74	±0.28	9.01	±1.08			
Biofilm	33	-25.09	±2.72	3.04	±1.88	8.27	±21.69			
Sediment	21	-29.88	±4.82	3.61	±2.02	1.64	±1.05			
Vegetation	12	-29.52	±2.55	3.76	±1.53	0.62	±0.16 ^			
Zooplankton	15	-30.36	±5.26	6.62	±5.37	4.84	±3.91			
Shrimp	7	-27.38	±0.97	10.26	±1.94	2.67	±0.67			
Yabby	6	-25.15	±0.60	10.56	±1.64	2.65	±0.45			
2018	169	-26.45	±2.84	3.79	±2.25	20.68	±27.34			
Soil	22	-25.92	±1.35	3.03	±0.75	15.01	±3.10			
TSS	24	-27.38	±2.12	5.22	±1.35	7.31	±0.57			
Biofilm	21	-23.81	±2.60	3.58	±1.51	19.77	±7.58			
Sediment	31	-26.57	±2.19	4.71	±4.16	17.46	±16.19			
Vegetation	33	-26.48	±2.66	3.28	±1.12	54.37	±43.68			
Zooplankton	24	-28.09	±3.43	2.79	±1.61	6.19	±0.79			
Scat	8	-27.37	±1.38	3.36	±0.40	2.47	±0.83			
Shrimp	6	-25.21	±5.91	4.25	±0.85	3.00	±1.16			

Table 8: Summary of carbon and nitrogen isotopes (δ^{13} C and δ^{15} N) and carbon to nitrogen (C:N) ratios results (mean +/- SD) for each sample type and year.

^ The values measured in 2017 are unusually low, due to the variation in the type of vegetation measured in 2017 relative to 2018.

Table 9: ANOVA results testing the effect of year (2017 vs 2018) and sampling year and location (site type include: Main – main channel, Wet – wetlands, or Creeks – low or mid-flowing creeks adjustment to the main channel).

			Year			Site	type & year	
			F value	p value		F value	p value	
Source	Biofilm	d13C	2.934	0.0927		2.741	0.0295	*
		d15N	1.21	0.276		4.053	0.00386	**
		CN	5.136	0.0279	*	2.303	0.0602	
	TSS	d13C	28.3	3.74E-06	***	5.814	0.000441	***
		d15N	127.7	2.57E-14	***	24.98	4.60E-11	***
		CN	37.98	5.31E-07	***	9.796	1.26E-05	***
	Sediment	d13C	11.28	0.0015	**	3.868	0.00522	**
		d15N	1.144	0.29		4.111	0.00378	**
		CN	14.13	0.000522	***	2.571	0.0425	*
	Vegetation	d13C	11.7	0.00138	**	2.943	0.0319	*
		d15N	1.328	0.255		0.872	0.489	
		CN	20.44	5.16E-05	***	12.4	2.20E-06	***
	Soil	d13C	1.91	0.171		2.166	0.0682	
		d15N	3.098	0.0829		10.08	3.79E-07	***
		CN	77.28	8.06E-13	***	16.66	1.66E-10	***
Consumer	Zooplankton	d13C	2.67	0.111		1.273	0.299	
		d15N	10.7	0.00236	**	2.562	0.465	
		CN	2.336	0.135		110	<2e-16	***
	Crustacean	d13C	0.931	0.355		0.768	0.601	
		d15N	49.01	2.27E-05	***	21.99	0.000379	**
		CN	0.432	0.525		1.405	0.329	

			All data - sourc (n	ces and cons = 270)	sumers		All Sources (n = 218)				All consumers (n = 52)				
Data	Factor	df	$\delta^{ extsf{13}}C$	$\delta^{ extsf{15}}$ N	C:N	df	δ ¹³ C	$\delta^{{\scriptscriptstyle 15}}{\sf N}$	C:N	df	δ ¹³ C	$\delta^{{}^{15}}{\sf N}$	C:N		
All	Туре	9	1.01E-09	1.60E-10	7.33E-16	4	4.43E-09	7.97E-05	1.24E-12	4	0.162	1.95E-10	2.00E-16		
	Year	1	0.000174	0.13	5.36E-11	1	0.000153	2.20E-05	1.38E-11	1	0.455	6.67E-15	1.89E-05		
	Site	8	2.61E-09	0.401	0.491	8	6.12E-06	0.0056	0.296	8	0.681	0.00268	0.302		
	Site type	2	0.357	0.159	0.105	2	0.646	0.0508	0.0592	2	0.491	0.203	0.867		
	Type*Year	4	2.36E-09	4.79E-07	0.3227	4	0.22	0.069581	1.06E-08						
	Type*Site	48	0.525	7.63E-07	1.49E-11	31	9.40E-09	5.04E-06	0.0404	16	0.353	0.4	0.02488		
2017	Туре	8	1.31E-07	3.73E-09	0.3	4	2.97E-07	0.018	0.129	2	4.16E-05	0.00205	0.00791		
	Site	8	7.29E-05	0.338	0.00755	6	0.00825	2.81E-05	0.000125	5	0.439	0.0773	0.463		
	Site type	2	0.18	0.158	0.216	2	0.819	3.59E-05	0.178	2	0.639	0.0425	0.284		
	Type*Site	4	1.20E-08	4.40E-09	1.55E-05	21	1.20E-07	2.54E-06	4.47E-05	3	0.09379	0.21977	0.7154		
2018	Туре	7	1.80E-05	0.00052	<2e-16	4	1.05E-05	0.00215	2.14E-12	2	0.231	0.0694	9.71E-13		
	Site	7	0.0437	0.0111	0.97	7	0.076	0.0781	0.937	7	0.739	0.00971	0.691		
	Site type	2	0.742	0.00194	0.686	2	0.919	0.0118	0.691	2	0.648	0.0444	0.941		
	Type*Site	4	0.2176	0.58892	1	28	0.5129	0.24413	0.974	12	0.417	0.53	0.01489		

Table 10: Summary of ANOVA and ANCOVA results testing for factorial effects on stable carbon and nitrogen isotopes values (‰) and C:N ratios for all samples pooled and for all pooled sources and consumers. Site type include: Main – main channel, Wet – wetlands, or Creeks – low or mid-flowing creeks adjustment to the main channel).



Figure 46. C:N ratios for each basal resource: (A) biofilm, (B) sediment, (C) soil, (D) TSS, and (E) vegetation at different site types (Main – main channel, Wet – wetlands, or Creeks – low or mid-flowing creeks adjustment to the main channel)) in 2017 (grey boxplots) and 2018 (black boxplots). Note that the three sites within each group are not considered replicates, they are different representations of that habitat type.



Figure 47. Biplot of %C and %N for each basal resource sample type and site type in 2017 and 2018. Site type include: Main Channel, Wetlands, or Creeks – low or mid-flowing creeks adjustment to the main channel. Note that the three sites within each group are not considered replicates, they are different representations of that habitat type.

6.3.3 CONSUMER ISOTOPE SIGNATURES

Isotope values and C:N ratios of consumers were significantly influenced by sample type in 2017 but only C:N ratios in 2018 (Table 11). Highest of δ 13C and δ 15N values were reported in crustaceans and lowest values in Zooplankton (Table 11). C:N ratios were highest in zooplankton sampled in 2018. Year only influenced δ 15N values of crustaceans and Zooplankton which were significantly lower in 2018 than 2017. Other consumer groups weren't sampled in consecutive years though all consumers pooled showed significantly lower δ 15N values in 2018 than 2017 (with a mean difference of 5.02, Table 3-4). Site type significantly influenced δ 15N values, but not δ 13C values or C:N ratios, of all sources and all consumers in 2017 and 2018 (Table 11). δ 15N values of source samples in 2017 were significantly lower in creek sites compared to both main channel and wetlands sites (Tukey p<0.01). In 2018 δ 15N values of source samples were significantly higher in wetland sites compared to those from the main channel (Tukey p=0.08). For consumers, δ 15N values were significantly lower in main channel than wetland sites (Tukey p=0.03).

Site only influenced the δ 15N values of pooled consumers in 2018 where Coppermine Wetland was recognised as being significantly different to the other sites (Tukey p<0.04). In 2017, the consumer isotopic signature Lake Merretti was significantly different to Downstream Chowilla.

			All data - sour (n	ces and cons = 270)	umers		A (ll Sources n = 218)			Al		
Data	Factor	df	δ ¹³ C	δ^{15} N	C:N	df	$\delta^{ m 13}$ C	$\delta^{ ext{15}} N$	C:N	df	$\delta^{ extsf{13}}C$	δ^{15} N	C:N
All	Туре	9	1.01E-09	1.60E-10	7.33E-16	4	4.43E-09	7.97E-05	1.24E-12	4	0.162	1.95E-10	2.00E-16
	Year	1	0.000174	0.13	5.36E-11	1	0.000153	2.20E-05	1.38E-11	1	0.455	6.67E-15	1.89E-05
	Site	8	2.61E-09	0.401	0.491	8	6.12E-06	0.0056	0.296	8	0.681	0.00268	0.302
	Site type	2	0.357	0.159	0.105	2	0.646	0.0508	0.0592	2	0.491	0.203	0.867
	Type*Year	4	2.36E-09	4.79E-07	0.3227	4	0.22	0.069581	1.06E-08				
	Type*Site	48	0.525	7.63E-07	1.49E-11	31	9.40E-09	5.04E-06	0.0404	16	0.353	0.4	0.02488
2017	Туре	8	1.31E-07	3.73E-09	0.3	4	2.97E-07	0.018	0.129	2	4.16E-05	0.00205	0.00791
	Site	8	7.29E-05	0.338	0.00755	6	0.00825	2.81E-05	0.000125	5	0.439	0.0773	0.463
	Site type	2	0.18	0.158	0.216	2	0.819	3.59E-05	0.178	2	0.639	0.0425	0.284
	Type*Site	4	1.20E-08	4.40E-09	1.55E-05	21	1.20E-07	2.54E-06	4.47E-05	3	0.09379	0.21977	0.7154
2018	Туре	7	1.80E-05	0.00052	<2e-16	4	1.05E-05	0.00215	2.14E-12	2	0.231	0.0694	9.71E-13
	Site	7	0.0437	0.0111	0.97	7	0.076	0.0781	0.937	7	0.739	0.00971	0.691
	Site type	2	0.742	0.00194	0.686	2	0.919	0.0118	0.691	2	0.648	0.0444	0.941
_	Type*Site	4	0.2176	0.58892	1	28	0.5129	0.24413	0.974	12	0.417	0.53	0.01489

Table 11: Summary of ANOVA and ANCOVA results testing for factorial effects on stable carbon and nitrogen isotopes values (‰) and C:N ratios for all samples pooled and for all pooled sources and consumers. Site type include: Main – main channel, Wet – wetlands, or Creeks – low or mid-flowing creeks adjustment to the main channel).

6.3.4 BASAL ENERGY CONTRIBUTIONS TO CONSUMERS

Mixing model results, based on the δ^{13} C and δ^{15} N values, revealed a low to moderate precision when distinguishing source contributions to zooplankton and crustaceans with wide posterior distributions. There were clear differences in relative contributions of the different basal resources incorporated in zooplankton and crustaceans by year. The relative contribution of biofilm (scraped from submerged substrates) and vegetation components remained constant between the consumer groups each year. However, for the zooplankton group, much higher soil and lower TSS and sediment contributions were predicted across all sites in 2018 compared to 2017 with that component being compensated in 2018 by the soil (i.e. terrestrial) component. (Figure 48). Crustaceans had slightly higher TSS and lower sediment contributions in 2018 than 2017 (Figure 48). These yearly patterns for both zooplankton and crustaceans were mostly consistent across site types.



Figure 48. Basal resource contributions (mean % posterior) for zooplankton sampled in different years.



Figure 49. Basal resource contributions (mean % posterior) for crustaceans sampled in different years.



Figure 50. Biplots of (A) Zooplankton and (B) Crustacean (Yabbies and Shrimp combined) sampled at site types in 2017 and 2018. Site type include: Main – main channel, Wet – wetlands, or Creeks – low or mid-flowing creeks adjustment to the main channel. Where there were too few points no ellipse was calculated.

6.4 Summary

During the 2017-2018 study period, significant between year differences were observed for the carbon and nitrogen pools of both the water column and the sediment pools. Higher concentrations of carbon (both total and organic) and nitrogen (total, NO_3^- and NH_4^+) were measured along with lower suspended solids in 2017 than 2018 (Table 6). While the basal resources were isotopically similar for both years, the zooplankton were enriched for $\delta^{15}N$ in 2017 suggesting they are feeding at higher trophic levels (Figure 44). The shift in measured C:N ratio suggests mobilisation of a large carbon pool in 2018 relative to 2017.

As both the terrestrial and riverine pools of nitrogen decreased in 2018 (Figure 47), this suggests an overall ecosystem response to drying conditions, rather than as a result of the managed inundation in 2018. The mobilisation of carbon in into the biofilm pool (Figure 46A) may be a result of either an increase in ambient concetration/change in availability, or a result of the managed inundation promoting carbon mobilisation. *In-situ* processing and accumulation of carbon appears to be occurring within the weir pool, as demonstrated by the increased C:N measured in 2018 (Figure 46B). Sediment desiccation causes the decline of bacterial activity and biomass and reactivation occurs when rewetting (Austin et al. 2004; Manzoni & Porporato 2011), hence the managed inundation in 2018, is likely to have enhanced sediment C and N mineralisation.

The data from this study may indicate that the 2016/17 high flow event had effects on the food webs which were still quantifiable in 2017, and the influence of the managed inundation may be relatively small compared to the whole of system drying phase that was ongoing throughout 2018. There was a substantial shift in the primary source of basal resources supporting zooplankton between 2017 and 2018 sampling. In 2017, the dominant inputs were predominantly autochthonous (TSS = 34%, soil = 23% and sediment = 25%, (Figure 45). In 2018, when there was a small managed inundation, soil became the dominant input (72%) and TSS and sediment decreased to 11% and 7% respectively (Figure 45). We propose that this may represent a line of evidence that there was a shift from autochthonous material being the dominant basal resource supporting zooplankton in the 2017 sampling period, to allochthonous material in the 2018 sampling period. This hypothesis is based on: (i) the TSS component is water sample filtered through GF/F filter, hence contains sediment, phytoplankton, micro-zooplankton etc.), (ii) the sediment component is the 0-5 cm sediment core containing benthic biofilm growing at the sediment-water interface as well as the underlying benthos, and (iii) the soil component is the unsorted upper soil horizon (0-5cm depth) from above the standing water line, which contains fragments of bark, leaves and other CPOM. However, a more robust sampling design, specifically targeting this hypothesis, is required to validate this suggestion.

7 Summary

In response to degradation of floodplain vegetation (e.g. *E. camaldulensis* and *E. largifloriens* woodlands) due to reduced inundation frequency throughout the lower River Murray, there has been increasing focus on using existing weirs, and constructing new regulators, to facilitate increases in frequency and duration of floodplain inundation. Such increase in inundation through the management of riverine and floodplain structures has the potential to threaten in-channel processes, such as dispersal of propagules (e.g. seeds and plankton), and impact on desirable trophic pathways (e.g. energy sources and types of phytoplankton).

This work has outlined how empirical analyses were used to quantify how changes in hydraulics influence inchannel processes. This information has been integrated in the Source hydrological model, which can then be used to evaluate potential operational scenarios.

The specific findings of this project are summarised below.

7.1 Dissolved oxygen dynamics

- Extensive field work has quantified the variability in standing load of litter and litter fall rates for dominant vegetation types on the lower River Murray floodplain.
- This information has been used to derive statistical distributions of parameters required to simulate changes in DO concentrations from regulator operations.
- Scenarios were simulated to demonstrate the application of the parameter distributions in the hydrological model, suggesting that
 - Current operations under consideration maintain DO levels above 6 mg.L⁻¹.
 - Impacts from extreme operations beyond current operational rules were largely contained to the site scale.

7.2 Phytoplankton abundance

- A high quality data set of phytoplankton cell counts extending back to 1946 has been developed.
- Machine learning techniques were applied in an attempt to forecast cell counts, and ultimately algal blooms, from inputs including velocity, water temperature and salinity.
- The results demonstrated that the technology of machine learning is capable to analyse the big data set of cyanobacteria data available for the River Murray for predictions.
- The models demonstrated the potential to represent recorded patterns in the cell counts, but not to the extent where successful bloom detection was expected.
- Further work on selecting predictors and algorithm calibration may improve model outputs, in particular for the upstream sites (Lock 5 and Lock 9) where the historical record included events with higher cyanobacteria biomass.
- A mixing criterion was implemented in the Source model to provide an indication of when reduced velocity, increased depth, or increased thermal energy will result in an increased risk of persistent stratification, and thus a higher risk of cyanobacteria blooms.

7.3 Propagule transport

- A large field campaign was undertaken in October and November of 2017 and 2018 to monitor changes in propagules in the Chowilla Anabranch, with a focus on seeds, Murray cod larvae and zooplankton abundance and diversity.
- The seed deposition investigation found sediment deposition was significantly greater in association with regulated in-channel inundation than during an in-channel flow pulse.

- Despite the large differences in sediment deposited, there was no significant difference in seed density between regulated in-channel inundation and an in-channel flow pulse. Mechanisms for seed input to the seed bank include from hydrochory, anemochory, zoochory and seed rain.
- Flow delivery or regulator operation should be timed to coincide with seed fall of eucalypts to maximise chances of recruitment.
- Murray cod larvae were associated with littoral habitats with mean velocities <0.1 m.s⁻¹, but high velocity difference (>0.3 m.s⁻¹). This suggests a preference of Murray cod larvae for slackwater habitats within broader fast-flowing reaches.
- Velocities >0.2 m.s⁻¹ are expected to entrain and transport rotifers.
- Velocity metrics of the proportion of the reach exceeding 0.2 m.s⁻¹ and 0.3 m.s⁻¹ were implemented in the Source model, which can then be interpreted with the understanding developed of the relationship to dispersal and retention of zooplankton and Murray cod larvae.
- The modelling results highlighted the difference between regulator operations at lower flows and higher flows for the in-channel lotic conditions, as quantified by these metrics.

7.4 Food web dynamics

- Sampling was undertaken at nine sites in November 2017 and November 2018, and for each site water column, biofilms, soils, sediment (benthos), phytoplankton and zooplankton, and crustaceans were sampled.
- Each sample was analysed for dissolved organic carbon (DOC), total carbon (TC), dissolved organic nitrogen (DON), total nitrogen (TN), nitrate (NO₃⁻), and ammonium (NH₄⁺).
- Stable isotope analysis was also undertaken trace patterns of energy flow and food web linkages.
- Statistical modelling was undertaken to test for statistically significant differences between years, sites and samples for different trophic levels, as well as to identify the main factorial effects and different basal energy sources between trophic levels.
- Higher concentrations of carbon (both total and organic) and nitrogen (total, NO₃⁻ and NH₄⁺) were measured along with lower suspended solids in 2017 than 2018.
- Isotopic differences were observed between the years for the terrestrial vegetation, as well as the suspended solid load and the benthic sediments, suggesting a terrestrial shift in response to climate in 2017 relative to 2018.
- Zooplankton were enriched for 15N in 2017 suggesting they were feeding at higher trophic levels in 2017 compared to 2018.
- As both the terrestrial and riverine pools of nitrogen decreased in 2018, this suggests an overall ecosystem response to drying conditions since the 2016/17 high flow event, rather than as a result of the managed inundation in 2018.
- Sediment desiccation causes the decline of bacterial activity and biomass and reactivation occurs when rewetting, hence the managed inundation in 2018, is likely to have enhanced sediment C and N mineralisation.

This report has presented a summary of new research focused on the gap in understanding on changes to inchannel processes from the operation of large infrastructure designed to enhance floodplain inundation. This will enable a more a more holistic evaluation of environmental water delivery and infrastructure operation, extending analysis beyond inundated area to both risks and benefits occurring across the ecosystems. The implementation of the findings from this research into the Source model will enable further integrated assessment of operational scenarios, from the site to the whole of South Australian River Murray scale.

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