The role of allochthonous dissolved organic carbon in supporting food webs in Australian lowland rivers

Matthew Balzer

A thesis in fulfilment of the requirements for the degree of

Doctor of Philosophy

August 2021

Freshwater Estuarine Research Group

School of Life Sciences

University of Technology Sydney

Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text. I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This research is supported by an Australian Government Research Training Program.

Production Note:
Signature removed prior to publication.

Signature Date 27/07/21

Acknowledgements

The list of people I need to thank for help, support and encouragement throughout this PhD is too long to put here, and words probably won't do it justice anyway. Nevertheless there are a number of people I really do owe a great deal of thanks to.

A massive debt of gratitude is owed to my supervisors Doug Westhorpe, Yoshi Kobayashi, Wade Hadwen, James Hitchcock and Simon Mitrovic whose patience and support has been nothing short of incredible. Their generous sharing of knowledge and encouragement for experimental ideas and writing has been a constant booster, particularly for the last year. Being able to have a group of supervisors that I can catch up with over a coffee or beer and talk about music, life and whatever else comes up has been amazing and made this entire PhD incomparably better. I owe particular thanks to Simon, taking a chance on a mug like me has been life changing, I used to work in factories and now I'm finishing a PhD. But more than that, your constant willingness to help and go far beyond the call of duty even when you are painfully overworked has been greatly appreciated and made this all possible, and I will always be incredibly grateful for that. Plus you're a great hang and have good taste in music.

I would also like to acknowledge all past and present members of the freshwater research group that I have been lucky enough to work with. From helping out in the field in the brutal heat to (even worse) counting algae in the lab everyone's help has always been brilliant. More importantly though I've loved working with everyone and its been a privilege to work with such a great group of friendly and genuine people, most of whom I consider either friends or very good friends. I would like to acknowledge special thanks for Laura and Lauren whose years (!) of help in the field made this PhD possible and enjoyable. And to Jordo for the last 6 wildly intense months, the work was good but the company was better, thanks for it all, I couldn't have picked a better mate to do it all with.

Finally, I simply could not have done any of this without my wife, Aleisha. I can't even put into words how grateful I am to have had you by my side for this, and everything else.

Dedicated to my mum and dad.

Preface

This thesis consists of six chapters. Chapters 2 to 5 have been written as separate articles that are in preparation for submission to peer reviewed scientific journals. These chapters are included in their pre-submission form, and as such use the terms we/our when discussing results and hypotheses. To prevent unnecessary duplication of references, a single reference list has been provided at the end of this thesis.

This thesis is a compilation of my own work, carried out with guidance from my supervisors and others. I conceptualized this research, conducted all data collection and analysis and wrote the manuscript. Contributions by supervisors and others are as follows:

Chapter 2: *Variable flow sizes and their effects on carbon, nutrients, phytoplankton and zooplankton in a highly regulated lowland river*

S. Mitrovic provided conceptual advice, guidance, reviewed manuscript and assisted with fieldwork

D. Westhorpe provided conceptual advice, reviewed manuscript and assisted with fieldwork

J. Hitchcock provided conceptual advice, guidance, reviewed manuscript

T. Kobayashi provided conceptual advice, reviewed manuscript

Chapter 3: *Hydrology controls of primary energy sources supporting zooplankton growth in a lowland river.*

W. Hadwen provided conceptual advice, reviewed manuscript

S. Mitrovic provided conceptual advice, reviewed manuscript and assisted with fieldwork

J. Hitchcock provided conceptual advice, reviewed manuscript

T. Kobayashi provided conceptual advice, reviewed manuscript

D. Westhorpe provided conceptual advice and assisted with fieldwork

Chapter 4: *Allochthonous dissolved organic matter support for freshwater zooplankton and mixotrophs in a mesocosm experiment*

S. Mitrovic provided conceptual advice, guidance, reviewed manuscript and assisted with fieldwork

J. Hitchcock provided conceptual advice, guidance, reviewed manuscript and assisted with fieldwork

T. Kobayashi provided conceptual advice and reviewed manuscript

C. Boys provided conceptual advice and assisted with fieldwork

W. Hadwen provided conceptual advice and reviewed manuscript

R. Delmas assisted in phytoplankton enumeration

Chapter 5: *Bioavailable DOC additions increase mixotroph and ciliate production in riverine microcosms.*

L. O'Brien provided conceptual advice and assisted with fieldwork

S. Mitrovic provided conceptual advice and reviewed manuscript

J. Hitchcock provided conceptual advice and reviewed manuscript

W. Hadwen provided conceptual advice

Table of Contents

Table of Figures

List of Tables

Abstract

The flow regime of a river plays a critical role in maintaining the health and processes within a river ecosystem. However, due to high water requirements for human needs such as agriculture, many rivers are regulated with dams and weirs. Regulation greatly reduces large flow events and in-channel flows resulting in a severe impact on the natural flow regime of a river, reducing allochthonous carbon loads otherwise mobilised during flows. Environmental flows are a key tool in mitigating the effects of regulation by reinstating a portion of the natural flow regime. However, there is still a knowledge gap between the role of these flows in mobilising allochthonous dissolved organic carbon (DOC) and the subsequent response of the lower food web.

Field monitoring on the Namoi River over 2.5 years revealed discharge was positively correlated with increases in nutrient and DOC concentrations with overbank flows mobilising very high levels of all measured nutrients. Phytoplankton and zooplankton also increased during and/or after flow events indicating flows increased food web production potentially via both heterotrophic and autotrophic production. Carbon stable isotope (^{13}C) analysis revealed allochthonous energy was supporting zooplankton communities even during small flow events which changed to autochthonous sources when the river ceased to flow.

Two manipulative studies were run using mesocosm and microcosm techniques. These studies showed pulses of DOC and dissolved organic matter (DOM) can greatly boost food web production with mixotrophic algae and zooplankton increasing significantly following terrestrial floodplain leachate additions in the mesocosm experiment. This was further seen in the phytoplankton communities of the microcosm experiment as mixotrophs and ciliates clearly increased following DOC (as glucose) +nutrient additions, making up for any autotrophic biovolume lost due to competition with bacteria.

This thesis contributes to the growing body of evidence suggesting allochthonous carbon may be an important energy source for riverine food webs. In particular, it suggests allochthonous organic matter may be considerably bioavailable and increase production via both heterotrophic and autotrophic production and greatly increase resources for higher consumers. Protecting flows that mobilise allochthonous DOM through environmental flows will be highly beneficial for riverine communities, particularly in highly regulated lowland rivers.

Chapter 1: General introduction

1.1 Research Scope and significance

Freshwater ecosystems and aquatic biodiversity are threatened globally by a range of human induced processes such as regulation, pollution, degradation of habitat, over-exploitation and climate change (Dudgeon et al., 2006). River regulation has had broad and destructive effects on freshwater ecosystems (Vorosmarty et al., 2010), however, regulation is also a key tool in sustaining human life and activities (Graf, 1999). The anthropogenic importance of river regulation coupled with the possible long-term effects of climate change such as more intense droughts and floods make the management and protection of freshwater ecosystems more urgent (Arthington, 2015).

The food webs of inland rivers are strongly influenced by their flow regimes. Flow plays a significant role in defining whether carbon from in-stream (autochthonous) photosynthesis or from terrestrial (allochthonous) organic matter is the dominant energy source for riverine productivity (Westhorpe and Mitrovic, 2012). Phytoplankton play a critical role as a basal resource for food webs, directly supporting higher trophic levels as well as indirectly supporting higher production via the microbial loop and heterotrophic grazer chain (Kritzberg et al., 2004). High flows may disadvantage phytoplankton communities as it transports populations downstream and decreases light for photosynthesis by increasing turbidity (May et al., 2003; Oliver et al., 2010). However, flow events may be highly beneficial for bacterial communities as high DOC concentrations associated with increased discharge de-couple bacteria from phytoplankton production. This can lead to bacteria out-competing phytoplankton for resources and dominating systems following flow events by using terrestrially derived carbon to support metazoan growth through heterotrophic production (Jansson et al., 2000; Mitrovic et al., 2014). This change in carbon source may lead to significant bottom-up effects in freshwater food webs, as they may affect both abundance and community assemblage of higher trophic levels such as zooplankton and fish.

Environmental flows (e-flows) are used as the key tool for mitigating the effects of regulation on river ecosystems by improving nutrient transport and connectivity between rivers, wetlands and floodplains (Arthington et al., 2015). Environmental flow regimes (EFR) when designed appropriately have been found to increase fish spawning and water bird breeding, mobilize nutrients and improve ecosystem health (Arthington and Pusey, 2003). Large

environmental flows that inundate floodplains can mobilize substantial amounts of terrestrial dissolved organic matter (Westhorpe and Mitrovic 2012). To date understanding what EFR are needed to promote ecosystem production in lowland river systems remains difficult to determine. This is in-part because there remains a large amount of uncertainty about the ecological relationships linking hydrology and bioenergetics responses in stream. This project aims to fill the key knowledge gap between environmental flows in lowland rivers and their ecological response to increased DOC and nutrients. By understanding the relationship between flow and ecosystem responses we may be able to predict future changes to food webs caused by different sizes of flows and changes to EFR rules and policies.

1.2 River flows and importance of freshes and floods

The flow regime of a river can be considered a master variable, controlling many fundamental aspects of river and ecosystem function (Power et al., 1995). The flow regime of a river includes a broad range of flow sizes, from large floods to base flow and cease to flow conditions. These different flow conditions exhibit different physical and biological stressors on riverine ecosystems (Hart and Finelli, 1999). Natural flow regimes vary with climatic conditions and geography, coupled with the surrounding land-use this makes the flow regime of every river unique to some extent (Poff and Zimmerman, 2010). However, similar basic characteristics (e.g. geomorphology and climate, Figure 1.1) make it possible to characterise flow regimes and develop general principles for the ecological responses of similar rivers (Bunn and Arthington, 2002).

The flow regimes of Australian semi-arid and lowland river systems are some of the most variable in the world (Puckridge, 1998). The extreme variability of flows in Australian dryland rivers leads to “boom and bust” conditions. “Boom” periods of high flow result in high resource availability and significantly increased food web productivity whereas “bust” periods are characterised by resource limitation and disconnection during low flow or cease to flow periods (Arthington and Balcombe, 2011). High and low flow conditions exert distinctly different selective pressures on riverine biotic communities and consequently have significant effects on the function of riverine food webs. In Australian lowland rivers, flood events inundate large areas of floodplain, mobilising large amounts of terrestrial organic matter and biota into the river (Westhorpe and Mitrovic, 2012; Nielsen et al., 2016). Medium size flow events, sometimes referred to as freshes, are smaller increases in river discharge that do not break the banks of the river. These events may also lead to increases in organic

carbon and nutrient concentrations but often at lower total loads than during high flow events (Woodward et al., 2015). In contrast, many Australian lowland rivers, particularly in the semi-arid and arid zones exhibit long periods of low flow or cease to flow periods where rivers are reduced to a string of disconnected waterholes (Bunn et al., 2003). These extremes exhibit fundamentally different physical and environmental conditions, leading to highly variable conditions for in-stream food webs. These different conditions have led to several different ecological concepts for the function of riverine food webs during different flow conditions.

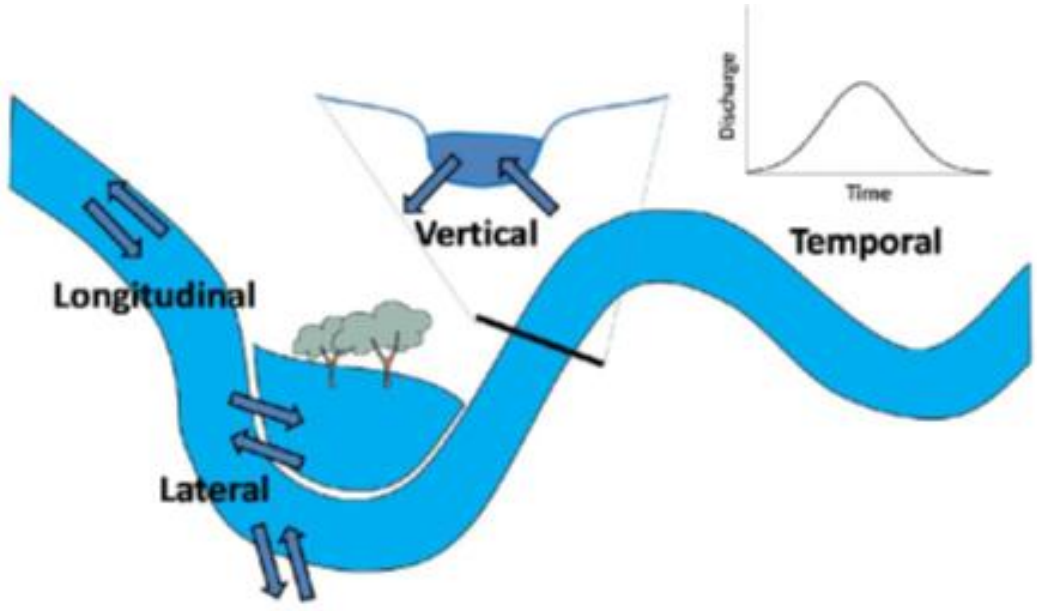

Figure 1.1 Examples of connections between the river and surrounding landscape; longitudinal, lateral, vertical and temporal (Ward, 1989).

1.3 River ecology

Hydrological conditions may fundamentally affect the way basal resources are mobilised and used in freshwater ecosystems (Burford et al., 2008; Fellows et al., 2009). In dryland river systems, a range of conceptual models can be applied to explore how aquatic food webs are fuelled. Junk's 'flood pulse concept' (FPC) suggests the importance of lateral connectivity with floodplain inundation for mobilising large pulses of organic matter resulting in long-term support of riverine food webs following flood events (Junk et al., 1989). Vannote's 'river continuum concept' (RCC) suggests longitudinal movement of organic matter from upstream sources support high levels of food web production in the downstream reaches of large rivers (Vannote et al., 1980). In contrast, Thorp and Delong's (1994) 'riverine

productivity model' suggests that autochthonous production via phytoplankton and local riparian vegetation support the bulk of metazoan growth in rivers. These contrasting models of riverine production and their application across a wide range of settings highlights the dynamic nature of freshwater riverine food webs. The hydrological variability of rivers may make it difficult to uniformly apply any conceptual models to food web productivity across landscape scale systems (Hoeinghaus et al., 2007).

Ultimately these river ecology concepts represent the different characteristics of flow and reflect the 2 major sources of organic matter (energy) supporting production in rivers; allochthonous organic matter, which derives from external/terrestrial sources such as floodplain detritus/riparian vegetation and autochthonous organic matter, which derives from in-stream photosynthesis such as phytoplankton, benthic algae, periphyton and submerged macrophytes.

1.4 Basal food resources

Autochthonous DOC enters aquatic ecosystems through the photosynthesis of algae and aquatic macrophytes (Wetzel, 1983; Bertilsson and Jones, 2003). During growth and upon death, algal cells and macrophytes release photosynthetically derived carbon into the water as dissolved organic matter (DOM; Azam and Cho, 1987; Bains and Pace, 1991). Autochthonous DOM is also incorporated into higher trophic levels more directly as zooplankton graze on phytoplankton and release autochthonous carbon enriched faeces (Hygum et al., 1997).

The quantity and quality of autochthonous DOC is affected and regulated by the ability of producers to photosynthesize. As such it is possible to directly tie the availability of limiting nutrients (N and P) and light levels to the amount of autochthonous carbon within a system (Hama and Honjo, 1987; Goes et al., 1999). Catchment characteristics such as flow regime and turbidity also have substantial impacts on the primary production within a river as high flow and turbidity can significantly reduce light availability and therefore primary production (Devercelli, 2010; Roach, 2013). As the majority of autochthonous DOC enters food webs via phytoplankton, biotic stressors such as grazing by zooplankton and macroinvertebrates as well as competition for nutrients with bacteria and other algal communities can have a large impact on the availability of autochthonous carbon in a system (Hygum et al., 1997; Carney et al. 2016). The sensitivity of algae to changes in water quality and flow variability make autochthonous carbon more readily available and often the dominant carbon source during

low flow periods or in lentic systems (Thorp and Delong, 2002; Bunn et al., 2003; Oliver and Merrick, 2006).

The majority of allochthonous carbon enters rivers episodically during large rain events and floods (Raymond and Saiers, 2010; Watkins et al, 2010). These events can trigger significant increases in DOC concentrations as they cause large inflows of organic matter from the surrounding catchment (Hinton et al., 1997; Aitkenhead-Peterson et al., 2003; Westhorpe and Mitrovic, 2012). Positive relationships between stream discharge and DOC have commonly been found (Henson et al., 2007, Griffiths et al., 2011). Previous studies have found that these rain and flood events contribute disproportionally large amounts of DOC into riverine systems (Easthouse et al., 1992; Hinton et al., 1997; Buffam et al., 2001), with floods generating up to 90 times more DOC than base flow conditions (Dalzell et al., 2005). The variability and magnitude of flood events also play a critical role in regulating allochthonous DOC and nutrient levels entering a system (Robertson et al., 1999; Westhorpe et al., 2008). Dalzell et al., (2007) suggests that hydrological variability is as important to mobilizing DOC as is geographic variability.

1.5 Primary and secondary production under autochthonous and allochthonous carbon conditions

Phytoplankton and bacteria form the base of most pelagic riverine food webs (Stahl et al., 2012; Figure 1.2). Both can be limited by inorganic nutrient concentrations such as nitrogen (N) and phosphorus (P) (Hecky and Kilham, 1988; Almeida et al., 2005) and as heterotrophs, bacteria are also often limited by dissolved organic carbon (DOC) concentrations (Westhorpe et al., 2010). Bacteria are consequently 'coupled' to phytoplankton as they use autochthonous exudates to fulfill their DOC requirements (Cole et al., 1988; Baines and Pace, 1991; Fouilland et al., 2014). However, during periods of high DOC concentrations such as flood events and leaf fall seasons, bacteria may be 'decoupled' from phytoplankton growth (Jansson et al., 2000; Carney et al., 2016). When bacteria are not limited by DOC they directly compete for limiting nutrients with phytoplankton (Drakare et al., 2002). Due to their larger surface to volume ratio and affinity for phosphorus bacteria generally have a competitive advantage over phytoplankton for acquiring limiting nutrients (Jansson et al., 2006). Often these pulses of carbon occur simultaneously to large declines in light availability due to turbidity, reducing photosynthesis and further increasing the advantage of bacteria over phytoplankton (Hitchcock and Mitrovic, 2013). This competition often leads to

large increases in bacterial concentrations while phytoplankton growth may be suppressed, leading to significant changes in basal producers of riverine food webs (Carney et al., 2016).

Changes to basal production may fundamentally affect higher consumers within riverine food webs (Azam et al., 1983; Nicolle et al., 2012). Energy from bacteria enters foodwebs via the microbial loop (Azam et al, 1983) and must pass through several extra food chain links (Figure 1.2) to reach consumers such as zooplankton compared to phytoplankton (Berglund et al., 2007; Degerman et al., 2018). This lengthened energy pathway results in lower transfer efficiency compared to phytoplankton grazing as approximately 70% of carbon is lost at each trophic link (Straile, 1997). Previous studies have found heterotrophic food webs to be up to 10 times less efficient than autotrophic pathways (Berglund et al., 2007). Ultimately this may lead to significantly lower metazoan production when food webs are based on bacterial rather than autotrophic production (Brett et al., 2009; Degerman et al., 2018).

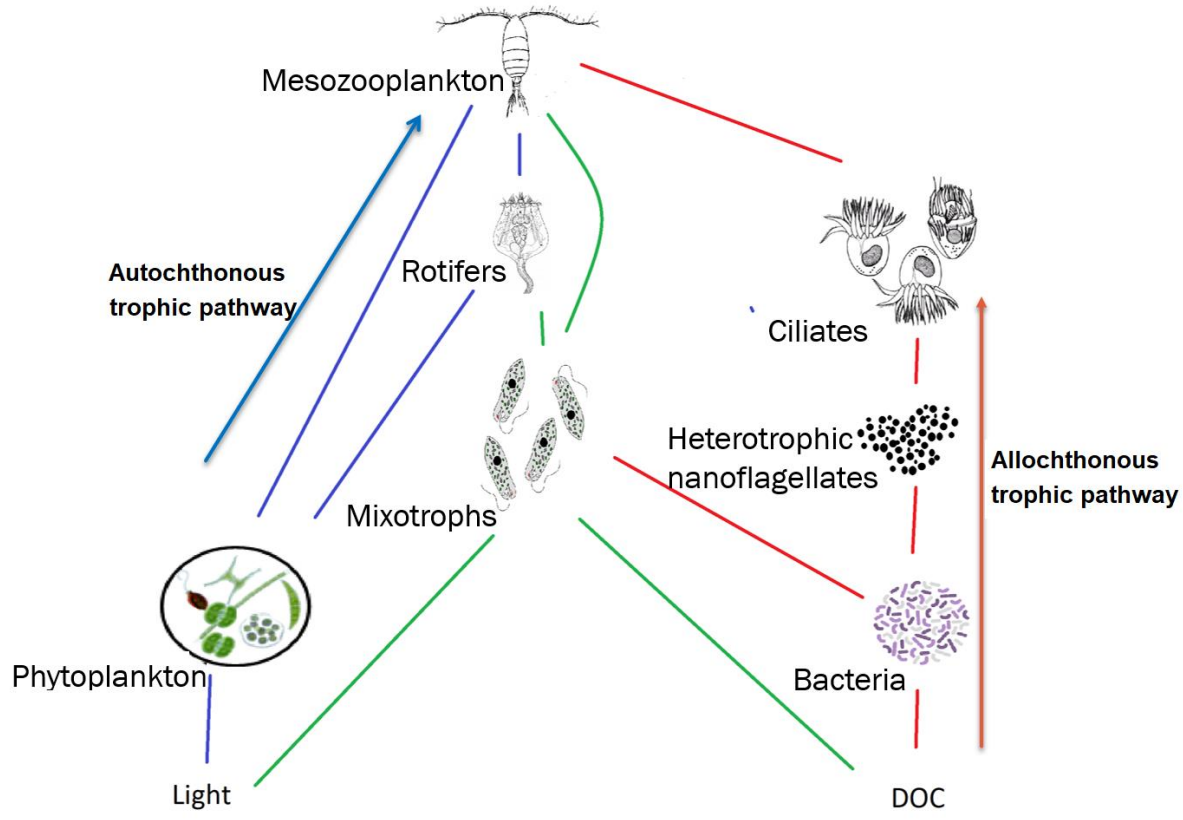

Figure 1.2 Simplified conceptual map of trophic pathways of autochthonous (blue) and allochthonous (red) energy. Green lines represent energy via both pathways (mixotrophy).

1.6 River regulation

Regulating rivers with dams and weirs has significant impacts on the flow regime, nutrient dynamics and biodiversity of rivers and is consequently considered a key threatening process to freshwater biodiversity globally (Poff, 1997; King et al., 2009). River regulation alters key ecological, geomorphic and physical processes as well as local food webs and community assemblages (Ligon et al., 1995; Poff and Zimmerman, 2010). Such broad ecological changes have led to the extensive deterioration of freshwater ecosystems and the floodplains surrounding them in many large river catchments (Arthington, 2006; Poff, 2007). Furthermore, through reducing flood frequency and magnitude, river regulation has significantly reduced floodplain connectivity with rivers (Richter et al., 2003; Page et al., 2005), reducing allochthonous carbon and nutrient pulses (Kingsford, 2000) as well as restricting fish passage between freshwater systems (Rolls, 2012). By fragmenting the river continuum with dams and weirs organic matter loads are also altered, often reduced or reset to upstream concentrations (Ward and Standford, 1995; Wipfli et al., 2007). These reductions in organic matter loads may alter primary and secondary production in the pelagic zone with phytoplankton, bacteria and zooplankton communities losing crucial basal resources for growth (Bott and Kaplan, 1985; Tockner et al., 2000). As a result, food for larval and juvenile fish may be significantly reduced (Humphries et al., 1999). In Australia this has led to the need to sustainably balance agricultural and urban water consumption with water used for the environment (Grafton and Hussey, 2007).

1.7 Environmental flows

Environmental flows are used to minimize the impact of river regulation on freshwater ecosystems such as by artificially replicating local flow events (Rolls et al., 2012). Appropriately managing these flows from dams and reservoirs is considered an important tool in sustaining ecosystem services and biodiversity in rivers (Arthington and Pusey, 2003; Shafroth et al., 2010). Successful use of flows requires a good understanding of the effects of flow and flood events on the geomorphic and biotic processes within a river (Shafroth et al., 2010) and has led to a broad range of methods being used to determine the efficiency and efficacy of environmental flows (Arthington and Pusey, 2003; Richter et al., 2006).

Restoring the natural flow regime is the fundamental motive for assigning flows in regulated rivers (Poff et al., 1997). It is now widely accepted that rivers require a variable regime of flow sizes to sustain basic ecosystem health (Poff et al., 2010). Further, effective restoration

requires an understanding of the local flow regime and its interactions with the surrounding ecosystem. This makes arbitrary flow rules unreliable as flow-ecology responses are highly variable and often regionally specific (Poff and Zimmerman, 2010; Sanderson et al., 2012). To address the variability of flow requirements the ELOHA (ecological limits of hydrological alteration) system (Poff et al., 2010) has been devised in an attempt to develop basic environmental flow frameworks for specific regions, reducing the time required to develop environmental flow regimes for individual rivers.

Several different aquatic ecosystems including rivers, wetlands, floodplains and estuaries receive and benefit from environmental flows (King et al., 2010). This has led to e-flows being used at a range of scales to suit purposes from small scale habitat restoration to entire river and basin scale restoration (Arthington, 2015). These different scales are used to target specific ecological processes and organisms such as the recruitment of riparian vegetation, fish spawning, waterbird breeding and nutrient and organic carbon mobilization (Poff and Zimmerman, 2010; Baldwin et al., 2016). Environmental thresholds such as fish biomass, abundance or assemblage can also be used as ecological metrics for the measurement of flow efficacy (Poff et al., 2010).

How effective environmental flows are depends heavily on the quality of assessment, appropriate implementation, and an effective policy framework (Pahl-Wostl et al., 2013). Several studies have found that environmental flows can mobilize large portions of dissolved organic carbon (Westhorpe and Mitrovic, 2012), improve fish biomass and growth (Rolls et al., 2012) and restore connectivity between rivers and floodplains (Baldwin et al., 2016). Accordingly understanding how to utilise these assets effectively could prove crucial in conserving and restoring riverine health, biodiversity and socio-ecological sustainability long term (Arthington, 2015).

1.8 Namoi River and significance

The Namoi River is in north central NSW, with the catchment covering approximately 43,000 km^2. Roughly two thirds of the Namoi River runs through the semi-arid zone of NSW, receiving an average rainfall of 400mm annually. Two major dams regulate flows on the Namoi; Keepit dam (capacity: 425,000ML) on the Namoi itself and Chaffey Dam (capacity: 62,000ML) on the Peel River, a major tributary of the Namoi. The Namoi feeds into the Barwon-Darling River, forming part of the Murray-Darling Basin drainage system. The Namoi River is a highly regulated system with a significantly altered flow regime due to the

two major storages and extensive water extraction. Large scale extraction is common on the mid to lower reaches for cotton irrigation which is a major industry in the region.

Such high levels of irrigation and extraction on the Namoi River have led to the need for water sharing plans to conserve water for the environment while balancing the needs of industrial agriculture. A major part of the Water Sharing Plans developed for the regulated Namoi River were supplementary flow sharing rules which dictate the amount of water shared between the environment and irrigators during natural flow events. They are divided into 2 ratios, a 90:10 (environment:extraction) operating from July 1 to October 31 and 50:50 from November 1 to June 30. The justification for these 2 different ratios is based on a natural increase in flows between July and November on the Namoi River and this being a key time period for fish passage and breeding. The 90:10 rules have been used in an attempt to return the Namoi River to closer to its natural flow regime during these periods. In July 2016 this period of 90:10 flow protection was lowered to 50:50 to increase access to water for the irrigation industry. These changes were applied on a trial basis until 2019 with the outcomes of this trial to be used to inform longer term supplementary water access rules. The monitoring studies of this body of research were undertaken in-part to understand how these potential changes in flow sizes due to larger extraction from the 90:10 to 50:50 flow rule changes may affect food webs within the Namoi River.

1.9 Research gaps

It has been suggested that future research should examine the broader food web responses of environmental flows and address the key knowledge gap of understanding ecological responses to differences in flow magnitude and duration (Poff and Zimmerman, 2010; Rolls et al., 2012). To fill this knowledge gap, a multi-stepped approach to further research is required to examine several important aspects of food web ecology. Firstly, how flow events effect nutrients and carbon dynamics in rivers across a range of flow sizes and environmental conditions. Secondly, the role these carbon sources and concentrations play in contributing to basal production and competition between phytoplankton and bacteria. Finally, how these changes to basal resources effect food quality and quantity for higher consumers such as zooplankton and how this effects the transfer efficiency from basal resource to higher consumers. By studying these food web dynamics we may further our understanding of how different flow events fundamentally effect ecological and food web processes. Previous research has focussed heavily on the role of allochthonous carbon in supporting production in

lakes (Carpenter, 2005; Karlsson et al., 2007; Tanentzap et al., 2017). However, few have attempted to clearly understand the role allochthonous inputs play in supporting production in rivers (Berggren et al., 2018). Further, emerging evidence suggests additional trophic pathways for allochthonous carbon may make it more efficient and a higher quality than previously thought due to the role of mixotrophic plankton and trophic upgrading (Hiltunen et al., 2017; Hansson et al., 2019). Combining these areas of research is likely to be crucial in increasing our understanding of how allochthonous carbon influences production in Australian rivers and how environmental flow events may be used more efficiently for increases in food web production. As the Australian lowland rivers such as the Namoi River often have few to no riffle areas for macroinvertebrate growth this thesis focus's on the pelagic zone of riverine food webs.

Aims and Hypotheses

The overarching aim of this thesis was to better understand how allochthonous dissolved organic matter effects the structure and function of the lower food webs (bacteria, phytoplankton and zooplankton) of Australian lowland rivers. This thesis was separated into two distinct sections: Two monitoring studies on the Namoi River examining the effects of flow events and organic carbon on riverine food webs, and two experimental studies aiming to investigate knowledge gaps raised during the monitoring studies. The specific aims of each chapter and study are summarised below:

Chapter 2: Variable flow sizes and their effects on carbon, nutrients, phytoplankton and zooplankton in a highly regulated lowland river.

This study aimed to understand the role of discharge on mobilising nutrients and DOC into the Namoi River and the consequent effects on phytoplankton and zooplankton production.

Hypothesis 1: High flow events would mobilise high concentrations of inorganic nutrients and DOC compared to those of medium sized events and low flow conditions.

Hypothesis 2: As food resources change with flow conditions, zooplankton communities would shift to groups more suited to specific feeding on components of the microbial loop and detritus compared to phytoplankton grazing during low flow.

Chapter 3: Hydrology controls of primary energy sources supporting zooplankton growth in a lowland river.

This study aimed to examine how allochthonous carbon supported zooplankton production across flowing and cease to flow (waterhole) conditions using ^{13}C stable isotopes as an indicator of terrestrial or autochthonous production.

Hypothesis 1: During flow periods both sites would derive carbon from similar sources, likely those dominated by allochthonous production, whereas during cease to flow periods autochthonous carbon would be the primary energy source for zooplankton.

Hypothesis 2: Zooplankton and phytoplankton communities would be significantly different between flow and cease to flow conditions, changing towards communities more suited to using either allochthonous or autochthonous production.

Chapter 4: Allochthonous dissolved organic matter support for freshwater zooplankton and mixotrophs in a mesocosm experiment

This mesocosm experiment aimed to understand how dissolved allochthonous materials in the form of a leachate support food web production when confounding effects of floods such as zooplankton eggbank hatching and longitudinal transport are removed.

Hypothesis 1: Additions of Allochthonous dissolved organic matter will increase zooplankton production with higher concentrations supporting more zooplankton. Further, $\delta^{13}C$ values of zooplankton would be closer to the leachate $\delta^{13}C$ values than the control, reflecting DOM assimilation.

Hypothesis 2: DOM addition would alter the structure of the phytoplankton community with relative abundance of mixotroph taxa higher with DOM addition and chlorophytes taxa lower

Chapter 5: Bioavailable DOC additions increase mixotroph and ciliate production in riverine microcosms.

This microcosm experiment aimed to understand how bacterial and phytoplankton competition changed with bioavailable DOC additions (as glucose) and how mixotrophic algae and ciliates respond to increased bacterial production.

Hypothesis 1: Bacteria would out-compete phytoplankton for limiting resources following additions of bioavailable DOC.

Hypothesis 2: Mixotrophic algae would increase in response to increases in DOC and bacteria potentially subsidising losses in autotrophic production.

Chapter 6: General discussion

To provide a general synthesis of the results from all chapters and apply them to broader knowledge gaps in the understanding of allochthonous carbon, including management recommendations and suggestions for future research.

Chapter 2: Variable flow sizes and their effects on carbon, nutrients, phytoplankton and zooplankton in a highly regulated lowland river

2.1 Abstract

The rivers of the Murray-Darling Basin in eastern Australia have been significantly impacted by regulation and extraction for irrigation. Regulation has led to altered flow regimes across the basin reducing nutrient loads, flood events and floodplain connectivity. Previous studies have found flow events to be effective in restoring connectivity with the floodplain and delivering water and nutrients further downstream. However, there is still a knowledge gap between the role of these flows in mobilising nutrients and the subsequent response of the lower food web such as phytoplankton and zooplankton. To partially fill this knowledge gap a monthly monitoring study was conducted on the Namoi River in north Central NSW from June 2016 to February 2018. Nutrient and dissolved organic carbon (DOC) concentrations were positively correlated with river discharge, and zooplankton concentrations were highest post flow events. Chlorophyll-*a* (a surrogate measure of phytoplankton biomass), DOC and discharge were the most influential drivers of change in zooplankton communities. River conditions preceding a flow event also appeared important as flow events following long periods of low flow had effects similar to, or stronger than, those of larger flow events that were preceded by more frequent flows. Further, our results suggested flow events increased production by both heterotrophic and autotrophic pathways. This led to significant boosts in zooplankton production compared to base flow conditions across all measured flow events. We suggest even small flow events can be important in increasing basal and zooplankton production, particularly during drought conditions.

2.2 Introduction

The importance of the flow regime on the ecology and health of a river ecosystem is well documented (Bunn and Arthington, 2002; Poff and Zimmerman, 2010). The flow regimes in Australian semi-arid and lowland river systems are some of the most variable in the world (Puckridge, 1998). The extreme variability of flows in Australian dryland rivers leads to "boom and bust" conditions within the in-stream food web. "Boom" periods of high flow

result in high resource availability and significantly increased food web productivity whereas "bust" periods are characterised by resource limitation and disconnection during low flow or cease to flow conditions (Bunn and Arthington, 2002). Understanding how food webs react to changes in river discharge may be an important tool in understanding ecosystem function in lowland rivers.

The role of flow events has long been conceived as important to productivity of lowland river food webs (Junk et al 1989; Poff and Zimmerman, 2010). Flow events can be described as a rise in river height or discharge above base levels, often associated with rainfall events and releases from dams (Puckeridge et al. 1998). High flow events, such as floods, are large-scale disturbances that break the banks of the river, creating connectivity between the riverine environment and the surrounding floodplain. These increases in connectivity can lead to the mobilisation of allochthonous organic matter and nutrients such as nitrogen and phosphorus (Westhorpe and Mitrovic, 2012). Medium size flow events, sometimes referred to as freshes, can be conceptualised as increases in river discharge and height that do not break the banks of the river. These events may also lead to increases in organic carbon and nutrient concentrations but often at lower total loads than during high flow events (Woodward et al., 2015). Increased discharge coupled with decreased light from higher concentrations of suspended sediment during flow events has been found to suppress primary production in many rivers (Allan and Castillo, 2007; Devercelli, 2010; Townsend and Douglas, 2017).

The alternating dynamics between floodplain-derived organic matter during high flow events and primary production during low flow can lead to distinctly different groups of producers at the base of the food web and shift rivers from being net autotrophic to net heterotrophic (Gawne et al., 2007; Carney et al., 2016). During high flows bacterial production may dominate the food web, utilising dissolved organic carbon (DOC) transported from the floodplain while phytoplankton are supressed due to dilution and reduced light (Drakare, 2002). During low flows, phytoplankton growth (autochthonous production) often dominates compared to the relatively short periods of flood pulses (Bunn et al., 2003). These changes between primary producers may lead to a significant bottom-up effect as different secondary consumers may dominate as the amount and source of food resources shifts (Hunter and Price, 1992).

Zooplankton are the major consumers of planktonic organisms in freshwater systems, with heterogeneous assemblages of different taxa feeding in different niches (Kobayashi, 1996;

1998; Shiel, 1995). Consequently, zooplankton are a crucial link in transferring energy from producers to higher trophic levels (Kobayashi and Church, 2003; Ning et al., 2010). Zooplankton groups such as rotifers, copepods and cladocerans are particularly important for the recruitment of Australian native fish, making up a significant part of fish diets during their larval and juvenile stages (Rowland, 1996; Humphries, 1999; King et al., 2009). How important flow events are in supporting increased food web production remains contested (Junk et al 1989; Thorp and Delong, 1994). Some studies have shown increases in zooplankton abundance concomitant with flow events (Ning et al., 2013; Furst et al., 2014). Contrastingly, others have suggested that flow events may not be important as the resulting allochthonous food resources are of poor quality (Thorp and Delong, 2002; Brett et al., 2009). It currently remains unclear what changes in secondary production can be expected following flow events in lowland systems.

The regulation of rivers has significant impacts on the flow regime, nutrient dynamics and biodiversity of rivers and is considered a key threatening process to freshwater biodiversity globally (Poff, 1997; Kingsford, 2000; King et al., 2009). These reductions in nutrient and organic matter loads may alter primary and secondary production, potentially significantly reducing food for larval and juvenile fish (Humphries et al., 1999). To mitigate the effects of regulation, restoring or protecting flow events that inundate floodplains and in-channel benches may be crucial in maintaining the health of rivers (Westhorpe and Mitrovic 2012; Arthington, 2015; Townsend et al., 2017). However, there is still a major knowledge gap in the relationship between flow events, the lower food web and food resources for higher trophic levels (Poff and Zimmerman, 2010; Rolls et al., 2012).

The aim of this study was to understand how organic carbon and nutrient concentrations, phytoplankton and zooplankton respond to different magnitude flow events in a lowland river system. To achieve this we conducted a two-year observational study on the Namoi River, NSW, Australia, during which time a number of flow events occurred. We hypothesized that: i) high flow events would mobilise high concentrations of inorganic nutrients and DOC compared to those of medium sized events and low flow conditions, ii) during or after flow events phytoplankton concentrations would be reduced due to competition for nutrients with bacteria and high turbidity, and iii) as food resources change with flow conditions, zooplankton communities would shift to groups more suited to specific feeding on components of the microbial loop and detritus compared to phytoplankton grazing during low flow.

2.3 Methods

The Namoi River is a highly regulated system with a significantly altered flow regime due to two major storages and extensive water extraction. Situated in north central NSW, the Namoi catchment receives an average rainfall of 400mm annually with two-thirds of the catchment running through the semi-arid zone. Large scale extraction is common on the mid to lower reaches for cotton irrigation which is a major industry in the region. The Namoi River flows into the Barwon-Darling system which is the major river system for semi-arid NSW.

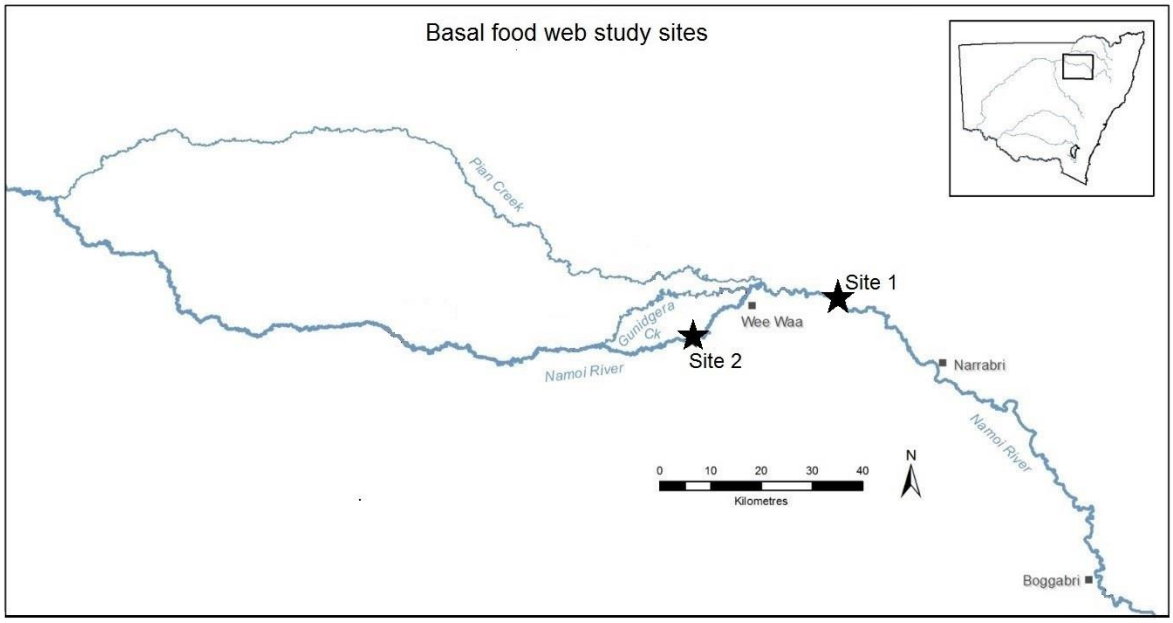

Figure 2.1 Map of the mid-lower reaches of the Namoi River displaying where the 2 sampling sites were located. The river flows right to left making Site 1 the most upstream and Site 2 the most downstream.

Two study sites were monitored on the main channel of the Namoi River (Figure 2.1). Samples were taken monthly from September 2016 to February 2018 at each study site (n=18 for both sites). The two sites were Site 1, near Department of Primary Industry Cotton centre 30°12'46.8"S 149°35'56.4"E; and Site 2, at Redbank farm 50km downstream of Site 1, 30°17'06.6"S 149°20'14.8"E. Both sites were located in the middle to lower section of the river. This area was chosen as its flow regime is more affected by water extraction and is downstream of major storages. Discharge was obtained from two gauging stations operated by the NSW Department of Primary Industries (waterinfo.nsw.gov.au), Mollee (419039), located 12km upstream of Site 1 and Weeta Weir (419068), located directly at Site 2.

At each site, water samples were collected for the determination of water quality and concentrations of phytoplankton and zooplankton. All instruments and sample bottles were

rinsed three times with *in-situ* river water to minimize contamination. Samples were taken using buckets and sub-sampled for nutrients, DOC and Chlorophyll-*a* (Chl-*a*). Each bucket sample was taken 20 to 30 metres apart and any stream sediment or detritus was avoided. All samples were taken in polyethylene containers, placed in a portable Engel fridge/freezer and frozen. Dissolved oxygen, water temperature, electrical conductivity, and pH were measured *in situ* using a Hydrolab field hand-meter Surveyor and MS5 minisonde probe.

Samples for DOC, oxidised nitrogen (NOx) and filterable reactive phosphorus (FRP) were filtered using 0.45 µm pore-sized cellulose acetate membrane syringe filters. Samples for total nitrogen (TN) and total phosphorus (TP) were unfiltered. Duplicates were taken of each nutrient and DOC sample. DOC samples were analysed using the High Temperature Combustion Method (APHA, 2005) and all N and P samples were analysed using a segmented flow analyser (OI Analytical Model FS3100, Xylem USA) according to standard methods (APHA, 2005).

Phytoplankton biomass (3 replicates) was measured by chlorophyll-*a* (Chl-*a*) analysis using a 500 mL volume of water for each sample, filtered through a 0.7 µm pore-sized glass fibre filter using a Mitivac vacuum hand pump. Filters were wrapped in aluminium foil and frozen until analysis using the boiling ethanol extraction method (International Standards Organisation, 1994).

Zooplankton samples (3 replicates each site) were collected from the pelagic zone with each replicate taken 20 to 30 metres apart. River water samples (70 L) were bucket poured through a 35 µm plankton net, concentrated into a sample bottle and preserved with 70% ethanol. For this study all copepods (adults and late stage copepodites) and cladocerans were classified as mesozooplankton. Mesozooplankton were counted and identified to order level for copepods and family level for cladocerans using Bogorov counting chambers and a dissecting microscope at a magnification of ×8. Nauplii and rotifers were counted using a Sedgewick-Rafter counting cell on a compound microscope at a magnification of ×200. Rotifers were identified to family level. The taxonomic key of Shiel (1995) was used for identification of mesozooplankton and rotifers. Rotifer concentrations were presented and analysed in individuals L^{-1} whereas mesozooplankton were presented and analysed in individuals per m^3.

Graphical plots and statistical analyses

Graphical plots of data were made using Sigma Plot software. Regression analyses were run to examine relationships between discharge and environmental factors such as DOC, TN and

TP. Quadratic regressions were chosen after visual inspection of graphical plots and when considering the potential for a curvilinear relationship in the context of within channel flows and flow size vs flow frequency. Linear regressions were also run when the quadratic term in the regressions was found to be insignificant. Before regressions were run data was tested for normality using Shapiro-Wilks test.

To test which environmental factors explained variances in zooplankton taxa abundance and assemblages, a redundancy analysis was performed using CANOCO 4.5 (Braak and Smilauer, 2002). Separate analyses were performed for Site 1 and Site 2. At both sites, copepods, cladocerans and rotifers were combined (all in ind m^3) to account for factors such as competition and predation and data was square-root transformed. All samples and variables were subject to Shapiro-Wilks test. To account for large variation and inflation factors all environmental variables were standardized using z-scores which describe the position of the raw score in comparison to the mean based off the standard deviation. The explanatory environmental variables were selected using automatic forward selection. The variables used in all analyses included discharge on day, mean 7 and 14 day antecedent discharge, 7 and 14 day max discharge, days since flow event, days pre flow event, discharge on day, DOC, TN, TP and Chl-*a.* Discharge data was obtained from gauging stations at each site operated by the NSW Department of Primary Industries (waterinfo.nsw.gov.au). Factors such as FRP, NOx and dissolved oxygen were not included in the final analysis due to high covariance with TP, TN and chl-*a*, respectively. At Site 2 additional flow variables (Site 1 discharge on day, Site 1 thirty-day max) were added to the analysis to account for any upstream influences on downstream zooplankton communities. Monte-Carlo permutation (999 permutations without restriction) was used to test the significance of canonical axis and environmental variables on zooplankton communities. Other variables measured but not shown were temperature, pH, turbidity and conductivity; these variables either showed very high covariance with other factors or were strongly insignificant.

To compare differences between different hydrological conditions we conceptualised flow periods as high, medium, and low flow. High flow periods consisted of any flows greater than 500 ML d^{-1} measured from when discharge was double the initial rate and lasting 2 months from start of flow to account for all potential changes to the food web or nutrients. Low flow was any period <200ML d^{-1} not within two months of a flow event. Medium flow periods were classified as the any periods between 200 and 500ML d^{-1} or any periods at the start or end of a high flow period not within the low flow range.

Permutational analysis of variance with pairwise comparisons (PRIMER 6.0 +PERMANOVA; Anderson et al., 2008) was used to analyse the differences in environmental variables (DOC, TN, TP, Chl-*a*), zooplankton and rotifer community structure during high, medium and low flow periods across both sites. Environmental data was based on Euclidean similarity matrices and ln(x+1) transformed. Zooplankton data was based on Bray-Curtis similarity matrices, and all zooplankton data sets were transformed using square root transformations before analysis. All data was subject to PERMDISP analysis and visual inspection using draftman's plots to test for normality before statistical tests were performed. Similarity percentage analysis (SIMPER) was used to analyse changes within zooplankton assemblage during these flow groups using a 90% threshold of species contribution (Clarke and Warrick, 2001). Non-metric Multidimensional scaling (nMDS) plots were used to visualise changes between the flow groups in mesozooplankton, rotifers and environmental variables (Clarke and Warrick, 2001).

2.4 Results

Flow conditions

The first half of 2016 was very dry within the catchment with several cease-to-flow periods. A high rainfall period during winter across central Northern NSW led to several flow events from June onwards and a large flood event in late September 2016 peaking at 28,899 ML d^{-1} at Site 1. From March 2017 river discharge levels were consistently low (50-200 ML d^{-1}) until October 2017 where a small flow occurred (1294 ML d^{-1} at Site 1) which was followed by 3 months of flows >1000ML d^{-1}. Typically flow magnitude was much higher at Site 1 than at Site 2 (Figure 2.2) as water is extracted or diverted for irrigation purposes between these two sites (mean discharge range at Site 2: 33-44% of mean discharge at Site 1).

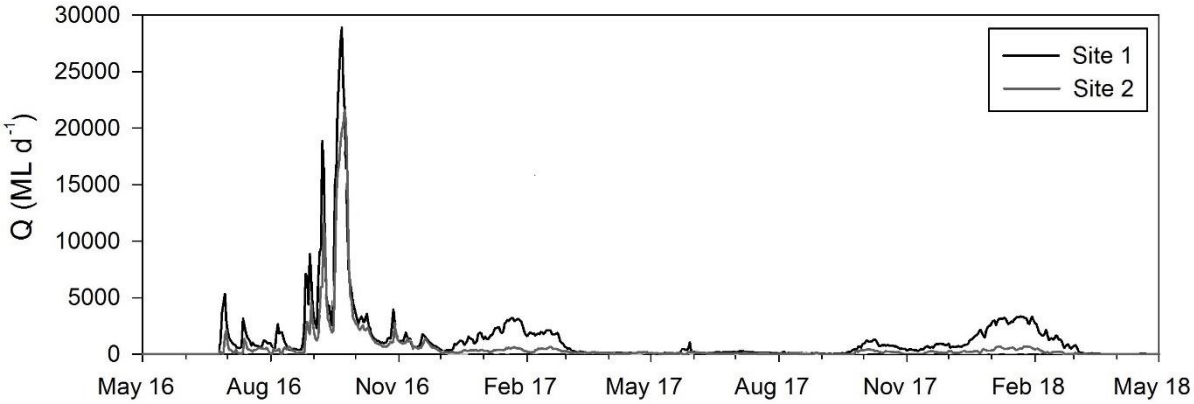

Figure 2.2 Hydrographs showing daily average discharge (ML d^{-1}) for the 2 sampling sites on the Namoi River across the sampling period June 2016 to February 2018. Site 1 is in black, Site 2 grey.

DOC, nutrient and chlorophyll-a concentrations

Both sites followed a similar temporal trend with high variability in DOC across the sampling period. DOC concentrations peaked during September 2016, January 2017 and from October 2017 to February 2018 which coincided with high and medium sized flow events. DOC concentrations remained similar across sites despite reduced discharge (*Q*) at Site 2 compared to Site 1 (Figure 2.3). Quadratic regressions (Table 2.1) showed that DOC at Site 1 was significantly related to discharge (p=0.0218, R^2=0.3997). DOC and discharge at Site 2 was not related to discharge at Site 2 but was significantly related to discharge when compared to discharge at Site 1 (p=0.0294, R^2=0.3957).

Total nitrogen (TN) and total phosphorus (TP) concentrations (Figure 2.3 C-F) showed high variability across the sampling period with a similar range at both sites. TN and TP concentrations peaked at both sites during the September 2016 high flow event and were lowest during the low flow period from June to August 2017. Both TN and TP also increased at both sites during the medium-sized flow events in January and October 2017. TN was significantly related to flow at Site 1 (p=0.0028, R^2=0.5440) and Site 2 (p=0.0024, R^2 =0.5772). TP was strongly related to flow at Site 2 (p=0.0001, R^2=0.8963) but weakly related at Site 1 (p=0.0444, R^2=0.3398). Filtered nutrients (oxidised nitrogen and filterable reactive phosphorus) showed weaker relationships to discharge than total nutrients. At Site 1 NOx was significantly but weakly related to discharge (p=0.0144 R^2=0.3259) however FRP showed no significant relationship. Filtered nutrients at Site 2 were both significantly related to discharge (NOx: p=0.0028, FRP p=0.0399) with FRP showing a strong correlation to discharge (R^2 =0.7457). Site 2 filtered nutrients were not correlated to upstream discharge levels.

At both sites chlorophyll-a (Chl-*a*) concentrations were highest in November 2016 following the September flows (Figure 2.3). Chl-*a* was also high in February and April 2017 at Site 2. During the flow event in October 2017, Chl-*a* concentrations more than tripled from the previous month, 23 days after the start of the flow (4.75 to 15ug L^{-1} at Site 1 and 2.65 to 17.5ug L^{-1} for Site 2).

Non-metric MDS showed high and medium flow groups separated strongly from the low flow group (Figure 2.4). Permutational Analysis of Variance (Table 2.2) supported this, finding DOC, TN, TP and Chl-*a* concentrations were significantly different between flow groups (p=0.001, f=6.288) but not between sites (p>0.05). At Site 1 high and medium flow

groups were significantly different from the low flow group (p=0.016 and p=0.034, respectively). At Site 2 environmental factors were only significantly different between medium and low flow groups (p=0.042), High and medium flow periods were not significantly different from each other at either site.

Table 2.1 Quadratic regression results of flow vs DOC, TN and TP. Downstream (Site 2) nutrient concentrations were also compared to upstream (Site 2 vs Q at site 1) discharge levels to account for irrigation extraction

Regressions	**Site 1**		**Site 2**		**Site 2 vs Q at Site 1**	
	R^2	*P*	R^2	*P*	R^2	*P*
DOC	*0.3997*	*0.0218*	0.1118	0.4362	*0.3957*	*0.0294*
TN	*0.5440*	*0.0028*	*0.5772*	*0.0024*	*0.4655*	*0.0125*
TP	*0.3398*	*0.0444*	*0.8963*	*0.0001*	*0.4153*	*0.0234*
NOx	*0.3259*	*0.0144*	*0.3322*	*0.0028*	0.0465	0.0612
FRP	0.2718	0.0699	*0.7457*	*0.0399*	0.3547	0.1909

Table 2.2 PERMANOVA with pairwise comparisons for environmental (Chl-a, DOC, TN, TP) concentrations, mesozooplankton assemblage and abundance and rotifer assemblage and abundance. Main test results use pseudo-f statistic whereas t- tests between flow groups use t-statistic.

PERMANOVA	**TEST**	**Environmental**		**Mesozooplankton**		**Rotifers**	
		f/t	*p*	f/t	*p*	f/t	*p*
MAIN TEST	SITES	0.068	0.914	1.118	0.346	8.715	0.001
	FLOW	6.288	0.001	12.851	0.001	11.542	0.001
	site×flow	0.035	0.905	2.881	0.004	2.715	0.001
SITE 1	H-M	0.556	0.682	2.413	0.001	1.778	0.010
	H-L	2.421	0.016	2.620	0.001	2.421	0.001
	M-L	2.394	0.034	1.466	0.064	1.973	0.001
SITE 2	H-M	1.088	0.313	2.917	0.001	2.781	0.001
	H-L	1.652	0.107	4.614	0.001	3.872	0.001
	M-L	1.958	0.042	2.048	0.005	2.925	0.001

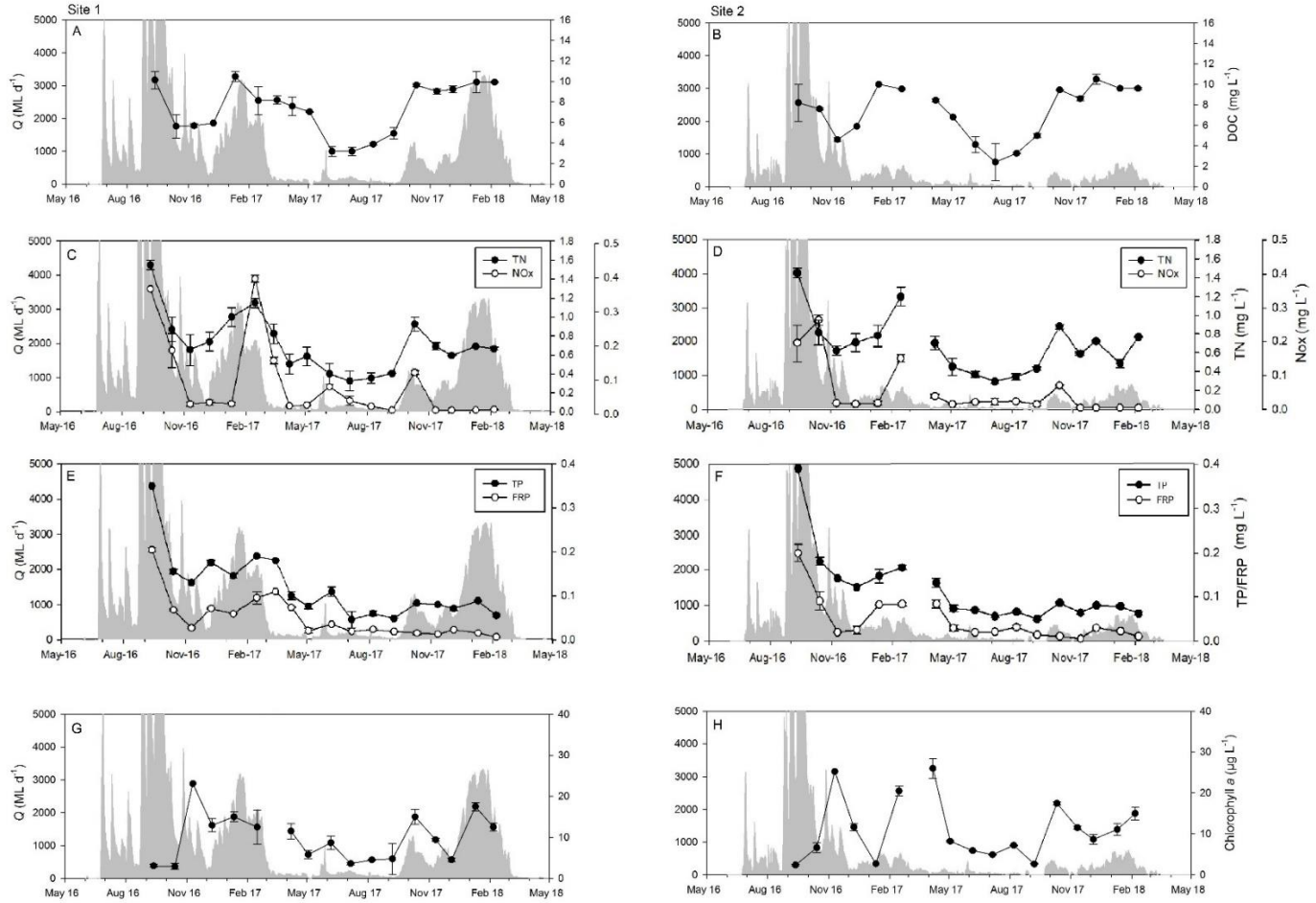

Figure 2.3 Monitoring data from the Namoi River for the sampling period June 2016 to February 2018. Discharge is shown in grey. Site 1 data is shown in the left column, Site 2 on the right. Mean concentrations of DOC (mg L^{-1}, A-B), TN and NOx (C-D, mg L^{-1}), TP and FRP (E-F mg L^{-1}) and Chl-a (G-H, µg L^{-1}) are all shown with standard error of the mean.

Zooplankton

A total of 17 rotifer families were identified during this study, *Brachionus spp.* was the most common, occurring in 100% of samples at both sites. Rotifers were overwhelmingly the most abundant zooplankton group, typically two orders of magnitude higher in concentration than nauplii and mesozooplankton. High variation in rotifer abundance was seen across the study period with a peak (939 ind. L^{-1}) more than 10 times higher than the lowest concentration (6 ind. L^{-1}). At both sites, rotifer concentrations increased after the September 2016 flood. The mean concentrations of total rotifers at Site 1 peaked post flood in December 2016 (339 ind. L^{-1}). At Site 2 concentrations were similar (372 ind. d^{-1}) during December 2016 but peaked during the October 2017 flow event (939 ind. L^{-1}) where high rotifer concentrations (>200 ind. L^{-1}) continued until January 2018. Non-metric MDS showed that high and low flow groups separated strongly in community structure (Figure 2.4). PERMANOVA with pairwise comparisons (Table 2.2) supported this, identifying significant differences between flow periods ($p=0.001$, $f=11.542$,) and sites ($p=0.001$, $f=8.715$). All flow groups were significantly different ($p\leq0.010$) at both sites. Similarity percentage analysis (Figure 2.7) found an average dissimilarity of 80.75% in rotifer community structure between high and low flow periods. At high flow *Keratella (32%), Brachionus (23%)* and *Filinia (15%)* were the most dominant genera whereas at low flow *Synchaeta (34%), Brachionus (18%)* and *Trichocerca (17%)* were the most dominant (Figure 7).

Concentrations of nauplii showed a similar pattern to rotifers and increased by a factor of 3 at Site 1 (2185 ind. m^{-3}) and Site 2 (3014 ind. m^{-3}) in the two months after the September 2016 high flow flood event (Figure 2.5). At both sites nauplii concentrations also increased during the October 2017 medium flow event leading to the highest concentrations for the sampling period at Site 2 (3128 ind. m^{-3}). The copepod community consisted of 4 taxonomic groups (Cyclopoids: *Mesocyclops spp*, *Thermocyclops spp* Calanoids*: Boeckella spp, Calamoecia spp*) which was heavily dominated by cyclopoids, occurring in 100% of samples at both sites. Calanoids were present in 47% of samples, occurring from October 2016 to January 2017 and September 2017 to December 2017 at both sites. The average ratio of cyclopoids to calanoids was 12(±3):1 across both sites. Total copepods showed high variation in abundance throughout the sampling period peaking at 6614 ind. m^{-3} in December 2016 and lowest at 29 ind. m^{-3} in August 2017. Copepods followed a very similar trend to nauplii with concentrations increasing in the two months following the September 2016 flood event leading to very high concentrations at Site 1 (6614 ind m^{-3}) and Site 2 (1121 ind m^{-3}) in

December 2016. High concentrations were also recorded at Site 2 (713 ind m^{-3}) during and after the October 2017 flow event. Cladocerans followed similar trends to copepods, peaking in December 2016 at Site 1 (1478 ind. m^{-3}) and in November 2017 at Site 2 (657 ind m^{-3}). The average ratio of copepods to cladocerans was 2.5(±0.4):1. Five genera (*Daphnia, Ceriodaphnia, Chydorus, Bosmina, Moina*) of cladocerans were identified across the study period, and *Chydorus* spp. was the most common at both sites, occurring in 65% (Site 1) and 82% (Site 2) of samples respectively. Non-metric MDS showed a clear separation of mesozooplankton groups between high and low flow periods (Figure 2.10). PERMANOVA with pairwise comparisons (Table 2.2) supported this, identifying a significant difference between flow groups ($p=0.001$) and no significant difference between sites ($p=0.346$). At Site 1, zooplankton were significantly different at high flow compared to medium ($p=0.001$) and low ($p=0.001$) but not between medium and low flow groups ($p>0.05$). At Site 2 zooplankton communities were significantly different between all flow groups ($p\leq0.005$).

Redundancy analysis explained a total of 52% of variation in the zooplankton community at Site 1 and 62% at Site 2 (Figure 2.6). At Site 1 all rotifers including *Brachionus, Filinia* and *Synchaeta* were strongly positively related to Chl-*a* and TN concentrations. *Keratella* showed a positive correlation with TP and a negative relationship to daily discharge whereas *Lecane* and *Ascomorpha* were closely positively correlated to daily discharge. Cyclopoids and nauplii were positively correlated to Chl-*a* concentrations while calanoids and cladocerans were positively correlated to TP concentrations and negatively correlated to daily discharge. Similar patterns were seen at Site 2 with the first 2 canonical axes explaining 55% of total variation. Chl-*a* was very similar to Site 1 and explained 36% of total variation, correlating positively to 65% of all rotifer families with *Brachionus, Filinia* and *Keratella* most closely related. *Polyarthra, Asplanchna* and *Lecane* were positively correlated to both Chl-*a* and DOC which played a much stronger role at Site 2 explaining 11% of total variation. *Synchaeta* and *Lepadella* were closely positively correlated to DOC and *Trichocera* was positively correlated to DOC at both sites. Calanoids and nauplii were closely positively related to Chl-*a* concentrations. Cyclopoids and cladocerans appeared positively correlated to both Chl-*a* and DOC concentrations and negatively related to daily discharge.

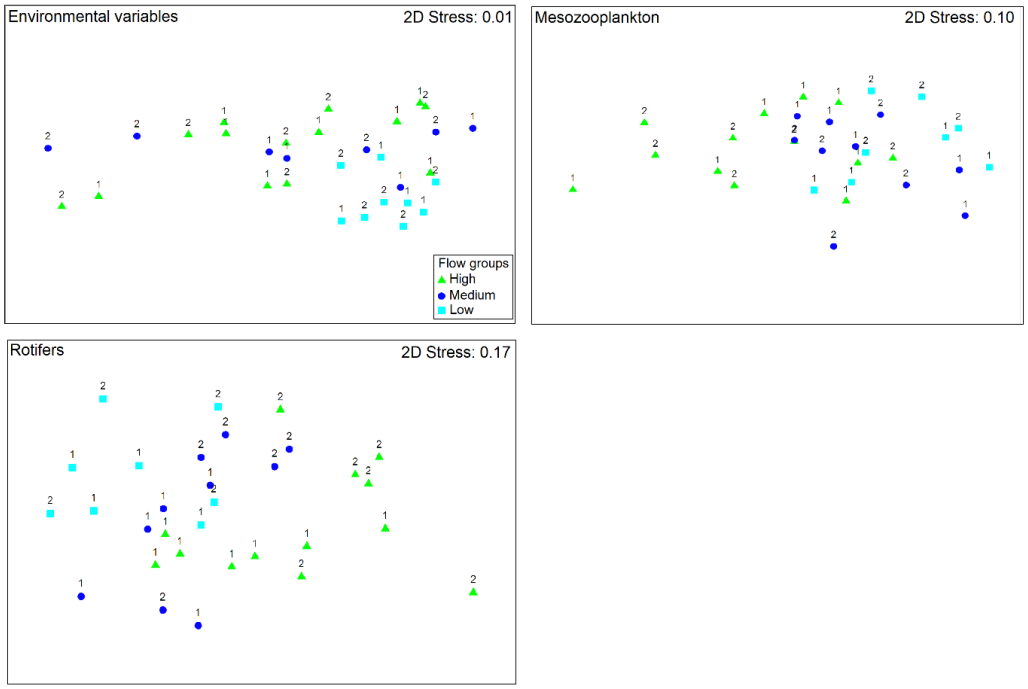

Figure 2.4 nMDS plots of environmental variables (DOC, TN, TP and Chl-a), rotifer community assemblage and mesozooplankton assemblage and at high (green triangles), medium (dark blue circles) and low (light blue squares) flow periods. The numbers above each point represent sites 1 and 2.

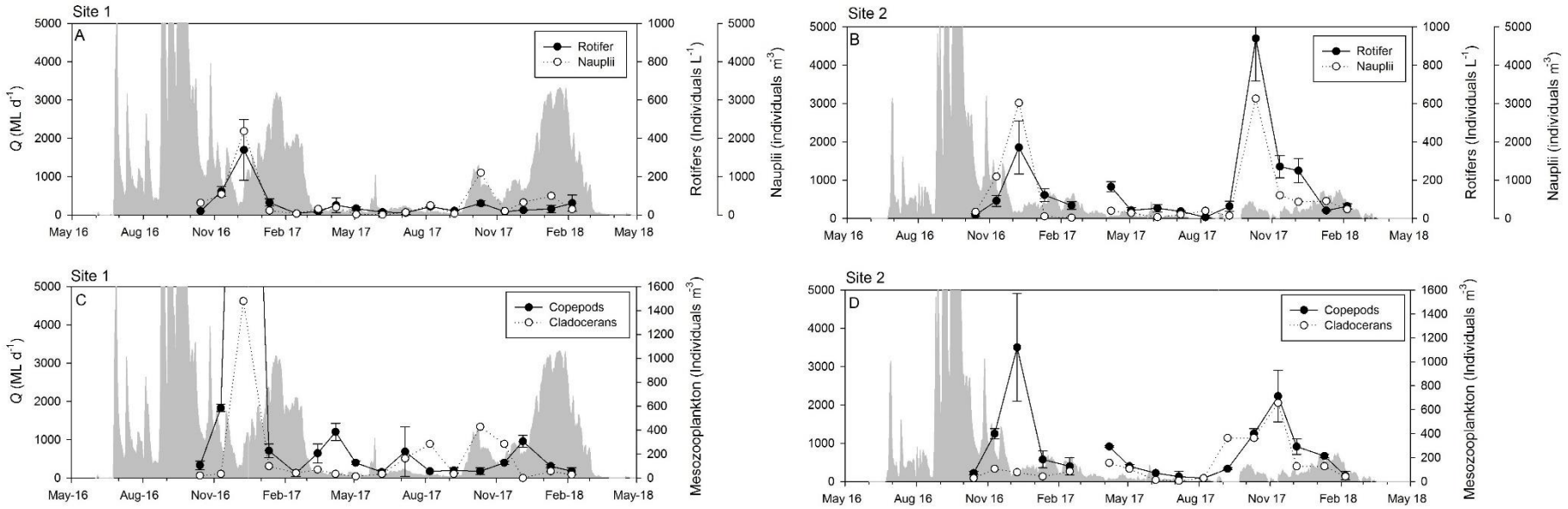

Figure 2.5 Monitoring data for zooplankton concentrations on the Namoi River from June 2016 to February 2018. A-B shows mean rotifer (individuals L^{-1}) and Nauplii (individuals m^{-3}) concentrations with standard error of the mean. Rotifers are in black while nauplii are on a secondary axis in white. C-D shows mean mesozooplankton concentrations (individuals m^{-3}) with standard error of the mean. Copepods are in black while cladocerans are in white. Zooplankton concentration peaks were not included to allow visibility of low concentration periods. Rotifers are 1000x higher in concentration than all other zooplankton.

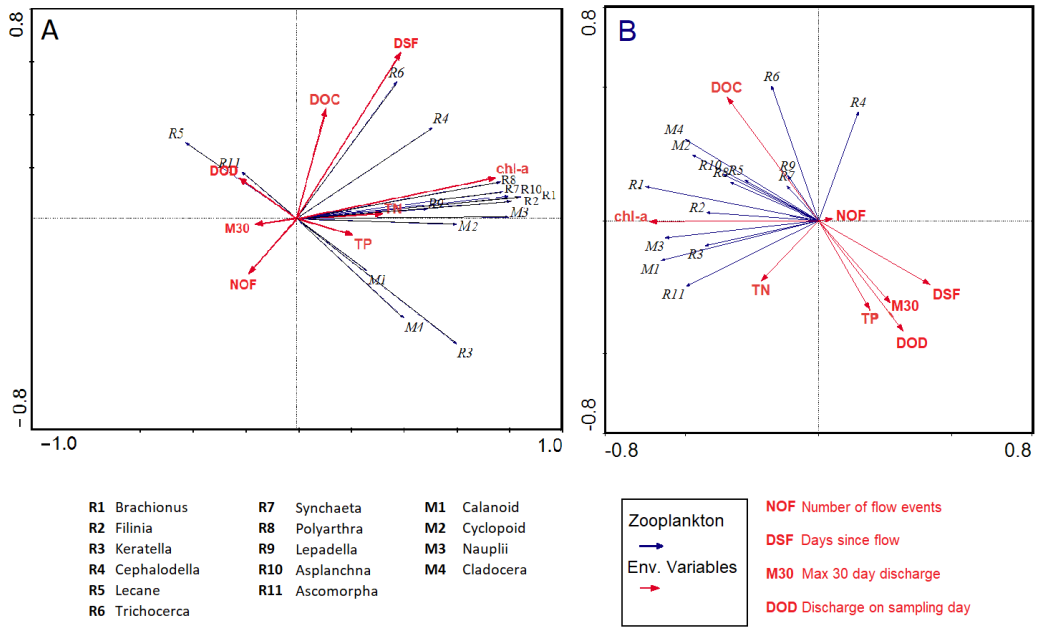

Figure 2.6 Redundancy analysis for all major zooplankton groups at Site 1 (A) and 2 (B).

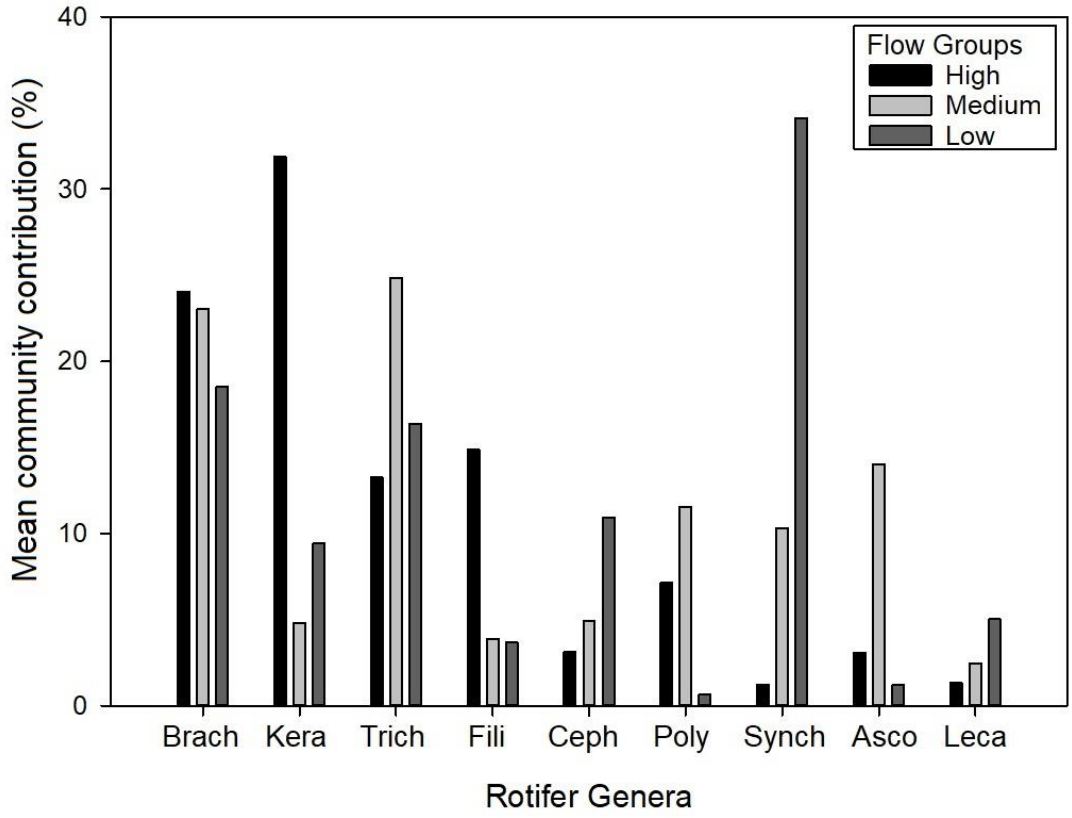

Figure 2.7 SIMPER analysis of rotifer communities at high, medium and low flow periods. Brach: Brachionus, Kera: Keratella, Trich: Trichocerca, Fili: Filinia, Ceph: Cephalodella, Poly: Polyarthra; Synch: Synchaeta; Asco: Ascomorpha, and Leca: Lecane

2.5 Discussion

The aim of this study was to understand how organic carbon, nutrients, phytoplankton and zooplankton dynamics changed following flow events in a lowland river. We found discharge played an important role in increasing nutrients and DOC concentrations and structuring the zooplankton communities throughout the year. Nitrogen, phosphorus and DOC were positively correlated to discharge and increases in zooplankton abundance occurred after the high flow event in September 2016 and the medium flow event in October 2017. Low-flow conditions were typically concomitant with low concentrations of nutrients, Chl-*a* and zooplankton. Further, zooplankton communities were significantly different between high and low flow periods (p=0.001). The high abundance of zooplankton after the high flow event suggests the differences in resources made available by high flow events may play a role in structuring the zooplankton communities and providing resources for higher trophic levels.

Nutrients, carbon and phytoplankton

Discharge at Site 1 was positively correlated to very similar levels of change in DOC concentrations at both sites (S1: R^2=0.3997; S2: R^2=0.3957). This is similar to previous research on the Namoi River finding positive relationships between DOC and discharge during a period of major flooding (Westhorpe and Mitrovic 2012). Studies on other lowland rivers in Australia such as the Edwards, Murray and Lachlan Rivers in south eastern Australia have also found DOC to increase considerably after high flow events, particularly following floodplain inundation (Cook et al., 2015; Nielsen et al., 2016; Moran et al., 2014; Rees et al., 2020). Studies from South America (Depretis and Kemp, 1993), North America (Dalzell et al., 2005; 2007) and Central Europe (Hein et al, 2003) have also found DOC concentrations increased during or after large flow pulses. As large DOC pulses most often occurred after floodplain inundation (Westhorpe et al., 2008) or heavy rainfall (Raymond and Saiers, 2010), DOC mobilisation in rivers is highly episodic. The 'flood pulse concept' of Junk et al., (1989) suggests the importance of large episodic pulses of allochthonous matter as an energy source for riverine production. These findings have been observed in Australian semi-arid rivers, where high flow events and floodplain inundation have been shown to provide important pulses of energy for connected riverine food webs (Cook et al., 2015; Wallace and Furst, 2016).

Nitrogen and phosphorus concentrations were positively correlated with discharge which is consistent with previous studies on the Namoi River (Westhorpe et al., 2008). Further, N+P concentrations peaked during the September flood event indicating large pulses of allochthonous nutrients like those hypothesised in the flood pulse concept (Junk et al., 1989) and seen during flood events on the Murray River, Australia (Nielsen et al, 2016; Rees et al., 2020). Medium-sized flow pulses (February and Octocber 2017) also appeared to increase nutrient concentrations, particularly total nutrients. In a study on the Gwydir River, NSW, Australia, in-channel pulses were positively correlated with increased nutrient loads, leading to positive relationships between increased riverbank inundation and increases in FRP and NOx concentrations (Woodward et al., 2015). Relationships between discharge and total nutrients were always stronger than NOx and FRP, although patterns were broadly similar (Table 1). The higher concentrations of total nutrients compared to filtered inorganic nutrients suggests that the nitrogen and phosphorus mobilised during the high flow event may be dominated by organic nutrients or nutrients in particulate form associated with detritus and sediment (Hessen et al., 2003; 2006). These particulate portions of the TN and TP loads are less immediately available for uptake and production by bacteria and phytoplankton compared to NOx and FRP (Beardall et al., 2001; Gillor et al., 2010). However, a portion of this particulate matter may be readily consumable for higher trophic levels (Hessen, 2006; Hladyz, et al., 2012).

Nitrogen and phosphorus are important nutrients for the growth of phytoplankton and bacterial communities which form the base of freshwater food webs (Hecky and Kilham 1988; Stahl et al., 2013). Thus, concentrations of available nitrogen and phosphorus can be crucial for supporting and increasing overall productivity. Westhorpe et al. (2010) found nitrogen and phosphorus additions to *in situ* microcosms in the Namoi significantly increased Chl-*a* concentrations (an indicator of phytoplankton biomass) and when combined with DOC additions significantly boosted bacterial production. This suggests that increases in river discharge that raise DOC and nutrient concentrations may result in enhanced primary and/or heterotrophic production (Gawne et al., 2007; Carney et al, 2016; Cook et al., 2015). This is supported by changes in Chl-*a* concentration during and after flow events throughout this study. Phytoplankton biomass increased considerably following the September 2016 flood event once high-flow conditions had decreased. Post-flood booms in phytoplankton biomass have been seen previously following floodplain inundation on the Murray River (Nielsen et al., 2016). In this study phytoplankton biomass also increased during smaller flows which

often coincided with increases in nitrogen and phosphorus concentration (February 2017, October 2017, January 2018). These smaller increases in phytoplankton occurred during the flow event rather than afterwards, opposite to the post-flood phytoplankton boom of September 2016. Further, despite nitrogen and phosphorus concentrations peaking during the flood event, Chl-*a* concentrations were at their lowest (S1:3.05µg L^{-1}, S2:2.60µg L^{-1}), possibly due to high turbidity (Irigoien and Castel, 1997), dilution (Townsend et al., 2017) and bacterial competition (Blomqvist et al., 2001; Drakare et al., 2002; Carney et al., 2016), which are common during large overbank flows. However, during smaller flows the effects of light limitation by turbidity and dilution/advection may be much lower and allow phytoplankton communities to utilise nitrogen and phosphorus made available by the flow event, increasing overall biomass. These findings suggest nutrient pulses related to flow events may significantly increase phytoplankton biomass either during or after a flow event depending on the size, nutrient loads and turbidity of the flow event.

Zooplankton

The extreme variability in zooplankton concentrations seen during this study is consistent with the boom and bust ecology of semi-arid lowland rivers in Australia (Shiel et al., 2006; Sternberg et al., 2008; Arthington and Balcombe, 2011). Rotifers and mesozooplankton increased following flow events at both sites, but the magnitude of these increases was highly variable. Following the September 2016 flood, copepods and cladocerans peaked in concentration for the study period at Site 1. Nauplii and rotifers also increased at both sites following this flood event. At Site 2 mesozooplankton and rotifers increased significantly during and after the October 2017 flow event leading to the peak rotifer and nauplii concentrations for the study. Previous studies have found both rotifer and mesozooplankton abundance increased considerably after flood events (Jenkins and Boulton, 2003; Shiel et al., 2006; Ning et al., 2013; Furst et al., 2014), at times increasing orders of magnitude compared to base flow conditions (Nielsen et al., 2016; Rees et al., 2020). Inundation of zooplankton egg banks and downstream transport during flow events may account for a large proportion of the observed increases in zooplankton abundance during this study (Jenkins and Boulton, 2003). Emergence from egg banks may take up to 2 weeks following inundation whereas downstream transport may result in immediate increases of adults to local zooplankton populations (Jenkins and Boulton, 2003). Flow pulses may also increase zooplankton populations indirectly as *in-situ* communities react to increases in food availability caused by flow events (Junk et al., 1989; Poff et al., 1997). TN and TP both increased with discharge

during this study indicating either direct or indirect increases in food resources such as detritus, bacteria and phytoplankton. Thus, the pulses in zooplankton following flow events are at least partially related to increases in resources, similar to those seen on the Kiewa and Ovens River (Ning et al., 2013). These flow-pulse booms in zooplankton have been found to also increase the taxon richness of local zooplankton communities, the effects of which may last up to a month post-flow (Shiel et al., 2006; James et al., 2008; Ning et al., 2013).

Zooplankton community abundance and composition were significantly different between high and low flow periods (PERMANOVA, p=0.001 at both sites). Similarity percentage analysis indicated large shifts in the rotifer community between high and low flow periods from *Keratella, Brachionus* and *Filinia* at high flow to *Synchaeta, Brachionus* and *Trichocerca* at low flow. Changes in the zooplankton community following flow events may reflect changes in available food resources as trophic strategies more suited to utilising post-flow resources dominate. Redundancy analysis found changes in zooplankton abundance and structure were strongly correlated to Chl-*a* concentrations at both sites. Booms in zooplankton often coincide with increases in phytoplankton concentration (Basu and Pick., 1997; Shiel et al., 2006); this was seen in this study during November and December 2016 at Site 1 and November 2016 and October 2017 at Site 2. These findings suggest phytoplankton growth and consumption may be the main source of energy used by zooplankton within the Namoi River, particularly in its mid-lower reaches. This is consistent with the river productivity model (Thorp and Delong, 1994) and other studies in freshwater systems (Thorp and Delong, 2002: Oliver and Merrick, 2006), which have found autochthonous production to fuel the bulk of metazoan production in rivers.

DOC was also significantly correlated to changes in the rotifer and copepod community, albeit it less strongly than phytoplankton. We found during and after high flow events bacterivorous rotifer genera (Arndt, 1993) in-particular *Brachionus*, *Keratella* and *Filinia* dominated the rotifer community. Further, *Brachionus* and *Keratella* have been found to significantly increase in abundance after DOC additions in a mesocosm study on the Namoi River, suggesting the ability to indirectly exploit DOC inputs via heterotrophic pathways (Mitrovic et al., 2014). In contrast, bacterivory in some of the rotifer genera that dominated during low flow periods such as *Synchaeta* and *Trichocera* has been found to be insignificant due to their larger filter feeding size (Boon and Shiel, 1990; Arndt, 1993). In the mesozooplankton, cyclopoid copepods and the cladoceran *Chydorus* increased markedly during flow events. Cyclopoids are raptorial feeders and known to prefer protists such as

ciliates and soft-bodied rotifers as food sources (Jurgen and Jeppesen, 2000; Barnett et al., 2007), leading to a 'semi-dependence' on DOC for nutrition (Berggren et al., 2014). *Chydorus* spp. dominated the cladoceran community during flow events and were responsible for the increase in cladoceran concentrations at both sites during the October 17 flow event. *Chydorus* abundance has previously been positively correlated with both DOC concentration and bacterial biomass, further suggesting a relation to organic matter from flow events (Hitchcock et al., 2016). Several previous studies have found DOC to play an important though highly variable role in supporting secondary production in freshwater food webs (Carpenter et al., 2005; Mitrovic et al., 2014; Berggren et al., 2018). The findings in this study suggest whether DOC may play a significant role in contributing to secondary production is dependent on the zooplankton species and flow conditions. As with DOC relationships to discharge, the role of DOC in supporting the lower food web appears both highly episodic and dependent on the bioavailability the DOC mobilised. During flow events DOC appeared to indirectly contribute to increased zooplankton production, complimentary to phytoplankton growth. However, during low flow periods DOC appeared to play a minor role in zooplankton production. Further, much of the evidence of DOC supporting food web production presented in this paper is indirect and more direct research is required to understand the role of DOC in supporting lowland river food webs.

Booms in fish populations have been linked to flood events and the energy pulses related to floodplain inundation (Junk et al., 1989; Puckeridge et al. 1998). These increases in fish recruitment are likely the result of large increases in biomass such as phytoplankton, bacteria and zooplankton in lower trophic levels (Costelloe et al., 2005). Several native Australian fish (Silver Perch *Bidyanus Bidyanus*, Golden Perch *Macquaria Ambigua* and Murray Cod *Maccullochella peelii*) are reliant on zooplankton as a key food source throughout their larval and juvenile growth stages (Humphries, 1999; King, 2005). Thus, booms in zooplankton abundance after flow events have been linked to pulses in native fish density (Balcombe et al., 2005). A feeding experiment on larval golden perch found the concentration of available zooplankton following fish hatching played a direct role on their survival, with concentrations <100 ind. L^{-1} leading to a mortality rate of 98.7% (Rowland, 1996). Further, Rowland (1996) also found a strong positive relationship (R^2=0.98) between zooplankton concentration and larval golden perch survival. Therefore, the abundance (Rowland, 1996) and diversity (Lichti et al., 2017) of zooplankton at the time of hatching may play a critical role in the recruitment of native fish species. From the results seen in this and other studies

(Shiel et al., 2006), flow events may affect zooplankton populations for weeks to months following an event. Post-flow zooplankton communities may therefore feed recently spawned fish for the entirety of their larval stage, potentially playing a crucial role in the broader survival and recruitment of the species.

Conclusion

Flow events were positively correlated to increases in DOC and nutrient concentrations and significant changes in zooplankton communities. We hypothesised that increases in discharge would result in high DOC and nutrient concentrations leading to a reduction in phytoplankton concentrations due to bacterial competition. However, higher flows appeared to result in increased production via both heterotrophic and autotrophic pathways. Ultimately, this led to large increases in resources for zooplankton. Resource and zooplankton booms during and after flows were not always related to the size of the flow event. The timing and antecedent conditions leading to a flow event appeared important in how large the food web response was to the flow. Therefore, the level of inundation, timing, and preceding conditions may play crucial roles in the impact flow events have on instream food webs. These factors should be considered in models for the effective management of flow events in lowland rivers.

Chapter 3: Hydrology controls of primary energy sources supporting zooplankton growth in a lowland river

3.1 Abstract

Australian rivers exhibit highly variable flow regimes, punctuated with large-scale flood events and long-term cease-to-flow periods. Given the link between flow and delivery of organic matter to rivers, the sources of energy supporting food webs is likely to change during these periods and have considerable effects on the functioning of riverine food webs. In order to understand how autochthonous and allochthonous sources of particulate organic matter (POM) support food webs under different hydrological regimes, the lower food web (producers and primary consumers) of two locations with permanent waterholes on the Namoi River were investigated monthly for 18 months. Specifically, zooplankton $\delta^{13}C$ stable isotopes and POM $\delta^{13}C$ were taken for analysis of the role of different carbon sources in sustaining primary consumers. PERMANOVA was used to measure differences between sites and flow periods and regression analysis was used for the estimation of relationships between zooplankton $\delta^{13}C$ and POM $\delta^{13}C$. During flow periods, carbon sources were very similar at both sites and were indicative of food webs that utilise terrestrial carbon as the primary energy source. During cease-to-flow periods, the waterhole with no local riparian vegetation had extremely high variation in zooplankton $\delta^{13}C$ and POM $\delta^{13}C$, indicating predominant use of phytoplankton as an energy source. In the vegetated site, terrestrial inputs appeared to offer a constant and consistent energy source which was reflected in the zooplankton $\delta^{13}C$ signatures and the prevalence of zooplankton and mixotrophs which use DOC and the microbial loop as a food source. These results highlight how energy sources supporting food webs are highly variable across hydrological and environmental conditions. Without high levels of terrestrial inputs from local riparian vegetation or during periods of high flows, waterhole food webs become driven by autochthonous sources of carbon. This research highlights the connection between hydrology and carbon flows in riverine ecosystems that are highly variable and are likely to become more variable due to the impacts of climate change. Significantly, riparian vegetation around persistent waterholes may be important in not just maintaining habitat and microclimate quality, but also as a means of sustaining aquatic biota across long periods of no flow.

3.2 Introduction

Lentic and lotic freshwater ecosystems exert distinctly different physical (or abiotic) conditions on food webs and trophic pathways (Hart and Finelli, 1999; Fellows et al., 2009). For example, flow, turbulence and turbidity play important roles in the feeding behaviours and survival of biota in lotic habitats relative to those in lentic habitats. In particular, hydrological conditions may fundamentally affect the way basal resources are mobilised and used in freshwater ecosystems (Burford et al., 2008; Fellows et al., 2009). In dryland river systems, a range of conceptual models can be applied to explore how aquatic food webs are supported. Junk's 'flood pulse concept' (FPC) suggests the importance of floodplain inundation for mobilising large pulses of organic matter resulting in long-term support of riverine food webs following flood events (Junk et al., 1989). Vannote's 'river continuum concept' suggests longitudinal movement of organic matter from upstream sources support high levels of food web production in the downstream reaches of large rivers (RCC, 1980). In contrast, Thorp and Delong's (1994) 'riverine productivity model' suggests that autochthonous production via phytoplankton and local riparian vegetation support the bulk of metazoan growth in rivers. These contrasting models of riverine production and their application across a wide range of settings highlights the dynamic nature of freshwater riverine food webs. The hydrological variability of rivers may make it difficult to uniformly apply any conceptual models to food web productivity across land-scape scales and systems (Hoeinghaus et al., 2007).

In semi-arid Australia, lowland rivers exhibit highly variable flow regimes from large flood events to long periods of below average flow (Puckridge, 1998). During long droughts many of these rivers cease to flow (CTF) for long periods (months to years) and are reduced to a string of disconnected waterholes (Bunn and Davies, 1999). These waterholes are crucial refuges for aquatic and terrestrial wildlife that depend on permanent water bodies for survival (Bunn et al., 2003). Refuge waterholes exhibit lentic conditions different from the in-channel lotic conditions of a flowing river (Leigh et al., 2010; Sheldon et al., 2010). During CTF periods, the longitudinal and lateral connections to the floodplain are severed, whereby there is little to no organic matter mobilisation (Kelleway et al., 2010; Sheldon et al., 2010). Under these conditions, the food web may be strongly supported by local sources of carbon, either allochthonous riparian inputs (Reid et al., 2008; Leigh et al., 2010) or autochthonous algal production (Fellows et al., 2009), as suggested in the RPM (Thorp and Delong, 1994). However, Burford et al., (2008) suggests floodplain carbon may significantly contribute to

heterotrophic production in waterholes via fish migration and death. Thus, the sources of organic carbon supporting food webs in these rivers may change considerably between hydrological and environmental conditions.

Many researchers have documented how climate change is likely to increase the occurrence and duration of CTF periods in lowland rivers (Sheldon et al., 2010; Balcombe et al., 2011). These changes are anticipated to have considerable impacts on the dynamics of food webs during flow and CTF periods. Identifying changes (and causes) in trophic pathways is essential for understanding ecosystem function in ephemeral rivers both now and with a view to future flow regimes (Bunn et al., 2003; Hladyz et al., 2012). Carbon stable isotope analysis is a widely used technique for examining linkages between carbon sources (terrestrial and aquatic) and higher consumers (Huryn et al., 2001; Hadwen et al., 2010; Medeiros and Arthington, 2010; Berggren et al., 2018). The different geographies, riparian zones and flow conditions of a river may fundamentally affect the source of carbon used by local food webs (Fellow et al., 2009; Sheldon et al., 2010; Mazumder et al., 2012). This has led to considerable uncertainty across studies in how these systems function, particularly during CTF conditions. Previous studies in Australian lowland rivers have found allochthonous carbon assimilation by the lower food web to be highly variable between flow conditions and through time (Hladyz et al., 2012; Hunt et al., 2012). Reid et al. (2008) suggested that carbon from riparian vegetation played a major role as an energy source for food webs during highly variable flow conditions (including cease to flow) in lowland intermittent streams. However, studies of waterholes in Cooper Creek, Central Australia, found benthic algae or phytoplankton were the primary energy source for the majority of the food web during CTF conditions, suggesting autochthonous production accounted for >55% of biomass in some primary and secondary consumers (Bunn et al., 2003; Leigh et al., 2010).

Zooplankton are the major planktonic consumers in freshwater ecosystems and are a major link between basal resources and higher trophic consumers (Kobayashi et al., 1998). The major crustacean zooplankton groups in freshwater systems are Cladocera, Calanoida and Cyclopoida. Each group uses vastly different feeding strategies for the consumption of resources (Berggren et al., 2014) and have been shown to use and assimilate sources of autochthonous and allochthonous carbon in different ways (Berggren et al., 2014). The turbulence of flowing rivers has been found to make zooplankton reliant on opportunistic feeding on seston (Berggren et al., 2018). However, as turbulence abates during low flow conditions, feeding strategies and patterns of energy transfer may change. Previous studies in

lentic ecosystems in North America and Europe have found allochthonous inputs support high levels of zooplankton and higher consumer biomass (Carpenter et al., 2005; Karlsson et al., 2012). Though there are some generalizable patterns in how lotic and lentic ecosystems function, there is considerably less known about systems that fluctuate between both (Madeiros and Arthington, 2010; Pusey et al., 2020). The carbon sources driving riverine zooplankton across fluctuations in lotic and lentic conditions have been particularly understudied (Pusey et al., 2020). Consequently, a major knowledge gap exists in understanding the dynamics of basal food resources supporting river food webs across these periods.

In this study we surveyed the energy sources and secondary consumers at two sites on the highly regulated Namoi River in north central NSW. Sampling was conducted monthly for 16 months, covering a period with a range of flow-event magnitudes and CTF periods. Significantly, one site had an intact riparian corridor while the other was more cleared and influenced by grazing. We focused on ^{13}C signatures of zooplankton communities to explore how these may respond to changes in the availability and accessibility of different primary carbon sources between flowing and CTF conditions. We focussed on zooplankton as they may show changes in ^{13}C isotope signatures more quickly than those of higher consumers such as fish (Berggren et al., 2014). Specifically, we analysed zooplankton ^{13}C samples and compared them to suspended particulate organic matter ^{13}C signatures (seston) and algae community composition as potential food sources. We hypothesised that during flow periods both sites would derive carbon from similar sources, likely those dominated by allochthonous production, whereas during CTF periods autochthonous carbon would be the primary energy source for zooplankton. The changes in energy sources would be reflected in zooplankton communities dominated by taxa more suited to using either microbial production or phytoplankton growth pathways, as well as changes in phytoplankton concentrations and community composition.

3.3 Methods

Two study sites were monitored on the main channel of the Namoi River (Figure 3.1). Samples were taken monthly at both sites from August 2017 until November 2018. The two sites were Milloo, 30°18'12.8"S, 149°08'09.9"E and Bugilbone, 30°16'20.3"S, 148°49'08.9"E, approximately 40km downstream of Milloo. Both sites were located in the middle to lower reaches of the river. These sites were chosen because both represent deep permanent water-

refuges and CTF periods are relatively common in this section of the Namoi River. Discharge data was obtained from two gauging stations operated by the NSW Department of Primary Industries (waterinfo.nsw.gov.au), "Bullawa" station, located 3km upstream of Milloo and "Bugilbone" station, located 0.3km upstream of Bugilbone. During CTF conditions the waterhole at Milloo had a maximum length of 41.6 m, width of 16.7 m and depth of 1.8-1.0m depending on the time since the previous flow event. The waterhole at Bugilbone had a maximum length of 32.3 m, width of 12.2 m and depth of 1.5-0.9 m depending on time since previous flow event. Riparian vegetation cover was very high (80-100% cover, DPIE, 2019) at Milloo and predominately comprised of *Eucalyptus camaldulensis* and *E. coolabah.* Riparian cover at Bugilbone was much lower (20-40%, DPIE, 2019) with a similar tree assemblage to Milloo.

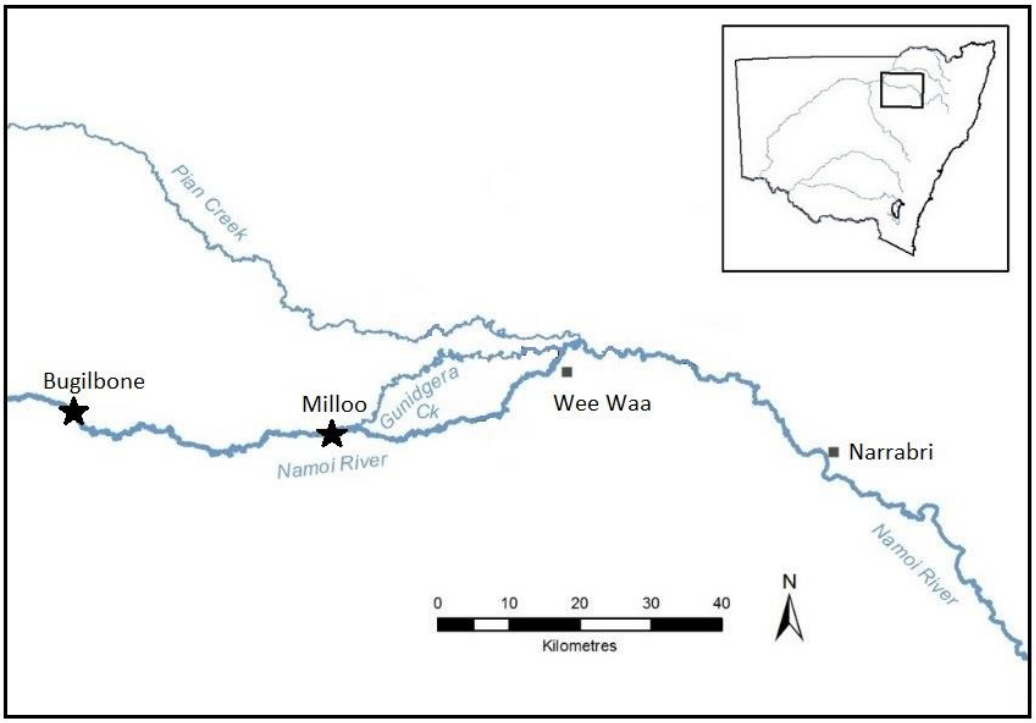

Figure 3.1 Site locations of Milloo (upstream) and Bugilbone (downstream) on the Namoi Rover in North central NSW. The Namoi River flows from right to left

At each site, water samples were collected for the determination of water quality and concentrations of phytoplankton and zooplankton. All instruments and sample bottles were rinsed 3 times with river water from the site to minimize the risk of cross-site contamination. Samples were collecting using buckets and sub-sampled for nutrients, DOC and phytoplankton. Each bucket sample was taken within the same section of the river during flow and CTF conditions and care was taken to avoid collection of any stream sediment or

detritus. All samples were stored in polyethylene containers, placed in a portable Engel fridge/freezer and frozen. Dissolved oxygen, water temperature, electrical conductivity, and pH were measured *in situ* using a Hydrolab field hand-meter Surveyor and MS5 minisonde probe.

Samples for DOC, oxidised nitrogen (NOx) and filterable reactive phosphorus (FRP) were filtered using 0.45 µm pore-sized cellulose acetate membrane syringe filters. Duplicates were taken of each nutrient and DOC sample. DOC samples were analysed using the High Temperature Combustion Method (APHA, 2005) and all N and P samples were analysed using a segmented flow analyser (OI Analytical Model FS3100, Xylem USA) according to standard methods (APHA, 2005).

To understand the broader ecological effects influencing phytoplankton communities, phytoplankton taxa were separated into broad functional groups similar to those of Karlsson et al. (2007) and measured in biovolume to account for large variance in cell size and abundance. Samples (100 mL each) for phytoplankton analysis were taken using a polyethylene terephthalate (PET) bottle filled from a bucket and preserved using 3-mL Lugol's iodine solution. Samples were counted at 200x magnification on a compound microscope using Sedgewick Rafter counting chambers. Measurements for biovolume were taken using an Olympus DP72 camera and cellSens Standard software (version 1.3). Twenty individuals of each species were measured to achieve a reliable average. Algae were identified using the keys of Prescott (1978) and Entwisle et al. (1997).

Zooplankton samples (3 replicates each site) were collected from the pelagic zone with each replicate taken approximately 5 m apart at a site between 13:00-16:00 hrs on each sampling day. Using a bucket, river water samples (70 L each replicate) were poured through a 35 µm plankton net. The zooplankton samples retained on the net were concentrated into a sample bottle and preserved with 70% ethanol. Mesozooplankton were counted and identified to order level for copepods and family level for cladocerans using light microscopy and Bogorov counting chambers at a magnification of ×8. The taxonomic key of Shiel (1995) was used for zooplankton identification.

Zooplankton samples for $\delta^{13}C$ analysis were taken using a 150µm plankton net (1m mouth diameter) towed through the water column for 15 min or until sufficient biomass for analysis was achieved. The net was pushed constantly upstream (during flowing conditions) and across the channel ensuring the mouth of the net was always in front of the sampler, facing

upstream to avoid zooplankton being washed out of the net by the current. Samples were then kept for 6-8 hrs in 0.7 µm filtered river water to purge zooplankton gut contents and were preserved with >80% ethanol. Seston was measured as suspended particulate organic matter (POM). POM samples were taken using river water prefiltered at 150 µm to remove any large zooplankton, then filtered onto pre-combusted GFC filter papers and frozen for analysis. To prepare for the analysis of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, zooplankton samples were rinsed thoroughly three times with reverse osmosis water to ensure all ethanol was removed. Samples were then picked using a dissecting microscope at 8 x magnification. Zooplankton were sorted into appropriate taxonomic groups, then further cleaned for impurities such as organic matter detritus, incorrectly-sorted zooplankton or filamentous algae using regent-grade water, and placed into 5-mm silver capsules. Samples were acidified using 1 mol L^{-1} HCL to remove any inorganic carbon from samples and dried at 60°C for 24 hrs. Samples were analysed at Griffith University using a continuous flow isotope mass spectrometer (GV Isoprime Eurovector EA 3000, Manchester, UK). Results were determined using IAEA-CH-6 ($\delta^{13}C$) and IAEA-N1, IAEA-N2 ($\delta^{15}N$) as standard reference material for carbon and nitrogen respectively. Zooplankton $\delta^{13}C$ values were corrected for fatty acids when C:N ratios were greater than 3.5 using the equations of Post (2007).

Graphical plots and statistical analyses

Graphical plots of data were made using Sigma Plot software. Linear regression analyses were run to examine relationships between POM $\delta^{13}C$ signatures and zooplankton $\delta^{13}C$ signatures. Before regressions were run, data were tested for normality using the Shapiro-Wilks test. Flow conditions were separated into two groups, cease to flow, defined as <1 ML d^{-1} and flow (>1 ML d^{-1}) (DPIE 2020). For statistical analysis of zooplankton ^{13}C signatures zooplankton were separated into flow groups (Flow and Cease-to-flow) within each site and compared across flow groups and sites, all zooplankton taxa were combined within these groups due to the difficulties associated with collecting zooplankton taxa at concentrations high enough to sample for stable isotope analysis. Consequently, no multivariate analyses between $\delta^{13}C$ values of zooplankton taxa occurred. Permutational multivariate analysis of variance with pairwise comparisons using PRIMER 6.0 +PERMANOVA (Anderson et al., 2008) was used to analyse data for significant differences between flow categories and sites. All data were checked for normal distribution and homogeneity of variance using PERMDISP and the Shapiro-Wilks test. Bray-Curtis distances were used for phytoplankton and zooplankton data. Species (count) data were transformed using a square-root

transformation. Similarity percentage analysis (SIMPER) was used to analyse changes between sites and flow categories within the zooplankton and phytoplankton assemblages using a 99% threshold of species contribution (Clarke and Warrick, 2001).

3.4 Results

Two distinct flow periods occurred between August 2017 and November 2018 at both sites on the Namoi River. Discharge from August 2017 until March 2018 was highly variable (0-500ML d^{-1} , average 148 ML d^{-1} at Milloo; 0-363 ML d^{-1}, average 85 ML d^{-1} at Bugilbone) with one short cease to flow (CTF) period for 15 days at the end of September 2017 occurring at both sites. Discharge was zero for >100 days at both sites from March 2018 until July 2018, when a short (24 days) flow pulse reached both sites peaking at 203 ML d^{-1} at Milloo and 145 ML d^{-1} at Bugilbone. A second smaller flow event occurred shortly after the first at Milloo reaching 37 ML d^{-1}. However this did not reach Bugilbone. This was followed by another CTF period of 91 and 120 days, at Milloo and Bugilbone, respectively, lasting until the end of November 2018 when another small flow pulse arrived at both sites (253 ML d^{-1} at Milloo, 194 ML d^{-1} at Bugilbone).

Water quality

Water quality parameters (Table 3.1) were very similar between sites. Dissolved organic carbon (DOC) concentrations were generally lower when the river was flowing compared to cease to flow conditions. Dissolved nutrients (NOx and FRP) at Milloo were much higher during flow periods compared to cease to flow (CTF) conditions and generally higher than nutrient concentrations at Bugilbone. Dissolved oxygen averaged >80% at both sites for all conditions with DO at Milloo being higher during CTF conditions than at Bugilbone. Turbidity at Milloo was much lower compared to Bugilbone which had an average turbidity (84.1 NTU) more than double that of Milloo (32.5 NTU) during CTF conditions.

Table 3.1 Water quality parameters (mean ± standard error) for DOC, NOx, SRP, dissolved oxygen (DO) and turbidity at flow and cease-to-flow (CTF) conditions at the Milloo and Bugilbone sites. n=36 for each parameter at each site.

	Milloo		**Bugilbone**	
	Flow	CTF	Flow	CTF
DOC (mg L^{-1})	8.402 ±0.845	10.23 ±2.95	9.067 ±0.946	10.20 ±1.213
NOx (mg L^{-1})	0.041 ±0.027	0.012 ±0.003	0.009 ±0.002	0.010 ±0.005
FRP (mg L^{-1})	0.019 ±0.006	0.006 ±0.001	0.012 ±0.003	0.006 ±0.001
DO (%)	89.49 ±3.01	96.35 ±14.04	91.6 ±1.71	81.7 ±5.71
Turbidity (NTU)	42.7 ±6.87	32.5 ±1.54	65.1 ±7.38	84.1 ±30.34

Zooplankton Community

PERMANOVA analysis found zooplankton communities at both sites were significantly different between flow conditions (p=0.002 at Milloo, p=0.007 at Bugilbone) but were not significantly different between sites (p>0.05) (Table 3.2). Zooplankton concentrations (Figure 3.2 A-B.) at both sites were typically 1 to 2 orders of magnitude higher during CTF conditions than during flow periods. Copepods averaged 170 ind. m^{-3} at Milloo and 269 ind. m^{-3} at Bugilbone during flow periods, Cladocerans averaged 486 ind. m^{-3} at Milloo and 412 ind. m^{-3} at Bugilbone. Similarity percentage (SIMPER) analysis found at both sites cyclopoids were the dominant copepod and chydorids were the dominant cladocerans during flow periods. During CTF periods, calanoid copepods reached high concentrations at both sites but were higher on average at Bugilbone (31,523 ind. m^{-3}) than at Milloo (16,350 ind. m^{-3}). Conversely, cyclopoid concentrations were always 4-10 times higher at Milloo (mean: 11,828 ind. m^{-3}) than at Bugilbone (mean: 2,212 ind. m^{-3}). *Daphnia* concentrations were extremely variable at both sites with concentrations ranging from 85 to 118,000 ind. m^{-3} at Milloo and from below the detection limit (14) to 51,571 ind. m^{-3} at Bugilbone. SIMPER analysis found during CTF conditions cyclopoids and *Daphnia* were the most dominant copepod and cladoceran groups respectively at Milloo, whereas calanoids and *Daphnia* were the most dominant at Bugilbone.

Phytoplankton community

Total phytoplankton biovolume (Figure 3.2 C-D) peaked at Milloo at 26 mm^3 L^{-1} during the February 2017 flow events and at >30 mm^3 L^{-1} in August 2018 during CTF conditions at Bugilbone. Phytoplankton biovolume was similar at both sites during flow (Milloo: 12.82 ±1.99 mm^3 L^{-1}, Bugilbone: 15.72 ±0.91 mm^3 L^{-1}), but was much higher on average at Bugilbone (24.05 ±2.51 mm^3 L^{-1}) than at Milloo (14.76 ± 2.14 mm^3 L^{-1}) during CTF conditions. Phytoplankton community composition (Figure 3.2 E-F) was significantly different between flow groups (p=0.006, F=5.189; Table 3.4) but not between sites (p>0.05). Generally, mixotrophs and cyanobacteria dominated the biomass across both sites and flow groups. However, mixotrophs were much higher at Milloo under CTF conditions (45.33% ±4.77 community comp vs 30.07% ±1.98 at Bugilbone) and cyanobacteria were higher at Bugilbone under CTF conditions (48.40% ±3.46 community comp vs 40.26% ±2.55 at Milloo). During flowing periods diatoms were similar at both sites averaging 28.22% ±1.76 and 23.95% ±1.89 community composition at Milloo and Bugilbone compared to 6.24% ±2.21 and 12.02% ±2.99 during CTF. Chlorophytes always made the lowest contribution to phytoplankton biomass across sites and flow groups with a range of 7.92% ±1.98 to 9.50% ±1.82 community contribution.

Table 3.2 PERMANOVA main test results for zooplankton and phytoplankton community analysis between sites and flow periods.

PERMANOVA		***p***	***F***	**perms**
Zooplankton	Site	0.792	0.437	999
	Flow	0.001	11.484	999
	Site × flow	0.279	1.272	999
Phytoplankton	Site	0.152	1.877	999
	Flow	0.003	5.187	999
	Site × flow	0.695	0.522	999

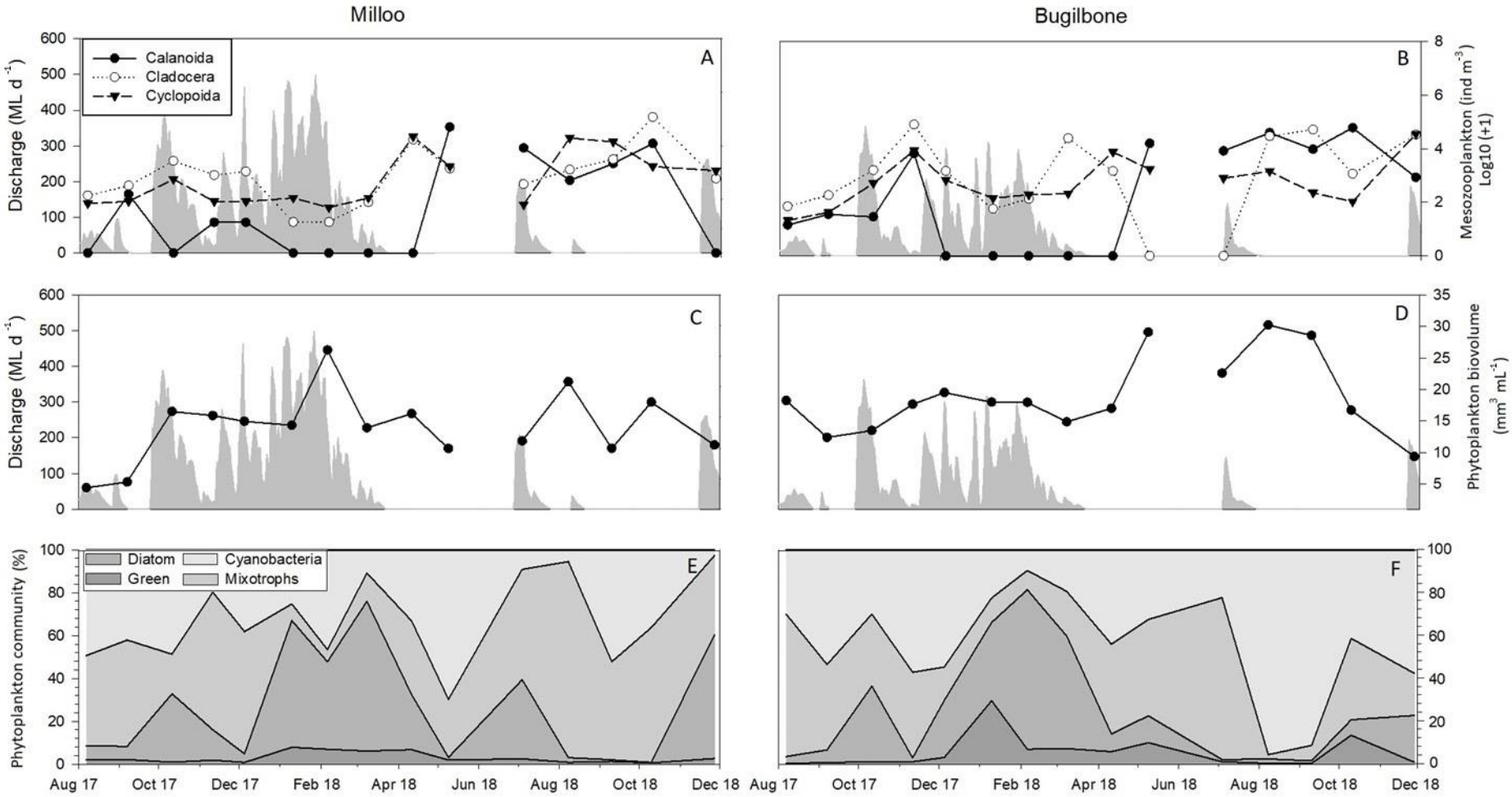

Figure 3.2 Zooplankton (A-B, Log10 transformed), total phytoplankton biovolume (C-D) and relative phytoplankton community composition (E-F) over the sampling period, the hydrograph for both sites is shaded grey.

Terrestrial and in-stream particulate organic matter

The $\delta^{13}C$ signatures of floodplain detritus had a small range of -29.5‰ to -28.1‰ and an average of -28.6‰. These $\delta^{13}C$ signatures were indistinguishable from fresh and dry redgum leaves and there was no difference observed between sites, highlighting the uniformity of terrestrial carbon signatures throughout the study area. In contrast, in-stream suspended particulate organic matter (POM) $\delta^{13}C$ signatures (Figure 3.3 A-B) varied across sites and hydrological conditions. The overall range of POM $\delta^{13}C$ signatures was lower at Milloo (-26‰ to -31‰) than at Bugilbone (-24‰ to -31.8‰), although both sites exhibited the largest variation during the cease to flow periods in 2018. POM ^{13}C values at Milloo were not significantly different from terrestrial detritus during flow (p=0.098) or during CTF (p=0.534) and were not significantly different across site flow conditions (p=0.334), averaging -29.2‰ and -28.8‰ respectively. Changes in POM $\delta^{13}C$ values were significantly related to phytoplankton biovolume during flow periods (p=0.0188, R^2=0.7006) and were near significant during cease to flow periods (p=0.0735, R^2=0.854). POM $\delta^{13}C$ values at Bugilbone were significantly different from terrestrial signatures at flow (p=0.002) and cease to flow (CTF) (p=0.007) stages, averaging -30.1‰ (±0.40) and -27.5‰ (±0.92) respectively. POM $\delta^{13}C$ values at Bugilbone were also significantly different from Milloo POM $\delta^{13}C$ during both flow categories and between flow conditions within Bugilbone (p=0.004). Changes to POM $\delta^{13}C$ at Bugilbone were significantly related to total phytoplankton biovolume across all flow periods (p=0.0137, R^2=0.5530), but not between individual flow groups (p>0.05). Phytoplankton community composition was not significantly related to changes in POM $\delta^{13}C$ values at either site (p>0.05).

Table 3.3 Permutational analysis of variance of POM 13C signatures between terrestrial detritus and POM (TDPOM) at flow and cease to flow conditions at Milloo (MF: flowing, MCTF: cease to flow) and Bugilbone (BF: Flowing, BCTF: Cease to flow).

PERMANOVA	Site/flow	*p*	t	perms
TDPOM vs	MF	>0.05	1.7576	866
	MCTF	>0.05	0.1148	982
	BF	0.002	3.7236	990
	BCTF	0.007	2.1949	975
MF vs	MCTF	>0.05	0.8886	993
	BF	0.046	2.1782	992
	BCTF	0.010	2.6121	997
MCTF vs	BF	0.029	2.7172	989
	BC	0.014	1.5432	996
Bugilbone flow vs	BC	0.004	3.4413	993

Zooplankton

Zooplankton $\delta^{13}C$ signatures (Figure 3.3 C-D) had broadly similar patterns to POM $\delta^{13}C$. Zooplankton $\delta^{13}C$ values were relatively stable at Milloo across all flow conditions (Flow range: -31.8 to -27.1‰, avg: -28.79‰; CTF range: -31.3 to -27.9‰, average:-29.41‰) compared to Bugilbone which had a similar range to Milloo during flow (-32.0 to -27.5 ‰, average:-29.23‰) but a larger range (-32.9 to -22.1‰) and more enriched average (-27.10‰) during CTF conditions.

Permutational analysis of variance (PERMANOVA; Table 3.3) indicated no significant difference in zooplankton $\delta^{13}C$ signatures between sites during flow periods (p= 0.178) or between flow groups at Milloo (p=0.465). During cease to flow periods zooplankton ^{13}C values at Bugilbone were significantly more enriched than zooplankton at Milloo (p=0.022) and during flow periods within Bugilbone (p=0.003). Linear regression analyses (Figure 3.4) showed that $\delta^{13}C$ signatures of all zooplankton taxa at Bugilbone were significantly related to local POM $\delta^{13}C$ signatures, with cladocerans more strongly related (p=0.0057, R^2=0.944) than calanoids (p=0.0049, R^2=0.650) and cyclopoids (p=0.0096 R^2=0.588). Neither calanoid nor cladoceran (p>0.05) $\delta^{13}C$ were significantly related to POM $\delta^{13}C$ at Milloo; however cyclopoid $\delta^{13}C$ s were strongly related to POM $\delta^{13}C$ (p=0.0038, R^2=0.838).

Changes in zooplankton $\delta^{13}C$ signatures during CTF conditions were strongly related to total phytoplankton biovolume at Milloo (p=0.0031, R^2=0.96) but not at Bugilbone (p>0.05). Neither discharge nor DOC concentrations were significantly related to zooplankton/POM

^{13}C signatures at either site (p>0.05). Zooplankton δ ^{13}C signatures were not significantly different when compared to different phytoplankton community compositions (p>0.05) at either site.

Table 3.4 PERMANOVA analysis of zooplankton carbon stable isotopes between flow groups and sites

PERMANOVA (^{13}C)		***p***	**t**	**F**	**perms**
Main test	Site	0.046	-	4.278	996
	Flow	0.018	-	6.352	998
	Site × flow	0.003	-	10.389	992
Flow vs CTF	Milloo	0.465	0.745	-	997
	Bugilbone	0.003	3.559	-	997
Between sites	Flow	0.178	1.347	-	995
	Pool	0.022	2.531	-	998

δ ^{15}N isotopes (Figure 3.3 E-F) were more clearly separated at Milloo than at Bugilbone and were not significantly different between sites during flow (Table 3.5) but were significantly different during CTF conditions (p=0.011, t=3.1468). At Milloo copepod zooplankton δ ^{15}N signatures were consistently more enriched (6-10‰) with an average of 8.5 compared to δ ^{15}N signatures of cladocerans which ranged from 4-6‰, averaging 5.5. Calanoid and cyclopoid δ ^{15}N values were not significantly different (p>0.05); however, both were significantly different from cladoceran δ ^{15}N values (p=0.002, t=4.318; p=0.006, t=3.108, respectively) for both flow groups. At Bugilbone δ ^{15}N separated less clearly between zooplankton taxa with cyclopoid copepods and cladocerans consistently overlapping across flow groups. Calanoid δ^{15}N values were significantly different from cladoceran δ ^{15}N values (p=0.024, t=2.414) but not cyclopoid δ ^{15}N values (p>0.05), and cyclopoid and cladoceran ^{15}N values were not significantly different from each other (p>0.05). Calanoid δ ^{15}N values were, however, typically higher than cyclopoid and cladoceran δ ^{15}N values, particularly under CTF conditions. Zooplankton δ ^{15}N values were significantly different between flow groups at Bugilbone (p=0.046, t=2.077) but not at Milloo (p>0.05).

Table 3.5 PERMANOVA with pairwise comparisons for δ 15N value of zooplankton across sites, flow conditions and zooplankton taxa

PERMANOVA (^{15}N)		***p***	**t**	**perms**
Flow vs CTF	Milloo	0.465	0.745	997
	Bugilbone	0.046	2.077	997
Between sites	Flow	0.892	0.220	998
	Pool	0.011	3.147	997
Between taxa (Milloo)	Cal vs Cyc	0.396	0.899	999
	Cal vs Clad	0.002	4.318	999
	Cyc vs Clad	0.009	3.108	999
Between taxa (Bugilbone)	Cal vs Cyc	0.064	1.967	999
	Cal vs Clad	0.024	2.414	997
	Cyc vs Clad	0.140	1.529	998

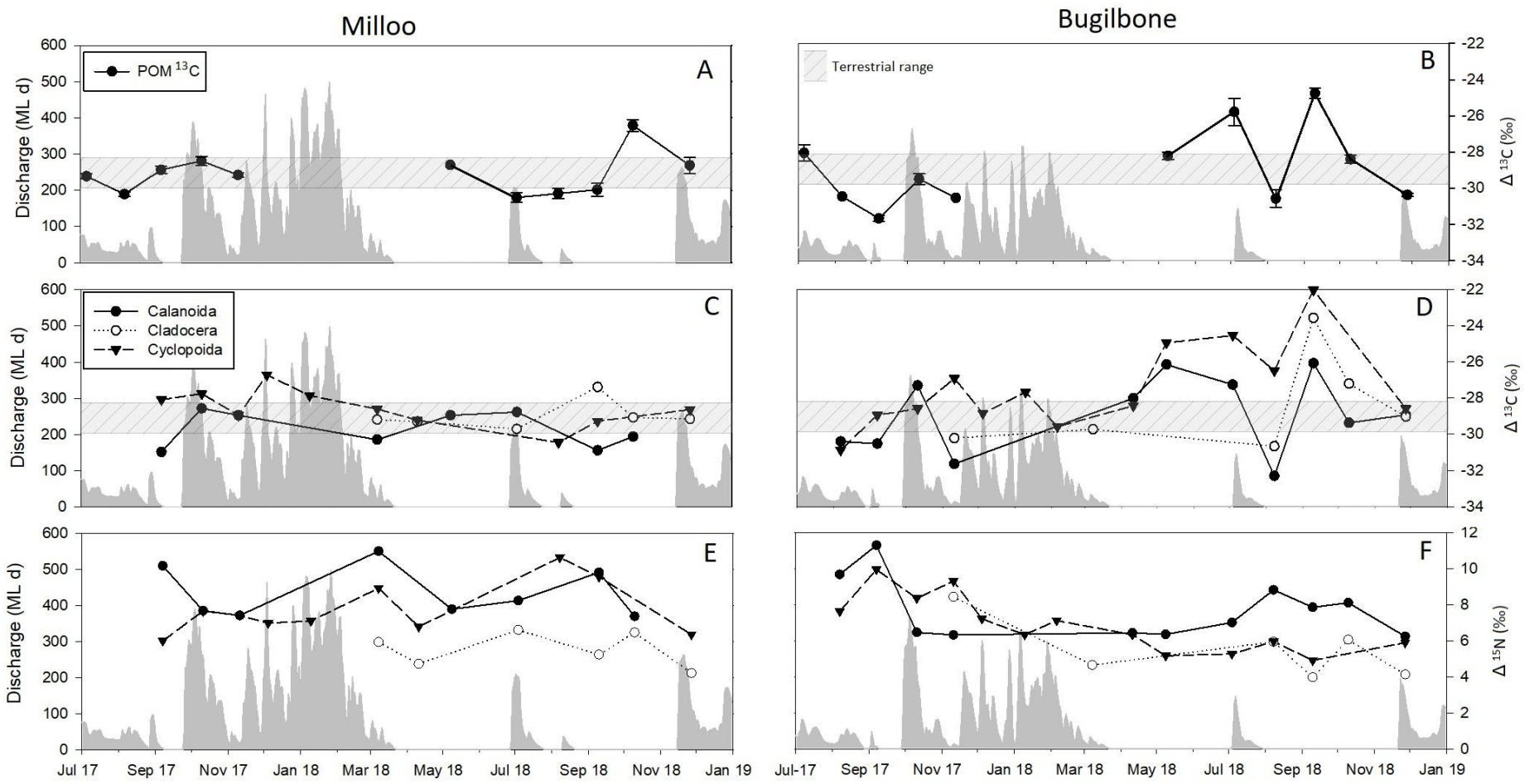

Figure 3.3 ^{13}C signatures for particulate organic matter (A-B) and zooplankton (C-D) and, ^{15}N for zooplankton (E-F). Discharge (ML d^{-1}) for each site is shown in shaded grey: Milloo (left column) and Bugilbone (right column).

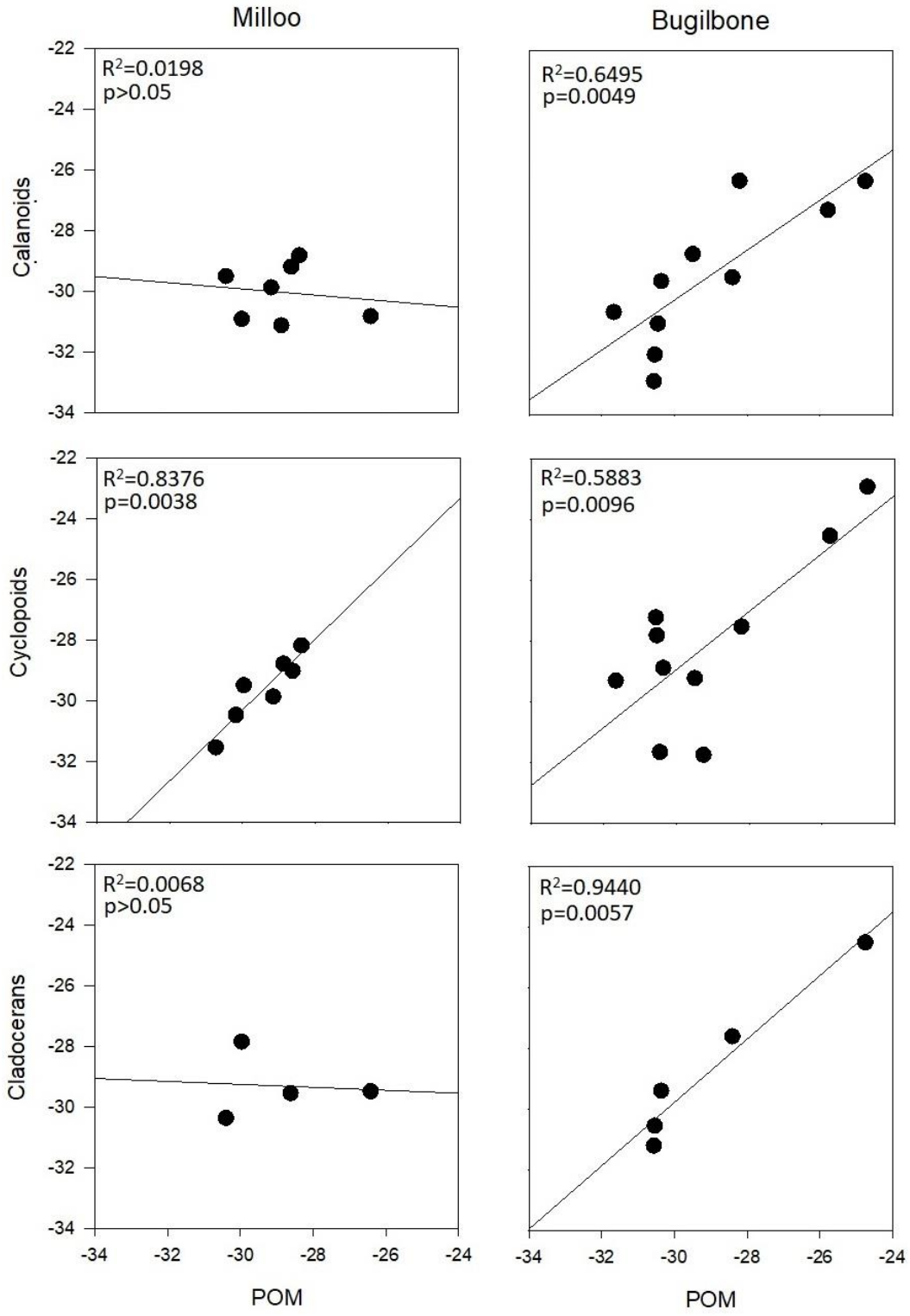

Figure 3.4 POM $\delta^{13}C$ vs zooplankton taxa $\delta^{13}C$ linear regressions with R^2 and p-value at Milloo (left column) and Bugilbone (right Column).

3.5 Discussion

This study examined two sites on the Namoi River during a period with flowing and cease to flow (CTF) conditions, offering an insight into the energy sources used by the lower food web across a range of hydrological conditions. The $\delta^{13}C$ signatures of terrestrial organic matter were very similar across sites with no temporal variation and were comparable to those of previous studies in eastern Australia (Reid et al., 2008; Hadwen et al. 2010a, Medeiros and Arthington, 2011; Rees 2020). We therefore asserted that any particulate organic matter or zooplankton $\delta^{13}C$ values that were more enriched or depleted than the terrestrial range were indicators that phytoplankton dominated the POM or zooplankton growth at that time (Hadwen et al., 2010a; 2010b). We found $\delta^{13}C$ values of zooplankton to be highly dynamic, varying significantly between sites and flow conditions. On the basis of the isotope evidence, suspended POM was a major food source and driver of change in zooplankton $\delta^{13}C$ signatures at both sites. However, the composition of suspended POM varied through time. Specifically, differences were evident between sites and especially flow conditions, with signatures aligning with terrestrial $\delta^{13}C$ signatures during flow periods and less so during periods of CTF. Furthermore, $\delta^{13}C$ signatures of POM were significantly related to changes in phytoplankton biovolume. Flow drove large changes in the phytoplankton and zooplankton communities at both sites and the $\delta^{13}C$ values of zooplankton at Bugilbone. Interestingly, riparian vegetation may also have been important, offering a stable energy source across flow conditions at Milloo. These results support our hypothesis that the source of energy driving food web production is significantly influenced by hydrological conditions.

During flow periods zooplankton $\delta^{13}C$ signatures were not significantly different between sites and were within or close to the range of terrestrial organic carbon at both sites (Figure 3.3). This similarity across sites may be a function of instream turbulence during flows, reducing the ability for zooplankton to selectively feed and potentially feeding indiscriminately on the POM pool (Berggren et al., 2018). POM or seston has been found to be an important carbon source for Australian riverine food webs at high flow (Hladyz et al., 2012), including when the POM is predominately composed of allochthonous organic matter (Roach et al., 2009). Changes in phytoplankton composition had no significant effect on zooplankton or POM $\delta^{13}C$ values during flow. This suggests POM was primarily composed of allochthonous matter during flow, particularly at Milloo where POM $\delta^{13}C$ was not significantly different from terrestrial detritus values. Zooplankton allochthony is in part

driven by high heterotrophic production relative to phytoplankton concentration (Karlsson et al., 2003). During flow periods it is likely that zooplankton are primarily using allochthonous energy to support production both due to the disruptive impacts of flow on phytoplankton productivity and the abundance of terrestrial DOC in flows (Berggren et al., 2018). Despite very low local riparian or allochthonous inputs, zooplankton $\delta^{13}C$ values were not significantly different from Milloo during flow and were often within the range of terrestrial matter. Longitudinal movement of organic matter from upstream sources (riparian and within channel) may have provided resources for downstream zooplankton during periods of higher flow and turbulence. Thus, downstream movement of organic material (RCC) may have played an important role in driving zooplankton $\delta^{13}C$ values at Bugilbone during flow periods (Vannote et al., 1980). Similarly to Milloo, phytoplankton composition had no significant effect on the $\delta^{13}C$ values of zooplankton at Bugilbone and phytoplankton biovolume only weakly effected POM signatures.

In sharp contrast to periods of flow the two sites showed clearly different patterns in the $\delta^{13}C$ values of zooplankton and POM at cease to flow conditions. Once flows had ceased, zooplankton $\delta^{13}C$ values at Bugilbone separated clearly from those at Milloo which did not change from flow conditions. An explanation for this may be the dense riparian vegetation at Milloo compared to Bugilbone. The constant leaf fall and riparian inputs at Milloo may have offered a more consistent nutrient and energy source for the lower food web compared to the cleared floodplain of Bugilbone. Riparian canopy cover may also decrease light intensity for in-stream algal production due to shading, reducing total phytoplankton biovolume (Bunn et al., 1999). Evidence for this reduction in photosynthesis may be seen in the lower total phytoplankton biovolume at Milloo compared to the cleared vegetation at Bugilbone during CTF conditions. Previous studies have found local riparian vegetation to play an important role as a reliable and stable energy source for waterholes during long dry periods (Reid et al., 2008; Kelleway et al. 2010; Leigh et al., 2010). Further, food web structure at Milloo was suggestive of constant terrestrial organic matter inputs (supplied by leaf fall from dense canopy cover), as bacterivorous mixotroph concentrations suggested potentially higher production via the microbial loop compared to Bugilbone. In contrast, POM and zooplankton $\delta^{13}C$ values at Bugilbone were significantly different from both Milloo and between flow conditions. One possible explanation for this may be the lack of a consistent energy source such as riparian vegetation at Bugilbone and thus large changes in the dominant energy source between flow conditions. Phytoplankton likely played a major role in these diversions

away from terrestrial vegetation signatures as enriched $\delta^{13}C$ values correspond to high photosynthesis (Macleod and Barton, 1998). This is supported by much higher total phytoplankton biomass at Bugilbone (Figure 3.2D) and previous research finding waterholes with cleared riparian vegetation are dominated by autochthonous production owing to reduced inputs and increased light availability (Bunn et al., 1999). Further, Hadwen et al., (2010) found drought periods increased the relative importance of local autochthonous production for higher consumers. Ultimately, the presence of riparian vegetation may have been the main factor in the difference in zooplankton and POM $\delta^{13}C$ values between sites. Constant inputs of organic matter from riparian canopy cover may further decrease phytoplankton production as bacteria use allochthonous DOM and out-compete phytoplankton for resources (Carney et al., 2016). Thus, the basal resources supporting waterhole foodwebs may be significantly altered by the presence (or absence) of riparian vegetation (Reid et al., 2008).

$\delta^{15}N$ values offered further insights into the food web stability between flow groups and sites. Zooplankton $\delta^{15}N$ values at Milloo showed food web structure remained similar across both flow groups with calanoids and cyclopoids occupying a higher trophic level compared to *Daphnia* (assuming fractionation between 2.5 and 3.4% per trophic level, Post 2002). Oppositely at Bugilbone, $\delta^{15}N$ values were highly variable and significantly different between flow groups with no clear patterns during periods of flow. This suggests that without a stable energy source food webs are highly susceptible to the disturbances of flow as suggested by Berggren et al., (2018).

Zooplankton $\delta^{13}C$ relationships to POM $\delta^{13}C$ varied significantly between sites and taxa. Calanoids, cyclopoids and cladocerans use distinctly different feeding strategies and thus, consume allochthonous organic matter in different ways (Berggren et al., 2014). Cyclopoids are generally raptorial feeders preying on microzooplankton as a major food source and are consequently considered semi-dependent on allochthonous materials (Barnett et al., 2007; Berggren et al., 2010). This may explain the stronger relationship to POM and higher concentration of cyclopoids at Milloo where allochthonous inputs and consequently microbial production may have been higher. Oppositely, many calanoids are selective suspension feeders, often reliant on phytoplankton for the bulk of their diet (Berggren et al., 2015), mostly consuming allochthonous carbon via POM or mixotrophic flagellates (Pace et al., 2004; Trochine et al., 2015). Calanoids were only significantly related to POM $\delta^{13}C$ at Bugilbone where allochthonous inputs were low and autotrophs likely formed the bulk of the

POM, particularly during CTF conditions. This suggests that calanoids may have been selectively feeding on phytoplankton and flagellates at Milloo, as seen previously in low flow rivers (Berggren et al., 2018). Similarly, *Daphnia* were only significantly related to POM δ ^{13}C values at Bugilbone. Cladocerans have been found to feed directly on bacteria (Kamjunke et al., 1999) and thus can incorporate allochthonous matter more efficiently than copepods feeding on higher trophic levels (Berggren et al., 2014). However, *Daphnia* have been found to grow poorly with low survival rates when provided with a mainly bacterial or terrestrial food source (Brett et al., 2009). This has been suggested due to the poorer quality of bacteria as a food source, lacking essential fatty acids that can only be synthesised via photosynthesis (Harwood and Russell, 1984; Taipale et al., 2012). Indeed, previous studies have found calanoids and cladocerans to have an upper limit in allochthonous matter consumption (Karlsson et al., 2012). This may explain why they were only related to POM δ ^{13}C at Bugilbone where phytoplankton biovolume was much higher, dominating the seston compared to Milloo. Nevertheless, calanoids and *Daphnia* were supported in high numbers at both sites, suggesting resources still support growth despite being dominated by allochthonous materials.

A previous study in floodplain lagoons of the Macintyre River, Australia, suggested zooplankton received most of their energy from autochthonous production (Medeiros and Arthington, 2011). Our results suggest the relative importance of allochthonous and autochthonous support is taxa-dependent as seen by Berggren et al, (2014). Further, zooplankton were strongly influenced by local inputs of allochthonous organic matter and used different carbon sources between waterholes (CTF) and flow conditions. Similarly, Bunn et al. (2003) suggested calanoids and cladocerans were primarily supported by phytoplankton in the waterholes of Cooper Creek, Australia. Our results draw similar conclusions for these taxa during CTF periods, particularly at Bugilbone where riparian vegetation is largely absent. However, during flow events, when POM δ ^{13}C more closely reflected terrestrial organic matter, Cladocera and calanoids appeared to assimilate allochthonous OM, suggesting a dynamic capacity for switching between autochthony and allochthony across different taxa.

The findings of this study support several different river productivity concepts depending on the hydrological conditions present, as suggested by Hoeinghaus (2007). During periods of flow longitudinal movement of organic matter (Vannote et al., 1980) appeared to mobilise allochthonous carbon sources for the downstream food webs, particularly at Bugilbone. Due

to low flow conditions throughout the study it was difficult to accurately test the flood pulse concept (Junk et al., 1989) as no overbank flows occurred. However, the potential for carbon pulses to significantly boost growth appeared plausible as zooplankton $\delta^{13}C$ signatures often reflected terrestrial signatures during flow periods. During CTF conditions both sites appeared to strongly reflect aspects of the riverine productivity model (Thorp and Delong, 1994). As autochthonous production at Bugilbone and local riparian production coupled with phytoplankton at Milloo appeared to play significant roles in supporting food webs.

It has been previously suggested that the use of allochthonous carbon is fundamentally different between rivers and lakes (Berggren et al., 2018). Our study examined these lentic and lotic conditions at the local scale within the same river. We drew similar conclusions to Berggren et al. (2018) suggesting that allochthony may range from very high during flow events to almost zero during CTF conditions depending on local riparian vegetation. Previous studies have suggested food webs based on a reliable source of energy such as riparian vegetation inputs are more resilient to disturbances (Closs and Lake, 1994). This resilience may be particularly advantageous for food webs in rivers susceptible to CTF conditions during droughts (Closs and Lake, 1994; Reid et al., 2008). Given the importance of zooplankton to the diets of small-bodied fish in Australian waterholes, often forming the bulk of their diet (Medeiros and Arthington, 2008; 2011) it is likely the effects of flow on basal resources and consequent effects on zooplankton production will reach fish populations (Jardine et al., 2015) and have a considerable effect on the production of higher trophic levels. Increasing and protecting riparian zones around inland rivers, particularly around known waterholes may greatly increase their resilience to climate change and drought. Flow events that flush-out and connect waterholes to downstream organic matter may further increase the long-term survival of these river refuges.

This study has several limitations that further research should address. Examining the range of phytoplankton $\delta^{13}C$ values that occur in Australian lowland rivers would be an important step in understanding the role of allochthonous vs autochthonous carbon in these rivers. Further studies addressing the $\delta^{13}C$ signatures of zooplankton at flow and cease to flow conditions across a range of river catchments, environments and over a longer time scale would provide a deeper understanding of some of the research gaps investigated in this research.

Chapter 4: Allochthonous dissolved organic matter support large increases in riverine mixotrophs and zooplankton in a mesocosm experiment

4.1 Abstract

There is still considerable debate as to whether the allochthonous dissolved organic matter mobilised during large flow events plays an important role in supporting secondary production in riverine food webs. Understanding how food webs respond to large pulses of terrestrial DOM (tDOM) is important to conceptualise how flow events effect food webs. A mesocosm experiment (1000L) using 3 concentrations of leachate made from floodplain dissolved organic matter (1mg C L^{-1}, 4mg C L^{-1}, 8mg C L^{-1} and a control) was run for 34 days in a dam filled with water from Gunbower Creek in Northern Victoria, Australia. Nutrients, phytoplankton, zooplankton and ^{13}C stable isotopes were collected to examine how floodplain nutrients effect production and community structure within the lower food web typical of an Australian lowland river. All leachate additions led to very high concentrations of zooplankton and mixotrophic algae compared to the control. Mixotrophs dominated the algal biovolume of all leachate additions until day 20 and appeared to drive changes in $\delta^{13}C$ signatures of POM which were significantly related to changes in zooplankton $\delta^{13}C$ signatures. Obligate autotrophs were not significantly reduced by tDOM additions and also appeared important as a food source as reflected in the ^{13}C signatures of zooplankton and POM after day 10. The results of this study showed the ability of phytoplankton and zooplankton communities in lowland rivers to respond quickly to changes in resource availability and quality. Ultimately our data suggests allochthonous DOM may be bioavailable and support production through a number of different trophic pathways including mixotrophy, offering a large boost to production via both autotrophy and heterotrophy. We suggest that mixotrophy may be an important pathway for allochthonous organic matter to enter riverine food webs and support secondary production.

4.2 Introduction

Energy in lowland riverine food webs can be conceptualised as originating from 2 sources, within system photosynthesis, primarily from phytoplankton (autochthonous production) and external terrestrial organic matter (allochthonous production). The majority of allochthonous organic matter is delivered to Australian lowland rivers during flood events as increased

hydrological connectivity with floodplain environments mobilises large amounts of terrestrial dissolved organic matter (tDOM) (Westhorpe and Mitrovic, 2012). These energy pulses have been hypothesised as important resource subsidies which sustain riverine food webs and the productivity of aquatic ecosystems (Junk et al. 1989; Burford et al., 2010). However, there is considerable debate as to whether allochthonous organic matter, in particular the dissolved portion, supports production, especially at higher trophic levels in freshwater food webs (Cole et al., 2011; Thorp and Delong, 2002; Brett et al., 2012). Much of the debate to date has focused on resource quality, whereby allochthonous organic matter is considered to be of a lower quality than autochthonous matter, due to its higher recalcitrance and lower fatty acid content (Brett et al., 2009). Furthermore, for tDOM to support higher trophic levels several extra links in the food chain are required to reach higher trophic levels (due to uptake via the microbial loop), greatly reducing its carbon transfer efficiency compared to the autochthonous energy pathway (Sommer et al., 2002; Brett et al., 2009).

Despite the prevailing view that tDOM is a poor quality carbon source, the quality of allochthonous DOM is variable (Berggren et al., 2010) with environmental conditions such as rain events and periods since terrestrial wetting greatly effecting the bioavailability of allochthonous organic matter (Baldwin et al., 2016). Less than 40% of DOC released from red gum detritus is readily bioavailable (Baldwin, 1999) whereas the recalcitrant portion breaks down more slowly (Gawne, 2007). The two portions may both play important roles in supporting food webs as an initial pulse of microbial production during flood events followed by a slow release which may maintain food web stability during environmental variations (Wetzel, 1995). Furthermore, the high volume of tDOM mobilised during flood events may mean that even if transfer efficiencies are low, there may still be significant subsidies to higher trophic levels of food webs (Pace et al., 2005; Tanentzap et al., 2017). Ultimately, the impact of allochthonous subsidies on riverine food webs may be highly dependent on the quality and quantity of tDOM, coupled with the local environmental conditions during flow events, making the potential for tDOM subsidies highly variable between ecosystems (Marcarelli et al., 2011).

Thorp and Delong (1994) suggested in their Riverine Productivity model, that during base flow conditions energy mobilised by phytoplankton through photosynthesis forms the base of lotic foodwebs. However, during times of high tDOM concentrations such as during flood events, bacteria may become dominant as they are decoupled from autochthonous production while phytoplankton growth is simultaneously suppressed by low light availability (Drakare

et al., 2002; Jansson et al., 2007; Carney et al., 2016). This potential dominance by bacterioplankton (Jansson et al., 2000) can also lead to a consequent reduction in the stoichiometric quality of autotrophs due to nutrient limitation, leading to reductions in food quality for metazoan consumers (Danger et al., 2007). Emerging evidence suggests mixotrophic microalgae that are able to obtain energy through both photosynthesis and phagotrophic consumption of bacteria may efficiently link allochthonous DOM to higher consumers (Flynn et al., 2013; Hansson et al., 2019). Mixotrophy may be advantageous during or following flood events when bacterial abundance may be high and light levels are reduced due to coloured DOM and suspended sediments (Kamjunke, 2007). Further, mixotrophs are considered an ideal food sources for zooplankton due to their nutrient stoichiometry being closer to that required by zooplankton (Katechakis, 2005; Hansson et al., 2019). Thus, mixotrophic microalgae may provide an alternate pathway through which allochthonous carbon can support higher trophic levels (Flynn et al., 2013).

Zooplankton are the main consumers of planktonic organisms in freshwater food webs, making them a crucial link between the lower food web and higher consumers (Kobayashi et al., 1998). The level of allochthonous support of zooplankton may vary significantly with different feeding behaviours (Berggren et al., 2014). Experimental evidence has found *Daphnia sp*. were unable to survive on bacteria fed tDOM alone and required phytoplankton for basic survival and reproduction (Brett et al., 2009). However, subsequent studies have shown that bacteria can supplement up to 50% of the phytoplankton diet with no adverse effects (Wenzel et al., 2012; McMeans et al., 2015). Furthermore, Degerman et al., 2018 found additions of glucose (DOC) lowered food web efficiency but still resulted in a net increase in zooplankton production. Mesocosm studies have found tDOM additions, in the form of leachates, increased rotifer and copepod production considerably in the Namoi and Bega Rivers (Mitrovic et al., 2014; Hitchcock et al., 2016). Hitchcock et al. (2016) further found calanoid $\delta^{13}C$ signatures were more similar to that of the leachate treatments as leachate concentration increased, indicating that these zooplankton were assimilating the added allochthonous carbon. Field data using ^{13}C stable isotopes has also indicated potentially high allochthonous carbon utilization in higher trophic levels, with some zooplankton using up to 50% (Pace et al., 2005) of carbon from allochthonous sources and fish up to 20% (Jones et al., 2018). These studies highlight the variable and potentially significant role of allochthonous carbon processing by bacteria in supporting metazoan freshwater food webs.

This study aimed to better understand how allochthonous dissolved organic matter inputs influence the structure and production of planktonic aquatic food webs. To understand the role of tDOM on riverine food webs we tested 3 hypotheses:

(I) Pulses of allochthonous dissolved organic matter would increase zooplankton production relative to the concentration of the DOM added, with higher concentrations supporting more production.

(II) That the $\delta^{13}C$ values of zooplankton would be closer to the leachate $\delta^{13}C$ values than the control, reflecting tDOM assimilation by the end of the experiment.

(III) Autochthonous production, specifically chlorophytes, would be reduced by pulses of tDOM, such that mixotrophs would dominate the algal community.

To test these hypotheses, we used mesocosms similar to those of previous studies (Karlsson et al., 2007; Faithfull et al., 2012, Mitrovic et al., 2014; Hitchcock et al., 2016) to experimentally manipulate a riverine food web with tDOM leachate additions. This experiment ran for 34 days, which is long enough to examine the full effects of a flood pulse and zooplankton generation time responses, as demonstrated in previous field research (Shiel et al., 2006).

4.3 Methods

Gunbower Creek, 35°47'45.4"S 144°13'16.0"E is a major tributary of the Murray River. Floodplain inundation, resulting in large pulses of allochthonous organic matter, is relatively common on Gunbower Creek during and after large flow events (Nielsen et al., 2015). River redgums (*Eucalyputus camaldulensis*) are the most common riparian vegetation in the area which is typical of floodplains on Australian lowland rivers (Westhorpe and Mitrovic, 2012). The mesocosm experiment was performed in a private PVC-lined dam (2.5m deep, 10m wide, 50m long) set next to the Gunbower Creek and which was filled directly from the creek using irrigation pumps. The dam was refilled 5 days prior to the experiment and topped up again the day prior to filling the mesocosms. Pumps at either end of the dam circulated water and prevented stratification throughout the waterbody.

To prepare the leachate, floodplain materials were collected from 8 randomly distributed $1m^2$ quadrats on the floodplain of the Murrumbidgee River 20km west of Gundagai NSW. All loose materials within the quadrat were collected, most of the organic material collected was dry, comprising of decaying leaves and sticks and some fresh grasses. The most common vegetation was river redgum (*Eucalytpus camaldulensis*) followed by casuarina

(*Casuarina cunninghamiana*), weeping willow (*Salix Babylonica*) and various grasses. All human litter such as plastics and glass bottles were removed before bagging. The leachate was made using a similar technique to that of Mitrovic et al. (2014). Floodplain materials were placed in two 70L bins and soaked in 100L of reverse osmosis water for 2 weeks at 4°C in the dark. Floodplain materials were swapped in/out of the bins every 3 days to maximise leachate concentration. The resulting leachates were then filtered through a series of filter sizes (10um and 1.3um) to 0.5µm using a vacuum pump and glass fibre filter papers. Once filtration was complete the leachate was homogenised and frozen at -20°C.

The mesocosms were built using bulk bags with a waterproof PVC liner, each bag measured 90cm by 90cm wide and were 160cm deep. Mesocosms were secured into three groups of four, representing the three treatments and control in triplicate. Each group of mesocosm bags was held together using a reinforced PVC pipe framework 200cmx200cmx200cm with the mesocosm bags held firmly inside this framework using rope similar to that of Hitchcock et al., (2016). Each frame was submerged until only the top 30cm of each mesocosm bag was above water and held in the water column using floats. This led to an end total volume of 1000L for each mesocosm. The frame of each group was anchored at 4 points to both sides of the dam to stop any potential drifting from wind. The grouped mesocosms were also tied to each other to make a continuous line to minimise any variation in light environment. To stop birds and leaves or branches entering the mesocosms, each were covered with wire (with a $1cm^2$ aperture).

The experiment ran for 34 days from the 31st of October until the 4th of December 2019. Mesocosm bags were filled one day before commencing the experiment using an electric pump and hose with a 4cm aperture. A flow rate was calculated for the pump and the filling of each bag was timed to ensure an equal volume of water in each mesocosm. Day 0 samples were taken after all bags were filled and before the leachate was added. The leachate was then added on the 31st of October (referred to as day 0). Leachates were added in 3 concentrations based off the dissolved organic carbon content of the leachate; low (1mgC L^{-1}), medium (4mgC L^{-1}), High (8mgC L^{-1}) all performed in triplicate including a control (no leachate addition). These concentrations were chosen to represent 3 different sized flow events and the consequent levels of allochthonous inputs from a small ‘fresh’ up until an overbank flood. All mesocosms, including controls, were mixed thoroughly after leachate addition.

Sampling

To measure changes in photosynthesis and respiration dissolved oxygen and temperature were measured using an HACH HQ20 LDO probe. Measurements were taken on days 0, 1, 3, 5, 6, 12, 20, 27 and 34.

Nutrients and chlorophyll-a

Samples of nutrients and chlorophyll-a (Chl-*a*) were collected on days 0, 1, 4, 8, 20 and 34. DOC, SRP and NOx samples were collected in pre-washed and sample rinsed 250 mL PET bottles, filtered to 0.45µm using cellulose acetate syringe filters and then frozen. Samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA, 2005). Samples for chlorophyll *a* (Chl-*a*) were determined by filtering 250 mL of water onto GF/C filters. Filters were frozen until subsequent determination by boiling ethanol extraction according to Standard Methods (APHA, 2005). Half of each Chl-*a* filter paper was removed before analysis and stored for $\delta^{13}C$ isotope analysis of particulate organic matter (POM).

Phytoplankton and protozoa

Phytoplankton were separated into broad functional groups similar to those of Karlsson et al., (2007). Composite water column samples for phytoplankton and ciliate analysis were taken using a bendable 1m (4cm aperture) long plastic pipe, with samples preserved using 3mL Lugol's iodine solution. Samples were taken on days 0, 1, 2, 4, 6, 8, 12, 20, 27 and 34 and counted at 200x magnification on a compound microscope using Sedgwick rafter counting cells. Measurements for biovolume were taken using an Olympus DP72 camera and cellSens Standard software (version 1.3). 20 individuals of each species were measured to achieve a reliable average. Algae were identified using the keys of Prescott, 1978 and Entwisle et al., 1997. Amoeba and Ciliates were counted with phytoplankton samples and identified using the key of Patterson, 1996.

Zooplankton

Zooplankton samples were collected for enumeration and stable isotope analysis on days 0, 1, 4, 8, 12, 20, 27, 34. To do this, 10L of mesocosm water was passed through a 53µm mesh and decanted into a pre-rinsed PET bottle. Zooplankton were purged for 4 hrs using 0.5µm filtered mesocosm water then preserved with >70% ethanol v/v. For enumeration, zooplankton were concentrated to 250x and a subsample (25% of total sample) counted on a

Sedgwick rafter cell at 100x magnification using a compound microscope. Zooplankton were identified using the key of Shiel (1995). Samples were returned to bottles and preserved using the original ethanol from each bottle. Zooplankton samples for $\delta^{13}C$ analysis were rinsed thoroughly three times with reverse osmosis water to ensure all ethanol was removed. Samples were then picked using a dissecting microscope at 8x magnification. Zooplankton were picked into appropriate taxa, then further cleaned for impurities such as organic matter, incorrect zooplankton group or filamentous algae, before being placed into 5mm silver capsules. Samples were acidified using 1mol L^{-1} HCL to remove any inorganic carbon from samples and dried at 60°C for 24 hrs. Zooplankton samples were pooled between replicates to ensure enough biomass for analysis, replicates were pooled for all samples to avoid variation between replicates biasing results. Samples were analysed at Griffith University using a continuous flow isotope mass spectrometer (GV Isoprime Eurovector EA 3000, Manchester, UK). Results were determined using IAEA-CH-6 as standard reference material.

Statistical analysis

Permutational multivariate analysis of variance using PRIMER 6.0 +PERMANOVA (Anderson et al., 2008) was used to analyse data for significant differences between treatments. Pairwise comparisons within PERMANOVA were used to test for significant differences between treatments within sampling days similarly to Hitchcock et al. (2016). All data was checked for normal distribution and homogeneity of variance using PERMDISP and draftman's plots. Euclidean distances were used for environmental variables and transformed using Log10 to account for skewed distribution and then normalised. Bray-Curtis distances were used for phytoplankton, mixotrophs and zooplankton and analyses run separately for individual taxa or functional groups. Species data was transformed using a square-root transformation. As zooplankton $\delta^{13}C$ data was pooled, no statistical analysis was conducted on the results. Instead, linear regressions were used to compare POM $\delta^{13}C$ signatures to zooplankton $\delta^{13}C$ and the ratio of mixotrophs to autotrophs ratio using Sigmaplot software. All regressions were checked for normal distribution using Shapiro-Wilk's test. The ratio of mixotrophs to autotrophs was calculated using the total biovolume of potential mixotrophs vs the total biovolume of all obligate autotrophic algae. This ratio data was then Log10 transformed to account for skewness.

4.4 Results

Dissolved organic carbon and nutrient concentrations (Figure 4.1.) increased relative to leachate additions and returned to starting concentrations by day 27. SRP concentrations in the high DOM treatment remained higher than all other treatments for the duration of the experiment. DO was negatively correlated to leachate additions, showing the biggest decrease immediately after leachate addition on day 1 in the high DOM treatment (20%). DO did not return to levels similar to the control until day 20. Chlorophyll-a (chl-a) concentrations in the leachate addition were significantly different from the control until day 34, although at times concentrations were higher and other times much lower. Chl-a concentrations in the medium and low treatments were not significantly different throughout the study. Chl-*a* was lowest in the high tDOM treatment until day 20, after which it was not significantly different from medium and low treatments. During the first 9 days of the experiment, Chl-*a* was highest in the control and significantly different from all other treatments. By Day 20, Chl-*a* decreased sharply in the control treatment, falling to a level below that of all other treatments. Measures of Chl-*a* on day 34 revealed similar levels across all treatments at the end of the experiment.

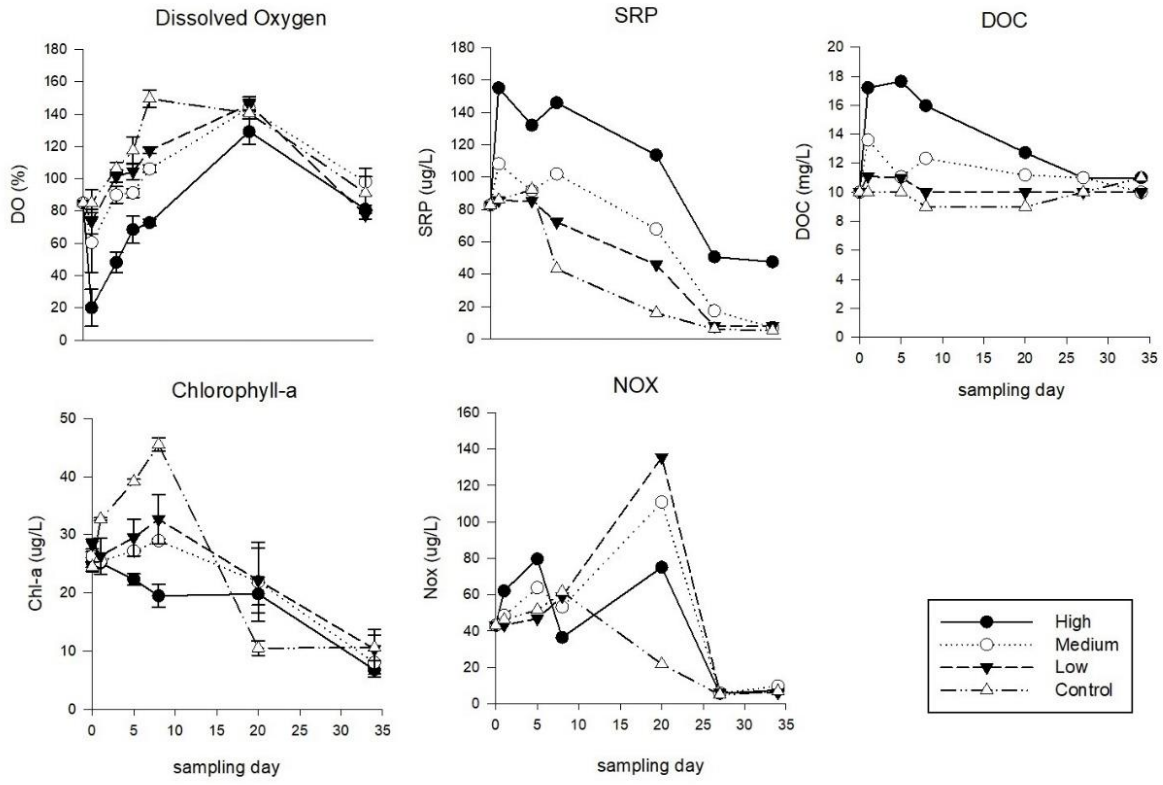

Figure 4.1 Dissolved oxygen (%), Soluble reactive phosphorus (SRP, ug L^{-1}), Dissolved organic carbon (DOC, mg L^{-1}), Chlorophyll-a (ug L^{-1}) and nitrogen oxides (ug L^{-1}), in all treatments across the sampling period. Treatments are identified by black circle (high), white

circles (medium), black triangles (low) and white triangles (control). Error bars represent the standard error of the mean.

Chlorophytes and mixotrophs were the most abundant algal groups throughout the study and at times were one to two orders of magnitude higher in biovolume than diatoms and cyanophytes (Figure 4.2). The biovolume of all algal groups differed significantly across time and the time x treatment interaction (p=0.001, all groups; Table 4.1), however only mixotroph and cyanophyte biovolume was significantly different between treatments (p:0001; Table 4.1). Chlorophyte and diatom biovolume were not significantly different between treatments (p>0.05) with Chlorophyte biovolume (Figure 4.1) generally increasing in all treatments from day 0 to 34. Mixotroph biovolume (Figure 4.1D) increased in all leachate treatments immediately after carbon additions, peaking in the high treatment at 7.4 mm^3 L^{-1} on day 12, which was 6.0 mm^3 L^{-1} higher than the control (1.3) and 2.2 mm^3 L^{-1} higher than the medium and low treatments, which were not significantly different from each other (p>0.05). Mixotroph biovolume was higher than all other algal groups in the medium and low treatments until day 12 and in the high treatment until day 20. Mixotroph biovolume in the high treatment was significantly different from the control by day 4 (p=0.014) and day 12 vs medium (p=0.018) and vs low (p=0.010) treatments. The low and medium treatments were significantly different from control by day 4 (low; p=0.033) and day 6 (medium; p=0.009) and returned to control levels on day 20 (p>0.05). The medium and low treatments were not significantly different from each other at any time point (p>0.05). Chlorophytes dominated the biovolume throughout the entire study in the control and in the tDOM treatments following reductions in mixotrophs, after day 20.

Cyanophyte biovolume (Figure 4.2B) was highest in the control group, peaking at 0.305 mm^3 L^{-1} on day 27. Leachate addition treatments led to lower cyanobacterial biovolume, reducing from day 0 levels until not being detected in the high treatment at day 8 and at days 27 and 34 in the low and medium treatments, respectively. Diatom biovolume (Figure 4.2C) was not significantly different between treatments and remained low throughout the duration of the experiment.

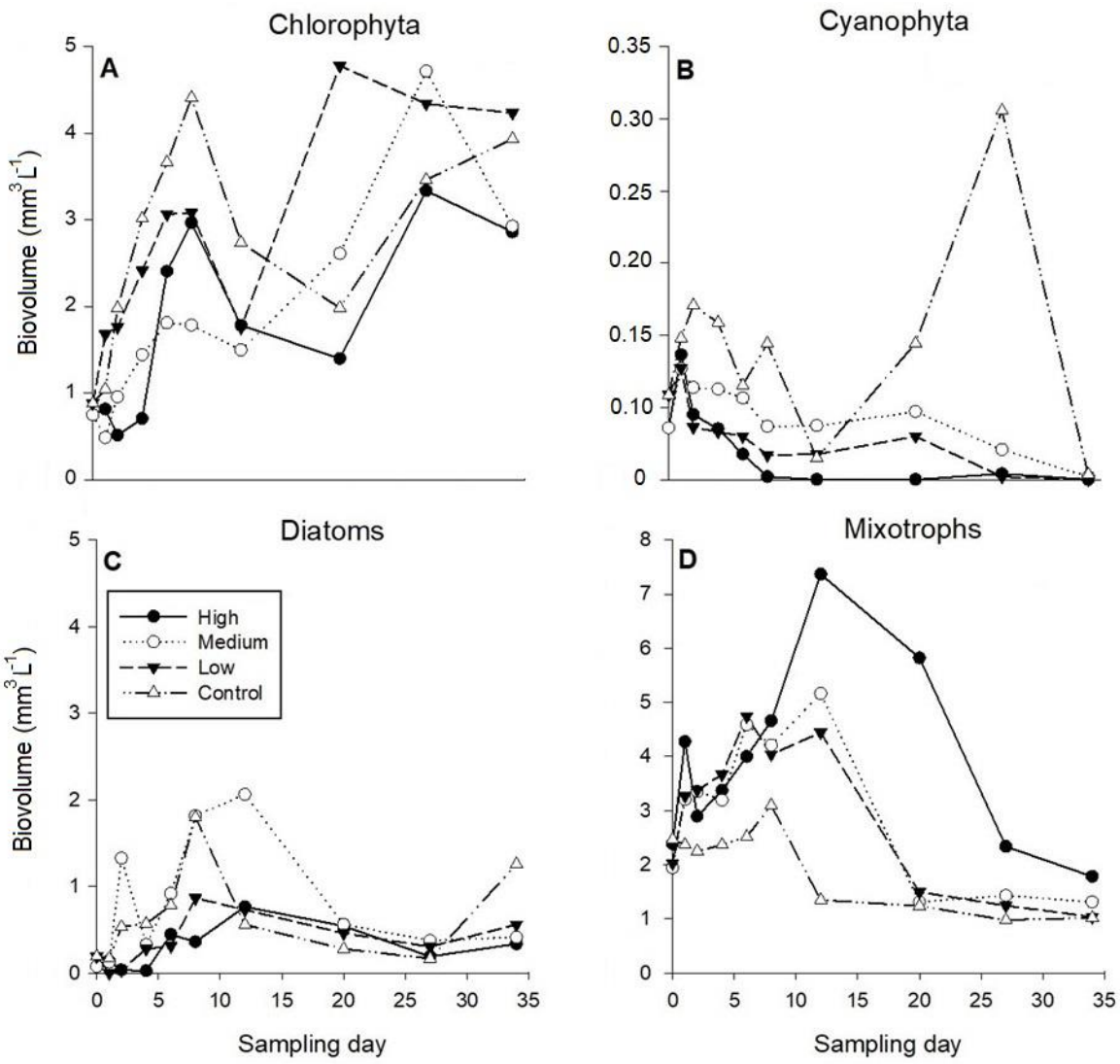

Figure 4.2 Total biovolume (mm^3 L^{-1}) of algal groups (A:chlotophytes, B: cyanophytes, C: diatoms, D:mixotrophs) in each treatment over time. Treatments are shown by black circle (high), white circle (medium), black triangle (low) and white triangle (control). Standard error bars removed for clarity.

The biovolumes of all mixotrophic genera and ciliates varied significantly through time (p=0.001, all groups). *Trachelomonas, Cryptomonas, Chroomonas* and Amoeba were also all significantly different between treatments (p=0.001; Table 4.1) with leachate additions resulting in higher concentrations of mixotrophs compared to the control (Figure 4.3A-D). *Trachelomonas* concentrations (Figure 4.3A) immediately increased following leachate addition. Low and medium treatments were significantly different from the control from days 1-12 peaking at 900 cells mL^{-1} (low) and 1100 cells mL^{-1} (medium) on days 6 and 12 then reducing to levels similar to the control by day 20. *Trachelomonas* in the high treatment peaked at 1200 cells mL^{-1} from days 8 to 20, over 5 times higher than the control. The high

DOM treatment was significantly different from the control from day 4 to 27 and from medium and low on days 8 and 20-34. *Trachelomonas* concentrations in the control did not change from day 0 levels until day 12 when they decreased to 200 cells mL^{-1} from approximately 600 cells mL^{-1}.

Cryptomonas and *Chroomonas* (Figure 4.3B-C) followed a similar pattern to *Trachelomonas*, with very large peaks in concentration on days 12 to 20 in the high treatment ($p<0.01$, compared to control) and days 8 to 12 in the medium and low treatments ($p<0.01$, compared to control). The high treatment was significantly different from medium and low treatments for both mixotrophs from day 4 to 20 ($p<0.05$). Like *Trachelomonas*, medium and low treatments were at similar levels to the control by day 20, and by day 27 all treatments were at a similar concentration.

The amoeba community was composed entirely of individuals from the *Saccamoeba* genus. Amoeba concentrations (Figure 4.3D) were significantly higher in all leachate treatments vs the control ($p=0.001$). Amoeba concentrations increases in all leachate treatments until day 12 after which concentrations in the high treatment remained between 80 and 100 cells mL^{-1} whereas medium and low treatments dropped to much lower concentrations by day 20. Amoeba concentrations in the high treatment were significantly different to the control from day 2 until the end of the experiment ($p<0.05$). Medium and low treatments were significantly different to the control from days 4 until 27. Ciliates (Figure 4.3E) were composed primarily of oligotrich ciliates and were not significantly different between treatments ($p>0.05$) though were across time ($p<0.05$).

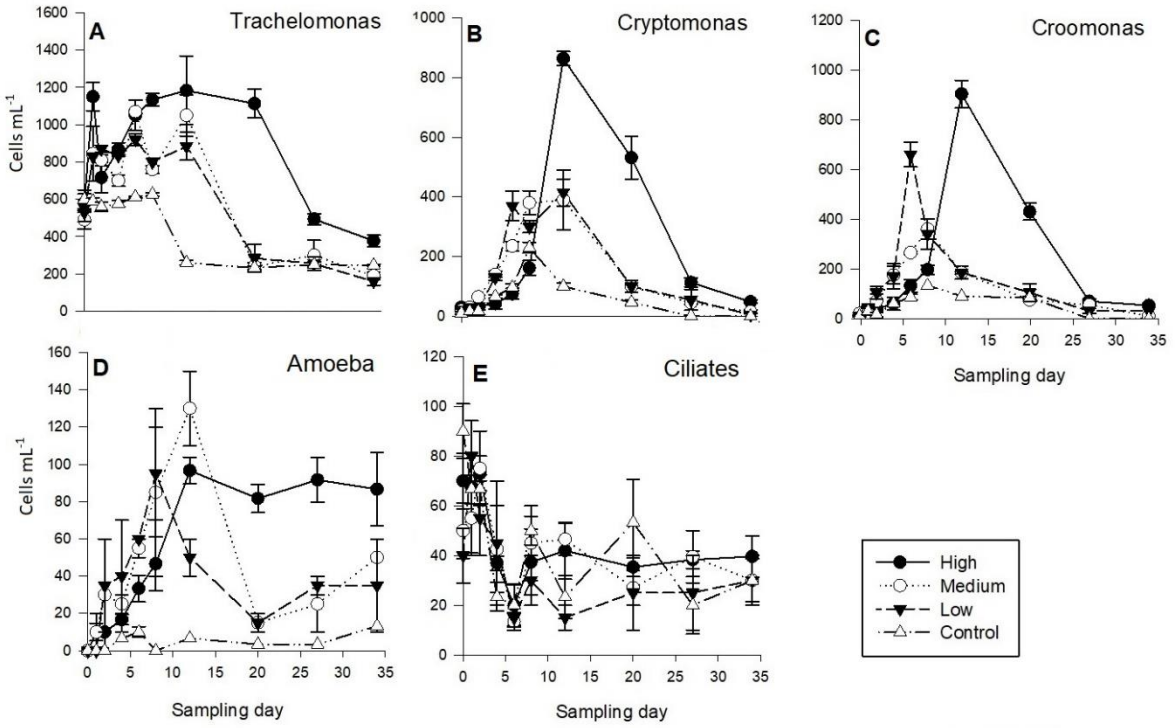

Figure 4.3 Mean concentration (cells mL^{-1}) of mixotrophic algae, amoeba and ciliates in each treatment over time with standard error of the mean. Treatments are indicated by black circles (high), white circles (medium), black triangle (low) and white triangles (control).

All zooplankton groups, apart from *Ceriodaphnia*, responded significantly across treatments (p=0.001) and time (p=0.001), with the effect of treatments varying strongly through time. All leachate additions led to significantly higher abundances of nauplii, copepodites, cyclopoids and *Daphnia* compared to the control. In contrast, calanoid copepod abundance was highest in the control group and *Ceriodaphnia* concentrations were not consistently different between control and leachate additions.

Nauplii and copepodite concentrations (Figure 4.4 A-B) followed similar patterns to each other, increasing sharply between days 1 and 5. Nauplii peaked in all leachate treatments on day 12 (800 to 1200 ind L^{-1}) and were two to three times higher than the control (400 ind L^{-1}). Nauplii concentrations in all leachate treatments were significantly different from the control from days 5 to 20 (p<0.01). Copepodites also peaked on day 12 in the medium (375 ind L^{-1}) and low (250 ind L^{-1}) treatments with the high treatment peaking later on day 20 (325 ind L^{-1}), at ten times the concentration of the control. Concentrations were significantly different between the control and the high treatment on days 5 and 20, the medium treatment on days 5-34 and the low treatment on days 12-20. By day 27, both nauplii and copepodite concentrations had reduced to levels close to that of the control.

Cyclopoid abundance (Figure 4.4C) increased until day 12 where it peaked in the control (100 ind L^{-1}), low (150 ind L^{-1}), and medium (220 ind L^{-1}) treatments. Cyclopoid concentrations were significantly different between the control and the medium treatment from days 1-27 and the low treatment on days 1, 12 and 27. After day 20, concentrations in the high treatment rose sharply to >400 ind L^{-1} until day 27, after which they declined to a level similar to those observed in all treatments. Cyclopoid concentrations were significantly different from the control in the high treatment on days 5-27, medium days 1-27 and in the low treatment on days 1, 12 and 27. Calanoid abundance (Figure 4.4D) was an order of magnitude lower than cyclopoids in all treatments. The control and low leachate treatments consistently supported the highest Calanoid abundance throughout the study, peaking in concentration on day 27 (low: 48 ind L^{-1}; Control: 39 ind L^{-1}) before reducing to the same levels as all treatments on day 34. The high leachate treatment always supported the lowest abundance of calanoids and was significantly different from the medium and low treatments on days 12-27 and from the control on days 20-27.

Daphnia concentrations (Figure 4.4E) were significantly different between leachate additions and the control on days 1, 5 and 20 ($p < 0.05$). All leachate treatments had similar abundances until day 12 when the low (180 ind L^{-1}) and medium (175 ind L^{-1}) treatments peaked at levels double that of the control (85 ind L^{-1}). From day 20 onwards *Daphnia* concentrations in the low and medium treatments were not significantly different from the control ($p>0.05$). The high treatment continued to increase, leading to a peak in concentrations (220 ind L^{-1}; $p=0.001$) on day 20 at approximately 10 times that of all other treatments. *Daphnia* concentrations in the high treatment then reduced until all treatments were at similar levels by day 34 and were not significantly different. *Ceriodaphnia* in all treatments followed a similar pattern, increasing to peak concentrations on day 12 with the highest concentrations in the control (>100 ind L^{-1}) and significantly different from all other treatments ($p<0.05$).

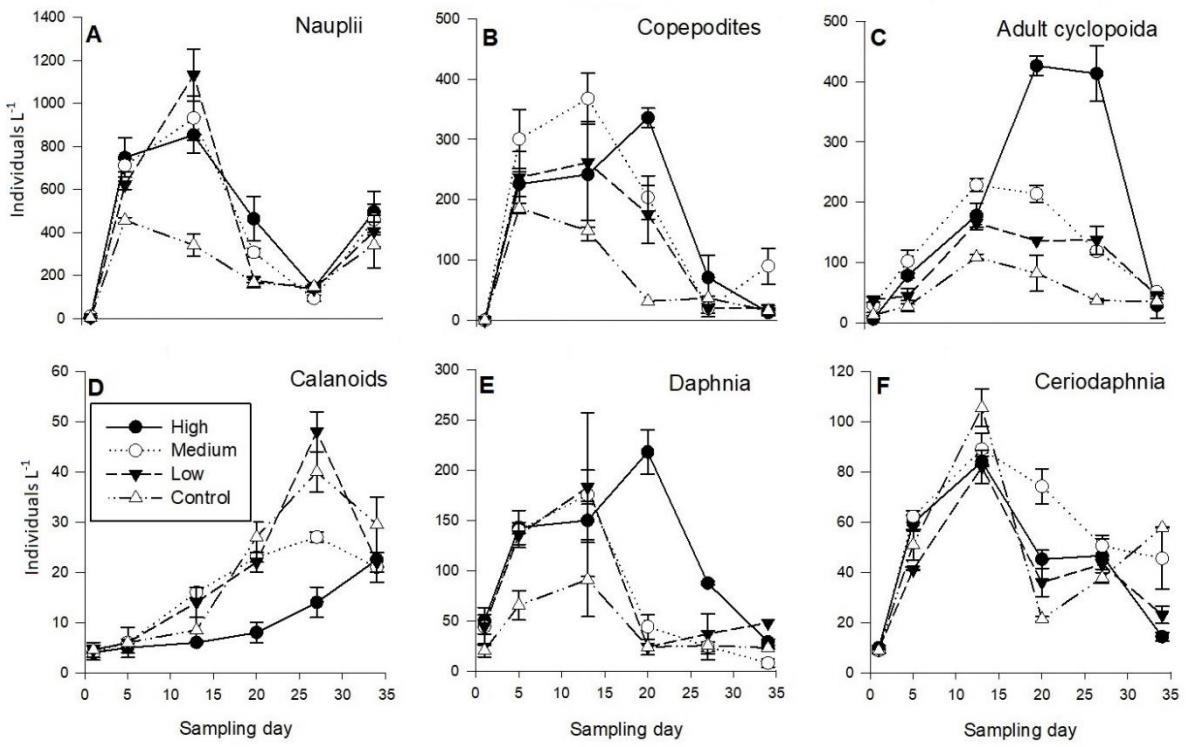

Figure 4.4 Mean zooplankton concentrations for each zooplankton group and treatment. Error bars indicate standard error of the mean.

Table 4.1 PERMANOVA main test results for differences between treatments.

	Group	Between Treatments			Between days			Days x Treatment		
		d.f	Pseudo-f	p	d.f	Pseudo-f	p	d.f	Pseudo-f	p
Functional groups	Chlorophyceae	3	32.95	>0.05	9	163.39	0.001	27	15.32	0.001
	Mixotrophs	3	100.87	0.001	9	98.71	0.001	27	12.78	0.001
	Diatoms	3	20.64	>0.05	9	27.94	0.001	27	10.45	0.001
	Cyanobacteria	3	11.889	0.001	9	10.7	0.001	27	4.87	0.001
Mixotroph groups	Trachelomonas	3	81.48	0.001	9	105.64	0.001	27	10.27	0.001
	Cryptomonas	3	5.01	0.001	9	12.92	0.001	27	3.34	0.001
	Croomonas	3	5.46	0.001	9	5.46	0.001	27	2.59	0.001
	Amoeba	3	7.17	0.001	9	3.48	0.001	27	1.23	0.001
	Ciliates	3	1.96	>0.05	9	7.01	0.001	27	1.19	0.001
Zooplankton	Daphnia	3	28.32	0.001	6	68.84	0.001	18	9.07	0.001
	Cyclopoids	3	12.10	0.001	6	40.15	0.001	18	3.10	0.001
	Calanoids	3	14.59	0.001	6	117.89	0.001	18	5.90	0.001
	Nauplii	3	12.20	0.001	6	679.31	0.001	18	7.86	0.001
	Bosmina	3	11.56	0.001	6	83.15	0.001	18	5.97	0.001
	Ceriodaphnia	3	6.06	>0.05	6	65.42	0.001	18	2.39	0.001
	Copepodites	3	16.78	0.001	6	401.5	0.001	18	11.87	0.001

Particulate organic matter (POM) δ^{13}C signatures changed immediately after leachate additions with the high leachate treatment dropping to -31.5‰ until day 12 and the medium treatment also briefly dropped to this level on day 1 (Figure 4.5A). POM δ^{13}C signatures showed a clear pattern with signatures reducing with increasing leachate concentration, with this pattern maintained for the entire study. POM δ^{13}C signatures increased sharply from days 5-12 in the control treatment, and in all leachate addition treatments from days 12 to 20. On day 12 the largest difference between POM signatures occurred as the high treatment (-32‰) was >8‰ lower than the control (-24‰).

Zooplankton δ^{13}C signatures (Figure 4.5) followed very similar patterns across all treatments, with signatures starting low, before increasing and peaking around day 20 or shortly thereafter. Between day 0 and 5 δ^{13}C signatures decreased in all zooplankton groups and treatments excluding a 1‰ increase between day 0 and 1 in *Daphnia* and *Ceriodaphnia* in the high treatment. After day 5 zooplankton in the high leachate treatment had the most depleted δ^{13}C signature and remained closer to the leachate signature (-27.5‰) than any other treatment. In contrast, all measured zooplankton groups in the control had the most enriched δ^{13}C signatures at each time point and were furthest from the leachate δ^{13}C signature. As with the POM, zooplankton δ^{13}C signatures showed a distinct pattern with more depleted δ^{13}C values in the treatments with the highest amount of leachate added. By day 12 cyclopoid δ^{13}C values had begun to clearly differentiate between the high carbon and control treatments, leading to the largest differences between treatments (>6‰) on day 27 with δ^{13}C signatures in the control at -18‰ and in the high treatment at -24‰. *Daphnia* and *Ceriodaphnia* δ^{13}C signatures (Figure 4.5C-D) followed very similar trends to cyclopoids though started with a more depleted signature at -27.9‰. Similarly to cyclopoids, *Daphnia* δ^{13}C values diverged after day 5 with the biggest difference falling on day 12 when signatures in the high leachate treatment were -28.2‰ compared to the control at -21.8‰.

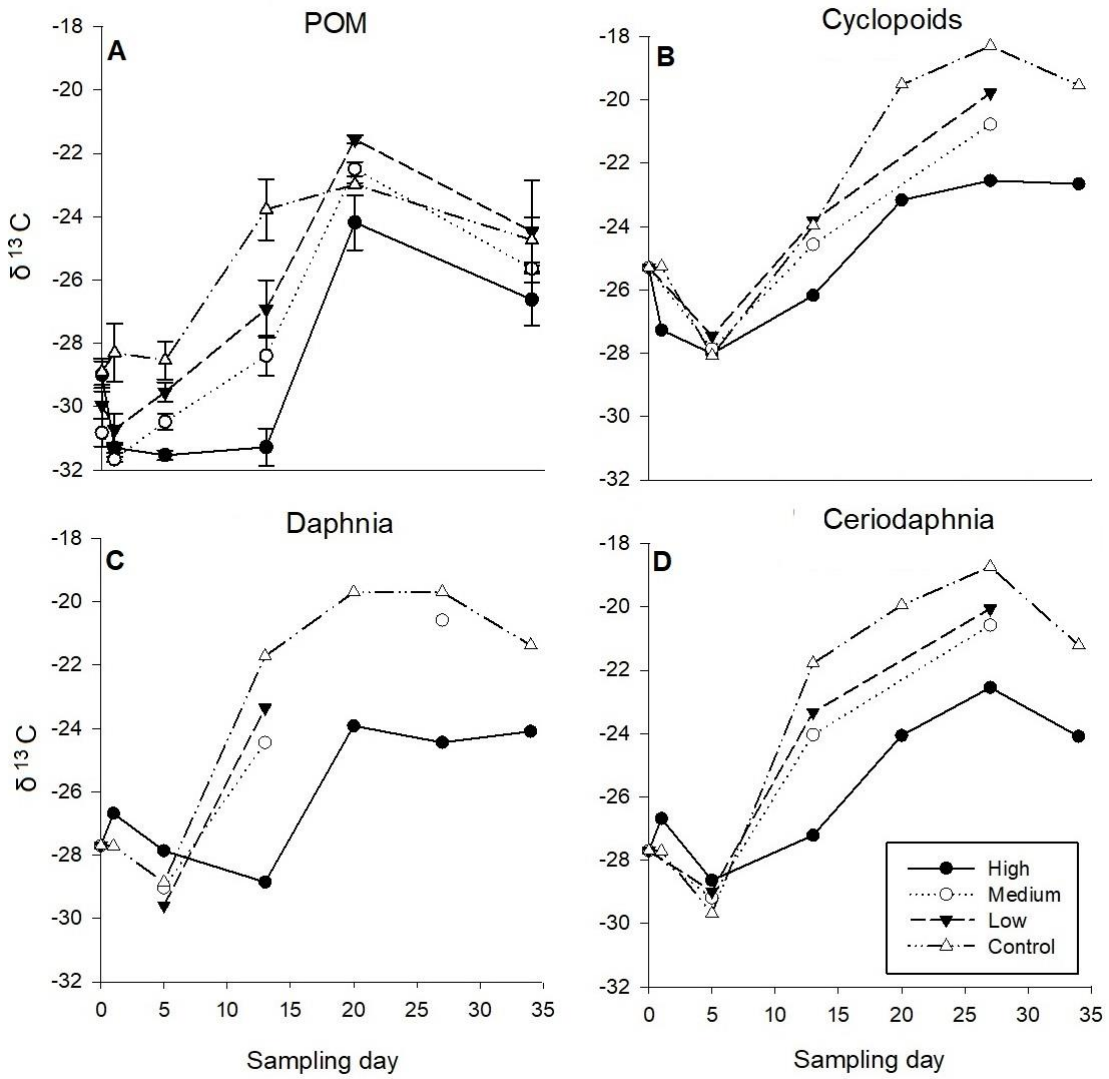

Figure 4.5 δ 13 C signatures of cyclopoids, Daphnia, Ceriodaphnia and particulate organic matter (POM). All zooplankton samples were pooled between replicates. Error bars on POM indicate Standard error of the mean.

Regression analysis found the ratio of mixotroph to obligate autotroph biovolume (Figure 4.6A) correlated strongly with particulate organic matter δ 13 C signatures (R^2= 0.6157, p<0.0001). A biovolume ratio >0 correlated with heavier particulate organic matter δ 13 C signatures (<-28‰) compared to those when autotrophic algae was more abundant than mixotrophs (<0) when POM δ 13 C signatures were >-28‰.

Total zooplankton δ 13 C signatures also related strongly with particulate organic matter signatures (R^2=0.7058, p<0.0001; Figure 4.6B). Regression analysis found cyclopoids in the high leachate treatment were strongly correlated to POM signatures (R^2=0.8233, p<0.0001) whereas cyclopoids in the control treatment were not significantly related to POM (p>0.05).

Daphnia and *Ceriodaphnia* δ^{13}C signatures in all treatments were significantly correlated to POM signatures (p<0.0001; R^2>0.85 all treatments).

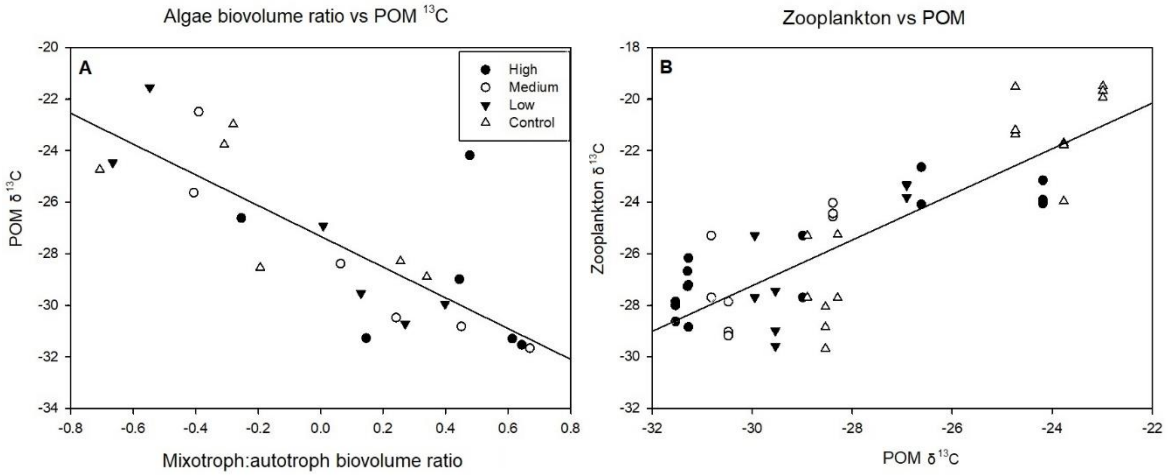

Figure 4.6 shows linear regression data from all sampling days for A: the biovolume ratio of mixotrophic:autotrophic phytoplankton vs the ^{13}C signature of particulate organic matter (POM) B: POM ^{13}C signature vs zooplankton ^{13}C signature (all taxa), separated into treatments: high (black circle), medium (white circle), low (black triangle), control (white triangle).

4.5 Discussion

This study aimed to understand how allochthonous organic matter influenced riverine food web structure and production. Our results expanded on those of previous similar mesocosm studies (Karlsson et al., 2007; Mitrovic et al., 2014; Hitchcock et al., 2016) and provide strong evidence of the important role of allochthonous sources of carbon in aquatic food webs. In terms of exploring a response to inputs of organic carbon, all additions of tDOM resulted in significant increases in mixotrophic flagellates and all zooplankton taxa abundance (except calanoids) compared to the control. Further, declines in concentrations of mixotrophs, cyclopoid spp. and *Daphnia* towards the end of the experiment and simultaneous increases in chlorophytes and calanoid spp. suggest the importance of allochthonous support as resources were depleted over time. These results support our first hypothesis that allochthonous inputs can significantly boost production in the lower food webs of aquatic ecosystems.

δ^{13}C values of zooplankton and POM in the high leachate treatment were closer to the range of the leachate than any other treatment, reflecting both the influence of tDOM additions on the POM pool and the use of leachate tDOM by higher trophic levels, supporting our second

hypothesis. However, the expected reduction in autochthonous production in response to leachate inputs was not observed. Instead, mixotrophs increased complimentarily to autotrophic production, dominating the algal biovolume in tDOM amendments, resulting in large net increases to phytoplankton biovolume and potentially playing a large role in driving changes in zooplankton growth by providing a trophic link between allochthonous carbon and primary consumers. Mixotroph biovolume was also significantly related to changes in POM ^{13}C values suggesting they were a potentially important path for tDOM uptake.

Phytoplankton and mixotroph responses

Changes in the algae community after the addition of tDOM offered two insights into the role of allochthonous carbon in phytoplankton dynamics. Firstly, mixotrophic algae can comprise the largest portion of the algae population after a carbon pulse and dominate the algal community following pulses of organic matter (Figure 4.2). Secondly, obligate autotrophs may respond more dynamically to allochthonous inputs than previously suggested as nutrients from tDOM support phytoplankton growth simultaneously to heterotrophic production. Our findings showed tDOM pulses can lead to large increases in heterotrophic production and, subsequently, can also stimulate autotrophic production, potentially leading to a range of available resources for consumers. These findings are similar to those of Faithfull et al (2011) who found autotrophic production was unaffected by glucose addition and did not reduce when mixotrophic algae dominated the community. This contrasts with previous studies showing bacterioplankton out-competed obligate autotrophs for nutrients in the presence of an allochthonous carbon source and significantly reduced the biomass of phytoplankton (Carney et al., 2016). Despite autotroph concentrations remaining stable, chlorophyll-a (chl-a) concentrations declined as tDOM additions increased. This may be a function of increasing concentrations of mixotrophs as they generally contain lower chl-a content than obligate autotrophs, or an indication of mixotrophs switching from photosynthesis to predominantly heterotrophic growth (Jones, 2000).

Mixotrophs were the dominant functional group (by biovolume) in all tDOM additions until day 12 (low, medium treatments) and 20 (high treatment), whereas chlorophytes dominated the algal biomass in the control treatment for the entire study. Blooms in *Trachelomonas* and later *Cryptomonad* populations in the tDOM treatments may have been a major resource supporting the increases in zooplankton populations. Previous studies have found mixotrophs may be a high-quality food source for zooplankton (Katechakis et al., 2005) and may be

preferentially preyed upon by some zooplankton taxa (Hansson et al., 2019). It has been suggested that mixotrophic flagellates may play a crucial role in mobilising allochthonous carbon for higher trophic levels and play a stabilising role between autotrophic and heterotrophic production and food quality (Flynn et al., 2013; Jager et al., 2014; Worden et al., 2015).

Mixotrophy appeared to play an important role in driving the POM $\delta^{13}C$ signature throughout the study. As the ratio of mixotrophic to autotrophic biovolume increased, $\delta^{13}C$ signatures of the POM were typically more depleted and closer to the leachate signature (R^2 = 0.621). This suggests that mixotrophs were using tDOM as an energy or nutrient source which resulted in POM signatures reflecting the leachate additions. Further, the POM $\delta^{13}C$ signature was strongly correlated to signatures of zooplankton. This is consistent with previous findings in Patagonian lakes where mixotrophy was a major driver of the $\delta^{13}C$ signature of POM and calanoid signatures in the system (Trochine et al., 2015). Without knowing the initial $\delta^{13}C$ values of mixotrophic algae, relationships between leachate 13c uptake and mixotrophic biovolume are based entirely off correlations and thus, somewhat speculative. However, this relationship between mixotrophy, POM and zooplankton suggests mixotrophy may play a significant role in linking allochthonous DOM and bacteria to higher trophic levels.

In the larger protists, only amoeba concentrations increased with tDOM additions with ciliate concentrations showing no significant difference between treatments. This may be due to intensive zooplankton predation as zooplankton exert strong top-down pressure on ciliate populations (Sommer and Sommer, 2006). In a similar mesocosm study, Degerman et al. (2018) found zooplankton predation greatly reduced ciliate populations despite abundant food (bacteria and nanoflagellates) for growth.

Zooplankton responses to tDOM

Terrestrial dissolved organic matter additions had a clear effect on the zooplankton community, similar to other studies in South East Australia (Mitrovic et al. 2014; Hitchcock et al. 2016) and Europe (Faithfull et al., 2011; Degerman et al., 2018). Additions of tDOM resulted in large increases in total zooplankton abundance, of which *Daphnia* and Cyclopoida spp. were the most pronounced. *Daphnia* growth and reproduction using tDOM via bacteria and mixotrophic flagellate intermediaries has been found to be equal to or better than when compared to completely autotrophic diets (Jager et al., 2014; Hiltunen et al., 2017; McMeans

et al., 2018). Evidence of allochthony in cyclopoids has been found in Swedish lakes (Berggren et al., 2014), Australian river mesocosm experiments (Mitrovic et al., 2014) and previous studies suggest cyclopoid production may be limited by tDOM due to their reliance on microbial food chains as a food source (Jurgens and Jeppesens, 2000; Berggren et al., 2010). *Daphnia* and cyclopoids reflect two distinctly different feeding behaviours for the consumption of allochthonous DOM (Berggren et al., 2014). As filter feeders *Daphnia* feed directly on bacteria and algae, removing several food chain links from the microbial loop, making the uptake of allochthonous DOM more efficient (Jager et al., 2014; Hiltunen et al., 2017). In contrast, raptorial cyclopoids utilise allochthonous DOM through consuming microzooplankton (rotifers/ciliates) at the top of the DOM-bacteria-nanoflagellate microbial pathway (Jurgen and Jeppesens, 2000; Karlsson; 2003; Pace, 2004). These different feeding strategies affect the efficiency of carbon transport to higher trophic levels as cyclopoids require extra links in the food web compared to *Daphnia* (Jansson et al., 2007; Karlsson et al., 2007). Other filter feeders/bacterivores such as copepod nauplii also increased immediately after the tDOM additions. As a result, a broad successional change was evident following leachate addition. Filter feeders able to immediately consume bacterioplankton (*Daphnia* and copepod nauplii) peaked early, followed later by dominance of raptorial omnivores which require higher level primary consumers (ciliates, rotifers, small microcrustaceans) to make tDOM available for consumption. This successional change in zooplankton was also evident in the $\delta^{13}C$ values of *Daphnia* and cyclopoids in the high tDOM treatment as *Daphnia* diverged from the control and moved towards the leachate signature earlier than the cyclopoids.

The $\delta^{13}C$ signatures of zooplankton were relative to the proportion of leachate added, similar to the findings of Karlsson (2007). Previous studies into zooplankton ^{13}C have found *Daphnia* and Cyclopoids used allochthonous DOM as an important food source in their diets (Berggren et al., 2014). In this study, *Daphnia* $\delta^{13}C$ signatures had clearly shifted away from the control by day 12 in the high treatment and were more depleted and much closer to the leachate $\delta^{13}C$ signature than all other treatments. *Daphnia* $\delta^{13}C$ values were significantly related to POM $\delta^{13}C$ values in all treatments; this is likely a function of non-selective filter feeding, directly consuming bacteria, POM and mixotrophs supported by tDOM. Interestingly, *Ceriodaphnia* which were not significantly different between treatments showed a similar pattern in $\delta^{13}C$ values to *Daphnia*, with $\delta^{13}C$ values more depleted and closer to leachate values relative to the size of leachate additions. Cyclopoids followed a

similar trend in overall $\delta^{13}C$ values over time, however, were only significantly related to POM in the high treatment and not significantly related to the control. This may be an indicator of the different make up of POM between the 2 treatments as cyclopoids are selective raptorial feeders (Jurgens and Jeppesens, 2000).

In contrast to previous studies (Karlsson et al., 2007; Hitchcock et al., 2016), calanoid abundance appeared to be negatively correlated to carbon addition. This correlation may be highly species dependent however, competition and predation on juvenile calanoids by cyclopoids in the high and medium carbon treatments may have caused reduced calanoid numbers. Increased abundances of cyclopoid copepods have been shown to exert top-down pressure through predation on calanoid adults and nauplii (Blumenshine and Hambright, 2003). Unfortunately, due to the low biomass of calanoids throughout this study there was not the required biomass to perform stable isotope analysis. Consequently, it remains unclear if calanoid production was supported by the allochthonous DOM addition in this study.

Ecological implications of allochthonous inputs

Additions of tDOM significantly increased the overall production within the mesocosms. Phytoplankton concentrations did not reduce following tDOM additions, instead increased levels of heterotrophic production were complimentary to phytoplankton growth, offering a large increase in resource availability to higher consumers. Mixotrophs dominated the algal biomass after tDOM addition, offering a second pathway for terrestrial matter to support consumers and an extra basal food option in addition to phytoplankton and the microbial-chain. The energy pathways supporting the food web in these experiments were dynamic and changed quickly based on the pulses of allochthonous material added. Bacterial, phytoplankton and zooplankton communities switched quickly between resources and zooplankton appeared able to use multiple trophic pathways for food resources. Ultimately, allochthonous resources appeared to support both autotrophic and heterotrophic production and greatly increased zooplankton and net phytoplankton growth throughout the lower food web. These results have important implications for our understanding of river ecology and conceptualising how food webs respond to allochthonous tDOM pulses that commonly occur during floods and storm flows.

Mixotrophy is a growing area of importance in river ecology, in highly dynamic environments such as lowland rivers where food web production may switch between allochthonous and autochthonous sources over short time scales. The ability to switch

between major energy sources may be crucial for energy transfer. Indeed, mixotrophy creates smooth transitions between photosynthesis and dissolved organic matter for supporting food webs (Worden et al., 2015), and is likely a quantitatively significant link between allochthonous organic matter and higher consumers (Hansson et al., 2019). Furthermore, mixotrophy may increase the transfer efficiency of food webs by offsetting the carbon lost during respiration in higher trophic levels with photosynthesis and reducing C:N/P mismatch between producers and consumers (Katechakis et al., 2005; Ward and Follows. 2016). Emerging evidence has shown mixotrophs may be a more stable and nutritious food source for zooplankton than obligate autotrophs (Hansson et al., 2019). Mixotrophy may therefore greatly increase the transfer efficiency and quality of tDOM to zooplankton compared to energy transfer from the microbial loop alone (Flynn et al., 2013; Hiltunen et al., 2017). Thus, by fuelling mixotrophic growth while maintaining autotrophic production allochthonous DOM may offer larger total foodweb subsidies than previously thought. In terms of flow events increasing tDOM concentrations our data suggests large pulses of tDOM can offer significant boosts to zooplankton production via both autochthonous and heterotrophic pathways. During these periods mixotrophs may be of particular importance for the transfer of terrestrial inputs to higher trophic levels and as a stable resource in highly variable environmental conditions.

Conclusion

This study has expanded on the results of several previous mesocosm experiments using terrestrial dissolved organic matter as an energy source for a freshwater food web. We found mixotrophic algae play a major role in mobilising tDOM for higher trophic levels. Further a trophic succession of filter feeders changing to raptorial species was evident as a pathway for allochthonous energy as zooplankton able to exploit bacterial populations emerged following leachate additions and were then predated upon by higher trophic levels such as copepods. Stable isotopes supported this and showed POM δ^{13}C signatures were correlated to the ratio of mixotroph vs obligate autotroph biovolume. POM and zooplankton δ^{13}C signatures were closely correlated suggesting mixotrophs played a major role in feeding zooplankton throughout the study. These results support our main hypothesis that pulses of allochthonous organic matter can significantly increase zooplankton growth. Further, our results suggest mixotrophy may be an important function for transfer of allochthonous energy to higher consumers in freshwater foodwebs. We contend that the use of allochthonous carbon in freshwater foodwebs is highly complex with multiple potential pathways of energy transfer to

higher trophic levels. Further research into the role of mixotrophic flagellates and their impact on consumers may greatly increase our underlying understanding of how allochthonous carbon is used by foodwebs.

Chapter 5: Bioavailable DOC additions increase mixotroph and ciliate production in riverine microcosms.

5.1 Abstract

Mixotrophic algae are increasingly being conceptualised as playing an important role in aquatic food webs, functioning both as primary producers and a link for the microbial loop to higher trophic levels. Emerging evidence suggests mixotrophy is potentially more common and more important in freshwater systems than previously thought. However, there remains a considerable knowledge gap in understanding how mixotroph production responds to resource pulses in freshwater systems. An *in-situ* microcosm experiment was run on the Lachlan River to test how the microplankton community responded to different inputs of organic carbon (as glucose) and nutrients (nitrogen and phosphorus) in the absence of grazing by larger zooplankton (> 63µm). The experiment ran for 5 days, with sampling every day for phytoplankton, ciliates, bacteria and chlorophyll-*a*. The Lachlan River was heavily nutrient limited leading to all treatments without a nutrient amendment being quite similar. Mixotrophs were clearly and significantly higher in treatments with carbon+nutrients compared to nutrients alone however had decreased by day 5, possibly due to grazing by ciliates. Both high and low DOC additions +nutrients suppressed chlorophyte and diatom biovolume strongly compared to nutrients without carbon. Ciliates were much higher in DOC+nutrient treatments and continued to increase until day 5. Ciliates in nutrient treatments were not different from non-nutrient treatments despite high bacterial abundance from days 3 to 5. Our data suggests mixotrophs may play an important role in making DOC and bacterial production available for consumption by higher trophic levels when present.

5.2 Introduction

Phytoplankton and bacteria form the base of most lowland riverine food webs (Stahl et al., 2013). Both can be limited by inorganic nutrient concentrations such as nitrogen (N) and phosphorus (P) (Hecky and Kilham, 1988; Almeida et al., 2005), however as heterotrophs, bacteria are also often limited by dissolved organic carbon (DOC) concentrations (Westhorpe et al., 2010). Bacteria are consequently 'coupled' to phytoplankton as they use autochthonous exudates to fulfill their DOC requirements (Cole et al., 1988; Fouilland et al., 2014). During periods of high allochthonous DOC/DOM inputs such as during flood events and during leaf fall, bacteria may be 'decoupled' from DOC production from phytoplankton growth (Jansson

et al., 2000; Carney et al., 2016). Once bacteria are not limited by DOC they directly compete for limiting nutrients with phytoplankton (Drakare et al, 2002). As DOC/DOM pulses often occur during flood or rain events, increased turbidity often occurs simultaneous to increased carbon loads, thus phytoplankton may be supressed by reduced light availability while competing for limiting nutrients with bacteria (Carney et al., 2016). Thus, bacterial production may dominate basal food webs following pulses of DOC and/or low light conditions (Jansson et al., 2006).

Changes to basal production can have a strong influence over the production and community structure of secondary consumers within riverine food webs (Azam et al., 1983; Nicolle et al., 2012). Energy from bacteria production enters foodwebs via the microbial loop (Azam et al, 1983) and must pass through several extra food chain links such as Heterotrophic nanoflagellates to reach consumers such as zooplankton compared to phytoplankton (Berglund et al., 2007; Degerman et al., 2018). This lengthened energy pathway results in lower transfer efficiency compared to phytoplankton grazing as roughly 70% of carbon is lost at each trophic link (Straile, 1997). Previous studies have found heterotrophic food webs to be up to 10 times less efficient than autotrophic pathways (Berglund et al., 2007). Ultimately this may lead to significantly lower metazoan production when food webs are dominated by bacterial rather than autotrophic production (Brett et al., 2009; Degerman et al., 2018).

Food web production is highly influenced by both resource availability and intracellular nutrient requirements of different taxa (Hessen and Faafeng, 2000). Obligate autotrophs have a large stoichiometric range of 100 -1000 (C:P) (Elser and Hasset, 1994) due in part to relationships between light availability and nutrient limitation (Sterner, 1997). Oppositely, bacteria and their consumers have relatively low C:P ratios (10-500) (Makino, 2003), however emerging evidence suggests this is dependent on nutrient availability and environmental conditions (Godwin et al, 2017). Bacterial competition for nutrients can reduce the quality of autotrophic algae as nutrients become limiting, increasing their carbon to nutrient ratio (Danger et al., 2007). Bacterial competition may therefore reduce both the quality and quantity of obligate autotrophs. However, previous studies suggest mixotrophic plankton may be able to exploit bacterial production and dominate systems when autotrophy is less successful (Sanders et al., 2001; Kamjunke et al., 2007).

Mixotrophic plankton (phytoplankton, flagellates and protists) are able to obtain energy via photosynthesis as well as through heterotrophic consumption of bacteria (phagotrophy) or

direct absorption of DOC (osmotrophy). Emerging evidence suggests mixotrophy is both more common and more important for aquatic food webs than previously thought (Flynn et al., 2013; Hansson et al., 2019). Mixotrophs may account for up to 60% of total bacterial consumption in some aquatic systems (Unrein et al., 2007). Bacteria are a nutrient rich energy source for mixotrophs due to their smaller C:N stoichiometry and high phosphorus content (Vadstein, 2000; Makino et al., 2003; Kamjunke et al., 2007). In consuming bacteria, mixotrophs may greatly increase the efficiency of carbon transfer as several links are removed from the heterotrophic energy pathway (Flynn et al., 2013; Ward and Follows, 2016). Mixotrophs can also be more stoichiometrically similar to metazoans than obligate autotrophs (Katechakis et al., 2005) and a superior and more stable food source for zooplankton consumers than obligate autotrophs (Jager et al., 2014; Hansson et al., 2019). Thus, there is the potential that mixotrophs increase the resource quality of bacterial production while still using both photosynthesis and phagotrophic energy pathways. Mixotrophy may play an in important role in carbon cycling and energy transfer for food webs in freshwater ecosystems (Flynn et al., 2013) however more studies are needed to better understand these roles.

The aim of this study was to test how mixotrophic plankton and the broader microplankton community respond to resource inputs of carbon and nutrients. To test this we performed a microcosm experiment on the Lachlan River in Central NSW monitoring the responses of bacteria, phytoplankton, ciliates and mixotrophs to additions different additions of glucose, nitrogen, and phosphorus. We hypothesised that i) bacteria and ciliate abundance would increase following additions of DOC; ii) Mixotrophic plankton abundance would increase in carbon additions and dominate the algal biomass

5.3 Methods

This experiment was conducted in the middle reaches of the Lachlan River in Central NSW, 33°36'35.9"S 148°28'33.0"E. The Lachlan River catchment is roughly 85,500 km^2 and flows from the Great Dividing Range to terminating in the Great Cumbung swamp (Moran et al., 2014). Similar to other lowland rivers in south east Australia flow is highly variable and heavily regulated (Kemp, 2010). The riparian vegetation at the site chosen for this study was relatively dense (40-60% canopy cover) and overwhelmingly dominated by *Eucalyptus camaldulensis.* Local land-use was heavily agricultural as is much of the Lachlan River. The experiment was started during base flow conditions, which had persisted for several weeks

before the beginning of the experiment. Water temperature ranged from 17 to 23°C averaging 20°C. Initial pH was 8.07±0.01 and dissolved oxygen was 85.1% ±6%. Turbidity was <30NTU for the duration of the experiment.

Experimental design

Microcosms were used to test the effects of carbon and nutrient additions on a lower food web community. The microcosms were 5L clear plastic bottles, filled to 4.5L to include an airspace for photosynthesis and to minimise anoxia. Microcosms were randomly placed and secured across a single rope. Styrofoam floats were used at even points along the rope to ensure microcosms remained just below the surface of the water column and cinderblocks held the experiment in place. All treatments were performed in triplicate. Treatments included two concentrations of DOC (5 and 10mg L^{-1}) added as glucose (sigma aldritch) and nutrients, nitrogen and phosphourus added as KNO_3 at 1mg L^{-1} and P: KH_2PO_4 at 0.6mg L^{-1} as used previously in Hitchcock et al. (2014). This resulted in 6 treatments: Control, high DOC (HDOC, 10mg L), low DOC (LDOC, 5mg L), nutrients alone, high DOC with nutrients (HDOC+N) and low DOC with nutrients (LDOC+N).

River water was filtered through a 65 µm plankton net to remove large zooplankton and collected in a 70L prewashed plastic tub. The water was then homogenised and poured into microcosm bottles, ensuring all bottles were filled evenly 1L at a time to minimise any heterogeneity in the phytoplankton or ciliate communities. Before microcosm bottles were filled, initial (day 0) samples were taken. Sampling occurred at the same time (3-4pm) as the initial samples every day for the duration of the experiment. All microcosms were gently mixed before sampling. Sampling for DO, chlorophyll-*a* (Chl-*a*) and bacteria occurred daily. Phytoplankton samples were taken on days 0, 1, 3 and 5. Nutrients, conductivity and pH were sampled on day 0 and day 5.

Sampling protocol

Conductivity, turbidity and pH were measured on days 0 and 5 using a calibrated Hydrolab surveyor and MS5 Sonde probe. Dissolved oxygen (percentage and mg L^{-1}) and temperature were measured in every microcosm bottle daily using a HACH HQ20 LDO probe. Nutrients (DOC, NOx and FRP) were sampled at day 0 and at day 5. Dissolved organic carbon (DOC), filterable reactive phosphorus (FRP) and dissolved oxides of nitrogen (NOx) samples were collected in pre-washed and sample rinsed 250 mL PET bottles, filtered to 0.45µm using cellulose acetate syringe filters and then frozen. DOC samples were analysed using the High

Temperature Combustion Method (APHA, 2005) and all N and P samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA, 2005).

Chlorophyll-*a* (chl-a) concentration was determined for analysis by collecting a 150 mL volume of water from each microcosm and filtered through a 0.7 µm pore-sized glass fibre filter using a Mitivac vacuum hand pump. Filters were wrapped in aluminium foil and frozen until analysis using the boiling ethanol extraction method (International Standards Organisation, 1994). To avoid dilution or contamination effects of adding water to microcosms, water taken for chl-a analysis was not replaced.

Samples for bacterial enumeration were sampled and analysed according to the methods in Carney et al., (2016). Briefly, 1mL of microcosm water was sampled, preserved with 80µL (2%) of glutaraldehyde and snap frozen with liquid nitrogen. Samples were analysed using a LSRII flow cytometer (Becton Dickinson, San Jose, CA, USA) and bacterial populations were discriminated according to cell side scatter (SSC) and SYBR green fluorescence (Seymour et al., 2007). In preparation for flow cytometric analysis, samples were stained with SYBR Green I nucleic acid stain and fluorescent reference beads (1µm in diameter) were added to each sample immediately before analyses in a final concentration of 10^5 mL^{-1}.

Phytoplankton and ciliate counts were taken (50mL) from each microcosm and preserved using Lugol's iodine solution (2mL). Samples were analysed using Sedgwick rafter counting cells and counted at 200x magnification on a compound microscope. Measurements for biovolume were taken using an Olympus DP72 camera and cellSens Standard software (version 1.3). Twenty individuals of each species were measured to achieve a reliable average. Phytoplankton were identified using the keys of Entwisle et al., (1997) and Prescott, (1978), protozoa were identified using the key of Patterson, (1996). We classified mixotrophic plankton as taxa previously found to be mixotrophic, for this study this included Cryptophytes, *Chlamydomonas, Euglenoids* and dinoflagellates. All diatoms were considered obligate autotrophs as were the chlorophytes in this study as there was no previous evidence suggesting the present taxa were mixotrophic.

Statistical analyses

PERMANOVA with Pairwise comparisons using PRIMER 6.0 +PERMANOVA (Anderson et al., 2008) was used to test for significant differences between treatments and days, similarly to that of Carney et al., (2016). Bacteria, chlorophyll-a, all phytoplankton groups

individually and as a community were analysed using Bray-Curtis similarities and transformed using a Log (x+1) transformation to account for skewness. All PERMANOVA tests were also subject to PERMDISP tests for homogeneity of variance and observation of distribution using draftsmen's plots. Similarity percentage analysis (SIMPER) was used to test for differences between community contribution amongst treatments and days using a 99% community threshold (Clarke and Warrick, 2001). Non-parametric multi-dimensional scaling plot was used to visualise differences between phytoplankton communities between treatments and days. All graphs were created using Sigmaplot software, excluding the nMDS plot which was created using PRIMER (Clarke and Warrick, 2001).

5.4 Results

Dissolved organic carbon and nutrient concentrations (Table 5.1) were all higher on day 0 than on day 5 in all treatments. DOC concentrations were higher on day 5 in both high carbon treatments (9.39 w/ nutrients and 9.69mg L^{-1} without nutrients) and LDOC+N (9.59 mg L^{-1}) compared to all other treatments. Soluble reactive phosphorus (SRP) concentrations were up to 2 orders of magnitude higher (range: 44.45 to 86.2 ug L^{-1}) in the nutrient additions on day 5 than treatments without nutrients (range: 0.045 to 0.73 ug L^{-1}).

Table 5.1 Ammonia (µg L^{-1}), soluble reactive phosphorus (µg L^{-1}), nitrogen oxides (µg L^{-1}) and DOC (mg L^{-1}) concentrations on day 0 (initial) and day 5 (all treatments). Standard error in brackets.

Treatment	Day	SRP (µg L^{-1})	Nox (µg L^{-1})	DOC (mg L^{-1})
Initial	0	8.29 (3.7)	20.05 (3.2)	13.32 (0.3)
Control	5	0.726 (0.1)	5.075 (0.2)	8.675 (0.1)
Low carbon	0	8.29 (3.7)	20.05 (3.2)	18.32 (0.5)
Low carbon	5	0.33 (0.8)	9.875 (0.4)	7.723 (0.3)
Low carbon + nutrients	0	608 (3.7)	1020 (3.2)	18.32 (0.5)
Low carbon + nutrients	5	86.2 (2.3)	9.105 (0.5)	9.5885 (0.1)
High Carbon	0	8.29 (3.7)	20.05 (3.2)	23.3 (0.7)
High carbon	5	0.0445 (0.1)	10.9 (0.1)	9.6925 (0.3)
High carbon + nutrients	0	608 (3.7)	1020 (3.2)	23.3 (0.7)
High carbon + nutrients	5	69.55 (6.9)	8.335 (0.2)	9.386 (0.1)
Nutrients	0	608 (3/7)	1020 (3.2)	13.32 (0.3)
Nutrients	5	44.45 (1.6)	9.115 (0.7)	7.7295 (0.7)

Dissolved oxygen concentrations (Figure 5.1) were significantly higher in all treatments with added nutrients from day 2 until the end of the experiment, peaking at 188% saturation on day 3 in the nutrient treatment. Within the nutrient treatments, dissolved oxygen concentrations increased inversely to carbon addition with the HDOC+N treatment always lower in dissolved oxygen concentrations than the LDOC+N and nutrients treatments. Both DOC additions without nutrients had lower dissolved oxygen concentrations (average: 98% ±1.1, both treatments) than the control (average 108% ±2.4) for the duration of the experiment. Chlorophyll-*a* (chl-*a*) (Figure 5.1) followed a very similar pattern to dissolved oxygen, increasing sharply in nutrient addition treatments compared to treatments without nutrient amendments. Chl-a concentrations were lower in both carbon treatments (average: 5.8± 0.6 high, 6.3±0.5 µg L^{-1} low) compared to the control (average: 12.3 ±0.6 µg L^{-1}), this also applied to nutrient treatments with HDOC+N always lower than LDOC+N and nutrients alone.

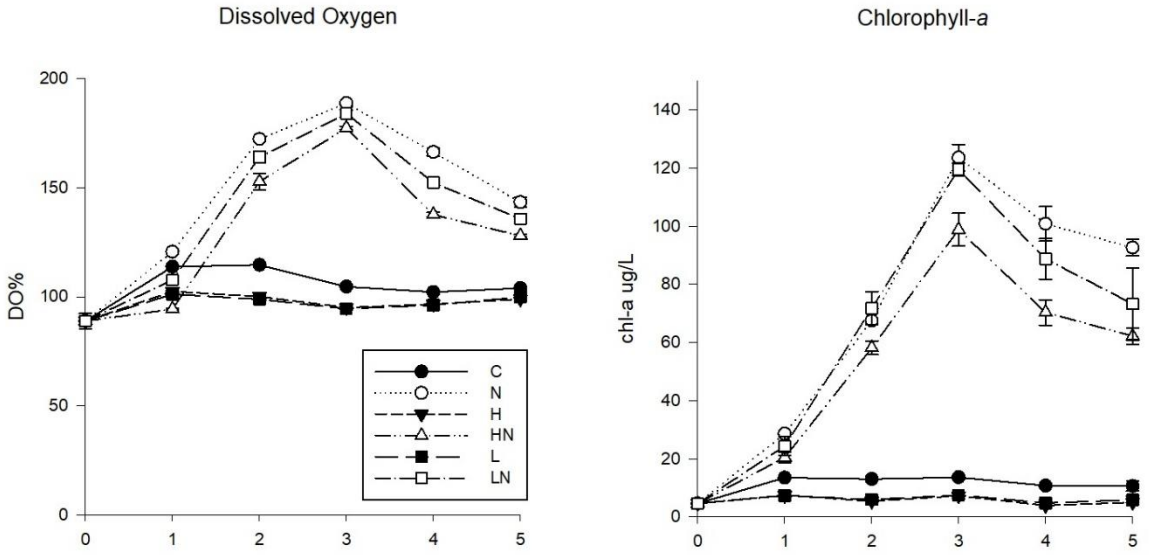

Figure 5.1 Dissolved oxygen (%) and chlorophyll-a (ug L) concentration in all treatments. black circles= control, white circles =nutrients, black triangles= high carbon, white triangles =high carbon and nutrients, black squares =low carbon and white squares= low carbon + nutrients. Error bars are standard error of the mean.

Total bacteria concentrations (Figure 5.2) increased sharply on day 1 in the HDOC+N, peaking at 1.4x10^7 cells mL^{-1} and remaining highest until day 3. LDOC+N had the second highest bacterial concentrations until day 3. Bacteria concentrations in the HDOC and LDOC treatments were higher (>4x10^6 cells mL^{-1}) than the control (2.6x10^6 cells mL^{-1}) and nutrient (2.5x10^6 cells mL^{-1}) addition on day one, however were equal to or lower than all other

treatments by day 2 and remained low (<2.5x10^6 cells mL^{-1}) for the duration of the experiment. Bacterial concentration was highest from day three to five in the nutrient treatment.

The ratio of high nucleic acid fluorescent (HNA) to low nucleic acid fluorescent (LNA) bacterial cells followed a similar trend as total cells, initially highest in the HDOC+N treatment followed by the nutrients without carbon treatment from days three to five. LDOC+N was consistently higher than all treatments without nutrients which were always lowest and not significantly different from each other (p>0.05).

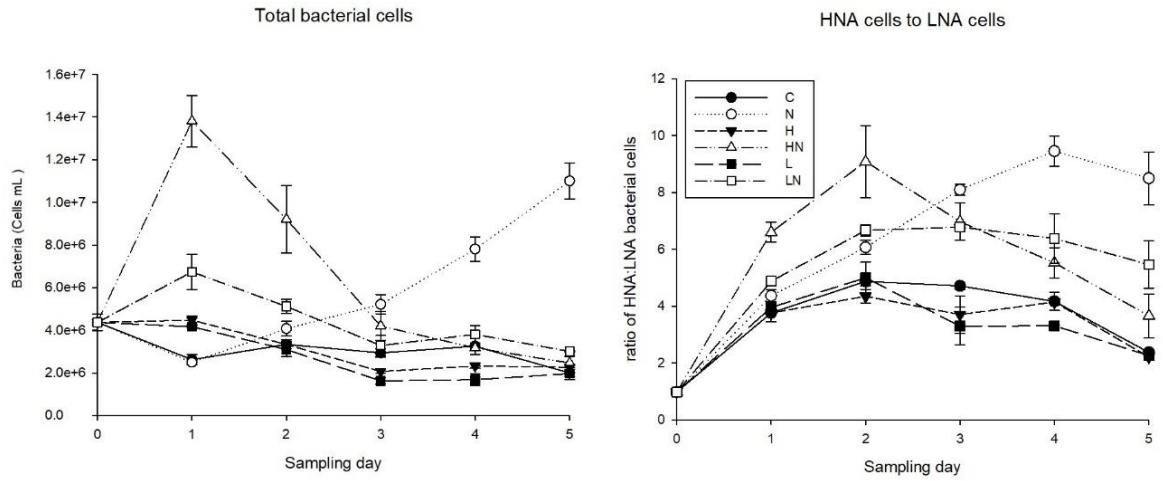

Figure 5.2 Total bacterial cell concentrations and the ratio of HNA to LNA emitting cells across all treatments. black circles= control, white circles =nutrients, black triangles= high carbon, white triangles =high carbon and nutrients, black squares =low carbon and white squares= low carbon + nutrients. Error bars are standard error of the mean.

The biovolume of obligate autotrophs (chlorophytes and diatoms, Figure 5.3A-B) followed very similar trends to each other although diatoms were up to 10-fold higher in biovolume than chlorophytes. All nutrient addition treatments led to much higher biovolumes of chlorophytes and diatoms compared to treatments without nutrients. Autotroph biovolume decreased with additions of carbon. Autotrophs in the HDOC+N treatment were significantly lower than the nutrient treatment for both chlorophytes and diatoms on day 5 (p=0.003 and 0.005, respectively). LDOC+N followed a similar trend, resulting in lower chlorophyte (p=0.005) and diatom (p=0.002) biovolumes compared to the nutrient addition throughout the study, though was always higher than the HDOC+N treatment. Chlorophyte biovolume was higher in the control (average: 0.29 ±0.09mm^3 L^{-1}) compared to the HDOC (0.09 ±0.02mm^3 L^{-1}) and LDOC (0.08 ±0.01mm^3 L^{-1}) treatments. Diatom biovolume followed a similar trend

and was higher in the control (average: 1.41 ±0.05mm^3 L^{-1}) compared to the HDOC (0.85 ±0.15mm^3 L^{-1}) and LDOC (1.09 ±0.20mm^3 L^{-1}) treatments and significantly different across all treatments (p<0.005) except HDOC vs LDOC (p=0.093). Cyanobacteria were present in only very small numbers and biovolume and have not been presented here.

Mixotroph biovolume (Figure 5.3C) was significantly higher (p=0.001) in the HDOC+N and LDOC+N treatments than all other treatments on day 3 peaking at 25mm^3 L^{-1} before dropping to levels similar to the nutrient treatment on day 5. Mixotroph biovolume was consistently low in all treatments without nutrient amendments (average: 2.08 ± 0.04mm^3 L^{-1}) and not significantly different amongst these treatments (p>0.05).

Ciliate biovolume (Figure 5.3D) was significantly higher in the HDOC+N and LDOC+N treatments than all other treatments from day 3 to 5, peaking on day 5 at 6 mm^3 L^{-1} in the high treatment (p<0.05). Ciliate biovolume was not significantly different between all other groups (p>0.05 for Control, nutrients, HDOC, LDOC).

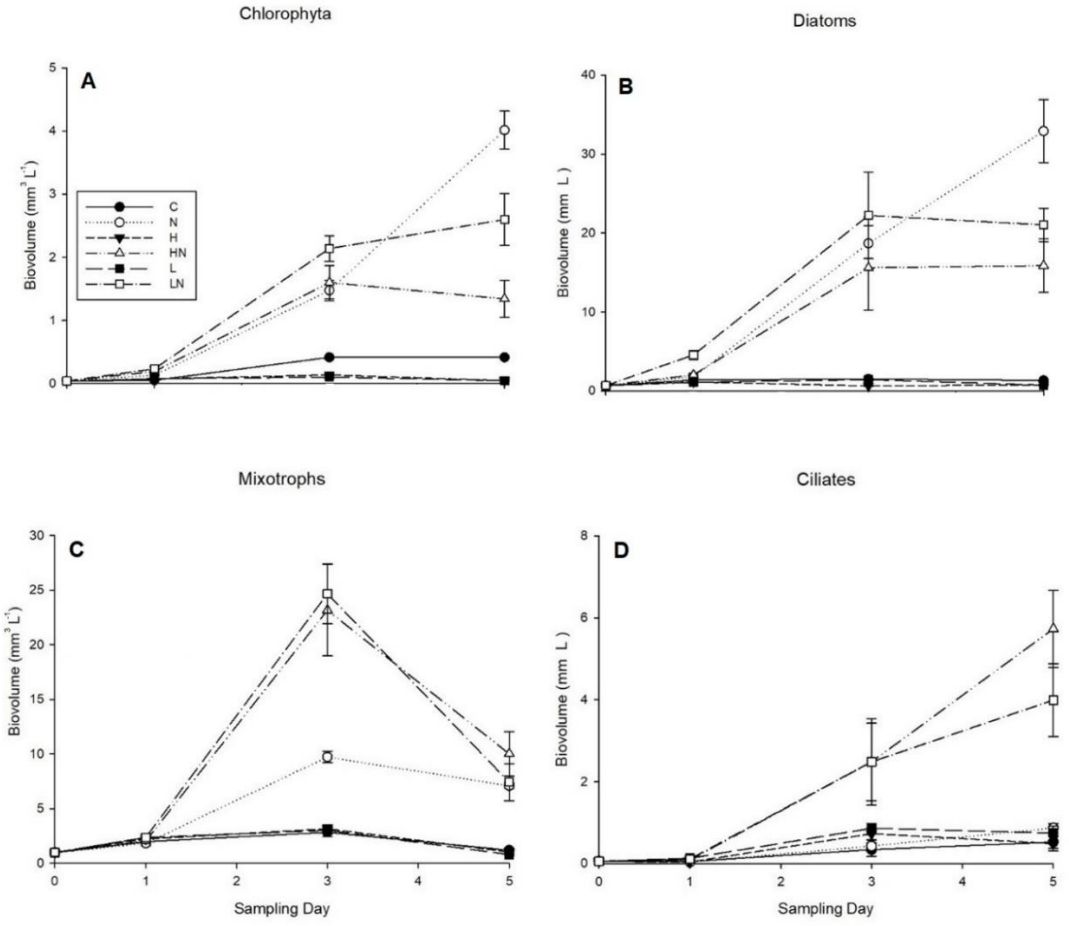

Figure 5.3 Biovolume (mm^3 L^{-1}) of chlorophytes (A), diatoms (B), mixotrophic algae (C) and ciliates (D) in all treatments over time. black circles= control, white circles =nutrients, black

triangles= high carbon, white triangles =high carbon+ nutrients, black squares =low carbon and white squares= low carbon+ nutrients. Error bars are standard error of the mean.

The phytoplankton community (Figure 5.4, Table 5.2) was significantly different between treatments (p=0.001) and time (p=0.001). nMDS analysis showed phytoplankton communities separated clearly between treatments with and without nutrient additions by day 3 (Figure 5.6). *Cyclotella* and *Nitzchia* were the most common genera of diatoms in all treatments. Chlorophytes were dominated by *Scenedesmus* in samples without nutrient amendments and *Scenedesmus, Cosmarium* and *Ankistrodesmus* in treatments with nutrient amendment. The dominant mixotrophic groups were *Chlamydomonas, Cryptomonas* and *Chroomonas*. Ciliates were dominated by oligotrich ciliate groups, primarily *Strombidium*. The community contribution (percent of total biovolume) of ciliates and mixotrophs was highest in the HDOC and LDOC treatments, however this was not significantly different from the control (Table 5.4). HDOC and LDOC also had the lowest contribution of chlorophytes and diatoms which was significantly different from all other treatments ($p<0.05$; Table 5.4). Ciliates also contributed to community biovolume more in the HDOC+N (24%) and LDOC+N (20%) treatments than in the nutrients (7%) and control (16%) treatments. Diatoms contributed the highest biomass to all treatments with a nutrient amendment however this declined inversely to DOC addition. Overall phytoplankton and protist communities were significantly different between all treatments (Table 5.3) from day 3 (except HDOC vs LDOC ($p>0.05$), HDOC+N vs LDOC+N ($p>0.05$) and nutrients vs HDOC+N, which was near significant (p=0.058)). This trend continued to day 5 with DOC and DOC+N treatments not significantly different ($p>0.05$). From day 3 all nutrient additions were significantly different from treatments without nutrients and all carbon additions were significantly different from treatments without carbon (except N vs HN on day 3 and C vs H on day 5 (Figure 5.4)). Total phytoplankton and protist biovolume (Figure 5.5) peaked for the study on day 3 in the LDOC+N treatment at >50mm^3 L^{-1}. HDOC+N was also high during this period, and on day 5 the nutrient treatment had the highest total biovolume of plankton at 45mm^3 L^{-1}. Total biovolume in the control was not significantly different from the high or low carbon additions ($p>0.05$).

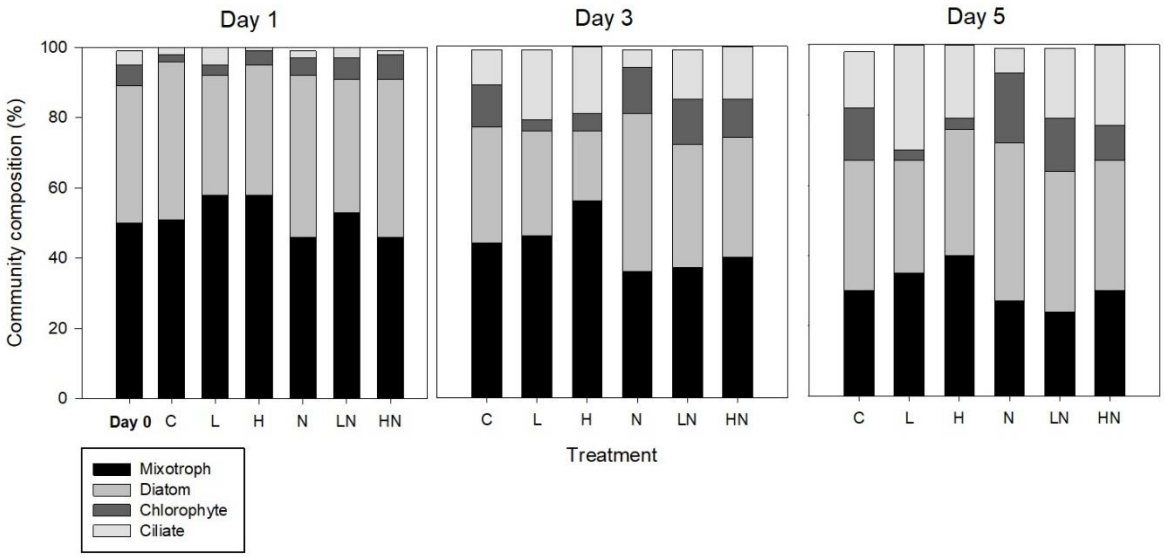

Figure 5.4 Phytoplankton and protozoa community composition by percent of total biovolume on days 1 (including day 0), 3 and 5. For clarity phytoplankton groups have been grouped into mixotrophs (bottom, black), diatoms (second from bottom, grey), chlorophytes (second from top, dark grey) and ciliates (top, light grey).

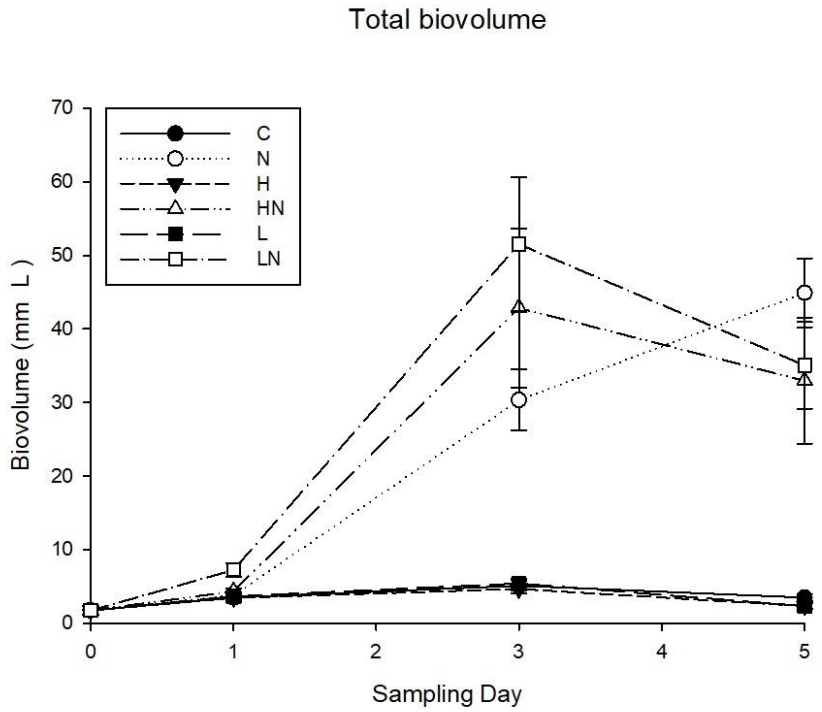

Figure 5.5 Total biovolume (mm3 L-1) of all measured phytoplankton and ciliate groups (chlorophytes, diatoms, mixotrophs and ciliates) in each treatment over time. black circles= control, white circles =nutrients, black triangles= high carbon, white triangles =high carbon and nutrients, black squares =low carbon and white squares= low carbon + nutrients. Error bars are standard error of the mean.

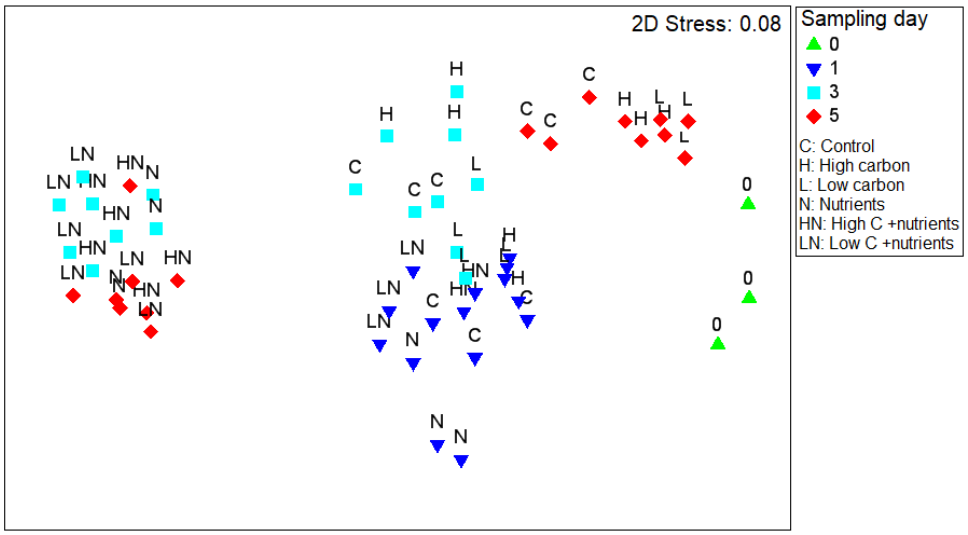

Figure 5.6. Non-parametric multidimensional scaling (nMDS) plot of phytoplankton community composition and biovolume. Data is separated by sampling day (Green triangle: day 0, blue triangle: day 1, blue square day 3 and red diamond day 5) and treatment (C,N,L,H,LN,HN). All data was log(+1) transformed before analysis.

Table 5.2 PERMANOVA main test comparing phytoplankton community across all treatments, days and the interaction between days and treatments. N=9.

PERMANOVA	**p-value**	**Pseudo-f**	**d.f.**	**perms**
Day	0.001	288.2	3	998
Treatment	0.001	92.14	5	998
Day x treatment	0.001	25.49	15	999

Table 5.3 PERMANOVA with pairwise comparisons analysis for changes in phytoplankton community comparing all treatments across days 1, 3 and 5. Numbers in bold show non-significant values.

Phytoplankton community	**p-value**		
Test	1	3	5
C vs N	**0.360**	0.005	0.001
C vs H	**0.527**	0.021	**0.059**
C vs HN	**0.115**	0.012	0.005
C vs L	**0.248**	0.030	0.029
C vs LN	0.040	0.002	0.002
N vs H	**0.119**	0.004	0.006
N vs HN	**0.433**	**0.058**	0.019
N vs L	0.047	0.002	0.003
N vs LN	0.040	0.009	0.033
H vs HN	**0.073**	0.009	0.004
H vs L	**0.385**	**0.068**	**0.275**
H vs LN	0.019	0.002	0.003
HN vs L	0.028	0.005	0.004
HN vs LN	0.024	**0.486**	**0.152**
L vs LN	0.006	0.001	0.003

Table 5.43 PERMANOVA with pairwise comparisons comparing significant differences between individual phytoplankton groups compared to treatments. Numbers in bold show non-significant values

	P-VALUE				
TEST	Chlorophyta	Diatoms	Mixotrophs	Ciliates	Total
C VS N	0.001	0.001	0.004	**0.308**	0.001
C VS H	0.001	0.001	**0.908**	**0.435**	**0.167**
C VS HN	0.001	0.001	0.001	0.025	0.001
C VS L	0.001	0.003	**0.627**	**0.075**	**0.384**
C VS LN	0.001	0.001	0.002	0.003	0.001
N VS H	0.001	0.001	0.002	**0.972**	0.001
N VS HN	0.003	0.005	0.012	0.031	**0.075**
N VS L	0.001	0.001	0.001	0.005	0.001
N VS LN	0.005	0.002	0.018	0.001	0.001
H VS HN	0.001	0.001	0.001	0.043	0.001
H VS L	**0.495**	**0.093**	**0.527**	0.022	0.034
H VS LN	0.001	0.001	0.001	0.001	0.001
HN VS L	0.001	0.001	0.001	0.025	0.001
HN VS LN	**0.065**	0.004	**0.810**	**0.546**	0.001
L VS LN	0.001	0.001	0.001	0.001	0.001

5.5 Discussion

We hypothesised that DOC additions would influence the structure of phytoplankton communities through changing bacterial growth and consequently increasing competition for nutrients and resources via the microbial loop. Production in the Lachlan River appeared heavily nutrient limited, leading to results clearly separating between treatments with and without nutrient amendments. We found carbon additions when coupled with nutrients resulted in increased bacterial growth which may have led to competition with autotrophs for limiting nutrients, consequently reducing autotrophic production in treatments with carbon and nutrient additions. Despite significant reductions in chlorophyte and diatom biovolume in DOC +nutrient additions, total biovolume was not significantly different from nutrient additions alone as mixotrophic plankton and ciliates greatly increased in biomass equal to or greater than any reductions in growth by obligate autotrophs. Our data suggests mixotrophic plankton growth may compensate for losses in autotrophic production due to bacterial competition in the presence of bioavailable carbon. Mixotrophy may make the energy transfer of DOC and microbial production a more efficient than previously thought (Berglund et al., 2007).

Bacterial and phytoplankton competition

DOC+nutrient additions led to significantly higher bacterial abundance and lower biovolumes of autotrophs compared to nutrients alone (Figure 5.3). Previous studies have found bacteria to increase at the expense of phytoplankton (Westhorpe et al., 2010; Carney et al., 2016). Bacteria may have a competitive advantage over phytoplankton for nutrient acquisition due to their higher surface area to volume ratio and affinity for phosphorus (Blomqvist et al., 2001; Jansson et al., 2006). These results are consistent with previous research finding bacteria were able to out compete obligate autotrophs for limiting nutrients when not limited by an organic carbon source (Hitchcock and Mitrovic, 2015; Carney et al., 2016). Further, Drakare et al., (2002) found phytoplankton concentrations were low following inflows of DOC despite high nutrient and light conditions in early summer, suggesting bacteria could out-compete phytoplankton even in ideal autotrophic growing conditions. Our results were consistent with previous microcosm experiments in Australian rivers finding chl-a concentrations decreased with increased bacterial production following additions of DOC (Westhorpe et al., 2010; Hitchcock and Mitrovic, 2013). We found chlorophytes and diatoms were clearly suppressed following DOC additions when coupled

with nutrient additions and chlorophytes alone showed inhibited growth following DOC additions without a nutrient amendment.

The greater bacterial production seen in the nutrient treatment after day 3 was likely due to increased autochthonous DOC production from high chlorophyte and diatom concentrations (Kritzberg et al., 2004). Bacteria appeared both nutrient and DOC limited in the Lachlan River as significant initial spikes in bacterial abundance only occurred in treatments containing both DOC and nutrient amendments. Carney et al., (2016) found distinctly different bacterial community assemblages occurred after additions of inorganic nutrients and an organic carbon leachate. We did not directly measure bacterial community changes, however emerging evidence suggests HNA (high nucleic acid fluorescent) and LNA (low nucleic acid fluorescent) bacterial cells may indicate different feeding groups within the bacterial community (Mojica et al., 2020). That would suggest different bacterial groups occurred between treatments with and without nutrients and between HDOC+N and LDOC+N. HNA:LNA ratios have also been used as a measure of active (HNA) vs inactive (LNA) bacterial cells (Gasol et al., 1999; Lebaron et al., 2001). Interestingly, this would suggest bacterial production remained high in the HDOC+N and LDOC+N treatments for the duration of the experiment. This suggests decreases in abundance were likely due to intensive grazing from mixotrophs and ciliates rather than exhaustion of DOC additions. This explanation may be more likely as some studies have found additions of carbon supported bacterial production for up 2 weeks as compared to the 2 days seen in the study (Drakare et al., 2002; Carney et al., 2016).

The apparent nutrient limitation of the Lachlan River (as seen in differences between algae biovolume in treatments with and without nutrients, Figures 5.2 and 5.3) appeared to significantly reduce the impact of the high and low DOC additions in treatments without nutrient amendments. Interestingly, reductions in chlorophyll-*a* and chlorophyte concentrations were still evident in samples without nutrient amendments and lasted for the duration of the experiment. These reductions were despite bacterial abundance and potentially activity in the HDOC and LDOC treatments either lower than or equal to the control by day 2. However, despite the same concentrations of DOC additions, reductions in autotrophs were far more pronounced with nutrient amendments than without. This may reflect similar conditions to that of Faithfull et al., (2011) who found glucose additions did not reduce phytoplankton production in a nutrient limited lake.

Mixotrophy and protists

The algal community composition changed significantly following DOC additions. Generally, DOC additions led to higher contributions to total biovolume by mixotrophs and ciliates and reductions in autotrophs. This is consistent with the findings of Carney et al., (2016) who found phytoplankton communities were dominated by mixotrophic cryptomonads following DOC additions to experimental mesocosms in a freshwater estuary. Broadly, autotrophs (chlorophytes and diatoms) were much higher in treatments without carbon additions, particularly in the nutrient treatment. Oppositely in DOC treatments without a nutrient amendment, ciliates and mixotrophs appeared particularly important for maintaining production while chlorophytes were reduced possibly by bacterial competition. In DOC+N treatments, high growth occurred via both heterotrophic and autotrophic pathways including very high mixotrophic and ciliate growth, despite large reductions in autotrophs. Emerging evidence suggests mixotrophy may be a crucial link between the microbial loop and higher trophic levels (Mitra et al., 2014; Hansson et al., 2019). Mixotrophy may ease the transition between heterotrophic and autotrophic production (Worden et al., 2015) offering a stabilising food source for higher trophic levels during times of resource flux (Hansson et al., 2019).

Mixotrophic biomass was 5-6 times higher than ciliate biomass in both DOC+N treatments, suggesting mixotrophy was more important for total growth under heterotrophic conditions than ciliates. This may demonstrate the ability of some mixotrophs (such as *Chlamydomonas*) to simultaneously use photosynthesis and heterotrophy for growth, rather than switching between the two (Tittel *et al.,* 2005). This ability to use both pathways may reduce competition for specific resources with specialist feeders. Previous studies have found mixotrophs may be at a disadvantage when competing against obligate autotrophs in high light and nutrient conditions, or specialist heterotrophs for bacterial prey (Jones, 2000; Fischer et al., 2017). However, their ability to use multiple sources of energy may make them more dominant during periods of nutrient limitation (Hansson *et al*., 2019). This may be reflected in the high contribution of mixotrophs to the initial community biovolume of this study and in treatments without nutrient amendments. It may also be reflected in the success of mixotrophs in the DOC+N treatments as they avoid direct competition with specialists by pooling resources required for photosynthesis as well as phagotrophy or osmotrophy. This ability to use both pathways simultaneously has been suggested to make mixotrophs an important resource for food webs across a wide range of ecological roles and environmental

conditions (Worden et al., 2015). Further, the presence of mixotrophs has been found to reduce the effects of bacterial competition on obligate autotrophs (Jager et al., 2014). Mixotrophy may therefore indirectly increase primary productivity by increasing the efficiency of the microbial loop and minimising reductions in autotrophic production as a consequence of bacterial competition.

Interestingly, in the DOC+N treatments bacteria were greater in the high treatment and autotrophs lower compared to the low DOC treatment, yet mixotroph and ciliate concentrations were very similar. This may be indicative of an optimal threshold of heterotrophy in the mixotrophs in this study as suggested (using mathematical models) by Crane and Grover, (2010). However, the gradient of phototrophic and heterotrophic strategies for mixotrophs typically falls along a large spectrum from mostly phototrophic to mostly heterotrophic (Flynn et al., 2013). The range within this spectrum likely changes across mixotrophic species and environmental conditions (Jones, 2000, Flynn et al., 2013).

Ciliate biovolume increased most clearly in the HDOC+N and LDOC+N treatments. Ciliate concentrations remained unchanged between the control and nutrient amendment despite large increases in phytoplankton biovolume in the nutrient amendment (Figure 5.3D). One explanation may be ciliates preferentially grazed on smaller mixotrophic flagellates rather than autotrophs. Mixotrophic biovolume decreased significantly between days 3 and 5 when ciliate concentrations increased in DOC+N treatments. In particular, *Chroomonas* biovolume was severely reduced during this period. Interestingly, ciliates did not respond to very high bacterial production in the nutrient treatment from day 3 to 5, suggesting a certain dependence on an additional resource to boost production e.g. an allochthonous carbon supply (Hiltunen et al., 2017)

Top-down pressure has been found to have significant effects on the basal food web of freshwater ecosystems (Degerman et al., 2018), with even low levels of zooplankton grazing having large impacts on the dynamics of a food web (Jager et al., 2014). Thus, it is likely these patterns in the lower food web would be significantly different if zooplankton were present in this study.

Community composition

A major finding from this study was the change in community and increased dominance of mixotrophs for DOC amendments. We found increases in mixotroph and ciliate production accounted for or surpassed any reductions in autotrophic production potentially caused by

bacterial competition via DOC additions or other processes. Notably, this was clearest when nutrient concentrations were high and growth increased sharply via both autotrophic and heterotrophic pathways, possibly limiting the effects of competition for nutrients on phytoplankton communities. However, total algal biovolume in the carbon treatments without nutrient additions were also not significantly different from the control due to small increases in mixotrophic and ciliate biovolume. This was despite autotrophic production being clearly higher in the control, as measured by Chl-*a* and chlorophyte biovolume. This mixotrophic subsidy of autochthonous production is likely to vary significantly between environmental conditions and local nutrient or organic carbon concentrations (Flynn et al., 2013). Nevertheless, our data suggests mixotrophy may play an important role in subsidising losses in autotrophic production with heterotrophic growth (Kamjunke and Tittel, 2009). Further, mixotrophy may be a crucial link in transferring energy from the microbial loop to higher trophic levels. This study adds to the growing body of research suggesting mixotrophic production may be a crucial energy pathway for freshwater food webs (Katechakis et al., 2005; Flynn et al., 2013; Jager et al., 2014; Hansson et al., 2019).

Conclusion

This study aimed to understand how additions of bioavailable DOC influenced planktonic communities including competition between bacteria and autotrophs and the role mixotrophy may play. We hypothesised that bacteria would out-compete phytoplankton for limiting resources and consequently reduce phytoplankton production as seen in previous studies (Jansson et al., 2006; Carney et al., 2016). Further, we hypothesised mixotrophic algae and ciliates would exploit increases in bacterial abundance and dominate the phytoplankton community following DOC additions. Our results showed the Lachlan River planktonic community was nutrient limited, resulting in only minor effects of carbon in treatments without nutrient additions. However, when DOC was coupled with nutrient additions large increases in both autotrophic and heterotrophic production were clear with considerable competition for resources between bacteria and obligate autotrophs. Mixotrophs and ciliates were much higher in biovolume in DOC+N treatments than any other treatment and contributed significant biomass to the lower food web. Given the nutrient and carbon limitation of phytoplankton and bacterial communities in the Lachlan River, inputs of these limiting resources, such as may occur during rain and flood events, are likely to greatly increase production via both autotrophic and heterotrophic pathways.

Chapter 6: General discussion

This thesis examined the effects of flow, dissolved organic carbon (DOC) and inorganic nutrients on the lower food webs of Australian lowland rivers. Monitoring studies were conducted to examine the relationship between river discharge, DOC and nutrient concentrations and consequent increases in phytoplankton and zooplankton concentrations. Further, monitoring of cease to flow conditions was undertaken to understand how (or if) switches from flowing river to cease to flow conditions influenced the degree to which allochthonous carbon sources supported food webs. Manipulative field experiments using mesocosms and microcosms were then carried out to further examine how dissolved organic carbon concentrations effect changes in food web structure (community composition and competition) and function (carbon transfer pathways). Collectively, these studies provided new insights into the importance of flow events, allochthonous carbon mobilisation and how this may affect riverine food webs.

6.1.1 Monitoring changes in resources and food webs

Field monitoring of the heavily regulated Namoi River revealed the nature of relationships between discharge and changes in nutrient and DOC concentrations (Chapter 2). Strong positive correlations were found between discharge and increases in DOC and nutrients with a large flood event contributing high total loads of carbon and nutrients into the river. Following these increases in flow and subsequent carbon and nutrient pulses, a phytoplankton response was observed, with increased concentrations. While flow events stimulated phytoplankton production, these increases occurred either during or after the flow pulses, depending on the size of the flow. For example, during the large flood event in September 2016, phytoplankton reduced to the lowest concentrations for the sampling period, before responding greatly, reaching some of the highest levels recorded for the sampling period once flows and turbidity had reduced. Zooplankton followed a similar trend, increasing in abundance during and after flow events, often aligning with peaks in phytoplankton and DOC concentrations.

Following the work exploring flow effects, I monitored distinctly different flow conditions as a drought reduced the Namoi River to a string of disconnected waterholes for long periods (2 periods >100 days each; Chapter 3). This gave an opportunity to evaluate how allochthonous support of zooplankton production changes between the drastically different states of a connected (flowing) river and a disconnected (pooling) river. Not surprisingly, significantly

different phytoplankton communities were sampled between the two flow conditions. In addition, I found the source of carbon supporting zooplankton production changed between flow conditions; allochthonous carbon appeared to support zooplankton during periods of flow, whereas autochthonous carbon supported zooplankton during the cease to flow conditions. Despite this general pattern, the presence of riparian vegetation also effected the sources of energy supporting zooplankton during cease to flow conditions. Specifically, allochthonous sources of carbon continued to support zooplankton production in the waterhole with dense riparian canopy cover during the cease to flow state. Importantly, these findings indicate that allochthonous carbon may support zooplankton even during low flow periods (1-500ML d^{-1}), suggesting that external sources of carbon do not only support food webs in response to larger flow events.

Several river ecology concepts were supported by the results from Chapters 2 and 3, showing the highly dynamic role allochthonous carbon plays in supporting and structuring Australian lowland riverine food webs. Chapter 2 strongly supported the flood pulse concept (Junk et al., 1989) and previous research on other rivers in Australia (Nielsen et al., 2016; Rees et al., 2020), North America (Dalzell et al., 2005; 2007) and Central Europe (Hein et al, 2003) which showed flood events can generate large pulses of production in riverine food webs. Further, the 'boom and bust' nature of zooplankton populations observed in Australian Rivers (Shiel et al., 2006; Sternberg et al., 2008) was also supported as both a function of hydrological factors (downstream transport and eggbank hatching; Jenkins and Boulton, 2003), and *in situ* growth from increased food resources (Poff et al., 1997). Interestingly, Chapter 3 found a range of ecological concepts applied at flow (RCC, Vannote et al, 1990) and cease to flow (RPM, Thorp and Delong, 1994) conditions. Further, Chapter 3 showed allochthonous carbon may play a role in supporting and structuring riverine food webs even during low flow conditions either via riparian inputs or downstream movements. The range of hydrological conditions studied throughout this thesis showed several ecological concepts can be applied to a single river. However, as seen in South American Rivers (Hoeinghaus, 2007), this hydrological variability makes it difficult to accurately apply a single conceptual model to food-web productivity across land-scape scale riverine ecosystems.

6.1.2 The relationships between discharge and carbon

Positive relationships between DOC and discharge, like those demonstrated in this thesis, have been previously found in Australian lowland rivers (Westhorpe and Mitrovic, 2012;

Woodward et al., 2015). Flood events have been found to be particularly important for mobilising DOC from the floodplain for instream processes (Gawne et al., 2007; Nielsen et al., 2016). While discharge and DOC were positively correlated in this study, the relationship was not as strong as expected with several other factors (proximity to previous flows, channel height, season) influencing DOC concentrations within the Namoi River.

The large flood event in September 2016 reflected the key processes proposed in the flood pulse concept (Junk et al., 1989), mobilising very high concentrations of carbon and nutrients. Considering the magnitude of discharge during this period (>30,000ML d^{-1}) the total load of DOC mobilised during this flood vastly exceeded all other measured periods during this study. As reported in previous studies (Buffam et al., 2001), the September 2016 flood event contributed disproportionately to the total carbon loads of the river system. Despite the magnitude of this influx, DOC concentrations increased even during smaller flow events. Given the greater frequency of smaller flow events in the Namoi River, the observed pattern suggests that a low-level pulse of organic carbon availability is maintained in the river during smaller flow events, with a switch to very large pulses of allochthonous dissolved organic carbon during flood events. Understanding and unpacking these two distinctly different sets of carbon conditions for riverine food webs is critical to understanding the relationship between DOC delivery, availability and utilisation in the lower food web.

Of particular significance in the Namoi River is the fact that irrigation extraction and diversion significantly reduce flow to downstream sites and generally prohibits floodplain inundation, except during the very highest flow events. The importance of longitudinal connections was evidenced by the fact that downstream DOC concentrations were related to upstream discharge values. Thus for this river system, reduction of floodplain wetting and connection to the main channel appeared to make the downstream transport (e.g. the RCC; Vannote, 1980) particularly important for delivering nutrients and carbon to downstream communities. However as previously stated, a relationship between overbank flows and subsequent high foodweb production was clear, suggesting flood pulses may be an important source of resource and production for the Namoi River. It is therefore possible that extraction and regulation of the Namoi River has changed how terrestrial organic matter is mobilised, changing from flood pulses and floodplain inputs to downstream movement as natural overbank flows have been severely reduced.

6.1.3 The relationship between discharge and planktonic food webs

Phytoplankton (as measured by chlorophyll-*a*) responded positively to flow events (perhaps influenced by increases in nutrient concentrations), with increases measured during and after flow events on the Namoi River. In this study, the 2016 flood event stimulated peak chlorophyll-a concentrations and altered the phytoplankton community composition. Previous studies have also found positive responses of phytoplankton to flow events (Townsend et al., 2017). Indeed, the phytoplankton community was significantly different between flowing and cease to flow conditions throughout the study, highlighting how flow, and responses to delivered DOC and nutrients, shape the composition of phytoplankton communities in rivers.

Zooplankton concentrations also increased during and after flow events. In this study zooplankton populations reached their highest concentrations following the 2016 flood (Chapter 2). Zooplankton populations have been found to increase considerably following floodplain inundation elsewhere in Australia (Nielsen et al., 2016; Rees et al., 2020) often increasing in taxon richness as well (Shiel et al., 2006; Ning et al., 2013). Inundation of sediment eggbanks of zooplankton may account for a large portion of increases in zooplankton seen following flow pulses in rivers (Jenkins and Boulton, 2003). Evidence for this hatching was seen in large increases in nauplii (juvenile copepods) during flow events. However, increases in food availability may also lead to *in-situ* increases in zooplankton populations (Ning et al., 2013). Indeed, increases in zooplankton often coincide with increases in phytoplankton concentrations (Basu and Pick, 1997) which was observed several times during this research.

Interestingly, the zooplankton taxa which dominated during and shortly after flows represented feeding groups which preferentially feed on food sources linked to the microbial loop (bacteria and ciliates) and DOC influxes (Berggren et al., 2014). Furthermore, the $\delta^{13}C$ values of zooplankton aligned strongly with allochthonous carbon sources during these periods, providing evidence that allochthonous carbon can support zooplankton directly through feeding via the microbial grazer chain (Chapter 3) during periods of flow.

Discharge is critically important in driving the structure and function of riverine food webs (Poff and Zimmerman, 2010). The results presented in this thesis (Chapter 2 and 3) suggest that increases in discharge lead to pulses in allochthonous resources for consumers and stimulate increased production via both the autotrophic and heterotrophic pathways.

Zooplankton responses to flow were particularly striking, with populations orders of magnitude higher shortly after flow events than they were during baseflow conditions. In addition to the contribution of allochthonous resources, flow events may also stimulate zooplankton responses as zooplankton eggbanks may hatch in the newly inundated sediment (Jenkins and Boulton, 2003).

6.1.4 Experimental studies reveal food web patterns

Manipulative food web experiments were undertaken to build on the observations from the monitoring components of the study (Chapters 2 and 3). Specifically, the response of populations and lower food web dynamics were explored in a mesocosm study, using a floodplain derived leachate (Chapter 4). Leachate additions led to large increases in mixotroph production while obligate autotrophs such as diatoms and chlorophytes remained unchanged. This, in turn, led to very large increases in zooplankton abundance with filter feeders such as nauplii and *Daphnia* responding immediately to leachate additions while cyclopoids, which feed on higher levels in the food web, increased around 2 weeks after additions. Analyses of $\delta^{13}C$ isotopes indicated that the size of leachate addition effected the amount of allochthonous carbon used by zooplankton, with zooplankton in the high leachate treatment having $\delta^{13}C$ values closer to that of the leachate than other treatments. Further, changes in zooplankton $\delta^{13}C$ values indicated they were able to quickly switch between using allochthonous and autochthonous sources depending on the abundance, and potentially the quality, of resources. Mixotrophs played an important role in mobilising allochthonous carbon for higher trophic levels. This was demonstrated by the significant relationship between the ratio of mixotroph to autotroph biovolume and the $\delta^{13}C$ values of particulate organic matter and the relationship between particulate organic matter and zooplankton $\delta^{13}C$ values.

While zooplankton clearly assimilated allochthonous DOC via mixotrophic pathways, a further understanding of how the phytoplankton community responded to bioavailable DOC additions was required. This final microcosm experiment focussed on the changes in mixotroph populations in response to changes in bacterial abundance and competition with obligate autotrophs without the influence of zooplankton grazing. In this study, DOC+nutrient additions led to large increases in bacteria, autotrophs, mixotrophs and ciliates (Chapter 5). However, phytoplankton biovolume decreased in the DOC+nutrient addition treatment when compared to the nutrients treatment alone and bacteria only increased initially

in treatments including both DOC and nutrients. Significantly, there was a very large increase in mixotrophs and ciliates in the DOC +nutrient treatments compared to the nutrients alone treatments. This increase in mixotrophs and ciliates accounted for the losses in obligate autotroph biovolumes observed in the treatments with DOC additions. These results highlight the potential importance of mixotrophs (and ciliates) as a key trophic link in transferring allochthonous carbon to higher consumers.

6.1.5 Food web responses to pulses of dissolved organic matter

Interestingly, $\delta^{13}C$ values of zooplankton suggested even relatively small pulses of carbon during low flow conditions played a role in supporting zooplankton production (Chapter 3). Similar findings have been reported by Berggren et al, (2018), who suggested that flows enable allochthonous carbon delivery and support of zooplankton production. Furthermore, carbon sources supporting zooplankton switched from allochthonous sources during flow to autochthonous sources during cease to flow conditions as suggested by previous studies on waterholes (Bunn et al., 2003; Burford et al. 2008). Importantly, even during low flow conditions (1 to 500ML d^{-1}) allochthonous carbon may be an important energy source for riverine food webs. This is consistent with the river continuum concept, suggesting upstream mobilisation of carbon and nutrients plays a vital role in supporting downstream communities (Vannote et al., 1980). As no flood events occurred during the stable isotope portion of this research, it was not possible to assess the *in situ* effects of floods on zooplankton $\delta^{13}C$ values.

While significant food web responses have been demonstrated in response to flow events (Chapter 2, Chapter 3), the mechanisms supporting these responses are not well understood. Indeed, responses to allochthonous resource supply were not able to be disentangled from important physical factors such turbidity, turbulence and hatching of zooplankton from sediment eggbanks. To further examine the effects of allochthonous DOM on food webs without these confounding factors a mesocosm (chapter 4) and *in-situ* microcosm (Chapter 5) experiment were run. Results from the mesocosm experiment were similar to previous mesocosms on the Namoi and Bega Rivers showing zooplankton abundance increasing in response to leachate additions (Mitrovic et al., 2014; Hitchcock et al., 2016). Previous studies have found the level that different zooplankton taxa use allochthonous materials may vary significantly depending on feeding groups (Berggren et al., 2014). However, autotroph communities were initially unaffected by allochthonous DOM additions (and increased later), resulting in high levels of resources for zooplankton communities via both pathways. This is

similar to the results of mesocosm studies in Europe, which have found that additions of glucose increased heterotrophic production while leaving autotrophs unaffected, resulting in zooplankton using energy from both pathways (Faithfull et al., 2011; Degerman et al., 2018).

To examine the basal effects of DOC (glucose) additions in the microcosm experiment, zooplankton (>63 um) were excluded to remove downward grazing pressure. DOC+nutrient additions led to significant increases in the biovolume of obligate autotrophs (chlorophytes and diatoms) as well as mixotrophs and ciliates. Bacterial abundance was significantly higher and autotroph production was clearly lower in treatments with DOC additions compared to nutrients alone, indicating bacterial competition reduced autotrophic biovolume (Drakare et al., 2002; Carney et al., 2016). However, this reduction in autotrophic biovolume was made up for by increases in the production of mixotrophs and ciliates in the DOC treatments. Mixotrophs also increased significantly in the mesocosm experiment, appearing to be a major driver of change between treatments and significantly related to changes in the $\delta^{13}C$ signature of particulate organic matter in the mesocosms. Ultimately, these experiments provide critical evidence that may be used to fill knowledge gaps around the use of allochthonous dissolved organic carbon in riverine foodwebs.

6.1.6 Importance of mixotrophy

In this study, mixotrophic plankton appeared to play a major role in mobilising carbon via the microbial loop for higher trophic levels. Emerging evidence suggests mixotrophy may be both more important and far more widespread in freshwater ecosystems than previously thought (Flynn et al., 2013; Hansson et al., 2019). In both experimental studies presented in this thesis, mixotrophs increased significantly following additions of carbon and nutrients compared to control conditions. Further, mixotrophic phytoplankton were much more dominant and at higher biomass in a waterhole with dense riparian vegetation (Chapter 3) than in a comparative waterhole without riparian inputs. This suggests mixotrophs may use allochthonous carbon sources and supply a stable energy source for higher consumers during highly variable hydrological conditions. These findings align with the conclusions of previous studies which have suggested mixotrophic plankton may be a superior and more stable food source for consumers than obligate heterotrophs (Katechakis et al., 2005; Jager et al., 2014; Hansson et al., 2019). This higher food quality in mixotrophs compared to obligate autotrophs (due to their similar C:N ratios to metazoan consumers) has also been suggested to

increase the transfer efficiency of carbon via mixotrophic compared to autotrophic pathways (Katechakis et al., 2005)

6.2 Management recommendations

Supplementary flow access and e-flow rules.

The field-based components of this study (Chapters 1 and 2) directly examined the effects of discharge on the food web of the Namoi River. These investigations were a part of a trial study into the effects of changing from the 90:10 to 50:50 (environment:extraction) supplementary flow access e-flow rules. These rules changed the allowance of extraction of natural flows from 90% protected for the environment and 10% access to irrigators to a 50:50 share. Flow events of all sizes had a demonstrable positive impact on instream nutrient and carbon levels and food web production. Under low flow conditions, nutrient, DOC and zooplankton concentrations remained low and the differences between flowing and cease to flow conditions appeared to effect the energy source supporting production in zooplankton. Overbank flows in particular appeared to trigger very large increases in DOC and nutrients in the Namoi River and these nutrients from the floodplain could support large increases in food web production (Chapter 4) as suggested in the flood pulse concept (Junk et al., 1989). Reductions in natural flows under the 50:50 access rules would likely significantly reduce food web production as less river channel and floodplain area would be inundated, reducing resources for primary and secondary production, and decreasing inundation of zooplankton eggbanks and potential hatching. As such, protecting natural flows with the 90:10 access rules would give a positive impact on ecosystem processes within the river.

Riparian vegetation and waterhole conditions.

While flow events drive substantial food web responses, riparian vegetation may support the food webs of river waterholes during cease to flow conditions (Chapter 3). Significant differences in plankton dominance were observed between a well vegetated and poorly vegetation waterhole during cease to flow conditions. The waterhole with low riparian coverage exhibited heavy dominance by cyanobacteria in the phytoplankton community, especially during the warmer cease to flow periods. Riparian vegetation and phytoplankton has previously been conceptualised in the riverine productivity model as important for supporting riverine food webs (Thorp and Delong, 1994). Protecting or increasing riparian vegetation around known permanent waterholes may be an important step in increasing the ecosystem health of these waterholes, not just through the provision of habitat and climate

controlling factors, but also through modification of carbon source delivery at the base of the food web. As drought and cease to flow conditions are expected to increase with climate change, maintaining the health of these waterholes may be crucial for the survival of terrestrial and aquatic life (Reid et al., 2008; Sheldon et al., 2010).

Timed flood pulses for native fish recruitment

The results of chapters 2 and 4 suggested large overbank flows and the nutrients derived from them may result in large increases in riverine productivity, particularly increases in zooplankton abundance. Given zooplankton form most of the diets of native larval and juvenile fish, these flood pulses may significantly increase resources for fish growth and survival after spawning events (Humphries et al., 1999). Using large flood pulses (e-flows) timed to occur either before or during spawning periods may significantly increase native fish recruitment as larval fish survival has been directly correlated to increased zooplankton abundance (Rowland, 1996).

6.3 Recommendations for further studies

Zooplankton eggbanks and channel inundation.

While there is clearly a positive relationship between discharge and increased zooplankton abundance (Chapter 2), it is difficult to differentiate between the physical effects of inundating zooplankton eggbanks as compared to increases in zooplankton due to *in-situ* growth from higher food resources. Studying how changes in river height and sinuosity (based off changes to flow conditions) effect the hatching of zooplankton from sediment eggbanks would provide two important insights into food web dynamics. Firstly, it will give direct information on how changes in river channel and floodplain inundation increase zooplankton directly through recruitment processes and secondly, it will offer insights into the direct effects of resources increasing recruitment and supporting higher numbers of zooplankton following flow events. Ultimately, both of these aspects of flow events are important to zooplankton populations and riverine food webs. Coupled studies, exploring zooplankton recruitment processes and carbon utilisation are needed to fully understand the direct and indirect influences of discharge on zooplankton communities.

Energy sources supporting zooplankton production between flow and cease to flow conditions.

Given the dynamic hydrology of Australia's inland rivers, this study has provided crucial insights into the dynamics of allochthonous and autochthonous support for zooplankton between flow and cease to flow conditions (Chapter 3). Indeed, this study is one of the first in Australia to directly look at the $\delta^{13}C$ isotopes of riverine zooplankton and how they change between flow and cease to flow conditions. While initial findings indicate strong capacity of zooplankton to switch between carbon sources depending on local and hydrological conditions, there is still considerable room for improvement of techniques and understanding how carbon sources support zooplankton (and potentially higher) production in lotic systems across vastly different environmental conditions. A focus on lower trophic levels such as phytoplankton, rotifers and ciliates to fully inform the likely food sources of zooplankton would be particularly beneficial. Further research into the direct and indirect role of riparian vegetation supporting riverine food webs, particularly during cease to flow conditions may also be extremely valuable for policy making and ecosystem modelling.

Efficiency of mixotroph use of allochthonous carbon and the microbial loop

A major finding of this thesis was the apparent importance of mixotrophic algae for mobilising allochthonous carbon via the microbial loop and supporting higher consumers (Chapter 4, Chapter 5). Though this research found mixotrophic algae responded strongly to increased floodplain DOM and DOC concentrations it was not clear how or if the transfer of these resources to higher trophic levels may have changed. Research directly measuring the differences in carbon transfer efficiency between the microbial loop and mixotrophic consumption of bacteria is still a key research gap in understanding how mixotrophs and allochthonous carbon effect food web dynamics. Understanding how mixotrophy may increase the upward transfer efficiency of carbon may fill a major point of contention around the value of allochthonous carbon as a resource for higher consumers.

Potential trophic upgrading of allochthonous carbon via mixotrophy and its role on polyunsaturated fatty acids (PUFA).

A major point of contention not examined in this thesis is the role of fatty acids (or lack thereof) in the food quality of allochthonous materials. Understanding how PUFA concentrations change with mixotrophy could add significant insights towards recognizing the importance of mixotrophs in mobilising allochthonous carbon, via the microbial loop, for

higher consumers. Further, research into the changes in food quality of mixotrophs as they switch between or simultaneously use autotrophic and heterotrophic pathways may also prove important. Similarly to the questions that remain around carbon transfer efficiency, understanding how food quality and the potential trophic upgrading of allochthonous carbon via mixotrophy effects higher trophic levels will go a considerable way towards filling another contentious knowledge gap in how allochthonous carbon supports higher trophic levels.

6.4 Conclusion

The aim of this thesis was to understand how flows, allochthonous dissolved organic matter and inorganic nutrients supported lowland river food webs. Broadly, the results of this thesis found aspects of all of the major river ecology concepts (flood pulse concept, Junk et al., (1989); river continuum concept, Vannote et al., (1980); and river productivity model, Thorp and Delong, (1994)) played a role in sustaining food webs in the Namoi River. Significantly, I found multiple lines of evidence suggesting that allochthonous DOM/DOC was able to increase production within the lower food web via overbank flows, downstream movement and riparian vegetation, depending on the hydrological conditions of the river. Monitoring studies on the Namoi River suggested flow events and the resources mobilised by them (at least partially) were able to significantly boost production in zooplankton which are a primary food source for juvenile and small bodied fish (Humphries, 1999). Manipulative studies provided further evidence for allochthonous carbon boosts to riverine food webs and identified mixotrophic plankton as a potentially major trophic link between basal resources, bacteria and higher trophic levels. River management policies should include the role of allochthonous inputs as a major source of energy and production for lowland river food webs. Further studies should focus on the role of carbon sources supporting zooplankton across different flow conditions and the transfer efficiency and quality of mixotrophic algae as a food source for higher consumers.

References

Aitkenhead-Peterson, J. A., McDowell, W. H., & Neff, J. C. (2003). Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters. In *Aquatic Ecosystems*. https://doi.org/10.1016/b978-012256371-3/50003-2

Allan, J. D., & Castillo, M. M. (2007). Stream ecology: Structure and function of running waters: Second edition. In *Stream Ecology: Structure and Function of Running Waters: Second Edition*. https://doi.org/10.1007-978-1-4020-5583-6

Almeida, M. A., Cunha, M. A., & Alcântara, F. (2005). Relationship of bacterioplankton production with primary production and respiration in a shallow estuarine system (Ria de Aveiro, NW Portugal). *Microbiological Research*. https://doi.org/10.1016/j.micres.2005.02.005

Anderson, M.J., Gorley, R. N., & Clarke, K. R. (2008). Permanova+ for Primer: guide to software and statistical methods. Plymouth, UK: PRIMER-E.

APHA, 2005. Standard methods for the examination of water and wastewater, 21 ed. American Public Health Association, Washington, D.C.

Arndt, H. (1993). Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) - a review. *Hydrobiologia*, *255–256*(1), 231–246. https://doi.org/10.1007/BF00025844

Arthington, A. H. (2015). Environmental flows: A scientific resource and policy framework for river conservation and restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *25*(2), 155–161. https://doi.org/10.1002/aqc.2560

Arthington, A. H., & Balcombe, S. R. (2011). Extreme flow variability and the “boom and bust” ecology of fish in arid-zone floodplain rivers: A case history with implications for environmental flows, conservation and management. *Ecohydrology*. https://doi.org/10.1002/eco.221

Arthington, A. H., Bunn, S. E., Poff, N. L. R., & Naiman, R. J. (2006). The challenge of providing environmental flow rules to sustain river ecosystems. *Ecological Applications*, *16*(4), 1311–1318. https://doi.org/10.1890/1051-0761(2006)016[1311:TCOPEF]2.0.CO;2

Arthington, A. H., & Pusey, B. J. (2003). Flow restoration and protection in Australian rivers. *River Research and Applications*. https://doi.org/10.1002/rra.745

Azam, F., & Cho, B. (1987). Bacterial utilization of organic matter in the sea. In *Ecology of Microbial Communities*.

Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., & Thingstad, F. (1983). The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps010257

Baines, S. B., & Pace, M. L. (1991). The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. *Limnology and Oceanography*, *36*(6), 1078–1090. https://doi.org/10.4319/lo.1991.36.6.1078

Balcombe, S. R., Bunn, S. E., McKenzie-Smith, F. J., & Davies, P. M. (2005). Variability of fish diets between dry and flood periods in an arid zone floodplain river. *Journal of Fish Biology*. https://doi.org/10.1111/j.1095-8649.2005.00858.x

Balcombe, Stephen R., Sheldon, F., Capon, S. J., Bond, N. R., Hadwen, W. L., Marsh, N., & Bernays, S. J. (2011). Climate-change threats to native fish in degraded rivers and floodplains of the MurrayDarling Basin, Australia. *Marine and Freshwater Research*, *62*(9), 1099–1114.

https://doi.org/10.1071/MF11059

Baldwin, D. S. (1999). Dissolved organic matter and phosphorus leached from fresh and 'terrestrially' aged river red gum leaves: implications for assessing river-floodplain interactions. *Freshwater Biology* **41**, 675–685.

Baldwin, D. S., Colloff, M. J., Mitrovic, S. M., Bond, N. R., & Wolfenden, B. (2016). Restoring dissolved organic carbon subsidies from floodplains to lowland river food webs: A role for environmental flows? *Marine and Freshwater Research*, *67*(9), 1387–1399. https://doi.org/10.1071/MF15382

Barnett, A. J., Finlay, K., & Beisner, B. E. (2007). Functional diversity of crustacean zooplankton communities: Towards a trait-based classification. *Freshwater Biology*. https://doi.org/10.1111/j.1365-2427.2007.01733.x

Basu, B. K., & Pick, F. R. (1997). Phytoplankton and zooplankton development in a lowland, temperate river. *Journal of Plankton Research*. https://doi.org/10.1093/plankt/19.2.237

Beardall, J., Young, E., & Roberts, S. (2001). Approaches for determining phytoplankton nutrient limitation. *Aquatic Sciences*. https://doi.org/10.1007/PL00001344

Berggren, M., Bengtson, P., Soares, A. R. A., & Karlsson, J. (2018). Terrestrial support of zooplankton biomass in northern rivers. *Limnology and Oceanography*, *63*(6), 2479–2492. https://doi.org/10.1002/lno.10954

Berggren, M., Ström, L., Laudon, H., Karlsson, J., Jonsson, A., Giesler, R., Bergström, A. K., & Jansson, M. (2010). Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecology Letters*, *13*(7), 870–880. https://doi.org/10.1111/j.1461-0248.2010.01483.x

Berggren, M., Ziegler, S. E., St-Gelais, N. F., Beisner, B. E., & Del Giorgio, P. A. (2014). Erratum: Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes (Ecology (2014) 95 (1947-1959). *Ecology*, *95*(11), 3230.

Berggren, Martin, Bergström, A. K., & Karlsson, J. (2015). Intraspecific autochthonous and allochthonous resource use by zooplankton in a humic lake during the transitions between winter, summer and fall. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0120575

Berglund, J., Müren, U., Båmstedt, U., & Andersson, A. (2007). Efficiency of a phytoplankton-based and a bacteria-based food web in a pelagic marine system. *Limnology and Oceanography*. https://doi.org/10.4319/lo.2007.52.1.0121

Bertilsson, S., & Jones, J. B. (2003). Supply of Dissolved Organic Matter to Aquatic Ecosystems : Autochthonous Sources. *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*.

Blomqvist, P., Jansson, M., Drakare, S., Bergström, A. K., & Brydsten, L. (2001). Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. *Microbial Ecology*. https://doi.org/10.1007/s002480000101

Blumenshine, S. C., & Hambright, K. D. (2003). Top-down control in pelagic systems: A role for invertebrate predation. *Hydrobiologia*, *491*, 347–356. https://doi.org/10.1023/A:1024422121048

Boon, P. I., & Shiel, R. J. (1990). Grazing on bacteria by zooplankton in australian billabongs. *Marine and Freshwater Research*. https://doi.org/10.1071/MF9900247

Bott, T. L., & Kaplan, L. A. (1985). Bacterial Biomass, Metabolic State, and Activity in Stream Sediments: Relation to Environmental Variables and Multiple Assay Comparisons. *Applied and Environmental Microbiology*. https://doi.org/10.1128/aem.50.2.508-522.1985

Brett, M. T., Arhonditsis, G. B., Chandra, S., & Kainz, M. J. (2012). Mass flux calculations show strong Allochthonous support of freshwater Zooplankton production is unlikely. *PLoS ONE*,

7(6), 1–10. https://doi.org/10.1371/journal.pone.0039508

Brett M.T., Kainz M.J., Taipale S.J., Seshan H. (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America*.

Buffam, I., Galloway, J. N., Blum, L. K., & McGlathery, K. J. (2001). A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream. *Biogeochemistry*, *53*(3), 269–306. https://doi.org/10.1023/A:1010643432253

Bunn, S. E., Davies, P. M., & Mosisch, T. D. (1999). Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology*. https://doi.org/10.1046/j.1365-2427.1999.00434.x

Bunn, Stuart E., & Arthington, A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management*, *30*(4), 492–507. https://doi.org/10.1007/s00267-002-2737-0

Bunn, Stuart E., Davies, P. M., & Winning, M. (2003). Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology*, *48*(4), 619–635. https://doi.org/10.1046/j.1365-2427.2003.01031.x

Burford, M. A., Cook, A. J., Fellows, C. S., Balcombe, S. R., & Bunn, S. E. (2008). Sources of carbon fuelling production in an arid floodplain river. *Marine and Freshwater Research*, *59*(3), 224–234. https://doi.org/10.1071/MF07159

Carney, R. L., Seymour, J. R., Westhorpe, D., & Mitrovic, S. M. (2016). Lotic bacterioplankton and phytoplankton community changes under dissolved organic-carbon amendment: Evidence for competition for nutrients. *Marine and Freshwater Research*, *67*(9), 1362–1373. https://doi.org/10.1071/MF15372

Carpenter, S. R., Cole, J. J., Pace, M. L., Van De Bogert, M., Bade, D. L., Bastviken, D., Gille, C. M., Hodgson, J. R., Kitchell, J. F., & Kritzberg, E. S. (2005). Ecosystem subsidies: Terrestrial support of aquatic food webs from 13C addition to contrasting lakes. *Ecology*, *86*(10), 2737–2750. https://doi.org/10.1890/04-1282

Clarke, K. R., & Warwick, R. M. (2001). Change in marine communities: an approach to statistical analysis and interpretation. (2nd ed.). Plymouth: PRIMER-E

Closs, G. P., & Lake, P. S. (1994). Spatial and temporal variation in the structure of an intermittent-stream food web. *Ecological Monographs*. https://doi.org/10.2307/2937053

Cole, J., Findlay, S., & Pace, M. (1988). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps043001

Cole, J. J., Carpenter, S. R., Kitchell, J., Pace, M. L., Solomon, C. T., & Weidel, B. (2011). Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(5), 1975–1980. https://doi.org/10.1073/pnas.1012807108

Cook, R. A., Gawne, B., Petrie, R., Baldwin, D. S., Rees, G. N., Nielsen, D. L., & Ning, N. S. P. (2015). River metabolism and carbon dynamics in response to flooding in a lowland river. *Marine and Freshwater Research*, *66*(10), 919–927. https://doi.org/10.1071/MF14199

Costelloe, J. F., Powling, J., Reid, J. R. W., Shiel, R. J., & Hudson, P. (2005). Algal diversity and assemblages in arid zone rivers of the Lake Eyre Basin, Australia. *River Research and Applications*. https://doi.org/10.1002/rra.851

Crane, K. W., & Grover, J. P. (2010). Coexistence of mixotrophs, autotrophs, and heterotrophs in planktonic microbial communities. *Journal of Theoretical Biology*, *262*(3), 517–527.

https://doi.org/10.1016/j.jtbi.2009.10.027

Dalzell, B. J., Filley, T. R., & Harbor, J. M. (2005). Flood pulse influences on terrestrial organic matter export from an agricultural watershed. *Journal of Geophysical Research: Biogeosciences*. https://doi.org/10.1029/2005jg000043

Dalzell, B. J., Filley, T. R., & Harbor, J. M. (2007). The role of hydrology in annual organic carbon loads and terrestrial organic matter export from a midwestern agricultural watershed. *Geochimica et Cosmochimica Acta*. https://doi.org/10.1016/j.gca.2006.12.009

Danger, M., Oumarou, C., Benest, D., & Lacroix, G. (2007). Bacteria can control stoichiometry and nutrient limitation of phytoplankton. *Functional Ecology*. https://doi.org/10.1111/j.1365-2435.2006.01222.x

Degerman, R., Lefébure, R., Byström, P., Båmstedt, U., Larsson, S., & Andersson, A. (2018). Food web interactions determine energy transfer efficiency and top consumer responses to inputs of dissolved organic carbon. *Hydrobiologia*, *805*(1), 131–146. https://doi.org/10.1007/s10750-017-3298-9

Depetris, P. J., & Kempre, S. (1993). Carbon dynamics and sources in the Parani River. *Limnology and Oceanography*. https://doi.org/10.4319/lo.1993.38.2.0382

Devercelli, M. (2010). Changes in phytoplankton morpho-functional groups induced by extreme hydroclimatic events in the Middle Paraná river (Argentina). *Hydrobiologia*. https://doi.org/10.1007/s10750-009-0020-6

DPIE 2019. Namoi Surface Water Resource Plan Risk Assessment. NSW Department of Planning, Industry, and Environment – Water, Parramatta, NSW, Australia.

DPIE 2020. Barwon-Darling Long Term Water Plan, Part B: Barwon-Darling planning units. Department of Planning, Industry and Environment, Parramatta, NSW, Australia.

Drakare, S., Blomqvist, P., Bergström, A. K., & Jansson, M. (2002). Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in humic Lake Örträsket. *Freshwater Biology*, *47*(1), 41–52. https://doi.org/10.1046/j.1365-2427.2002.00779.x

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. In *Biological Reviews of the Cambridge Philosophical Society*. https://doi.org/10.1017/S1464793105006950

Easthouse, K. B., Mulder, J., Christophersen, N., & Seip, H. M. (1992). Dissolved organic carbon fractions in soil and stream water during variable hydrological conditions at Birkenes, southern Norway. *Water Resources Research*. https://doi.org/10.1029/92WR00056

Entwisle T.J., Sonneman J.A., Lewis S.H., (1997) Freshwater algae in Australia, Sainty and Associates, Monash University, Australia

Elser, J. J., & Hassett, R. P. (1994). A stoichiometric analysis of the zooplankton-phytoplankton interaction in marine and freshwater ecosystems. *Nature*. https://doi.org/10.1038/370211a0

Faithfull, C., Huss, M., Vrede, T., Karlsson, J., & Bergström, A. K. (2012). Transfer of bacterial production based on labile carbon to higher trophic levels in an oligotrophic pelagic system. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*(1), 85–93. https://doi.org/10.1139/F2011-142

Faithfull, C. L., Huss, M., Vrede, T., & Bergström, A. K. (2011). Bottom-up carbon subsidies and top-down predation pressure interact to affect aquatic food web structure. *Oikos*, *120*(2), 311–320. https://doi.org/10.1111/j.1600-0706.2010.18683.x

Fellows, C. S., Bunn, S. E., Sheldon, F., & Beard, N. J. (2009). Benthic metabolism in two turbid dryland rivers. *Freshwater Biology*, *54*(2), 236–253. https://doi.org/10.1111/j.1365-2427.2008.02104.x

Fischer, R., Giebel, H. A., Hillebrand, H., & Ptacnik, R. (2017). Importance of mixotrophic bacterivory can be predicted by light and loss rates. *Oikos*. https://doi.org/10.1111/oik.03539

Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Granéli, E., & Burkholder, J. M. (2013). Misuse of the phytoplankton-zooplankton dichotomy: The need to assign organisms as mixotrophs within plankton functional types. *Journal of Plankton Research*, *35*(1), 3–11. https://doi.org/10.1093/plankt/fbs062

Fouilland, E., Tolosa, I., Bonnet, D., Bouvier, C., Bouvier, T., Bouvy, M., Got, P., Le Floc'h, E., Mostajir, B., Roques, C., Sempéré, R., Sime-Ngando, T., & Vidussi, F. (2014). Bacterial carbon dependence on freshly produced phytoplankton exudates under different nutrient availability and grazing pressure conditions in coastal marine waters. *FEMS Microbiology Ecology*, *87*(3), 757–769. https://doi.org/10.1111/1574-6941.12262

Furst, D. J., Aldridge, K. T., Shiel, R. J., Ganf, G. G., Mills, S., & Brookes, J. D. (2014). Floodplain connectivity facilitates significant export of zooplankton to the main River Murray channel during a flood event. *Inland Waters*, *4*(4), 413–424. https://doi.org/10.5268/IW-4.4.696

Gasol, J. M., Li Zweifel, U., Peters, F., Fuhrman, J. A., & Hagström, Å. (1999). Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Applied and Environmental Microbiology*. https://doi.org/10.1128/aem.65.10.4475-4483.1999

Gawne, B., Merrick, C., Williams, D. G., Rees, G., Oliver, R., Bowen, P. M., Treadwell, S., Beattie, G., Ellis, I., Frankenberg, J., & Lorenz, Z. (2007). Patterns of primary and heterotrophic productivity in an arid lowland river. *River Research and Applications*. https://doi.org/10.1002/rra.1033

Gillor, O., Hadas, O., Post, A. F., & Belkin, S. (2010). Phosphorus and nitrogen in a monomictic freshwater lake: Employing cyanobacterial bioreporters to gain new insights into nutrient bioavailability. *Freshwater Biology*, *55*(6), 1182–1190. https://doi.org/10.1111/j.1365-2427.2009.02342.x

Goes, J. I., Handa, N., Taguchi, S., Hama, T., & Saito, H. (1995). Impact of UV radiation on the production patterns and composition of dissolved free and combined amino acids in marine phytoplankton. *Journal of Plankton Research*. https://doi.org/10.1093/plankt/17.6.1337

Godwin, C.M., Whitaker, E.A. and Cotner, J.B., 2017. Growth rate and resource imbalance nteractively control biomass stoichiometry and elemental quotas of aquatic bacteria. *Ecology*, *98*(3), pp.820-829.

Graf, W. L. (1999). Dam nation: A geographic census of american dams and their large-scale hydrologic impacts. *Water Resources Research*. https://doi.org/10.1029/1999WR900016

Grafton, R. Q., & Hussey, K. (2007). Buying back the living Murray: At what price? *Australasian Journal of Environmental Management*. https://doi.org/10.1080/14486563.2007.10648704

Griffiths, N. A., Tank, J. L., Royer, T. V., Warrner, T. J., Frauendorf, T. C., Rosi-Marshall, E. J., & Whiles, M. R. (2012). Temporal variation in organic carbon spiraling in Midwestern agricultural streams. *Biogeochemistry*. https://doi.org/10.1007/s10533-011-9585-z

Hadwen, W. L., Fellows, C. S., Westhorpe, D. P., Rees, G. N., Mitrovic, S. M., Taylor, B., Baldwin, D. S., Silvester, E., & Croome, R. (2010a). Longitudinal trends in river functioning: Patterns of nutrient and carbon processing in three Australian rivers. *River Research and Applications*. https://doi.org/10.1002/rra.1321

Hadwen, W. L., Spears, M., & Kennard, M. J. (2010b). Temporal variability of benthic algal δ13C signatures influences assessments of carbon flows in stream food webs. *Hydrobiologia*, *651*(1), 239–251. https://doi.org/10.1007/s10750-010-0303-y

Hama, T., & Honjo, T. (1987). Photo synthetic products and nutrient availability in phytoplankton population from Gokasho Bay, Japan. *Journal of Experimental Marine Biology and Ecology*. https://doi.org/10.1016/0022-0981(87)90072-4

Hansson, T. H., Grossart, H. P., del Giorgio, P. A., St-Gelais, N. F., & Beisner, B. E. (2019). Environmental drivers of mixotrophs in boreal lakes. *Limnology and Oceanography*, *64*(4), 1688–1705. https://doi.org/10.1002/lno.11144

Hart, D. D., & Finelli, C. M. (1999). Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. *Annual Review of Ecology and Systematics*. https://doi.org/10.1146/annurev.ecolsys.30.1.363

Harwood, J. L., & Russell, N. J. (1984). Lipids in Plants and Microbes. In *Lipids in Plants and Microbes*. https://doi.org/10.1007/978-94-011-5989-0

Hecky, R. E., & Kilham, P. (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnology and Oceanography*. https://doi.org/10.4319/lo.1988.33.4_part_2.0796

Hein, T., Baranyi, C., Herndl, G. J., Wanek, W., & Schiemer, F. (2003). Allochthonous and autochthonous particulate organic matter in floodplains of the River Danube: The importance of hydrological connectivity. *Freshwater Biology*, *48*(2), 220–232. https://doi.org/10.1046/j.1365-2427.2003.00981.x

Henson, S. S., Ahearn, D. S., Dahlgren, R. A., Van Nieuwenhuyse, E., Tate, K. W., & Fleenor, W. E. (2007). Water quality response to a pulsed-flow event on the Mokelumne River, California. *River Research and Applications*. https://doi.org/10.1002/rra.973

Hessen, D. O. (2006). Determinants of seston C:P-ratio in lakes. *Freshwater Biology*, *51*(8), 1560–1569. https://doi.org/10.1111/j.1365-2427.2006.01594.x

Hessen, D. O., Andersen, T., Brettum, P., & Faafeng, B. A. (2003). Phytoplankton contribution to sestonic mass and elemental ratios in lakes: Implications for zooplankton nutrition. *Limnology and Oceanography*. https://doi.org/10.4319/lo.2003.48.3.1289

Hessen D.O. Faafeng B.O. (2000). Elemental ratios in freshwater seston: Implications for community structure and energy transfer in food webs. Arch. Hyrobiol. Special Issues Adv. Limnol. **55:** 349–363.

Hiltunen, M., Honkanen, M., Taipale, S., Strandberg, U., & Kankaala, P. (2017). Trophic upgrading via the microbial food web may link terrestrial dissolved organic matter to Daphnia. *Journal of Plankton Research*, *39*(6), 861–869. https://doi.org/10.1093/plankt/fbx050

Hinton, M. J., Schiff, S. L., & English, M. C. (1997). The significance of storms for the concentration and export of dissolved organic carbon from two Precambrian Shield catchments. *Biogeochemistry*, *36*(1), 67–88. https://doi.org/10.1023/A:1005779711821

Hitchcock, J. N., & Mitrovic, S. M. (2015). After the flood: changing dissolved organic carbon bioavailability and bacterial growth following inflows to estuaries. *Biogeochemistry*, *124*(1–3), 219–233. https://doi.org/10.1007/s10533-015-0094-3

Hitchcock, J. N., & Mitrovic, S. M. (2013). Different resource limitation by carbon, nitrogen and phosphorus between base flow and high flow conditions for estuarine bacteria and phytoplankton. *Estuarine, Coastal and Shelf Science*, *135*, 106–115. https://doi.org/10.1016/j.ecss.2013.05.001

Hitchcock, J. N., Mitrovic, S. M., Hadwen, W. L., Roelke, D. L., Growns, I. O., & Rohlfs, A. M. (2016). Terrestrial dissolved organic carbon subsidizes estuarine zooplankton: An in situ mesocosm study. *Limnology and Oceanography*, *61*(1), 254–267. https://doi.org/10.1002/lno.10207

Hladyz, S., Nielsen, D. L., Suter, P. J., & Krull, E. S. (2012). Temporal variations in organic carbon utilization by consumers in a lowland river. *River Research and Applications*, *28*(5), 513–528. https://doi.org/10.1002/rra.1467

Hoeinghaus, D. J., Winemiller, K. O., & Agostinho, A. A. (2007). Landscape-scale hydrologic characteristics differentiate patterns of carbon flow in large-river food webs. *Ecosystems*, *10*(6), 1019–1033. https://doi.org/10.1007/s10021-007-9075-2

Humphries, P., King, A. J., & Koehn, J. D. (1999). Fish, flows and flood plains: Links between freshwater fishes and their environment in the Murray-Darling River system, Australia. *Environmental Biology of Fishes*, *56*(1–2), 129–151. https://doi.org/10.1023/a:1007536009916

Hunt, R. J., Jardine, T. D., Hamilton, S. K., & Bunn, S. E. (2012). Temporal and spatial variation in ecosystem metabolism and food web carbon transfer in a wet-dry tropical river. *Freshwater Biology*, *57*(3), 435–450. https://doi.org/10.1111/j.1365-2427.2011.02708.x

Hunter, M. D., & Price, P. W. (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology*. https://doi.org/10.2307/1940152

Huryn, A. D., Riley, R. H., Young, R. G., Arbuckle, C. J., Peacock, K., & Lyon, G. (2001). Temporal shift in contribution of terrestrial organic matter to consumer production in a grassland river. *Freshwater Biology*, *46*(2), 213–226. https://doi.org/10.1046/j.1365-2427.2001.00648.x

Hygum, B. H., Petersen, J. W., & Søndergaard, M. (1997). Dissolved organic carbon released by zooplankton grazing activity - A high-quality substrate pool for bacteria. *Journal of Plankton Research*. https://doi.org/10.1093/plankt/19.1.97

Irigoien, X., & Castel, J. (1997). Light limitation and distribution of chlorophyll pigments in a highly turbid estuary: The Gironde (SW France). *Estuarine, Coastal and Shelf Science*. https://doi.org/10.1006/ecss.1996.0132

Jäger, C. G., Vrede, T., Persson, L., & Jansson, M. (2014). Interactions between metazoans, autotrophs, mixotrophs and bacterioplankton in nutrient-depleted high DOC environments: A long-term experiment. *Freshwater Biology*, *59*(8), 1596–1607. https://doi.org/10.1111/fwb.12366

James, C. S., Thoms, M. C., & Quinn, G. P. (2008). Zooplankton dynamics from inundation to drying in a complex ephemeral floodplain-wetland. *Aquatic Sciences*, *70*(3), 259–271. https://doi.org/10.1007/s00027-008-8034-0

Jansson, M., Bergstrom, A. K., Blomqvist, P., & Drakare, S. (2000). Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology*. https://doi.org/10.1890/0012-9658(2000)081[3250:AOCAPB]2.0.CO;2

Jansson, Mats, Bergström, A. K., Lymer, D., Vrede, K., & Karlsson, J. (2006). Bacterioplankton growth and nutrient use efficiencies under variable organic carbon and inorganic phosphorus ratios. *Microbial Ecology*. https://doi.org/10.1007/s00248-006-9013-4

Jansson, Mats, Persson, L., De Roos, A. M., Jones, R. I., & Tranvik, L. J. (2007). Terrestrial carbon and intraspecific size-variation shape lake ecosystems. In *Trends in Ecology and Evolution*. https://doi.org/10.1016/j.tree.2007.02.015

Jardine, T. D., Hadwen, W. L., Hamilton, S. K., Hladyz, S., Mitrovic, S. M., Kidd, K. A., Tsoi, W. Y., Spears, M., Westhorpe, D. P., Fry, V. M., Sheldon, F., & Bunn, S. E. (2014). Understanding and

overcoming baseline isotopic variability in running waters. *River Research and Applications*, *30*(2), 155–165. https://doi.org/10.1002/rra.2630

Jardine, Timothy D., Woods, R., Marshall, J., Fawcett, J., Lobegeiger, J., Valdez, D., & Kainz, M. J. (2015). Reconciling the role of organic matter pathways in aquatic food webs by measuring multiple tracers in individuals. *Ecology*. https://doi.org/10.1890/14-2153.1

Jenkins, K. M., & Boulton, A. J. (2003). Connectivity in a dryland river: Short-term aquatic microinvertebrate recruitment following floodplain inundation. *Ecology*, *84*(10), 2708–2723. https://doi.org/10.1890/02-0326

Jones, R. I. (2000). Mixotrophy in planktonic protists: An overview. *Freshwater Biology*, *45*(2), 219–226. https://doi.org/10.1046/j.1365-2427.2000.00672.x

Jones, R. I., Kankaala, P., Nykänen, H., Peura, S., Rask, M., & Vesala, S. (2018). Whole-Lake Sugar Addition Demonstrates Trophic Transfer of Dissolved Organic Carbon to Top Consumers. *Ecosystems*, *21*(3), 495–506. https://doi.org/10.1007/s10021-017-0164-6

Junk, Wolfgang J., Bayley, Peter B., Sparks, R. (1989). Junk et al. 1989 - the flood pulse concept in river-floodplain systems..pdf. In *Canadian Journal of Fisheries and Aquatic Science*.

Jürgens, K., & Jeppesen, E. (2000). The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. *Journal of Plankton Research*. https://doi.org/10.1093/plankt/22.6.1047

Kamjunke, N., Benndorf, A., Wilbert, C., Opitz, M., Kranich, J., Bollenbach, M., & Benndorf, J. (1999). Bacteria ingestion by Daphnia galeata in a biomanipulated reservoir: A mechanism stabilizing biomanipulation? *Hydrobiologia*. https://doi.org/10.1023/A:1003722318598

Kamjunke, N., Henrichs, T., & Gaedke, U. (2007). Phosphorus gain by bacterivory promotes the mixotrophic flagellate Dinobryon spp. during re-oligotrophication. *Journal of Plankton Research*. https://doi.org/10.1093/plankt/fbl054

Kamjunke, N., & Tittel, J. (2009). Mixotrophic algae constrain the loss of organic carbon by exudation1. *Journal of Phycology*, *45*(4), 807–811. https://doi.org/10.1111/j.1529-8817.2009.00707.x

Karlsson, J., Jonsson, A., & Jansson, M. (2001). Bacterioplankton production in lakes along an altitude gradient in the subarctic north of Sweden. *Microbial Ecology*. https://doi.org/10.1007/s00248-001-0009-9

Karlsson, Jan, Berggren, M., Ask, J., Byström, P., Jonsson, A., Laudon, H., & Jansson, M. (2012). Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. *Limnology and Oceanography*, *57*(4), 1042–1048. https://doi.org/10.4319/lo.2012.57.4.1042

Karlsson, Jan, Jonsson, A., Meili, M., & Jansson, M. (2003). Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography*. https://doi.org/10.4319/lo.2003.48.1.0269

Karlsson, Jan, Lymer, D., Vrede, K., & Jansson, M. (2007). Differences in efficiency of carbon transfer from dissolved organic carbon to two zooplankton groups: An enclosure experiment in an oligotrophic lake. *Aquatic Sciences*, *69*(1), 108–114. https://doi.org/10.1007/s00027-007-0913-2

Katechakis, A., Haseneder, T., Kling, R., & Stibor, H. (2005). Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light : Nutrient hypothesis might not hold for mixotrophs. *Limnology and Oceanography*, *50*(4), 1290–1299. https://doi.org/10.4319/lo.2005.50.4.1290

Katechakis, A., & Stibor, H. (2006). The mixotroph Ochromonas tuberculata may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. *Oecologia*. https://doi.org/10.1007/s00442-006-0413-4

Kelleway, J., Mazumder, D., Wilson, G. G., Saintilan, N., Knowles, L., Iles, J., & Kobayashi, T. (2010). Trophic structure of benthic resources and consumers varies across a regulated floodplain wetland. *Marine and Freshwater Research*. https://doi.org/10.1071/MF09113

Kemp, J. (2010). Downstream channel changes on a contracting, anabranching river: The Lachlan, southeastern Australia. *Geomorphology*. https://doi.org/10.1016/j.geomorph.2010.04.018

King, A. J. (2005). Ontogenetic dietary shifts of fishes in an Australian floodplain river. *Marine and Freshwater Research*. https://doi.org/10.1071/MF04117

King, A. J., Tonkin, Z., & Mahoney, J. (2009). Environmental flow enhances native fish spawning and recruitment in the Murray River, Australia. *River Research and Applications*. https://doi.org/10.1002/rra.1209

Kingsford, R. T. (2000). Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. In *Austral Ecology*. https://doi.org/10.1046/j.1442-9993.2000.01036.x

Kobayashi, T., Shiel, R. J., Gibbs, P., & Dixon, P. I. (1998). Freshwater zooplankton in the Hawkesbury-Nepean River: Comparison of community structure with other rivers. In *Hydrobiologia*. https://doi.org/10.1023/a:1003240511366

Kobayashi, Tsuyoshi, & Church, A. G. (2003). Role of nutrients and zooplankton grazing on phytoplankton growth in a temperate reservoir in New South Wales, Australia. *Marine and Freshwater Research*. https://doi.org/10.1071/MF02025

Kobayashi, Tsuyoshi, Gibbs, P., Dixon, P. I., & Shiel, R. J. (1996). Grazing by a river zooplankton community: Importance of microzooplankton. *Marine and Freshwater Research*. https://doi.org/10.1071/MF9961025

Kritzberg, E. S., Cole, J. J., Pace, M. L., Granéli, W., & Bade, D. L. (2004). Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake 13C addition experiments. *Limnology and Oceanography*, *49*(2), 588–596. https://doi.org/10.4319/lo.2004.49.2.0588

Lebaron, P., Servais, P., Agogué, H., Courties, C., & Joux, F. (2001). Does the High Nucleic Acid Content of Individual Bacterial Cells Allow Us to Discriminate between Active Cells and Inactive Cells in Aquatic Systems? *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.67.4.1775-1782.2001

Leigh, C., Burford, M. A., Sheldon, F., & Bunn, S. E. (2010). Dynamic stability in dry season food webs within tropical floodplain rivers. *Marine and Freshwater Research*, *61*(3), 357–368. https://doi.org/10.1071/MF09107

Lichti, D. A., Rinchard, J., & Kimmel, D. G. (2017). Changes in zooplankton community, and seston and zooplankton fatty acid profiles at the freshwater/saltwater interface of the Chowan River, North Carolina. *PeerJ*. https://doi.org/10.7717/peerj.3667

Ligon, F. K., Dietrich, W. E., & Trush, W. J. (1995). Downstream Ecological Effects of Dams. *BioScience*. https://doi.org/10.2307/1312557

MacLeod, N. A., & Barton, D. R. (1998). Effects of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. *Canadian Journal of Fisheries and Aquatic Sciences*. https://doi.org/10.1139/f98-075

Makino, W., Cotner, J. B., Sterner, R. W., & Elser, J. J. (2003). Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. *Functional*

Ecology. https://doi.org/10.1046/j.1365-2435.2003.00712.x

Maranger, R. J., Pace, M. L., Del Giorgio, P. A., Caraco, N. F., & Cole, J. J. (2005). Longitudinal spatial patterns of bacterial production and respiration in a large river-estuary: Implications for ecosystem carbon consumption. *Ecosystems*. https://doi.org/10.1007/s10021-003-0071-x

Marcarelli, A. M., Baxter, C. V., Mineau, M. M., & Hall, R. O. (2011). Quantity and quality: Unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology*. https://doi.org/10.1890/10-2240.1

May, C. L., Koseff, J. R., Lucas, L. V., Cloern, J. E., & Schoellhamer, D. H. (2003). Effects of spatial and temporal variability of turbidity on phytoplankton blooms. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps254111

Mazumder, D., Johansen, M., Saintilan, N., Iles, J., Kobayashi, T., Knowles, L., & Wen, L. (2012). Trophic shifts involving native and exotic fish during hydrologic recession in floodplain wetlands. *Wetlands*. https://doi.org/10.1007/s13157-011-0262-8

McMeans, B. C., Koussoroplis, A. M., Arts, M., & Kainz, M. J. (2015). Terrestrial dissolved organic matter supports growth and reproduction of Daphnia magna when algae are limiting. *Journal of Plankton Research*, *37*(6), 1201–1209. https://doi.org/10.1093/plankt/fbv083

Medeiros, E. S. F., & Arthington, A. H. (2008). The importance of zooplankton in the diets of three native fish species in floodplain waterholes of a dryland river, the Macintyre River, Australia. *Hydrobiologia*, *614*(1), 19–31. https://doi.org/10.1007/s10750-008-9533-7

Medeiros, E. S. F., & Arthington, A. H. (2011). Allochthonous and autochthonous carbon sources for fish in floodplain lagoons of an Australian dryland river. *Environmental Biology of Fishes*, *90*(1), 1–17. https://doi.org/10.1007/s10641-010-9706-x

Mitra, A., Flynn, K. J., Burkholder, J. M., Berge, T., Calbet, A., Raven, J. A., Granéli, E., Glibert, P. M., Hansen, P. J., Stoecker, D. K., Thingstad, F., Tillmann, U., Väge, S., Wilken, S., & Zubkov, M. V. (2014). The role of mixotrophic protists in the biological carbon pump. *Biogeosciences*. https://doi.org/10.5194/bg-11-995-2014

Mitrovic, S. M., Westhorpe, D. P., Kobayashi, T., Baldwin, D. S., Ryan, D., & Hitchcock, J. N. (2014). Short-term changes in zooplankton density and community structure in response to different sources of dissolved organic carbon in an unconstrained lowland river: Evidence for food web support. *Journal of Plankton Research*, *36*(6), 1488–1500. https://doi.org/10.1093/plankt/fbu072

Mojica, K. D. A., Carlson, C. A., & Behrenfeld, M. J. (2020). Regulation of Low and High Nucleic Acid Fluorescent Heterotrophic Prokaryote Subpopulations and Links to Viral-Induced Mortality Within Natural Prokaryote-Virus Communities. *Microbial Ecology*. https://doi.org/10.1007/s00248-019-01393-9

Moran, N. P., Ganf, G. G., Wallace, T. A., & Brookes, J. D. (2014). Flow variability and longitudinal characteristics of organic carbon in the Lachlan River, Australia. *Marine and Freshwater Research*, *65*(1), 50–58. https://doi.org/10.1071/MF12297

Nicolle, A., Hallgren, P., Von Einem, J., Kritzberg, E. S., Granéli, W., Persson, A., Brönmark, C., & Hansson, L. A. (2012). Predicted warming and browning affect timing and magnitude of plankton phenological events in lakes: A mesocosm study. *Freshwater Biology*. https://doi.org/10.1111/j.1365-2427.2012.02733.x

Nielsen, D. L., Cook, R. A., Ning, N., Gawne, B., & Petrie, R. (2016). Carbon and nutrient subsidies to a lowland river following floodplain inundation. *Marine and Freshwater Research*, *67*(9), 1302–1312. https://doi.org/10.1071/MF14390

Ning, N. S. P., Gawne, B., Cook, R. A., & Nielsen, D. L. (2013). Zooplankton dynamics in response

to the transition from drought to flooding in four Murray-Darling Basin rivers affected by differing levels of flow regulation. *Hydrobiologia*, *702*(1), 45–62. https://doi.org/10.1007/s10750-012-1306-7

Ning, N. S. P., Nielsen, D. L., Hillman, T. J., & Suter, P. J. (2010). The influence of planktivorous fish on zooplankton communities in riverine slackwaters. *Freshwater Biology*. https://doi.org/10.1111/j.1365-2427.2009.02283.x

Oliver, R. L., & Merrick, C. J. (2006). Partitioning of river metabolism identifies phytoplankton as a major contributor in the regulated Murray River (Australia). *Freshwater Biology*. https://doi.org/10.1111/j.1365-2427.2006.01562.x

Oliver, R. L., Mitrovic, S. M., & Rees, C. (2010). Influence of salinity on light conditions and phytoplankton growth in a turbid river. *River Research and Applications*. https://doi.org/10.1002/rra.1309

Pace, M. L., Cole, J. J., Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Van De Bogert, M. C., Bade, D. L., Kritzberg, E. S., & Bastviken, D. (2004). Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, *427*(6971), 240–243. https://doi.org/10.1038/nature02227

Page, K., Read, A., Frazier, P., & Mount, N. (2005). The effect of altered flow regime on the frequency and duration of bankfull discharge: Murrumbidgee river, Australia. *River Research and Applications*. https://doi.org/10.1002/rra.828

Pahl-Wostl, C., Arthington, A., Bogardi, J., Bunn, S. E., Hoff, H., Lebel, L., Nikitina, E., Palmer, M., Poff, L. R. N., Richards, K., Schlüter, M., Schulze, R., St-Hilaire, A., Tharme, R., Tockner, K., & Tsegai, D. (2013). Environmental flows and water governance: Managing sustainable water uses. *Current Opinion in Environmental Sustainability*, *5*(3–4), 341–351. https://doi.org/10.1016/j.cosust.2013.06.009

Patterson D.J., (1996) Free-living freshwater protozoa: a colour guide, Manson publishing, USA

Poff, N. L. R., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., Sparks, R. E., & Stromberg, J. C. (1997). The natural flow regime: A paradigm for river conservation and restoration. *BioScience*. https://doi.org/10.2307/1313099

Poff, N. L. R., Olden, J. D., Merritt, D. M., & Pepin, D. M. (2007). Homogenization of regional river dynamics by dams and global biodiversity implications. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(14), 5732–5737. https://doi.org/10.1073/pnas.0609812104

Poff, N. L., Richter, B. D., Arthington, A. H., Bunn, S. E., Naiman, R. J., Kendy, E., Acreman, M., Apse, C., Bledsoe, B. P., Freeman, M. C., Henriksen, J., Jacobson, R. B., Kennen, J. G., Merritt, D. M., O'Keeffe, J. H., Olden, J. D., Rogers, K., Tharme, R. E., & Warner, A. (2010). The ecological limits of hydrologic alteration (ELOHA): A new framework for developing regional environmental flow standards. *Freshwater Biology*, *55*(1), 147–170. https://doi.org/10.1111/j.1365-2427.2009.02204.x

Poff, N. L., & Zimmerman, J. K. H. (2010). Ecological responses to altered flow regimes: A literature review to inform the science and management of environmental flows. *Freshwater Biology*, *55*(1), 194–205. https://doi.org/10.1111/j.1365-2427.2009.02272.x

Post, D. M. (2002). The long and short of food-chain length. In *Trends in Ecology and Evolution*. https://doi.org/10.1016/S0169-5347(02)02455-2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*(1), 179–189. https://doi.org/10.1007/s00442-006-0630-x

Power, M. E., Sun, A., Parker, G., Dietrich, W. E., & Wootton, J. T. (1995). Hydraulic Food-Chain Models. *BioScience*. https://doi.org/10.2307/1312555

Prescott G.G, (1978) How to know the freshwater algae, 3rd edn, WCB?Mcgraw-Hill, USA

Puckridge, J. T., Sheldon, F., Walker, K. F., & Boulton, A. J. (1998). Flow variability and the ecology of large rivers. *Marine and Freshwater Research*. https://doi.org/10.1071/MF94161

Pusey, B. J., Pusey, B. J., Jardine, T. D., Beesley, L. S., Kennard, M. J., Ho, T. W., Bunn, S. E., & Douglas, M. M. (2020). Carbon sources supporting Australia's most widely distributed freshwater fish, Nematalosa erebi (Günther) (Clupeidae: Dorosomatinae). *Marine and Freshwater Research*, *June*. https://doi.org/10.1071/MF20014

Raymond, P. A., & Saiers, J. E. (2010). Event controlled DOC export from forested watersheds. *Biogeochemistry*, *100*(1), 197–209. https://doi.org/10.1007/s10533-010-9416-7

Rees, G. N., Cook, R. A., Ning, N. S. P., McInerney, P. J., Petrie, R. T., & Nielsen, D. L. (2020). Managed floodplain inundation maintains ecological function in lowland rivers. *Science of the Total Environment*, *727*, 138469. https://doi.org/10.1016/j.scitotenv.2020.138469

Reid, D. J., Quinn, G. P., Lake, P. S., & Reich, P. (2008). Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: A stable isotope study. *Freshwater Biology*, *53*(10), 2036–2050. https://doi.org/10.1111/j.1365-2427.2008.02025.x

Richter, B. D., Mathews, R., Harrison, D. L., & Wigington, R. (2003). Ecologically sustainable water management: Managing river flows for ecological integrity. *Ecological Applications*. https://doi.org/10.1890/1051-0761(2003)013[0206:ESWMMR]2.0.CO;2

Richter, B. D., Warner, A. T., Meyer, J. L., & Lutz, K. (2006). A collaborative and adaptive process for developing environmental flow recommendations. In *River Research and Applications*. https://doi.org/10.1002/rra.892

Roach, K. A. (2013). Environmental factors affecting incorporation of terrestrial material into large river food webs. *Freshwater Science*, *32*(1), 283–298. https://doi.org/10.1899/12-063.1

Roach, K. A., Winemiller, K. O., Layman, C. A., & Zeug, S. C. (2009). Consistent trophic patterns among fishes in lagoon and channel habitats of a tropical floodplain river: Evidence from stable isotopes. *Acta Oecologica*. https://doi.org/10.1016/j.actao.2009.03.007

Robertson, A. I., Bunn, S. E., Boon, P. I., & Walker, K. F. (1999). Sources, sinks and transformations of organic carbon in Australian floodplain rivers. In *Marine and Freshwater Research*. https://doi.org/10.1071/MF99112

Rolls, R. J., Boulton, A. J., Growns, I. O., Maxwell, S. E., Ryder, D. S., & Westhorpe, D. P. (2012). Effects of an experimental environmental flow release on the diet of fish in a regulated coastal Australian river. *Hydrobiologia*, *686*(1), 195–212. https://doi.org/10.1007/s10750-012-1012-5

Rowland, S. J. (1996). Development of techniques for the large-scale rearing of the larvae of the Australian freshwater fish golden perch, Macquaria ambigua (Richardson, 1845). *Marine and Freshwater Research*, *47*(2), 233–242. https://doi.org/10.1071/MF9960233

Sanders, R. W., Caron, D. A., Davidson, J. M., Dennett, M. R., & Moran, D. M. (2001). Nutrient acquisition and population growth of a mixotrophic alga in axenic and bacterized cultures. *Microbial Ecology*. https://doi.org/10.1007/s00248-001-1024-6

Sanderson, J. S., Rowan, N., Wilding, T., Bledsoe, B. P., Miller, W. J., & Poff, N. L. (2012). Getting to scale with environmental flow assessment: The watershed flow evaluation tool. *River Research and Applications*. https://doi.org/10.1002/rra.1542

Seymour, J., Seuront, L., and Mitchell, J. G. (2007). Microscale gradients of planktonic microbial communities above the sediment surface in a mangrove estuary. *Estuarine, Coastal and Shelf Science* **73**, 651–666. doi:10.1016/J.ECSS.2007.03.004

Shafroth, P. B., Wilcox, A. C., Lytle, D. A., Hickey, J. T., Andersen, D. C., Beauchamp, V. B., Hautzinger, A., McMullen, L. E., & Warner, A. (2010). Ecosystem effects of environmental flows: Modelling and experimental floods in a dryland river. *Freshwater Biology*, *55*(1), 68–85. https://doi.org/10.1111/j.1365-2427.2009.02271.x

Sheldon, F., & Fellows, C. S. (2010). Water quality in two Australian dryland rivers: Spatial and temporal variability and the role of flow. *Marine and Freshwater Research*, *61*(8), 864–874. https://doi.org/10.1071/MF09289

Shiel R.J., (1995) A guide to the identification of rotifers, cladocerans, and copepods from Australian inland waters. Cooperative research centre for freshwater ecology, Australia

Shiel, R. J., Costelloe, J. F., Reid, J. R. W., Hudson, P., & Pawling, J. (2006). Zooplankton diversity and assemblages in arid zone rivers of the Lake Eyre Basin, Australia. *Marine and Freshwater Research*, *57*(1), 49–60. https://doi.org/10.1071/MF05101

Sommer, U., & Sommer, F. (2006). Cladocerans versus copepods: The cause of contrasting top-down controls on freshwater and marine phytoplankton. *Oecologia*. https://doi.org/10.1007/s00442-005-0320-0

Sommer, U., Stibor, H., Katechakis, A., Sommer, F., & Hansen, T. (2002). Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. *Hydrobiologia*. https://doi.org/10.1023/A:1021340601986

Stahl, D. A., Flowers, J. J., Hullar, M., & Davidson, S. (2012). Structure and function of microbial communities. In *The Prokaryotes: Prokaryotic Communities and Ecophysiology*. https://doi.org/10.1007/978-3-642-30123-0_34

Sternberg, D., Balcombe, S., Marshall, J., & Lobegeiger, J. (2008). Food resource variability in an Australian dryland river: Evidence from the diet of two generalist native fish species. *Marine and Freshwater Research*, *59*(2), 137–144. https://doi.org/10.1071/MF07125

Sterner, R. W., Elser, J. J., Fee, E. J., Guildford, S. J., & Chrzanowski, T. H. (1997). The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *American Naturalist*. https://doi.org/10.1086/286088

Straile, D. (1997). Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. *Limnology and Oceanography*. https://doi.org/10.4319/lo.1997.42.6.1375

Taipale, S. J., Brett, M. T., Pulkkinen, K., & Kainz, M. J. (2012). The influence of bacteria-dominated diets on daphnia magna somatic growth, reproduction, and lipid composition. *FEMS Microbiology Ecology*, *82*(1), 50–62. https://doi.org/10.1111/j.1574-6941.2012.01406.x

Tanentzap, A. J., Kielstra, B. W., Wilkinson, G. M., Berggren, M., Craig, N., Del Giorgio, P. A., Grey, J., Gunn, J. M., Jones, S. E., Karlsson, J., Solomon, C. T., & Pace, M. L. (2017). Terrestrial support of lake food webs: Synthesis reveals controls over cross-ecosystem resource use. *Science Advances*. https://doi.org/10.1126/sciadv.1601765

Thorp, J. H., & Delong, M. D. (1994). The Riverine Productivity Model: An Heuristic View of Carbon Sources and Organic Processing in Large River Ecosystems. *Oikos*. https://doi.org/10.2307/3545642

Thorp, J. H., & Delong, M. D. (2002). Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. In *Oikos*. https://doi.org/10.1034/j.1600-0706.2002.960315.x

Tittel, J., Bissinger, V., Gaedke, U., & Kamjunke, N. (2005). Inorganic carbon limitation and mixotrophic growth in Chlamydomonas from an acidic mining lake. *Protist*. https://doi.org/10.1016/j.protis.2004.09.001

Tittel, J., & Kamjunke, N. (2004). Metabolism of dissolved organic carbon by planktonic bacteria and mixotrophic algae in lake neutralisation experiments. *Freshwater Biology*, *49*(8), 1062–1071. https://doi.org/10.1111/j.1365-2427.2004.01241.x

Tockner, K., Malard, F., & Ward, J. V. (2000). An extension of the flood pulse concept. *Hydrological Processes*, *14*(16–17), 2861–2883. https://doi.org/10.1002/1099-1085(200011/12)14:16/17<2861::AID-HYP124>3.0.CO;2-F

Townsend, S. A., & Douglas, M. M. (2017). Discharge-driven flood and seasonal patterns of phytoplankton biomass and composition of an Australian tropical savannah river. *Hydrobiologia*, *794*(1), 203–221. https://doi.org/10.1007/s10750-017-3094-6

Trochine, C., Villanueva, V. D., Navarro, M. B., Balseiroand, E., & Modenutti, B. (2015). The abundance of mixotrophic algae drives the carbon isotope composition of the copepod Boeckella gracilipes in shallow Patagonian lakes. *Journal of Plankton Research*, *37*(2), 441–451. https://doi.org/10.1093/plankt/fbv005

Unrein, F., Massana, R., Alonso-Sáez, L., & Gasol, J. M. (2007). Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. *Limnology and Oceanography*. https://doi.org/10.4319/lo.2007.52.1.0456

Vadstein, O. (2000). *Heterotrophic, Planktonic Bacteria and Cycling of Phosphorus*. https://doi.org/10.1007/978-1-4615-4187-5_4

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*. https://doi.org/10.1139/f80-017

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Liermann, C. R., & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*. https://doi.org/10.1038/nature09440

Wallace, T. A., & Furst, D. (2016). Open water metabolism and dissolved organic carbon in response to environmental watering in a lowland river-floodplain complex. *Marine and Freshwater Research*, *67*(9), 1346–1361. https://doi.org/10.1071/MF15318

Ward, B. A., & Follows, M. J. (2016). Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), 2958–2963. https://doi.org/10.1073/pnas.1517118113

Ward, J. V. (1989). The Four-Dimensional Nature of Lotic Ecosystems. *Journal of the North American Benthological Society*. https://doi.org/10.2307/1467397

Ward, J. V., & Stanford, J. A. (1995). The serial discontinuity concept: Extending the model to floodplain rivers. *Regulated Rivers: Research & Management*, *10*(2–4), 159–168. https://doi.org/10.1002/rrr.3450100211

Watkins, S. C., Quinn, G. P., & Gawne, B. (2010). Changes in organic-matter dynamics and physicochemistry, associated with riparian vegetation loss and river regulation in floodplain wetlands of the Murray River, Australia. *Marine and Freshwater Research*. https://doi.org/10.1071/MF09312

Wenzel, A., Bergström, A. K., Jansson, M., & Vrede, T. (2012). Survival, growth and reproduction of Daphnia galeata feeding on single and mixed Pseudomonas and Rhodomonas diets. *Freshwater Biology*, *57*(4), 835–846. https://doi.org/10.1111/j.1365-2427.2012.02751.x

Westhorpe, D. P., & Mitrovic, S. M. (2012). Dissolved organic carbon mobilisation in relation to variable discharges and environmental flows in a highly regulated lowland river. *Marine and Freshwater Research*, *63*(12), 1218–1230. https://doi.org/10.1071/MF12122

Westhorpe, D. P., Mitrovic S.M., Chessman B.C., (2008). Integrated monitoring of environmental flows: wetting terrestrial organic matter hypothesis. Technical Report: IMEF Phase 1, 1998–2005. NSW Department of Water and Energy, Australia.

Westhorpe, D. P., Mitrovic, S. M., Ryan, D., & Kobayashi, T. (2010). Limitation of lowland riverine bacterioplankton by dissolved organic carbon and inorganic nutrients. *Hydrobiologia*, *652*(1), 101–117. https://doi.org/10.1007/s10750-010-0322-8

Wetzel, R. G. (1995). Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology* **33**, 83–89.

Wipfli, M. S., Richardson, J. S., & Naiman, R. J. (2007). Ecological linkages between headwaters and downstream ecosystems: Transport of organic matter, invertebrates, and wood down headwater channels. *Journal of the American Water Resources Association*. https://doi.org/10.1111/j.1752-1688.2007.00007.x

Woodward, K. B., Fellows, C. S., Mitrovic, S. M., & Sheldon, F. (2015). Patterns and bioavailability of soil nutrients and carbon across a gradient of inundation frequencies in a lowland river channel, Murray-Darling Basin, Australia. *Agriculture, Ecosystems and Environment*, *205*, 1–8. https://doi.org/10.1016/j.agee.2015.02.019

Worden, A. Z., Follows, M. J., Giovannoni, S. J., Wilken, S., Zimmerman, A. E., & Keeling, P. J. (2015). Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. In *Science*. https://doi.org/10.1126/science.1257594

Zeug, S. C., & Winemiller, K. O. (2008). Evidence supporting the importance of terrestrial carbon in a large-river food web. *Ecology*, *89*(6), 1733–1743. https://doi.org/10.1890/07-1064.1

Appendix.

List of Rotifer and Mesozooplankton families identified during monitoring studies (Chapter 2 and 3).

Copepoda	**Cladocera**	**Rotifers**
Cyclopoida: *Mesocyclops spp*, *Thermocyclops spp* Calanoida: *Boeckella spp,* *Calamoecia spp*	*Daphia,* *Ceriodaphnia,* *Moina,* *Macrothrix,* *Bosmina,* *Chydorus,* *Acroperus*	*Brachionus,* *Keratella,* *Testudinella,* *Filinia,* *Dicranophorus,* *Lecane,* *Trichocerca,* *Cephalodella,* *Asplanchna,* *Synchaeta,* *Polyarthra,* *Ascomorpha,* *Hexarthra,* *Euchlanis,* *Lepadella,* *Bdelloid spp.*