Dissolved organic carbon in the lower Namoi River, New South Wales:

Determining responses to flow, loads and food web linkages

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Abstract

Like many lowland rivers around the world, the Namoi River in north-west New South Wales (NSW) has greatly reduced rates of flow due to effects of regulation and abstraction. This has likely led to reduced amounts of allochthonous dissolved organic carbon (DOC) entering main channels through less frequent wetting of benches, flood runners and floodplains. However, these changes in DOC have not been quantified. In addition, the benefits that increased delivery of DOC may have to aquatic food webs are not well understood for lowland rivers.

The NSW Government has focussed on the sustainable use of natural resources with a strong environmental protection component. Part of the legislative requirements was to supply environmental flows to rivers and to quantify ecological benefits. A scientific approach was developed, and monitoring of these releases began in 1999 under the Integrated Monitoring of Environmental Flows (IMEF) program.

In this study DOC concentrations were quantified over different flow regimes across three sites at a variety of temporal scales with export loads and diel variation determined. DOC concentrations over low flow periods were fairly similar between sites and ranged between 5 and 10 mg L^{-1}. DOC concentrations during a major flood increased substantially with a mean of 20.4 mg L^{-1} and a maximum of 44 mg L^{-1}. A significant ($\boldsymbol{P}$<0.05) positive linear relationship was found between median DOC concentration and flow. The relationship between DOC and flow was used to estimate DOC loads to the river under different modelled flow scenarios including without environmental flow, with environmental flow, and simulated natural flow. Environmental flows were found to increase DOC delivery relative to current flows and approximated that delivered under natural conditions.

To determine diel variability, DOC was sampled at 4 hr intervals from two sites across two distinct flow regimes. This included a large flood (mean flow 224 m^3 sec^{-1} and a peak flow of 376 m^3 sec^{-1}) sampled four hourly for ten consecutive days. DOC concentrations were significantly greater at night than during the day ($\boldsymbol{P}$<0.05) and the mean DOC concentration was 23.4 mg L^{-1} at night compared to 18.9 mg L^{-1} during daylight hours. The magnitude and duration of flow within this lowland river system and the mobilisation of large quantities of

allochthonous carbon appeared to play a role in increasing DOC concentration and the diel difference.

Experimental investigations were also carried out to determine the responses of the planktonic food web (i.e., bacterioplankton, phytoplankton and zooplankton) to various additions of DOC. ***In-situ*** microcosm results indicated that ambient DOC availability limited the bacterioplankton for the three seasons over which we conducted the experiments. When DOC was added alone, dissolved oxygen concentrations decreased primarily because of increased bacterial respiration and bacterioplankton growth generally increased relative to controls. Additions of DOC alone led to a pattern of decreased chlorophyll a concentration relative to that of the controls, except for willow leachate. Additions of inorganic nutrients alone increased chlorophyll ***a*** concentrations above that of the controls, indicating limitation of phytoplankton productivity.

Results from 70 L mesocosm experiments to determine zooplankton responses revealed that DOC addition with and without nutrients increased heterotrophic respiration and led to significant increases in bacterial biomass. In treatments with the natural leachate, zooplankton concentration and diversity increased relative to that of the controls. Amendment with glucose alone also resulted in their increased growth and diversity, but not to the same extent. Glucose with inorganic nutrients led to similar growth and diversity to that of the control.

This study has shown that environmental flows increase the delivery of allochthonous DOC to the river and should create conditions that more resemble natural conditions. Furthermore it supports the hypothesis that allochthonous sources of DOC delivered to a river with inflows will stimulate heterotrophic bacterioplankton and alter the food web leading to increased zooplankton concentration and diversity.

Structure of thesis

Please be advised that Chapters 3 to 6 have been written as papers for publication and therefore some repetition of sections (e.g., Materials and Methods) is necessary.

Chapter 5 has been published in the journal Hydrobiologia as follows:

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Chapters 3 and 6 are currently in preparation for submission to appropriate journals.

Chapter 1. Background to the study

1.1. Ecology of Australian Rivers

An understanding and appreciation of the importance streams and rivers play in human survival and ecological diversity is being recognised globally with many countries adopting legislative procedures and management strategies designed to preserve and restore many of the world's aquatic ecosystems. In Australia, European-style land use and management has brought about many detrimental changes to Australian river systems of which many are due to the failure to understand that floodplains are essential components of Australian river systems (White 2000). The interaction of physical and chemical processes in rivers influences biological processes at a range of scales (Boulton and Brock, 1999). Flow plays a vital role in both longitudinal and lateral transport of various energy source components and several models have been put forward (e.g., River Continuum Concept: Vannote ***et al***. 1980; Flood Pulse Concept: Junk ***et al.*** 1989) to help explain the role among organic matter (e.g., dissolved organic carbon), the biota and the physical habitat of river ecosystems.

It is generally accepted that organic matter in aquatic systems is derived from two distinct sources: autochthonous, primary production within the system or from allochthonous, terrestrial organic matter (TOM) washed into the system from the catchment. The relative proportions of these two sources of carbon will vary between water bodies and systems (Cole ***et al***. 2002). Wetzel (2003) states that in every detailed annual organic carbon budget of lake and river ecosystems, the heterotrophic metabolism of an ecosystem cannot be supported by organic matter generated by phytoplankton alone. Terrestrial ecosystem processes provide a major source of carbon to aquatic ecosystems (Finlay 2003). Littoral zone communities and allochthonous inputs provide a large portion of organic matter required to support total heterotrophic metabolism (Wetzel 2003). In addition, terrestrially derived carbon may be very important in higher trophic levels of food webs, leading to secondary production through invertebrates and fish (Grey ***et al***. 2001).

The phytoplankton community may also be influenced as the complete photolysis of the humic components of dissolved organic carbon (DOC) releases CO_2 into the water column, thereby stimulating phytoplankton production (Wetzel 2003). These processes are not restricted to the surface waters (UV-A & B irradiance) but affect much of the variable volume of the photic zone (Wetzel 2003). The availability of DOC also provides a food source (directly and indirectly) for meiofauna (e.g., various zooplankton, small chironomids and flatworms) and the biological activities of these creatures can influence metabolic yields through the ecosystem (Hakenkamp & Morin 2000).

Characteristic of lowland semi-arid floodplain systems is the high degree of variability in frequency of inundation of the various geomorphic elements (e.g., main channel, flood-runners and billabongs: Frazier and Page, 2006). The natural drying and flooding cycles of these systems is altered as a consequence of river regulation (Baldwin and Mitchell, 2000). These floodplains regulate river water quality, acting as landscape buffers, with periodic flooding influencing stream biogeochemistry as a source or sink of carbon, and inorganic nutrients from leaf litter and floodplain soil which are mediated by the soil microbial biomass (Robertson ***et al***. 1999; Baldwin and Mitchell 2000; Sánchez-Andrés ***et al***. 2010). However, river regulation and subsequent reduction in flood magnitude and frequency have led to a reduction in the amount of these carbon sources on floodplains and riparian habitats from being wetted and entering the river.

The Namoi River, like many Australian lowland rivers has been considerably altered through the presence of headwater dams, large-scale development on floodplains, licensed water extractors, flow regulation and the construction of levees resulting in loss of connectivity with the surrounding landscape (Thoms and Sheldon, 2000; Chessman 2003). It has undergone much change in its water usage patterns in recent times with flow volume as well as the regularity of small to moderate sized events markedly reduced from those that would have occurred before development of irrigation (Chessman 2003). As a result, this is likely to be reducing the concentrations and transported load of allochthonous DOC entering the river as

fewer benches and floodplains are wetted. This may be limiting the yields of aquatic organisms and diminishing the health of the river.

1.2. Water management in Australia

The promotion of the conservation of ecosystems as a public good first took place in 1992 at the Earth Summit in Rio de Janeiro at the global level. Since then, many countries have developed laws and policies providing water rights to river dependent species and ecosystems once basic human needs are met (Acreman and Dunbar, 2004). Various names have been assigned to the management of flow releases through time to enhance or maintain river health in a particular state, including environmental flow, in-stream flow, environmental allocation and ecological flow requirement (Gordon ***et al.*** 1994; Acreman and Dunbar, 2004).

Both State (NSW Water Reforms) and Federal (National Water Initiative) government initiatives have focussed on the sustainable use of natural resources with a strong environmental protection component. The current Legislative Conceptual Model dictates that ***"Sustainable water use ensures a healthier environment that enables sustainable farming and consequently, sustainable communities"***. This initiative has led to the development of the NSW Water Sharing Plans (WSP) for all regulated and unregulated surface water and groundwater sources in the state.

In 2006 the then Department of Natural Resources (DNR) tabled 'The Draft DNR Corporate Plan 2006-2009' and stated that the Department will make "***management decisions and develop policies that encourage the adoption of better management practices for the protection of the State's natural resources. We will base these decisions and policies on sound science and objective data***". The Natural Resource Commission (NRC) also tabled Performance Indicators to monitor and evaluate the NRC Surface Water Targets and the effectiveness of the water sharing plans.

Prior to this in 2005, the DNR also tabled the "Knowledge Strategy" which "***identified the knowledge needed to support the State in its key areas of natural resource management***". The relevant key lines of business described in the Corporate Action Plan included:

1. Healthy and productive landscapes; and

2. Sustainable allocation, conservation and use of water.

The provision to maintain particular quantities of water within some NSW rivers for environmental purposes has been in place for many years. However, a systematic approach to environmental water allocation across the State was first developed as part of the water reform process instigated by the NSW Government in 1997 (DLWC 1998; Thoms & Swirepik 1998). As part of this process, the government adopted twelve broad river flow objectives (RFOs). These embody key attributes of flow regimes and flow management that affect the condition of aquatic ecosystems. They are strongly oriented toward the partial restoration of the natural flow regime, a strategy advocated by many river ecologists (e.g. Power ***et al.*** 1996; Poff ***et al.*** 1997).

NSW river flow objectives

1. Protect natural water levels in river pools and wetlands during periods of no flow
2. Protect natural low flows
3. Protect or restore a portion of freshes and high flows
4. Maintain or restore the natural inundation patterns and distribution of floodwaters supporting natural wetland and floodplain ecosystems
5. Mimic the natural frequency, duration and seasonal nature of drying periods in naturally temporary streams
6. Maintain or mimic natural flow variability in all streams
7. Maintain the rates of rise and fall of river heights within natural bounds
8. Maintain groundwater within natural levels and variability, critical to surface flows or ecosystems
9. Minimise the impact of in-stream structures
10. Minimise downstream water quality impacts of storage releases
11. Ensure that the management of river flows provides the necessary means to address contingent environmental and water quality events
12. Maintain or rehabilitate estuarine processes and habitats

In response to the RFOs outlined above a number of environmental flow rules (grouped into six broad categories) were developed in 1998 by community-based river management committees (RMCs) in each of six valleys that have major rivers regulated by large storages operated by NSW State Water: (i.e., the Gwydir, Hunter, Lachlan, Macquarie, Murrumbidgee and Namoi valleys). These rules have been approved by the NSW Government, and implemented (via operating and licensing procedures) by the NSW Office of Water and its predecessors.

1.3. Water in the Namoi River

The WSP for the regulated sections of the Namoi River is the "***Water Sharing Plan for the Upper Namoi & Lower Namoi Regulated Water Sources 2003***", hereafter referred to as the ***Plan***. This Plan took effect on 1 July 2004 and ceases 10 years after that date. It is a legally binding document under the Water Management Act 2000.

The ***Plan*** has addressed environmental water issues by focusing on the supplementary environmental water and uncontrolled access provisions within the Lower Namoi River Water Source (downstream of Keepit Dam). These environmental water provisions essentially fall under off-allocation access rules (category 5) and work as follows:

> 'Off-allocation' periods are declared for inland rivers when reservoirs spill or high flows enter from unregulated tributaries. During such periods, irrigators can pump water without the quantity being debited from their annual entitlement. Off-allocation rules set flow thresholds for off-allocation access and restrict the amount of water permitted for extraction during such periods, or limited its timing.

Category 5 was applied to the Lower Namoi River, as flow variability is considered important for river health (Bunn & Arthington 2002; Richter ***et al***., 2003). The ***Plan*** aims at reserving a percentage of each flow event within the Lower Namoi River for the environment. The rules limit the extraction of intermediate and high flows that have entered the Lower Namoi from unregulated tributaries downstream of Keepit Dam when access to uncontrolled flows or by supplementary access licence holders is announced. The proportion of flows protected from extraction for a particular event is (DWE 2009):

- 90% of each event between 1 July and 31 October;
- 50% of each event between 1 November and 30 June.

A scientific approach was thus required to determine if the flow rules set by the respective RMCs had merit and could maintain / improve the health of NSW inland river systems as postulated by the NSW government's Water Reforms in 1997, and later re-enforced by the ***Plan*** and the NSW 2006-2009 Corporate Plan (e.g., sustainable allocation, conservation and use of water). The delivery of TOM, primarily in the form of DOC from surrounding landscapes (e.g., benches & floodplains) with managed flows that mimic those that would have naturally occurred prior to regulation was deemed a relevant ecological indicator in the conservation and sustainable allocation of water as DOC has the potential to enter the food web and stimulate heterotrophic production.

1.4. Integrated monitoring of environmental flows

The Integrated Monitoring of Environmental Flows (IMEF) Program is a scientific assessment of the response of major regulated rivers and associated wetlands to environmental water allocations in NSW. The IMEF has been progressively developed and implemented since 1998; Chessman (2003) and Chessman & Jones (2001) detail the design and scope of the program. The IMEF was instigated in order to provide RMCs, natural resource management agencies and the broader community with sound scientific feedback on the effects of environmental flow rules. This was intended to inform reviews of water allocations in the WSPs which are subject to periodic evaluation.

The IMEF objectives are:

- to investigate relationships between water regimes, biodiversity and ecosystem processes in the major regulated river systems (and the Barwon-Darling River);
- to assess responses in hydrology, habitats, biota and ecological processes associated with specific flow events targeted by environmental flow rules, and;
- to use the resulting knowledge to estimate likely long-term effects of environmental flow rules and provide information to assist in future adjustment of rules.

In order to assess the performance of the flow rules, the IMEF project team created a series of predictions (scientific hypotheses) about possible environmental benefits these types of rules may produce. The hypothesis that is applicable to Category 5 flows relates to organic carbon replenishment as follows:

"Protecting or restoring a portion of freshes and high flows, and otherwise maintaining natural flow variability (RFOs 3 and 6), through off-allocation use restrictions and dam releases, will increase the wetting of coarse terrestrial organic matter on river banks, benches and floodplains, and consequently increase microbial activity and the populations of animals that feed on detritus or on the microbes that use dissolved organic matter."

At the Namoi catchment scale, works have been implemented to address the above mentioned criteria. For example, environmental flows have been provided to the Namoi

River in an attempt to enhance terrestrial / aquatic linkages and thus improve the overall health of the riverine environment. Environmental flows achieve this by increasing the wetted area of banks (and associated riparian zones) and period of inundation, and thereby increase DOC concentrations in the river.

At present there are limited Australian data that clearly address the following issues:

1. Environmental flows (at key times in the year) will improve the overall health of riverine ecosystems (difficult to define 'health' based on DOC dynamics);
2. Detailed understanding of the ecological benefits (e.g., water quality) that these environmental flows provide to rivers and wetlands, and;
3. How the delivery and types of environmental flows can be modified to increase environmental benefits.

1.5. Constraints

As with any ***in-situ*** monitoring designs there are always constraints that can impede the overall outcomes. Therefore to achieve these hypotheses pre-planning is always crucial. However environmental factors such as weather can greatly reduce accessibility to aquatic sites with access limited due to floods and heavy rains. Conversely, the potential impacts of drought in a worst case scenario can result in lack of suitable flows to monitor (e.g., no inundation of river benches and surrounding floodplains where large quantities of TOM can accumulate).

Studies in Australia have shown that the timing of individual flow events can impact upon both the quality and quantity of carbon mobilised to the river. Baldwin and Mitchell (2000), state that the seasonal timing of flooding and drying events on floodplains is critical. This timing is relevant to the Lower Namoi River with flow / flood events in summer expected to provide a higher yield of nutrients and concomitantly heterotrophic production due to increased temperatures (Baldwin and Mitchell, 2000) and also higher rates of leaf-fall from native riparian trees. Changes to antecedent conditions with multiple high flows will also influence both the quality and quantity of carbon mobilised. Summer months are associated with highest rainfall in the catchment (CSIRO 2006). As previously mentioned, rain events (depending on the severity) can limit access to many sections of the lower Namoi River due to its soil type and relatively flat river floodplain topography (DLWC 2000).

I was fortunate enough over the course of this study to be able to gather data from a number of flow events of varying magnitudes to give an indication of the functioning and relationships that exist between discharge, energy sources (i.e., carbon) and river biota.

1.6 Aims & hypotheses of research

The purpose of this study will be to determine if the flow rules set for the lower Namoi River to protect a proportion of freshes and high flows will result in an influx of carbon into the river due to more frequent wetting of river banks, benches and in some cases floodplains than would otherwise occur. If this is achieved the next step will be to determine if this increase in allochthonous DOC influences the aquatic food web, specifically, do heterotrophic bacteria, phytoplankton and meiofauna such as zooplankton respond to increases in allochthonous DOC inputs. The DOC data collected will then be used to develop DOC delivery models run via the department's Integrated Quantity and Quality Model (IQQM) flow simulation models (Simons et al 1996) that can be used to investigate the impacts of water resource management policies by simulating and comparing streamflow under developed and 'natural' flow conditions (DLWC 2000). This helps predict the amount of DOC supplied to the river under different flow scenarios, such as with and without environmental flow allocations, to estimate potential delivery quantities and river health benefits from these flows. This study is divided into 3 broad aims:

- Aim 1. To determine the response of DOC concentrations (spatially & temporally) to changes in flow in the lower Namoi River.
- Aim 2. To determine responses of bacteria and phytoplankton to DOC increases.
- Aim 3. To determine whether increases in DOC influence the food web.

A specific hypothesis was developed for each aim as follows:

1. Increased flows down the Namoi River will result in increased DOC concentrations in the river water;
2. Increased DOC concentrations will lead to increased microbial responses (measured indirectly as bacterial respiration (dissolved oxygen consumption) and bacterial growth (relative surface area measurements));

3. Increased microbial responses will be transferred to higher organisms such as zooplankton.

Hypothesis-1 will be answered by:

- Monitoring in-stream DOC concentrations - spatially, seasonally and during storm events (including baseline data to determine background concentrations);

Hypotheses 2 & 3 will be answered by:

- Determining the ecological effects of increased DOC concentrations on river bacterioplankton, phytoplankton & zooplankton by both ***in-situ*** and laboratory carbon additions that simulate the effects of environmental water delivery.

Chapter 2. A review of the literature

2.1. Forms of dissolved organic matter

Almost all of the organic carbon on earth is created through photosynthesis, whether on land or in water (Ludwig 2001), with dissolved organic carbon (DOC) being an important carbon source in stream ecosystems (Meyer & Tate 1983). Dissolved organic matter (DOM) formation in aquatic ecosystems is often equated with organic carbon formation due to its overall abundance in the mass of DOM. There are two main forms of total carbon in aquatic environments, these are inorganic and organic. The inorganic form called dissolved inorganic carbon (DIC) occurs in the ionic form as various carbonate species (i.e., HCO_3^-, CO_3^{2-}, & H_2CO_3), acting as one of the main parameters controlling the pH of natural fresh waters; or in the form of dissolved free CO_2. Free CO_2 in surface freshwater can transfer carbon between organic and inorganic pools, return carbon to the atmosphere, and when in elevated concentrations may be utilised during photosynthesis or augmented by respiration of the biota during transport downstream (Hope ***et al***. 1994).

Forms of total organic carbon (TOC) are DOC and particulate organic carbon (POC) with the distinction between the two generally made based on whether or not it passes through a 0.45 to 0.7 µm pore size filter (Hope ***et al***. 1994; APHA 1998; Raymond & Bauer 2001). The construction of carbon budgets to determine the dynamics of aquatic ecosystems has revealed that the DOC fraction often represents the major pool or flux of organic carbon being transported through the system (Thomas 1997; McKnight ***et al***. 2003).

Collectively, the fulvic and humic acids are known as humic substances and are defined as a series of relatively high molecular weight, yellow to black coloured substances and represent a high percentage of DOC (Aitkenhead-Peterson ***et al.*** 2003). Fulvic acids are the largest component of the dissolved fraction (45-65%) in a typical riverine sample (McKnight ***et al***. 2003) and together with the humic acid fraction (responsible for much of the colour seen in many rivers) can represent 75% of the total DOC fraction (Hope ***et al***. 1994; McKnight ***et al***.

2003). Colloidal organic matter represents about 20% of the remaining dissolved fraction (Hope ***et al***. 1994).

DOM also contains a number of other elements, importantly nitrogen (N) being a key component of proteins and amino acids. The carbon to nitrogen ratio is comparatively low in most plants. As plants are often the ultimate source of the reduced carbon produced globally, heterotrophs including microbes are often nitrogen limited. It is suggested that in some aquatic systems the contribution of organic N by heterotrophs may be larger than that of autotrophs (Caraco & Cole 2003). Different limiting factors exist for the formation of organic N from inorganic N by autotrophs and heterotrophs. For example, light is an important limitation for most aquatic autotrophs, whilst organic carbon supply by both allochthonous and autochthonous sources often limits bacterial production. Additionally, heterotrophic organic N formation may depend on the C:N ratio of the organic matter (OM) being used (e.g. low N content of OM may result in higher formation of organic N by microbial heterotrophs). Therefore heterotrophic N formation may be greatest in systems with relatively low light and supply of OM with low N content, generally defined by a high ratio of terrestrial organic matter (TOM) to autochthonous loads (Caraco & Cole 2003). I predict that this would be the case in many first and second order forested streams. Other components of DOM include sugars, carboxylic acids, nucleic acids, peptides and humic substances potentially providing aquatic organisms with energy, with the total mass of DOM in aquatic systems exceeding that of living organisms (Thomas 1997).

2.2. Sources of DOC

An important factor in understanding the biotic functioning of a riverine system is determining the origin and quantity of OM that drives the system. Even during periods of high primary production (e.g. phytoplankton blooms) much of the food webs energy supply is potentially derived from non-living terrestrial sources (O'Connell ***et al***. 2000). DOM is the major form of OM in almost all aquatic ecosystems, playing a significant role in aquatic food webs, mediating the availability of dissolved nutrients and metals, and modifying the optical

properties of water bodies. The term 'DOM' is often used in carbon, energy and nutrient budgets for aquatic ecosystems, and as such, can have broad effects on food webs, heterotrophy and nutrient retention / release (Findlay & Sinsabaugh 2003). Broadly speaking, Findlay & Sinsabaugh (1999) propose three important components that affect source variability and standing stocks of DOM in aquatic ecosystems. These are: 1. the variability in patterns of DOM concentration that is dependent upon a combination of timing, magnitude and predictability of the sources in an ecosystem; 2. the wide range of hydrological processes transporting DOM internally and externally, and in turn an equally broad range of flow events acting to transport DOM from river reaches; and, 3. the multitude and complexity of compounds that make up DOM allows the biological and abiotic transforming processes to vary sufficiently for the quantity and quality of DOM to be controlled by which ever removal process dominates at a given time or place.

Autochthonous DOM is derived predominantly from algae (phytoplankton & periphyton) and macrophytes. It is regarded as a major source of metabolic substrates for heterotrophic microorganisms, influencing both the activity and composition of aquatic microbial communities. There are several pathways that mediate the release of DOM to the surrounding water: these include: cell senescence or lysis (viral infection); predation (sloppy feeding); and extracellular release (leakage and active excretion). Important regulatory factors of this DOM release are 1. nutrient deficiency resulting in increased DOM release when one or more nutrients are in low supply, and; 2. light intensity, resulting in increased DOM release with increased photolytic activity (Bertilsson & Jones 2003).

In general terms allochthonous DOM is accumulated as precipitation moves: 1) through the atmosphere (collecting pollen and organic dust particles); 2) washes through vegetation (pollen, dust, insect exudates and leaching from sources inside the leaf itself); 3) through ground cover and organic soil (dependent upon watershed slope, canopy cover, antecedent soil moisture and depth of water table); and 4) percolates downward through soil mineral

horizons (Aitkenhead-Peterson ***et al.*** 2003). This is also dependent on the factors previously outlined for ground cover and organic soil.

Organic matter in aquatic systems is derived from two distinct sources: autochthonous, primary production within the system and allochthonous TOM washed into the system from the watershed (Cole ***et al.*** 2002). Contributions also come from older material derived from terrestrial soils, and continental and marine sedimentary rocks (Raymond & Bauer 2001). Anthropogenic sources derived from agricultural (e.g. runoff & erosion), domestic and industrial activities (e.g. effluent discharge) can contribute to this pool (Hope ***et al***. 1994).

It is generally accepted that the ratio of allochthonous to autochthonous inputs into a stream is dictated by size (stream order) and vegetation cover. Forested upper catchment streams (first and second order) depend on inputs of carbon and nutrients from the surrounding catchment, particularly the fringing riparian zone. This is exacerbated as in-stream production is also often light-limited. However, further down the catchment (higher order streams) with sparse vegetation (and relative to stream width), direct terrestrial inputs are often reduced in comparison with in-stream primary production (Bunn ***et al***. 2003), so autochthonous sources become more important.

The source of DOC that fuels riverine ecosystems will vary depending on location within a catchment, and the state of the flow regime (i.e., regulated or natural) particularly in larger lowland systems where regulation has isolated rivers from their floodplains (Bunn ***et al***. 2003). Climatic conditions such as frequency of storm events will also influence DOC sources (Mulholland 2003). A study comparing two forested catchments in Ontario, Canada showed that riparian and wetland sources contributed most of the in-stream DOC during storms (Hinton ***et al***. 1998). A simultaneous study to determine the age of DOC sources using carbon isotopes (^{14}C & ^{13}C) from surface waters, groundwater and soils from the forested catchments revealed that DOC derived from the groundwater was an older recalcitrant pool (due to extensive recycling in the soil zone), whilst DOC from surface waters and wetlands was a younger more labile fraction derived from recently produced and

leached OM (Schiff ***et al***. 1997). The authors suggest that the relative proportion of these two sources will change seasonally due to changes in water flow paths and organic carbon dynamics.

A study by Hope ***et al***. (1997) estimating organic carbon fluxes in British rivers suggests that the soil carbon pool is the single most important factor in determining riverine DOC fluxes. The authors concluded that for British rivers the mean soil organic carbon storage in the catchment is an effective determinant of DOC production, adsorption and transport processes at the catchment scale based on 'non-storm' DOC fluxes. Raymond & Bauer (2001) suggest that the dominant source of both DOC and POC to most rivers is of terrestrial origin. Terrestrial ecosystem processes provide a major source of carbon to aquatic ecosystems (Finlay 2003) and this terrestrially derived carbon may be very important in food webs and can lead to secondary production of invertebrates and fish (Grey ***et al.*** 2001).

There have been many models developed that predict the spatial and temporal movement and cycling of biotic and abiotic components of the river ecosystem as they are continuously or periodically transported downstream. For example nutrient spiralling broadly refers to the longitudinal distance a nutrient atom travels while completing a cycle, going from abiotic form to biotic and back to abiotic (Webster and Ehrman, 1996). The following paragraphs focus on four models that I believe are relevant to this study, in that they cover both upstream and lateral sources of allochthonous carbon as well as localised within reach sources, all of which have the potential to contribute to the dynamic functioning of a lowland river ecosystem with their importance being dependent upon hydrologic patterns within the catchment.

The principal sources of organic carbon and associated energy that fuel the food webs of large rivers are much debated in the ecological literature. The first 'holistic' conceptual model to determine energy sources and flow was the River Continuum Concept (RCC), developed by Vannote ***et al***. (1980). The authors proposed that: "***the RCC provides a framework for integrating predictable and observable biological features of flowing water***

systems within the physical-geomorphic environment". Developed primarily from studies in temperate North America, the RCC proposes that the majority of the carbon supply to large rivers arrives with upstream water in the form of dissolved and fine particulate organic matter (FPOM), the product of upstream processing of inputs from the riparian zones of small streams (Vannote ***et al.*** 1980). Therefore large rivers (stream order / size >6) are predicted to be dependent upon the loss or leakage of upstream processing of organic matter (principally FPOM) for their primary source of energy; whilst the input of coarse particulate organic matter (CPOM) from adjacent riparian vegetation is deemed an insignificant contribution due to river width. In-stream primary production is often very limited in highly turbid deep lowland river systems where respiration exceeds production (Thorp & Delong 1994).

Based on the RCC model the serial discontinuity concept (SDC) was developed by Ward and Stanford in 1983. The SDC viewed impoundments as major disruptions of longitudinal gradients along the river continuum, resulting in shifts in the biotic and abiotic processes and patterns, the extent of displacement being dependent upon the variable of interest and also as a function of dam position along the river continuum (Ward & Stanford 1995). A refinement of the initial model was later developed based on three defined reaches (headwater-constrained; braided; and meandering - which in this case were assumed to be in longitudinal sequence). The headwater reach was similar to that proposed in the original SDC. However, the latter two reaches were introduced as the effects of river regulation had focused on within-channel processes and ignored the lateral connectivity with floodplains and their diverse array of terrestrial and aquatic habitats (Ward & Stanford 1995). The conclusion from the three-reach model (confined to the USA) was that lateral interactions between the river and the floodplain were critical to a holistic understanding of natural river ecosystems and alterations induced by regulation (Ward & Stanford 1995). Further studies on the efficacy of the SDC examined a number of regulated rivers from around the world and generally found that discontinuity distance and relative intensity of regulation varied from

river to river, with the resetting or recovery of key variables such as temperature and species richness observed at varying distances downstream from the dam. In some cases downstream anthropogenic effects prevented recovery (Stanford & Ward 2001).

The Flood Pulse Concept (FPC) of Junk ***et al.*** (1989) was developed as a complementary concept to that of the RCC, in an attempt to explain the relationship between the biota and the environment of an unmodified large river-floodplain system. The authors proposed that: "***the pulsing of the river discharge (the flood pulse), is the major force controlling biota in river-floodplains***." The emphasis is on the lateral exchange between the floodplain and the river channel and associated nutrient cycling within the floodplain that occurs during annual seasonal inundation (Junk ***et al.*** 1989). A number of caveats exist such as the variation in the quantity and quality of OM being delivered, which is dependent upon density of vegetation and frequency / duration of flood pulses and extent of inundation of the floodplain (Thorp & Delong 1994). Later studies by Sedell ***et al***. (1989) cited in Thorp & Delong (1994) concluded that systems with constricted channels should follow patterns predicted by the RCC; whereas systems with extensive floodplains should be primarily sourced by lateral linkages, placing downstream transportation of OM to a minor role. A study by Puckridge ***et al***. (1998) looking at flow variability and the ecology of large rivers noted that the FPC has some limitations and could be improved by: 1. looking at in-channel flow variation and not just overbank flows; 2. organisms adapted to unpredictable pulses (e.g. via opportunism and trophic generalism) and therefore a system is not necessarily reliant upon a predictable annual flood pulse; 3. large rivers being highly complex, therefore a systems response can not be based upon just a limited number of common hydrological variables such as frequency, rate of rise and fall, and duration of flow; and, 4. individual rivers have distinct patterns of flow variability that potentially play an important part in their ecology, therefore a broad concept may not always apply (Puckridge ***et al***. 1998).

A more recent concept proposed is the Riverine Productivity Model (RPM) of Thorp and Delong (1994); it maintains that in-stream photosynthesis and local riparian inputs are often

important in large rivers, particularly those with constricted channels. The main thrust of the initial RPM is that the bulk of organic matter assimilated by consumers is derived from a combination of local autochthonous production (e.g., phytoplankton, aquatic plants and biofilm) and direct inputs from the riparian zone (e.g., abscised leaves, POC and DOC) during times without flood pulses (Thorp & Delong 1994). More recently, Thorp and Delong (2002) have expanded on this model, suggesting that multicellular animals (metazoans) in most large rivers rely mainly on carbon sourced from algal photosynthesis and that C_3 and C_4 plants from the riparian zone are less important than originally hypothesised. They contend that terrestrial carbon inputs are processed mainly by the 'microbial loop' involving bacteria and fungi. Limited transfers can occur from this loop to the algal-grazer pathway, for example planktonic fishes feeding on rotifers, which in turn feed on heterotrophic microbes. Therefore, based on these new findings the revised testable RPM hypothesis is: "***the primary, annual energy source supporting metazoan production and species diversity in mid to higher-trophic levels of most rivers (≥ 4th order) is autochthonous primary production entering food webs via algal-grazer and decomposer pathways.***" In support of this hypothesis a study was undertaken (Delong ***et al.*** 2001) comparing three large rivers in the USA. Two rivers the Mississippi and Missouri experienced unseasonal flooding that lasted for several months, in contrast to the Ohio River which did not flood during the same period. Stable isotope ratios of carbon and nitrogen ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ respectively) collected from samples were used to compare between potential food sources and consumers. The overall findings were that the trophic structures of the two flooded rivers did not vary from that of the Ohio River as consumers continued to rely on sources of OM that were available in the absence of a flood (Delong ***et al.*** 2001).

2.3. DOC response to flows / flood events

Oceans are one of the biggest reservoirs of allochthonous OM (most of which is DOC) in the global carbon cycle, the main source of which is from rivers (Ludwig 2001). The export of DOC from rivers to the oceans is a function of runoff, and the DOC concentration and export

is simply a product of discharge-weighted mean annual concentration and runoff (Mulholland 2003). At a global scale there is a clear relationship between DOC export, latitude and climate. The factors driving this relationship are the organic matter percentage of soils and hydrological processes. The mobilisation and transport of soil organic carbon to aquatic ecosystems is positively related to the flux of water across the landscape, driven by the balance between precipitation and evapotranspiration (Mulholland 2003).

In 1981 the broad global estimates of organic carbon entering rivers was around 50% transported to the ocean, 25% oxidised within the system and 25% stored as POC in the system as sediment. Whilst numerous individual catchment studies (predominantly from the northern hemisphere) quantitatively estimate the annual export of organic carbon (as DOC & POC) from streams to vary from 1 to 500 kg C ha^{-1} yr^{-1}, with an overall mean of 56.2 kg C ha^{-1} yr^{-1} (Hope ***et al***. 1994). Three major sources of error need to be accounted for when estimating river carbon fluxes: these are: 1. the concentration of OM will vary with discharge and season, with variation greatest for particulates during storms in smaller catchments where both discharge and concentration fluctuate rapidly; 2. horizontal and vertical variations in the concentration of POC may be considerable, particularly in larger rivers, with some material being transported as bedload; and, 3. estimates of the flux of carbon as CO_2 are usually overlooked (Hope ***et al***. 1994).

Literature suggests that the concentration of DOC in most lotic systems varies between about 0.5 to 50 mg L^{-1}, with the primary influences on DOC concentrations being precipitation and the presence of wetlands (Hinton ***et al***. 1998; Mulholland 2003). In the upper catchments the flowpath of water through soil (e.g. peat) is also an important factor influencing DOC concentration, with water flowpaths at the land surface in contact with organic-rich soils having higher DOC concentrations than those receiving drainage along deeper flowpaths (Aitkenhead ***et. al***. 1999; Mulholland 2003). An increase in DOC concentration has been linked to an increase in discharge, in particular during floods and storm events (e.g., Hinton ***et al***. 1997; Hinton ***et al***. 1998; Buffam ***et al***. 2001; Henson ***et al***.

2007). A flow chart of how DOC concentrations can vary in response to increased flows in the lower Namoi River is presented in Figure 1. The flow chart provides a range of pathways or 'effects' that may lead to a change in DOC concentration within the river during and post an increase in discharge. Also presented are a number of co-variables that will influence DOC concentration including: timing / frequency of antecedent events; connectivity / availability of organic matter on the adjacent landscape; biological and physical responses (Figure 1). The overall delivery of DOC to the system is therefore dependent upon many variables (not withstanding the complex breakdown and assimilation pathways presented in Figure 2) and will vary in both space and time. Some studies have also reported a positive correlation with an increase in dissolved organic nitrogen (DON) during storm events, however, the measurement of DON concentrations in streams has been less frequently studied (Buffam ***et al***. 2001).

A number of studies has looked at the origin of DOC (and flow) entering a stream under varying flow conditions (e.g. Meyer & Tate 1983; Eckhardt & Moore 1990; Jardine ***et. al***. 1990). Jardine ***et al.*** (1990) quantified the subsurface transport of DOC through a forested hill slope during storm events and showed that the subsurface DOC may be a significant contributor of DOC to stream discharge; and depending on the duration and intensity of the storm the DOC concentrations on the ascending limb were similar to or greater than that of the descending limb. These results were very similar to the stream discharge DOC concentrations during storm events in the same watershed and is due to the relatively high sources of DOC in the A horizon and B horizon soils (Jardine ***et al***. 1990).

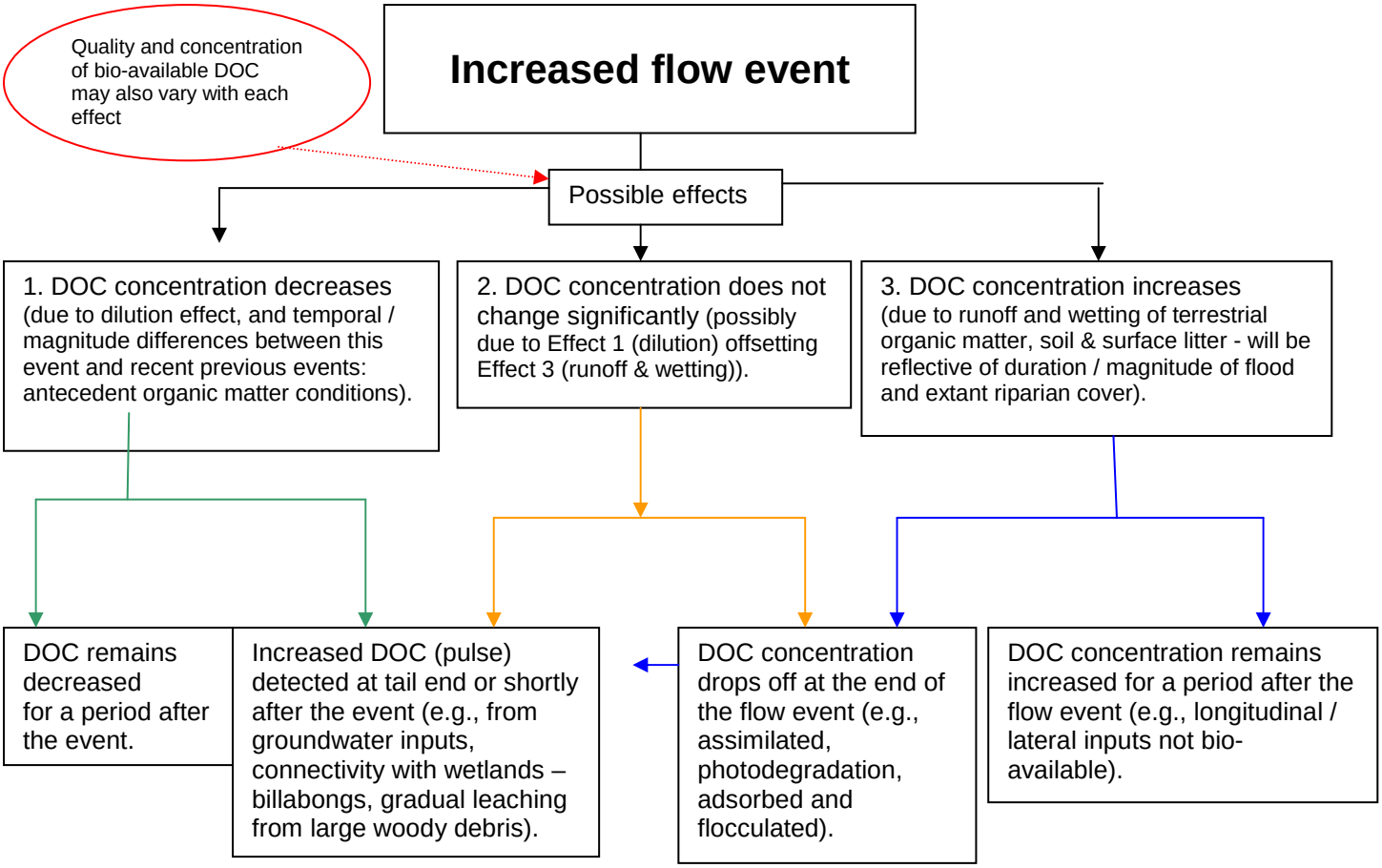

Figure 1: Conceptual flow chart outlining how dissolved organic carbon (DOC) concentrations may vary in the lower Namoi River during & post a flow event (compared with DOC concentrations prior to an event).

In southern Norway, Easthouse ***et al***. (1992) showed that the contribution of the dominant soil horizons to stream discharge varied considerably, being governed by storm intensity and antecedent hydrological conditions. At base flow, stream water originated in the deeper bog layers surrounding the stream, whilst a storm event after a prolonged dry period resulted in water originating from the B horizon and surface bogs. However, during very intense storms and upon soil saturation the O horizon was the significant source. The DOC also increased with peak discharge and was dominated by fulvic acids.

A study in Canada (Eckhardt & Moore 1990) looked at several catchment characteristics (e.g. wetland cover; catchment area; slope; forest cover; and drainage) and their influence

on the variations in DOC concentrations in streams draining 42 small catchments. DOC concentrations in streams showed consistent positive relationships with the catchment variable wetland cover within the catchment, the addition of the other variables did little to improve the regression model. Another study comparing cleared (2 years before the study) and non-cleared watersheds in North Carolina, USA, showed the importance of land use in affecting DOC-producing abilities of the vegetation and drainage of the land, with an overall decrease in stream DOC concentrations resulting from deforestation (Meyer & Tate, 1983). However, an interesting finding was that during storm events there was no difference in DOC concentrations versus discharge regressions for the two watersheds. The authors listed four potential sources for the increased DOC concentrations during storm events: 1. the flushing of terrestrial storage areas; 2. incident precipitation; 3. DOC leaching from POM in previously dry bed / channel areas; and, 4. flushing from the streambed and intermittent channels (Meyer & Tate, 1983).

Storm events can often contribute a large proportion of the total flow and therefore may represent a significant proportion of total DOC exported through a catchment (Easthouse ***et al***. 1992; Hinton ***et al***. 1997) and this is particularly relevant in well-drained catchments (Mulholland 2003). Hinton ***et al***. (1997) consistently obtained positive relationships between DOC and stream discharge during storm events in a number of sub-catchments in Central Ontario, Canada, with riparian and wetland areas being the major sources of stream DOC (Hinton ***et al***. 1998). The authors emphasised some important hydrological processes that control DOC dynamics only occur during storms and would go unnoticed over longer spaced sampling regimes (Hinton ***et al.*** 1998). A more recent study by Norström ***et al.*** (2010) in central Sweden compared two first-order streams in adjacent catchments, with the only major difference being the area of wetlands adjacent to the streams. The authors found that DOC concentrations in both streams positively correlated to precipitation, causing similar patterns in runoff. The DOC dynamics of wetland sub-catchments was also shown to be different from riparian sub-catchments during storms. In the wetlands, transport was

affected by leaching and flushing of DOC at the surface leading to lower DOC concentrations with successive storms, whilst in riparian soils groundwater flow paths were more important with stronger positive relationships between discharge and DOC concentrations observed (Hinton ***et al***. 1998).

A number of uncertainties arise when attempting to calculate DOC export based on DOC concentrations and discharge. Several methods exist for calculating nutrient fluxes within catchments including the period-weighted method, several regression-based methods, and the sample-interpolation method; with the method employed becoming important when the concentration is flow dependent (Hinton ***et al***. 1997). In the period-weighted method the average concentration of successive samples is multiplied by the volume of water passing the site during a specified interval (e.g., 24 hr), thus providing an average 24 hr DOC export load and also an average DOC export over the entire period of the flood event that was sampled. The total export is obtained by summing the export during individual sampling intervals. A caveat with this method is that whilst changes in flow volume are accounted for, changes in DOC concentration with stream discharge are not. To minimise this weakness (and where practicable) the authors suggest increasing the sampling regime particularly during an event, thus providing more precise estimates of export load. This weakness is accounted for in the regression-based methods as they take into account the changes in concentration with discharge. However, these methods do not use the measured concentration data to calculate export (concentrations are calculated from the regression) resulting in uncertainty in the DOC export estimates. The sample-interpolation method is a combination of both the previous methods. It assumes that DOC concentration varies linearly with discharge (Mulholland 2003) between successive samples and is calculated in three steps (see Hinton ***et al***. 1997 for a detailed explanation) and is only appropriate when multiple samples have been collected, particularly near each minimum and maximum in stream discharge, thus defining all changes in DOC concentration with discharge.

Climate change may have a significant effect on DOC export due to the strong correlation between DOC export and runoff (Hinton ***et al***. 1997; Mulholland 2003). In the United Kingdom a time series study observing changes in DOC concluded that climate (especially a severe drought) is a major driver in loss of carbon from upland peat catchments (Worrall & Burt 2004). Climatic impacts will also vary depending on geographical factors (e.g., latitude), for example aquatic ecosystems in cool wet temperate climates often have higher DOC concentrations due to preservation of soil OM, whilst more humid tropical climates generate greater runoff and thus greater fluxes of DOC into and through aquatic ecosystems. There are two potential consequences of climate change raised by Hinton ***et al***. (1997), these are an increase in the size and frequency of extreme events, with these changes potentially having a large effect on DOC export. Furthermore, the effect of climate change on the seasonal distribution of precipitation would differ among catchments, since runoff response to precipitation will vary across catchments. It is also evident that greater knowledge is required on the effects of catchment land-use (e.g. urbanisation and agriculture) and channel morphology (e.g. impoundment and channelisation) on DOC concentration and flux (Mulholland 2003; Worrall & Burt, 2004) particularly because DOC has such an important role in the structure and functioning of aquatic ecosystems (Mulholland 2003).

2.4. DOC and food webs

A fundamental role that DOM plays in aquatic environments is to serve as a source of carbon and energy to microbial food webs (Meyer & Tate 1983; Moran & Covert 2003). However, efforts in the past to understand DOM- microbial linkages have been hampered by the thousands of different compounds that comprise DOM (***cf***. Findlay & Sinsabaugh 1999). Heterotrophic bacterial metabolism (benthic and pelagic) is often recognised as a key component in mediating many of the transformations of carbon processing (into biomass and inorganic components) in aquatic ecosystems (Findlay & Sinsabaugh 1999; Findlay 2003; Rees ***et al***. 2005), with the bacteria feeding on DOM potentially being one of the primary food sources for secondary consumers such as zooplankton (Boon and Shiel 1990). In fact,

the utilisation of DOC is almost exclusively the domain of heterotrophic bacteria, due to their abundance, large surface-to–volume ratios (Drakare ***et al.*** 2002; Findlay 2003), and their ability to use the substrate at low concentrations (McDonald ***et al***. 2007). The heterogeneity and diversity of microbial communities makes it difficult to clearly define the pathways linked to DOM assimilation. However, these pathways can be broadly divided into four 'uptake' categories: 1. direct, 2. ectoenzyme-mediated, 3. photolysis-mediated and 4. sorption-mediated (Findlay & Sinsabaugh 1999). A schematic diagram (Figure 2) shows the complexity of carbon cycling, which is ultimately solar driven with carbon being derived from allochthonous and autochthonous sources (Boulton and Brock, 1999). Complexity arises when trying to determine the processes most relevant in driving the system. This will dependent upon: location within the catchment (e.g., upland vs lowland); vegetation characteristics (e.g., cleared vs uncleared); the type of water body (broadly: lentic and lotic) and the dominant functional groups that help breakdown and ultimately convert and utilise the energy sources available. In the lower Namoi River the main pathways of breakdown and conversion would include solar-mediated; physical processes; and biological in which heterotrophic bacteria would arguably play a crucial role within this aquatic ecosystem.

In recent decades some findings have helped elucidate the routes by which DOM enters the microbial food web. An important finding has been the recognition that macromolecules in the DOM pool can be photo-degraded to more biologically labile molecules and then directly assimilated by bacterioplankton (Moran & Covert 2003). Some DOM photoproducts are inorganic compounds (e.g., carbon monoxide & carbon dioxide) that represent direct photochemical mineralisation of carbon but generally have no direct effect on the microbial food web. Other DOM photoproducts are organic molecules that remain part of the DOM pool and have altered susceptibility to biological degradation (Moran & Covert 2003). The role of sunlight in the assimilation of DOC into aquatic ecosystems is through photosynthesis (algae & macrophytes) and photochemical reactions with DOM, with the photolysis of DOM releasing the nitrogen and phosphorus to algae and bacteria (McKnight ***et al***. 2003).

Similarly, DOC concentrations can fluctuate with a diel change due to photosynthetic fixation and subsequent extracellular release. Bacteria in streams have been shown to rapidly assimilate the input of DOC from periphyton with an increase in bacterial activity reported of 1.4 to 3.0 times from morning to afternoon in a creek in the USA (Bertilsson & Jones 2003). An earlier study looked at the effects of light (that resembled the UV of natural sunlight) on the availability of allochthonous DOM to pelagic bacteria in a humic lake in Sweden showed that both bacterial abundance and cell volume increased in response to DOM exposed to light. The conclusions were that the end products of photo-oxidation were probably low molecular weight compounds palatable to bacterioplankton, with the stimulatory effect occurring after irradiation of the water over ≤ 4-h (Lindell ***et al.*** 1995).

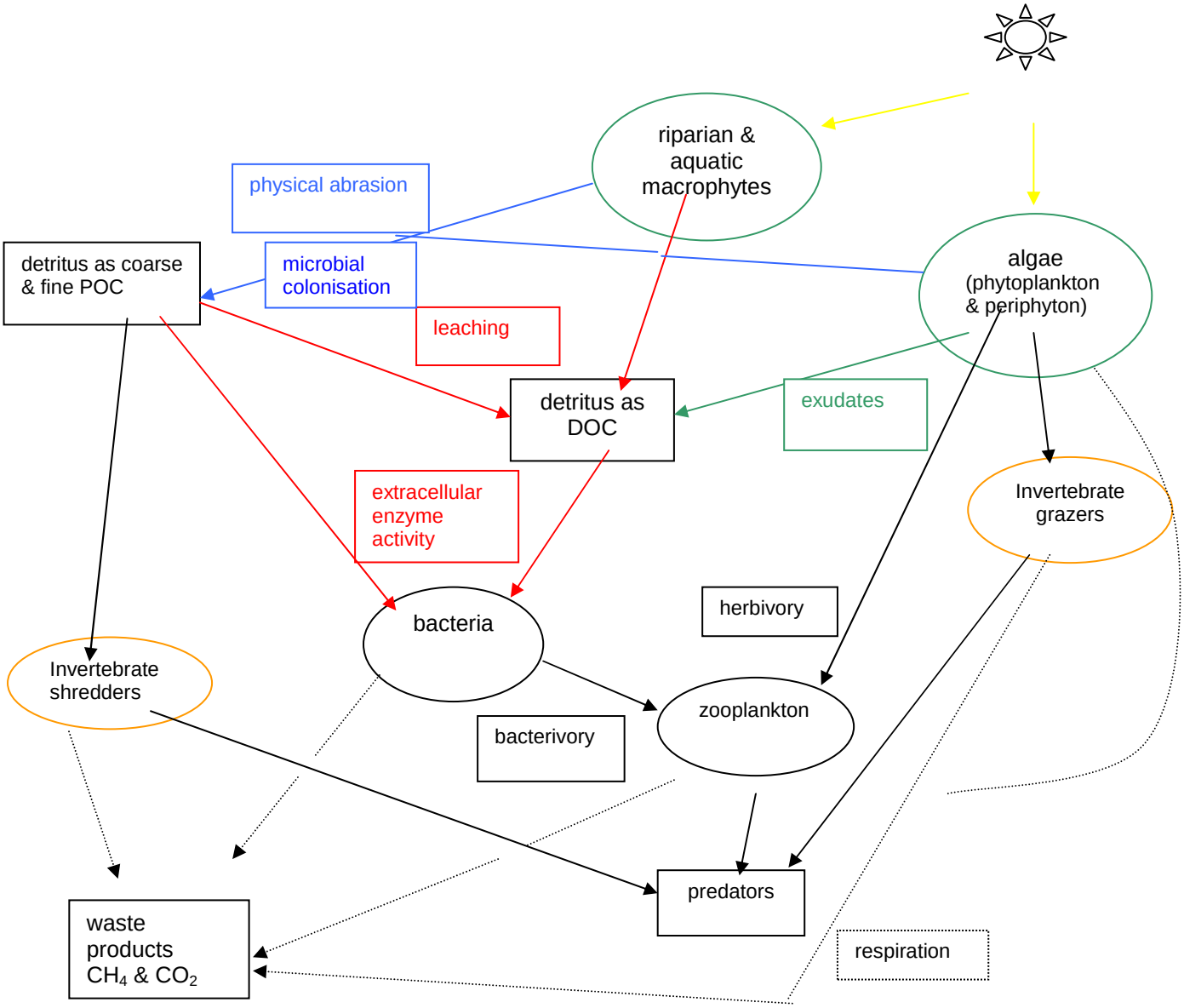

Figure 2: Carbon-cycling diagram, showing energy pathways, with bacteria playing a key role in several pathways. (adapted from Boulton and Brock (1999); and pers. com. P. Boon).

The role of periphyton communities has been shown to play a key role in carbon dynamics of streams and also potentially interacting with heterotrophic bacteria (Figure 2), such as the DOC released from photosynthesising algae being utilised by bacteria (Rier & Stevenson 2002). Biofilm matrices (when substrata available) are now seen as the preferred mode for bacterial growth with sessile populations predominant in almost all natural ecosystems (Fischer 2003). Rier & Stevenson (2002) looked at the following two key components of

algae-bacteria interactions in an oligotrophic stream in the USA: 1. carbon limitation (if bacterial biomass was positively associated with algal biomass when light is not limited and allochthonous sources are not labile); and, 2. nutrient competition (if bacteria in the presence of labile DOC (as glucose) would limit algal growth through nutrient competition when nitrogen and phosphorus are limiting). The authors found that for the carbon limitation experiment the bacteria responded positively to higher algal biomass; however not as a source of DOC, but from an increase in availability of substratum for colonisation. The second experiment (nutrient competition) revealed that bacteria in the presence of glucose were co-limited by inorganic nutrients, with algae not appearing to be negatively affected by competition with bacteria for the nitrogen and phosphorus.

Early studies on the uptake of DOM by heterotrophic bacteria have relied upon compounds such as glucose and amino acids. Glucose and amino acids play an important role in cycles of carbon, and are labile low-molecular-weight DOM that forms the building blocks of biopolymers and macromolecules found in organisms. Although concentrations are low, turnover rates can be fast enough that these compounds can support a large proportion of bacterial growth and comprise a large fraction of the labile DOM flux, with glucose taken up more quickly than other sugars in streams (Kirchman 2003). The minimal knowledge that we do have about sugar uptake indicates that glucose dominates the total monosaccharide flux, with concentrations of free glucose usually higher than those of other monosaccharides, and turnover of glucose is faster, with the possible exception of fructose. When comparing both dissolved free amino acids (DFAA) and glucose across environments (i.e., lentic, lotic and oceanic), assimilation of DFAA seems to support more bacterial production than glucose assimilation (Kirchman 2003).

Bioassay experiments are the most common and currently the best method in determining the uptake (bioreactivity) of DOM in natural water samples as it directly estimates the fraction of natural DOC that can be used by microbial assemblages under defined conditions (Benner 2003). The method involves incubating a filtered water sample containing DOM and

a natural bacterial assemblage. Experiments are typically incubated in the dark (thus limiting the interaction of any autotrophic organisms that may not have been removed during filtration) for a period of days to a week. The standard measurements obtained include bacterial production and DOC consumption in the form of DOC loss or respiration (drop in dissolved oxygen) thus giving an estimate of the bioavailability of DOM (Benner 2003). Despite the overall simplicity of bioassays there are a number of potential errors that can occur on the parameters measured. Storage of water samples can rapidly alter the microbial population structure; nutrients can be a limiting factor for the microbial utilisation of DOM, more importantly there are no standard protocols for bioassay experiments and finally the extent of DOM utilisation depends upon the duration and temperature of the experiment (Benner 2003). Furthermore, del Giorgio & Davis (2003) state that if bioassay experiments are used to estimate metabolic rates of DOM consumption then only the initial stage of the experiment can be used as bioassay experiments are static (not constantly replenished with DOC as with ***in situ*** experiments) and the labile fraction is therefore quickly depleted, resulting in a rapid decline in consumption rates. To determine the reliability of bioassays in determining the metabolic estimates of DOM consumption the authors looked at a number of bioassays with incubation times of <1.5 days. When these rates were compared with the range of total bacterial DOC consumption (sum of bacterial respiration (BR) & bacterial production (BP) based on 478 pairs of observations) the authors found that there was good agreement (within a factor of 2) between the median initial rates of consumption in bioassays and the median rates of consumption ***in situ*** calculated from BR & BP measurements. It was therefore concluded that short term bioassays can give reliable information relating to the bacteria-DOM interaction that occurs in natural waters (del Giorgio & Davis 2003).

2.5. DOC sources and utilisation in Australian Aquatic Environment

Australia is a flat continent, making lowland rivers the dominant river form and of this, the majority (83%), are inland systems with semi-arid to arid climatic regimes, and thus many exhibit “cease-to-flow” for periods of time (Thoms & Sheldon 2000). Despite the high

variability of flow, many inland rivers are major sources of water for irrigation, and stock and domestic supplies (e.g., Murray-Darling system) and feature extensive floodplains and networks of anastomising channels and distributaries (Bunn ***et al***. 2006). In Australia an understanding of the sources and the processing of carbon in lowland rivers is gradually emerging (e.g. Robertson ***et al***. 1999; Bunn ***et al***. 2003; Oliver & Merrick, 2006; Gawne ***et al***. 2007; Hadwen ***et al***. 2009). Evidence suggests that riparian trees such as ***Eucalyptus camaldulensis*** (river red gum) contribute large quantities of litter to large rivers and associated floodplains in south-eastern Australia (Briggs and Maher 1983; Francis and Sheldon 2002; Zander ***et al***. 2007). Some of this litter falls directly into water bodies at times of low flow, but much of it remains on dry floodplains (Robertson ***et al.*** 1999) and in-channel surfaces (Thoms and Sheldon 1996, 1997) until inundated by rises in river levels.

An early study by Boulton (1991) on the benefits of eucalyptus leaves as a food source revealed that dry or 'exposed' leaf litter was not heavily colonised by microbes and thus less palatable to macroinvertebrates than wetted leaf litter. The wetting of eucalypt leaves expedited the leaching of volatiles such as polyphenols and thus with time the leaves became more heavily colonised by microbes (whilst the dry leaves did not), which led to an increase in the density of macroinvertebrates colonising the leaves. This work was comparable with a number of other studies suggesting that between 20% and 30% of the leaf mass is leached within days from the litter (Boulton 1991; O'Connell ***et al***. 2000; Strauss & Lamberti 2002). A thorough review outlining the pathways and processes in the decomposition of leaf litter in streams is offered by Boulton and Boon (1991). A study by Glazebrook and Robertson (1999) near the Murray River in New South Wales (NSW) supported the findings of Boulton showing that the decomposition of river red gum leaves was greatest in flooded sites in autumn and slowest in non-flooded sites in winter. The greatest standing stocks of leaf litter as CPOM was also in autumn, with resultant carbon loss measured as TOC also greatest in the flooded sites in autumn (Glazebrook & Robertson 1999).

Several studies in Australia have looked at river red gum organic matter as a carbon source to aquatic ecosystems (e.g. Baldwin 1999; Glazebrook and Robertson 1999; O'Connell ***et al***. 2000; Francis & Sheldon 2002). Baldwin (1999) compared the release of DOM from aged and fresh red gum leaves under abiotic conditions (achieved by inhibiting microbial activity) and showed that DOM from aged leaves was almost linear with the amount of leaf material, suggesting abiotic release. However, the DOM extracted from fresh leaves reached saturation and the author suggested that hydrolytic enzymatic processes may also be involved. Two more important findings were that: 1. Low concentrations of DOM leached from fresh-leaves was readily consumed by microbes; however, increased concentrations of DOM (i.e. due to higher leaf biomass) resulted in limited microbial processing of the available carbon with nutrients (e.g., nitrogen and phosphorus) being the limiting factor; and 2. DOM taken from aged leaves is not as bioavailable as fresh leaves, with only 30% of the aged leaf DOM utilised by the bacteria (Baldwin 1999).

O'Connell ***et al.*** (2000) compared the release of DOC from river red gum litter components and soil at a floodplain near Albury in south-eastern NSW and found that the amount of DOC leached after 29 days decreased in the order: leaves>>twigs=bark>CPOM=soil. The authors concluded that even though CPOM and soil represent the greatest % standing stock on the floodplain they would contribute the least, whilst leaves would probably contribute the greatest source of DOC during inundation of the floodplain. A more detailed study comparing DOC release from leaves, twigs and bark again showed that significantly more DOC (***P***<0.05) was released from leaves, with no significant differences for maximum release of DOC (***P***>0.05) under aerobic or anaerobic conditions for any component of litter (O'Connell ***et al.*** 2000). With regard the microbial uptake of DOC the authors showed that microbes metabolised the most DOC from leaf leachate (~40%), followed by twig leachate (~30%) and bark leachate (~25%). In comparing spatial differences along the floodplain (high versus low flows) there was no significant difference (***P***>0.05) in the rate of release of DOC nor the microbial uptake of DOC (O'Connell ***et al.*** 2000).

A later study by Francis and Sheldon (2002) again compared the DOC release from various red gum litter types (leaves, twigs, gumnuts and bark) following inundation along a floodplain of the lower Darling River. Litter accumulation along floodplains of the lower Darling River is dominated by river red gum leaves, bark, twigs and gumnuts (Thoms & Sheldon 1997). Results showed that red gum leaves released the largest amount of DOC per gram dry weight and after 21 days the cumulative release of DOC from leaf litter was nearly ten times that of the other litter types, with most DOC released within the first 8 days of inundation for all litter types (Francis & Sheldon 2002). Weight loss of leaves from permanently flooded and intermittent flooded treatments showed little difference between treatments with most rapid weight loss occurring within the first 72 h (***cf***. Strauss & Lamberti 2002) to 1 week after inundation. DOC release from leaves was also rapid occurring within the first 24 h of flooding in both treatments (Francis & Sheldon 2002).

The studies described above support the fact that DOC from terrestrial organic matter (specifically river red gum leaves) is a potential food source for organisms in Australian lowland rivers. However, in some situations evidence from stable isotope studies suggest that algal production may be a more important source of carbon for higher trophic levels especially snails, crustaceans and fish, (Bunn and Davies 1999). Robertson ***et al***. (1999), state that when DOC from allochthonous sources is not available, a shift to in-channel algal production dominated carbon pools occurs and the heterotrophic community is growth limited and tied to DOC produced by autotrophs.

Bunn ***et al***. (2003) carried out a study to determine the sources of organic carbon (OC) supporting the food web in Cooper Creek (an arid zone floodplain river) in southwestern Queensland. The techniques used included the determination of benthic gross primary production (GPP) and respiration (R_{24}) using ***in situ*** chambers (e.g. Bunn ***et al***. 1999) and the stable isotopic signatures of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) to assess trophic interactions between primary producers (i.e., dominant riparian vegetation and periphyton), primary consumers (e.g., micro-organisms) and secondary consumers (e.g., crustaceans).

The findings showed that there was no evidence for the food web being directly supported by upstream organic matter inputs (longitudinal) or riparian (lateral) inputs and was therefore not consistent with the predictions of the RCC and FPC for large rivers. Autotrophic GPP was also very high in the waterholes despite high turbidity, and the benthic algae were the most important source of OC for consumers. These findings were initially unexpected considering the extensive riparian connectivity along the channels. The importance of benthic algae as a dominant food source was shown in the ^{13}C enriched signatures of several consumers (tracking the ^{13}C signatures of the algae), particularly shrimp (***Macrobrachium***), yabby (***Cherax***) and catfish (***Neosilurus***) with mixing model data suggesting only about 20-25% of biomass carbon of these species was derived from allochthonous sources (Bunn ***et al***. 2003). Furthermore, Bunn ***et al.*** (2003) state that "micro-algae are major drivers of food webs in large floodplain river systems. The factors influencing distribution, composition and production of micro-algae in these systems (e.g., flow regulation, light and general land management) potentially have a much higher impact on the food web than variations in the terrestrial carbon pool. These findings should influence the way large rivers are managed".

The importance of floodplain inundation in Australian dryland river systems, although still poorly understood in many systems, has the potential to provide carbon subsidies during times of overbank flooding (Burford ***et al***. 2008). A similar study to that of Bunn ***et al.*** (2003) was undertaken on the Murken waterhole during a flood in January 2004 with the floodplain surrounding the waterhole inundated for 38 days. The Murken waterhole is located within the complex anastomising channel system that typifies the Cooper Creek catchment (Burford ***et al***. 2008). The findings were consistent with that of the FPC, supporting the lateral river-floodplain exchanges of carbon; however, the linkages were not through the expected pathways (e.g., detrital matter deposited in the waterhole) but largely via fish biomass that had accumulated on the floodplain while consuming aquatic food sources. A conceptual model of a fish food web put forward by the authors suggests that: during the

flood fish move out into the floodplains and feed upon smaller fish that fed on terrestrial detritus; and, also on aquatic invertebrates that had fed upon terrestrial cyanobacteria. In the study it was estimated that 50% of the carbon in the waterhole fish originated from the floodplain. As the flood retreated and the waterhole became isolated, fecundity rates increased with the dead fish providing an indirect carbon floodplain food source to the waterhole ecosystem (Burford ***et al***. 2008). Bunn ***et al***. (2006) also discuss the importance of flood pulses in providing a boom of production on the floodplains and associated wetlands. They recorded significant aquatic algal production during periods of prolonged inundation, along with an increased abundance of both aquatic and terrestrial-floodplain derived sources providing a rich feeding ground for a variety of taxa including frogs, fish, crustaceans and birds.

A study looking at primary productivity in the Murray River in temperate south-eastern Australia indicated that this system could potentially be modelled on a combination of the three productivity concepts (RCC, FPC & RPM) depending on spatial and temporal variations (Gawne ***et al***. 2007). The authors concluded that the system was driven by a combination of autochthonous (i.e., phytoplankton & macrophytes) and allochthonous (riparian) sources, with both spatial and temporal (specifically seasonality) variation associated with each source. Phytoplankton production peaked in summer and autumn correlating with light levels, whilst a reduction in phytoplankton production at the Albury site was linked to the release of cold water from Hume Dam (***cf***. Oliver & Merrick, 2006), a finding consistent with the SDC. Riparian inputs varied with species composition, with litter fall highest in summer and autumn from native species and highest in winter from exotics. An increase in stream width (e.g. up to 100 m) resulted in less direct shading of the channel and a lower proportion of litter input. Macrophytes where driven by both seasonality and flow rates with greatest inputs (material converted to detritus) occurring in winter and spring. Geomorphology also plays a role, highlighted at the Barmah site where numerous benches provided increased habitat for macrophytes enabling a much greater contribution from these

producers (Gawne ***et al***. 2007). An earlier study by Oliver & Merrick (2006) measuring river metabolism at three sites (Albury (upstream site), Barmah & Hattah) along a 1000 km length of the Murray River concluded that autochthonous sources of carbon (predominantly phytoplankton) were the main drivers of the measured metabolism. Although significant amounts of allochthonous organic debris were evident in the river (from past floods) the metabolic contributions were not substantial due to the aged recalcitrant nature of the material. A similar study by Vink ***et al.*** (2005) quantifying ecosystem metabolism in the middle reaches of the Murrumbidgee River also showed phytoplankton dominated ecosystem production despite being phosphate limited. The authors concluded that the high contribution of phytoplankton in the Murrumbidgee system could be a consequence of flow regulation and resultant loss of riverine connectivity with adjacent floodplains (Vink ***et al.*** 2005).

A study by Rees ***et al***. (2005) quantifying bacterial abundance and production on the Murray River was undertaken using the same three sites by Oliver & Merrick (2006). The findings were that bacterial abundance was different across the three sites with a trend of increasing bacterial numbers downstream. Rather than being a trend along the river continuum (refer to the RCC previously) the authors suggest that the difference in numbers reflects localised conditions with the potential impact of hypolimnic releases of water from Hume Dam with low bacterial density (not quantified) accounting for the lower numbers at the Albury site. The increased bacterial numbers at the other two sites was potentially due to variations in carbon supply and / or may reflect a decrease in grazing pressure. Abundances and production across all sites were correlated with chlorophyll ***a*** and not with nutrients or carbon concentration. Rees ***et al***. (2005) concluded that a source of carbon is fundamental for fuelling bacterial production, and that under the current river management conditions of highly modified flows (via the presence of headwater dams and many main channel weirs and licensed water extractors) phytoplankton play an important role in fuelling bacterial production.

The potential impacts of water storages within Australia cannot be under-estimated with the previous two paragraphs alluding to the potential impact of the SDC on downstream productivity. A recent study by Baldwin ***et al*** (2010) found that the water quality in the Murray River downstream of Lake Hume was affected during conditions of extreme drawdown. The study revealed that the lake was a net exporter of carbon, nitrogen, phosphorus and iron during the study period. Most of the carbon, nitrogen and phosphorus were exported in the form of algal biomass. Furthermore, processes in the lake were also shown to influence the downstream algal community structure, with communities upstream of the lake being dominated by green algal species whilst communities within and downstream of the lake dominated by cyanobacteria (Baldwin ***et al.***, 2010).

Few studies have examined the fate of allochthonous carbon and its importance to secondary production in food webs (Kritzberg ***et al.,*** 2006). A recent study by Hitchcock ***et al.*** (2010) was performed to determine whether the addition of DOC (in the form of glucose), would be taken up by secondary consumers via heterotrophic bacteria in an estuarine section of the regulated Hunter River on the mid-north coast of New South Wales. Previous studies have shown that freshwater inflows to estuaries deliver DOC as well as inorganic nutrients, which may regulate estuarine productivity, through their effects on the growth of heterotrophic bacteria and phytoplankton (Aitkenhead-Peterson ***et al.,*** 2003; Mulholland, 2003; Thingstad, 2003). The first set of experiments (***in-situ*** 1.25 L bottles over 4 days, zooplankton removed) showed that bacterial growth was significantly (***P***<0.05) enhanced by the addition of DOC, primarily as a result of enhanced microbial respiration, compared to that of the control. Furthermore the addition of DOC plus inorganic nutrients (nitrogen & phosphorus) in some cases further enhanced bacterial growth. However, when inorganic nutrients alone were added bacterial growth was not enhanced suggesting that DOC is limiting, whilst the addition of DOC alone also led to a decrease in chlorophyll ***a*** concentration. The authors suggested that this is probably a result of competition between the heterotrophic bacteria and phytoplankton for the available inorganic nutrients. This

implies that the ambient concentrations of DOC in the Hunter Estuary were limiting the growth of the heterotrophic community both spatially and temporally over the course of the study (Hitchcock ***et al***. 2010). The larger 70 L container trial included the zooplankton and was run over a longer period (10-days) to allow time for the zooplankton to acclimate and respond to the treatments. Results showed that bacterial numbers increased initially (with no change in chlorophyll ***a***) and then the bacterial numbers dropped off during the latter days of the trial suggesting increased grazing pressure by zooplankton. The zooplankton (calanoid copepods) density was significantly higher (***P***<0.05) in the DOC treatment at day 10, compared to that of the control. The overall findings suggest that an increase in freshes and flows can potentially deliver increased concentrations of DOC to the estuary thus stimulating both primary (bacteria) and secondary (zooplankton) production (Hitchcock ***et al***. 2010).

A comparative study on the bioavailability and utilisation of bulk DOC and the fulvic acid fraction by heterotrophic bacteria was carried out in the Murrumbidgee River and Berry Jerry Lagoon (a meander cut-off lagoon near Wagga Wagga), in south-eastern Australia. The fulvic acid fraction of DOC was chosen as it represents the largest component of the dissolved fraction (45-65%) in a typical riverine sample and is soluble at all pH values (McKnight ***et al***. 2003). The results showed that bacterial abundance was significantly higher when exposed to bulk DOC than on just fulvic acid in both ecosystems after the data were normalised to carbon concentration. Bacteria grew almost immediately on the Murrumbidgee River DOC, whereas there was a 2-day lag phase before bacteria were able to utilise the fulvic acid and a 1-day lag phase existed for both DOC and fulvic acid from the lagoon. Bacterial growth rates followed the same path as abundances: i.e., river DOC > river fulvic acid > lagoon DOC > lagoon fulvic acid (McDonald ***et al***. 2007). The authors concluded that as bacteria grew more readily on the bulk DOC, the non-humic substances must play a role in the bioavailability of DOC in aquatic ecosystems; and as fulvic acids were less bioavailable the differences between the bioavailability of the DOC from the two habitats

is potentially due to the higher humic content of Berry Jerry Lagoon. The authors also suggest that linkages (as outlined in the FPC) between rivers and floodplain ecosystems are clearly important as the lagoon bacteria were able to utilise the river DOC immediately (McDonald ***et al***. 2007).

Chapter 3. Using flow management to increase delivery of allochthonous derived dissolved organic carbon to the highly regulated lower Namoi River

3.1. Introduction

The concentration of dissolved organic carbon (DOC) in most lotic systems varies between 0.5 to 50 mg l^{-1}, with the primary influences on DOC concentrations being runoff from precipitation and the presence of wetlands (Hinton ***et al.*** 1998; Mulholland 2003). Hinton et al. (1997) consistently obtained positive relationships between DOC concentration and stream discharge during rain events. Increases in DOC concentration have also been linked to increases in discharge, in particular during floods and large rainfall events (e.g. Hinton ***et al.*** 1997; Hinton ***et al.*** 1998; Buffam ***et al.*** 2001; Henson ***et al.*** 2007). As large rain events often contribute a large proportion of the annual total discharge, they may also represent a significant proportion of total DOC exported through a catchment (Easthouse ***et al.*** 1992; Hinton ***et al.*** 1997; Mulholland 2003).

It is now widely acknowledged that river regulation has profound effects upon a variety of riverine dependent ecosystem attributes including water quality (Growns ***et al.*** 2009); downstream nutrient and organic matter subsidies (Kelly 2001; Wipfli ***et al.*** 2007); disruption of the hydrologic cycle (Freeman ***et al.*** 2007); downstream habitat alteration (Ogbeibu and Oribhabor 2002); and loss of connectivity to dependent floodplain and wetland ecosystems (Poff ***et al.*** 1997; Kingsford 2000; Page ***et al.*** 2005). All these attributes can influence the quantity and quality of allochthonous DOC that enters rivers and suggests that the quantity (due to decreased discharge or decreased concentration) delivered has been reduced in regulated rivers. The seasonal timing of flooding and drying events on floodplains is important as flow events in summer are expected to provide a higher yield of allochthonous organic carbon and nutrients as increased temperatures lead to greater heterotrophic production (Baldwin and Mitchell 2000).

The Namoi River, Australia, like many lowland rivers around the world has been altered considerably through the presence of headwater dams, large-scale floodplain irrigation development and flow regulation devices such as weirs. This has resulted in a loss of connectivity with the surrounding landscape (Thoms and Sheldon 2000; Chessman 2003). Flow volumes as well as the frequency of small to moderate sized events have been markedly reduced from those before regulation (Chessman 2003). As a result, fewer benches and floodplains are wetted, and this is likely to reduce the concentration and load of allochthonous DOC entering the river. This may be altering the period of time that the river is allochthonously driven (Westhorpe ***et al.*** 2010) and may be limiting the yields and diversity of planktonic organisms and ecological functioning.

There is a recognised need to appropriately manage river floodplain ecosystems, acknowledging that their integrity is greatly impeded by river regulation and resultant loss of longitudinal connectivity, flow variability (e.g., flood pulses) and river-floodplain connectivity (Bayley 1995; Sparks 1995; Poff ***et al.*** 1997). Even in heavily regulated river systems such as the Danube (Austria) and Missouri (USA) there is a potential to restore floodplains and allow the flood pulse to maintain ecosystem function (Sparks 1995). In New South Wales, Australia, a systematic approach to environmental water allocation across the State was developed to improve river and wetland health (Thoms and Swirepik 1998). These flows are strongly oriented toward the partial restoration of the natural flow regime, a strategy advocated by many river ecologists (Power ***et al.*** 1996; Poff ***et al.*** 1997). In the Namoi River, rules that protect high flow events from extraction have been implemented when reservoirs spill or high flows generated from unregulated tributaries occur.

The purpose of this study was to determine how DOC concentrations responded to discharge and to develop relationships between discharge and DOC concentration in the Namoi River. These relationships were used to estimate loads delivered to the river under different modelled flow scenarios. The flow simulation models were run using the Integrated Quantity and Quality Model (IQQM) (Simons ***et al.*** 1996). This allowed prediction of the

amount of DOC supplied to the river under different flow scenarios, such as with and without environmental flow allocations, and also under simulated natural (low development) flows, to estimate potential delivery quantities and infer river health benefits from these flows. The working hypothesis being: environmental flows will increase the delivery (loads) of DOC to the lower Namoi River compared with the current regulated (pre-environmental) flows.

3.2 Materials and Methods

3.2.1. Catchment and sites

The Namoi catchment is located in central north New South Wales (NSW), covering an approximate area of 43 000 km^2 and forms part of the Murray-Darling Basin Drainage System (Figure 3). The Namoi River is a typical alluvial Australian inland river, originating in a comparatively wet upland area, and for the majority of its length flows through a semi-arid landscape that provides little additional discharge (Thoms and Sheldon 2000). Flows are regulated by Keepit Dam (storage capacity of $425x10^6$ m^3) on the Namoi River and Chaffey Dam (capacity $62x10^6$ m^3) on the Peel River.

Three sites were selected along the Namoi River within the mid to lower (riverine plain) landform zones (Figure 3) with an elevation ranging from <250 m at Boggabri to <150 m at Walgett, and an average annual rainfall of 400-600 mm (DLWC 2000). The riverine plains are characterised by a complex pattern of tributaries, anabranches and effluent channels, and are subject to extensive flooding (Thoms and Sheldon 2000). The dominant tree species at Boggabri and Bugilbone are native river red gums (***Eucalyptus camaldulensis***) and the exotic weeping willows (***Salix babylonica***) and at Walgett are river red gums and native wattle (***Acacia stenophylla***). River sediments comprise a mixture of approximately 60% sand, 30% clay and 10% silt (unpublished data). Turbidity ranges between 5-50 NTU gradually increasing downstream, and large runoff events can increase turbidity levels to over 1000 NTU (DLWC 2000). Phosphorus concentrations often exceed the acceptable water quality limits (50 µg L^{-1}; ANZECC 2000) for aquatic ecosystems and algal blooms are a major problem in the water storages of the Namoi catchment (DLWC 2000, Mitrovic ***et al.***

2003). Hydrographic data for the three sites on the Namoi River were obtained from gauging stations operated by the NSW Office of Water (NOW). Stage was recorded every 15 minutes and converted to m^3sec^{-1}.

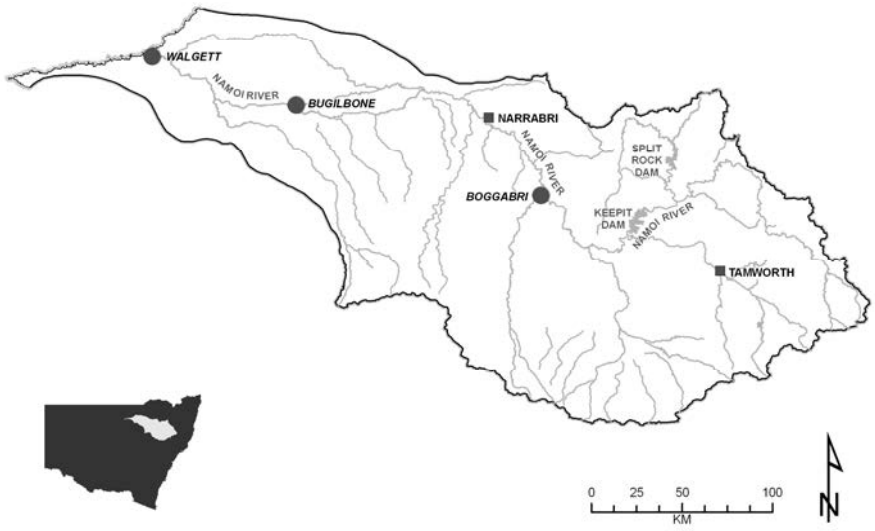

Figure 3: Map of the Namoi catchment in northern NSW, Australia, showing the three study sites ●; major towns and reservoirs.

3.2.2. DOC sampling

Routine seasonal samples were collected between September 1999 and March 2005 at Boggabri (26 times), Bugilbone (14 times) and Walgett (16 times) along the Namoi River. Sites were not sampled on the same temporal scale. However, independent replicate samples were taken at each site on more than one occasion to assess within-site variability at that time (replicates: ***n***=3-12).

Three flow events were sampled every 4-hours for DOC concentration. The first stages of the rising limb of the January-2004 flood event at Bugilbone were sampled 17 times (in triplicate first 6 times; duplicate the remainder) from 3:30 pm, 28th January to 07:30 am, 31st January 2004. The rising limb, peak and a portion of the falling limb stages of the second event upstream of Walgett were sampled 59 times (with triplicate samples taken 4 times to check for variation within each time) from 11:30 am, 13th December to 08:30 am, 23rd December 2004. The latter stages of the rising limb, the peak and the majority of the falling

limb stages of the third event at Bugilbone were sampled 36 times from 11:30 am, 06th December to 07:00 am, 12th December 2008. Manual sampling was undertaken during the Bugilbone-2004 event and a programmable Manning auto-sampler (Model 4901) was utilised over the course of the two longer flood events at Walgett-2004 and Bugilbone-2008. Due to lack of resources, complete sampling across all three flood events was not possible.

Samples for DOC analysis were collected midstream from 20 cm below the water surface, or midway in the water column in shallower waters. DOC samples were acidified with hydrochloric acid and refrigerated at 4°C until analysis in the laboratory. DOC was filtered prior to analysis using 0.45µm pore size Sartorius Minisart® filters (cellulose acetate membrane) attached to disposable plastic syringes. An unfiltered portion of the sample was used to determine TOC (total organic carbon). The method used to analyse DOC / TOC was the High Temperature Combustion Method, 5310 B (APHA 1998; Wetzel and Likens 2000). T-tests were run to determine relationships between DOC concentrations and discharge.

3.2.3. DOC export during flood events

Both average and total DOC exports were calculated based on the period-weighted method (Hinton ***et al.*** 1997). In this method the average concentration of successive samples is multiplied by the volume of water passing the site during a specified interval (e.g., 24-hr), thus providing an average 24-hr DOC export load and also an average DOC export over the entire period of the flood event that was sampled. The total export is obtained by summing the export during individual sampling intervals. A caveat with this method is that whilst changes in flow volume are accounted for, changes in DOC concentration with stream discharge are not (Hinton ***et al.*** 1997). Hinton ***et al.*** (1997) relied upon weekly data to determine DOC exports and concluded that increasing the sampling regime, particularly during a flow event, would provide more precise estimates of export load. To minimise this weakness in the method, we sampled DOC on a 4-hourly basis over the sampling duration of each event (as previously outlined).

3.2.4. Modelling of flow scenarios and DOC export

Models of river flow under different environmental flow scenarios were created for the study sites with the IQQM (Simons ***et al.*** 1996; DIPNR 2005). Scenarios with environmental flows, without environmental flows and simulated natural (low development) were modelled for the period July 2000 to June 2005. Modelled flows with environmental flows were used in preference to actual measured flows in order to increase the comparability of the two scenarios. The effects of some lower catchment tributaries are not represented well and this can result in small discrepancies between modelled and measured flows. Flows were also modelled over this period in the absence of major water impoundment and major extraction. These flows (termed the low-development scenario or simulated natural) are similar to natural flows but not identical because the models cannot remove the effects of minor extraction, changes in runoff patterns caused by clearing of catchment vegetation or flow changes caused by anthropogenic impacts on the morphology of river channels and drainage connections.

Modelling of DOC load to the river under different flow scenarios was based upon relationships found between flow and DOC concentrations across the three flood events as well as the routine seasonal sampling. Linear regression was used to determine the relationship between discharge and median DOC concentration over a range of flow categories (0.6, 1.1, 5, 11, 18, 54, 114, 232, 325 and 370 $m^3 sec^{-1}$) that encompassed low flow periods, moderate flows and floods. Median DOC concentrations were used in the linear regression as they tend to be less skewed and therefore have a greater central tendency when dealing with a range of concentrations than does the mean. The relationship was then used to model total DOC export under the three modelled flow scenarios over the period 2000 to 2005.

3.3. Results

3.3.1. DOC concentrations: Low flows and floods

Mean DOC concentrations (± s.e.) during low flows increased with distance downstream of Keepit Dam, with concentrations at the Boggabri, Bugilbone and Walgett sites being 7.3 (±0.2), 7.8 (±0.2) and 10.3 (±0.9) mg L^{-1}, respectively (Figure 4). Mean DOC as a percentage of mean TOC was consistent across all sites, ranging from 87% at Bugilbone to 90% at Boggabri (Table 1). Minimum DOC concentrations for the three sites were 4.0, 4.9 and 4.4 mg L^{-1}, respectively. The mean DOC concentrations (± s.e.) during the two Bugilbone flood events (2004 and 2008) were 10.5 ± 0.2 and 12.6 ± 0.2 mg L^{-1}, respectively (Figure 4). The maximum and minimum DOC concentrations recorded during both the Bugilbone flood events were 14 and 7.8 mg L^{-1} (2004) and 16.0 and 9.0 mg L^{-1} (2008), respectively. The December 2004 flood event sampled upstream of Walgett mobilised a greater concentration of DOC (Figure 4), with a mean of 20.4 mg L^{-1} (± 0.9). The maximum and minimum concentrations recorded were 44 mg L^{-1} and 7.1 mg L^{-1}, respectively. DOC concentrations for each flood event in relation to flow are presented in Figures 5 a, b and c; whilst Figures 5 d, e and f show each sampling occasion in relation to the entire flood event. For each event (Figure 5) DOC concentration responded differently as follows: Figure 5a shows a decrease in DOC concentration as discharge increases; Figure 5b shows an immediate and large increase in DOC concentration with a steady decline over time; whilst Figure 5c shows an increase in DOC concentration with discharge then a steady level, perhaps declining at the end. During each flood event mean DOC, as a percentage of mean TOC, showed similar patterns to the low flow data ranging from 88 – 94 % (Table 1). Parametric comparisons (T-tests) of DOC concentrations revealed that during high flow events DOC concentrations were significantly higher ($P<0.0001$) than concentrations during low flows.

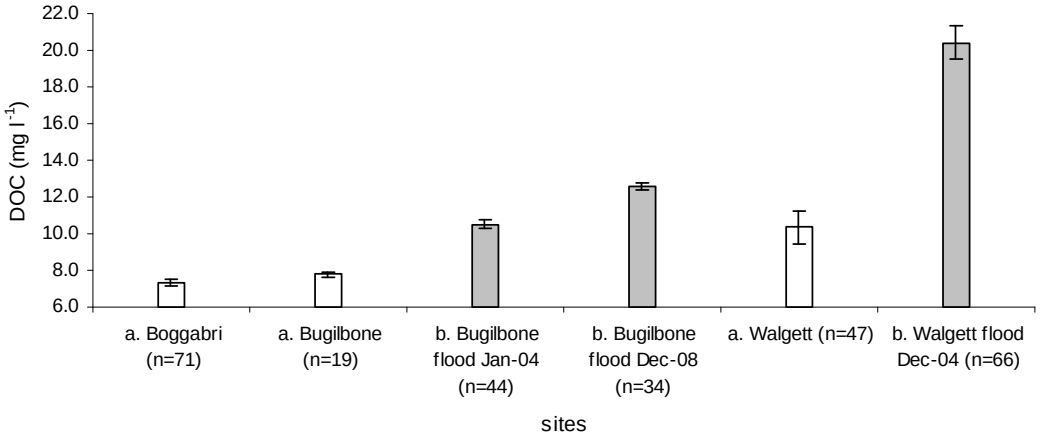

Figure 4: Mean DOC concentrations (± s.e.) in the lower Namoi River: a. background sampling (≤ 0.6 m^3 sec^{-1}) at 3 sites (Boggabri, Bugilbone & Walgett) moving longitudinally downstream between September 1999 – March 2005. b. 3 flood events: grey bars (Bugilbone & Walgett).

Table 1: Percentage of TOC that is DOC for different sites and flow conditions. nt = not tested. * 2008 flood

Flow	Boggabri	Bugilbone	Walgett
Low	90.3	87.1	89.2
Flood	nt	90.1 & 94*	88.3

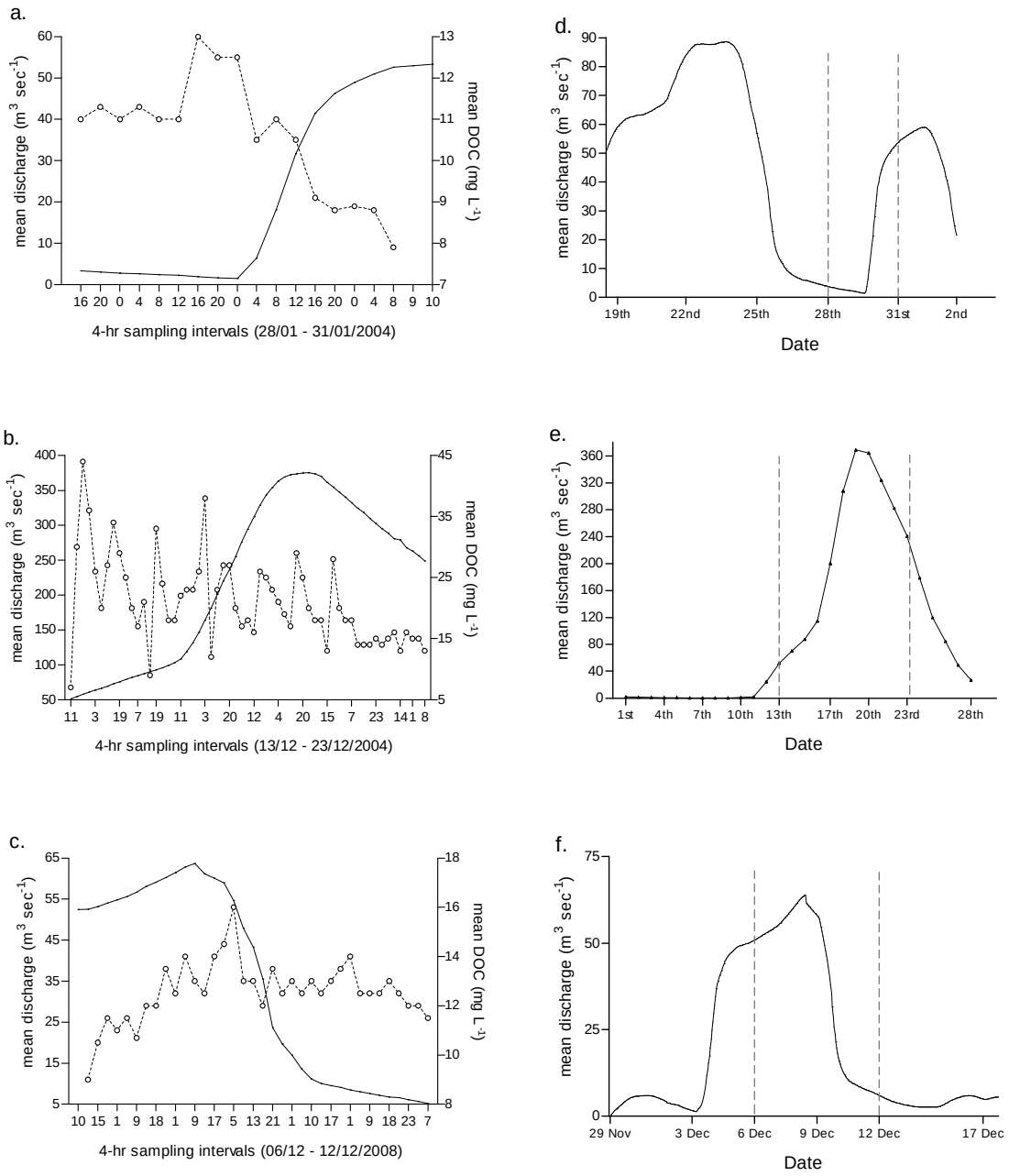

Figure 5: Mean discharge (m^3 sec^{-1}) and DOC concentrations (mg L^{-1}) measured every 4-hr in the lower Namoi River during three flood events: a. Bugilbone January 2004; b. Walgett December 2004; c. Bugilbone December 2008. d, e & f: expanded views of hydrographs in relation to each DOC sampling event corresponding to a, b & c respectively. Values on the 'x' axis for figures a, b & c are in 24-hr. Vertical dashed lines represent sampling period in relation to entire flow event.

3.3.2. DOC export loads

Daily DOC loads (tonnes) were estimated for the largest flood event (December 2004 at Walgett) from 13 – 23 December during the rising stage, peak and first four days of the falling limb of the hydrograph (Table 2). The mean daily load of DOC mobilised over the

sampling period of the flood event was estimated to be 399.8 tonnes (s.e. ± 59.5-tonnes, ***n***=11). This is greater than 100 times the average low flow daily load of DOC (3.4-tonnes: s.e. ± 1.3, ***n***=15) that was estimated on data from fifteen separate occasions between September 1999 and May 2002. The minimum and maximum mean flows on any day of the flood fell between 0.39 and 17.36 m^3sec^{-1} which were equivalent to the 80th and 14th percentile flows for the respective months sampled. The estimated total DOC exported over the period of the event was 4079 tonnes (Table 2). A linear regression to determine potential relationships between mean daily flow and mean daily DOC loads revealed a highly significant (***P***< 0.0001; r^2 = 0.85) relationship. The mean was used over the median as it had a high central tendency and confidence limits.

Table 2: Estimate of daily carbon (DOC) loads (tonnes) with corresponding mean daily discharge (m^3 sec^{-1}) and total DOC export over the sampling period of the flood event. ***n*** = 6 on 14th – 19th and 21st; 5: 20th & 22nd; 4: 13th; 3: 23rd December. * The mean discharge is the first 12-hr as DOC sampling was only taken in the morning.

Date	Mean DOC (tonnes)	Mean flow (m^3 sec^{-1})
13-12	130.24	51.43
14-12	161.52	69.67
15-12	156.71	87.20
16-12	212.40	113.46
17-12	437.47	199.35
18-12	540.97	307.93
19-12	713.95	369.17
20-12	613.28	364.95
21-12	435.50	325.19
22-12	362.13	283.20
23-12*	314.86	254.25
Total period	4079.03	

3.3.3. Modelling of DOC export with environmental flows

Linear regression analysis was performed on the median DOC concentration observed across different flow categories for all three sites and all sampling occasions. A significant (***P***< 0.05; r^2 = 0.54) positive relationship also existed. This linear regression model was Y= 0.000587*X + 4.43. A Y intercept of 4.43 mg L^{-1} DOC was selected as this was the average background DOC concentration across all three sites. This was used to predict DOC

concentration at a daily time step for mean daily flows generated by the IQQM models. Modelled flow scenarios were run using IQQM to generate outputs without environmental flow rules, with environmental flow rules in place, and simulated natural flows. The hydrograph (Figure 6) shows the flows generated by the IQQM model for the high flow period in 2000-01 and compares the discharge with environmental flows and without (data were log transformed to reduce the effect of outliers). Table 3 shows the total DOC load calculated for each scenario (June to July) for the years 2000-01 to 2004-05. The impact of environmental flow rules was greatest in 2000-01 where they more than doubled the DOC load to the river with an extra 74,000 tonnes exported. The environmental flow rules in this period approximated DOC delivery under natural conditions (Table 3). However, in subsequent years a lack of substantial rainfall and associated discharge resulted in the flow rules having little impact (Table 3).

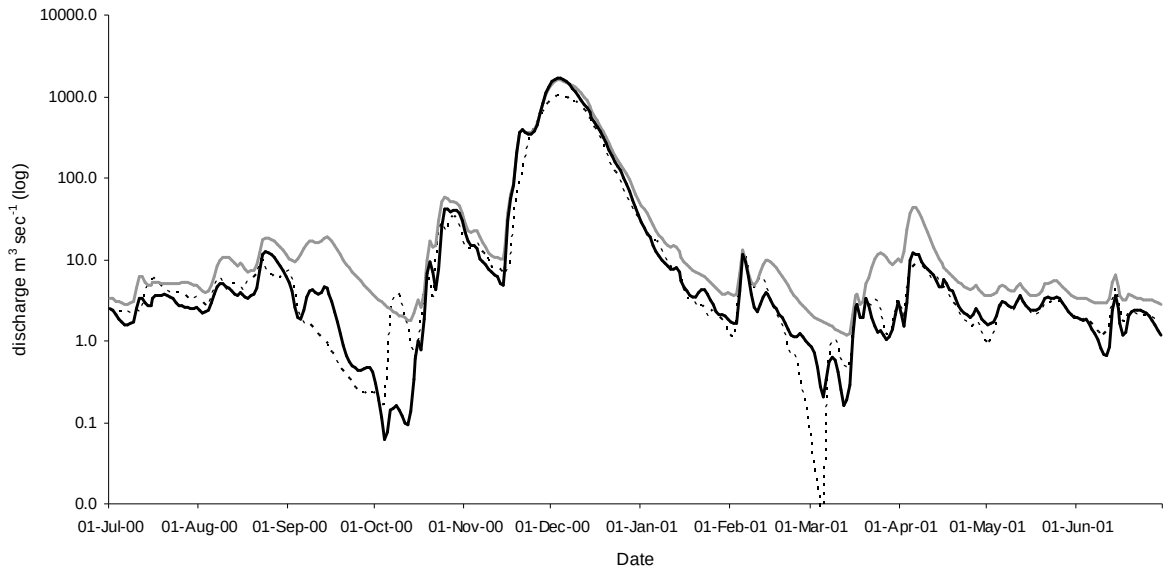

Figure 6: Flows generated by IQQM over 2000-2001 for scenarios: without environmental flows (dashed black line); with environmental flows (solid black line); and simulated natural flows (solid grey line).

Table 3: Yearly (June to July) DOC export loads (tonnes) for the lower Namoi River based on modelled scenarios (IQQM) of no environmental flows, with environmental flows and simulated natural flows. Load was determined daily and summed to give yearly DOC export.

DOC export Tonnes			
Years	No environmental flows	With environmental flows	Simulated natural
2000-01	68618	143316	149204
2001-02	367	363	887
2002-03	133	166	286
2003-04	971	1020	2393
2004-05	738	752	1547

3.4. Discussion

3.4.1. DOC concentrations

DOC samples obtained during the course of this study had concentrations of 4 - 44 mg L^{-1} that fell within the ranges (0.5 - 50 mg L^{-1}) found in most lotic systems with background DOC concentrations of semi-arid regions typically ranging from 1 to 3 mg L^{-1} (Mulholland 2003). Background DOC concentrations of approximately 4.4 mg L^{-1} were recorded during this study and the slightly higher DOC concentrations could be a reflection of landscape changes on floodplains adjacent to the river (e.g., clearing for agriculture and grazing) that influences the flow path of water across the landscape (Mulholland 2003). It was also evident that mean low flow DOC concentrations increased with longitudinal distance within the catchment (Figure 4). This may be a result of a build-up of refractory DOC originating from upstream sources and processes as described in the river continuum concept (Vannote ***et al.*** 1980). However, increases in organic carbon with longitudinal distance can also be a result of local site specific inputs. The upper site (Boggabri) has less than 25% riparian cover whilst the two downstream sites maintain approximately 50% cover respectively (DLWC 2000). This increased riparian cover may lead to a comparative increase in direct riparian inputs and associated in-stream production as described by the Riverine Productivity Model (Thorp and Delong 1994). Lateral connectivity of lowland rivers with floodplains is also a key contributor of carbon and nutrient inputs compared with that of downstream transport (Junk ***et al.*** 1989). The reasonably fast utilisation (days) of DOC by planktonic-bacteria (Baldwin 1999;

Robertson ***et al.*** 1999; Westhorpe ***et al.*** 2010) also supports the theory that local reach inputs are the main source of carbon. The water travel times between sites are about 6 days between Boggabri and Bugilbone, and about 4 days between Bugilbone and Walgett (Westhorpe ***et al.*** 2008). The literature shows that bacterial utilisation of DOC based on bacterial respiration and production measurements can occur within 1 to 2 days (c.f. del Giorgio and Davis 2003) further supporting local reach inputs.

3.4.2. Relationships between flow and DOC

The data show that flow events increased DOC concentrations considerably in the mid to lower Namoi River. A positive relationship between discharge and DOC concentrations was found ($r^2 = 0.54$) and large amounts of DOC were mobilised and exported when flood events occur (such as 4079 tonnes for the 2004 Walgett flood (Table 2)). Our findings are similar to those of studies by Hinton ***et al.*** (1998) and Henson ***et al.*** (2007) where the authors also found a positive relationship between DOC concentration and stream discharge during flow events in smaller streams and rivers. This also supports findings from other countries that have shown that DOC concentrations in rivers increase during storm and runoff events often by several orders in magnitude (e.g., Hinton ***et al.*** 1997; Aitkenhead-Peterson ***et al.*** 2003; Nortström ***et al.*** 2010). Further to this, the findings from this study indicate that antecedent discharge conditions in a lowland river also influence the concentration and pattern of DOC in relation to a particular flood event as presented in Figure 5. In Figure 5a the DOC concentration declines as discharge increases, however when antecedent flows are taken into account (Figure 5d) it can be seen that the initial elevated DOC concentration was probably in response to the previous larger flow event mobilising DOC and the subsequent smaller event that was measured led to a dilution effect taking place. When comparing Figures 5b and 5e it is also evident that a large amount of carbon was mobilised (Fig. 5b) in response to an increase in discharge and antecedent discharge patterns were not sufficient to previously mobilise the available carbon (Fig. 5e), however the relatively high discharge that was maintained for several days led to a gradual dilution in DOC concentration. The

third flow event (Figures 5c and 5f) like the previous flow event occurred after a period of minimal discharge and led to an increase in DOC concentration in response to an increase in discharge. These three sampling occasions highlight the influence that antecedent events can have in determining the availability and concentration of DOC that can be mobilised. Therefore discharge, periodicity, frequency and duration of prior flow events will be a key factor in determining the mobilisation of DOC within a lowland river. A schematic of possible outcomes relating to DOC concentration and discharge is presented in Figure 1(Chapter 2).

Positive relationships between DOC concentrations in streams and the number of wetlands in catchments have also been shown in Canada (Eckhardt and Moore 1990). As few wetlands occur on the lower Namoi River floodplain the leaching of organic matter from wetted floodplain areas is likely the main contributor to DOC to the river during floods. Meyer and Tate (1983) showed significant differences in DOC delivery between a 'cleared' and 'uncleared' watershed with higher DOC concentrations entering the river in the 'uncleared' watershed. Large areas of the lower Namoi catchment are cleared (~75%), however there are considerable areas of natural vegetation (~25%) scattered throughout the catchment (DLWC 1999). Despite the clearing, large quantities of DOC were mobilized during flow events. The large quantities of DOC detected in the river may be derived from a combination of both in-channel and watershed sources.

3.4.3. River Regulation and DOC

Numerous studies have reported on the ecological and biogeochemical importance of flow variability, frequency and connectivity of rivers with surrounding landscapes (Hornberger ***et al.*** 1994; Puckridge ***et al.*** 1998; Bunn and Arthington 2002; Page ***et al.*** 2005; Sánchez-Andrés ***et al.*** 2010). Despite this, traditional water management has often dampened natural flow variability, and altered seasonality to ensure steady and dependable water supplies for human purposes (Richter ***et al.*** 2003). In the Namoi River, flow regulation has greatly reduced the magnitude of flow events (Figure 6). Geomorphic features such as benches, flood-runners and flood plains are depositional zones of terrestrial organic matter (TOM)

(Thoms and Sheldon 2000). If flow magnitude is considerably suppressed, wetting of terrestrial landscapes will be reduced, resulting in less DOC mobilised, transformed and transported downstream.

To assess the performance of the environmental flows the quantity of DOC exported was modelled under various flow scenarios. As no 'control' rivers exist in the Namoi or adjacent catchments, the modelling approach was seen as the best option to assess the delivery of DOC to the river. This approach has been used elsewhere to estimate the benefits of flow management scenarios on large rivers that did not have suitable controls (Mitrovic ***et al.*** 2006). The hydrological modelling tool IQQM (Simons ***et al.*** 1996; DIPNR 2005) allowed flow hydrographs to be generated under the different flow scenarios. This was coupled to the linear regression relationship between DOC and flow to determine daily loads for each scenario and this was added to give yearly loads.

The modelling revealed that when large flow events occurred, such as in 2000-01, the environmental flow rules had a considerable effect. Much greater loads (143,316 tonnes) were delivered with environmental flows than would be delivered under the scenario where no environmental flows were allocated (68,618 tonnes) (Table 3). The DOC load under environmental flows was similar to that under simulated natural (low development) conditions (149,204 tonnes). In this year the environmental flows were effective at delivering DOC to the system. The other modelled years had generally low flows and lacked substantial flow events that resulted in only small increases in DOC export under environmental flows rules as compared to those without. Modelled mean annual flows during the latter years of this study were between 1.8 – 10 m^3 sec^{-1} compared with a mean annual flow of 88.8 m^3 sec^{-1} in 2000-01. The low flows were partly a result of a severe drought in south eastern Australia (Bond ***et al.*** 2008).

The alteration of natural flow regimes through river regulation is a major concern worldwide (Poff ***et al.*** 1997). Thoms and Sheldon (2000) documented a 48% reduction in long-term median annual flows and a 91% reduction in the magnitude of annual flood events over the

past 100 years in the Barwon-Darling River as a result of regulation. The Namoi is a major regulated tributary of this river. It is likely that climate change will reduce rainfall in the Namoi catchment in the future (CSIRO 2006) and these low flow periods could become more common. Lowland rivers in semi arid-areas are somewhat adapted to periods of no flow and dry phases as the functioning of these ecosystems has evolved (Thoms and Sheldon 2000). However, the periods of no and low flow are much greater under regulation than they are under natural flows as is evident in the hydrographs presented in Figure 6.

A number of international reports on carbon fluxes in rivers have shown that river discharge (and maintenance of natural flow regimes) is the major factor influencing the fluxes of POC and DOC in rivers (Robertson ***et al.*** 1999; Vink ***et al.*** 2005; Oliver and Merrick 2006). Other factors include primary production, litter pool sizes in watersheds, and the development of agriculture in catchments (Robertson ***et al.,*** 1999). The DOC results in this study (Table 3; Figure 4) support the maintenance of natural flow regimes as they can inject substantial quantities of DOC into aquatic ecosystems by wetting river benches, riparian zones and floodplains.

There is some speculation as to the ecological role of allochthonous DOC in aquatic systems with some studies showing autochthonous sources to be more important (Bunn ***et al.*** 2003). However, there is a growing body of evidence on the importance of allochthonous DOC sources in driving aquatic food webs (Thorp and Delong 2002; Carpenter ***et al.*** 2005; Cole ***et al.*** 2011). In the lower Namoi River, Westhorpe ***et al.*** (2010) using ***in situ*** microcosms showed that bacterioplankton outcompeted phytoplankton when labile allochthonous DOC was added suggesting DOC limitation. Results from a mesocosm study at Boggabri suggest that allochthonous DOC inputs are being utilised through the food chain to higher organisms such as zooplankton with the planktonic structure being altered (see Chapters 5 & 6).

Many countries have developed laws and policies providing water rights to river dependent species and ecosystems once basic human needs are met (Acreman and Dunbar 2004). The management of flow releases through time to enhance or maintain river health has been

called variously environmental flows, in-stream flows, environmental allocations and ecological flow requirements (Gordon ***et al.*** 1994; Acreman and Dunbar 2004). In 1998, environmental flow rules were developed for each of the catchments that have major rivers regulated by large storages in NSW such as the Namoi River. The flow rule that dictated the environmental flow in the Namoi River prior to 2004 (under the Water Act 1912) was the "off-allocation access rule and high-flow rule". These flow rules changed under the Water Management Act (2000) upon the release of the Lower Namoi Regulated River Water Sharing Plan in mid 2004 to the "supplementary access rule" with water users requiring a licence to access this water. This rule comes into effect when reservoirs spill or high flows enter from unregulated tributaries. During such periods, irrigators can pump water without the quantity being debited from their annual entitlement. Supplementary access rules set flow thresholds for access and restrict the amount of water permitted for extraction during such periods, or limit its timing.

Richter ***et al.*** (2003) suggest that the challenge of ecologically sustainable water management is to design and implement a water management program that stores and diverts water for human purposes in a manner that does not cause affected ecosystems to degrade or simplify. The implementation of hydrological modelling tools such as IQQM in conjunction with biogeochemical data such as DOC concentration and loads is an important step in determining environmental flow regimes that will maintain riverine ecosystem integrity whilst still being able to meet the needs of water users. To argue for increased environmental flows, evidence of their role in effective delivery of constituents and their ecological benefit are required. This information can also give scientists and managers new information about how to deliver water better to increase outcomes for ecosystems. For example: a regulated flow release (e.g., 20 m^3 sec^{-1}) that piggy-backed on to the Bugilbone 2008 discharge event (Figure 5) from an unregulated tributary and runoff may have provided a greater ecological benefit due to increased floodplain connectivity than releasing the parcel of water pre or post the unregulated inflow. Changes in rainfall and higher evaporation rates

due to climate change are likely to lead to less water for streams and rivers in the Namoi catchment (CSIRO 2006). This will place additional strains on the catchment's water resources. Due to current and increasing demand for water, the potential negative effects of climate change and drought, water managers will have to adopt new ways of managing water to maximize environmental benefit (Thoms and Sheldon 2000; Bunn and Arthington 2002; Richter ***et al.*** 2003; Acreman and Dunbar 2004).

The relationships between discharge and DOC and the quantities that may be delivered under different types of flow events in lowland rivers are not well understood. The present study has shown that DOC is positively related to discharge and that partial restoration of natural flows can result in increased delivery of DOC to a river. This has the potential to increase the periods of heterotrophic dominance towards a more natural regime (Westhorpe ***et al.*** 2010). The importance of allochthonous DOC in structuring food webs in aquatic systems is becoming more apparent (Carpenter ***et al.*** 2005; Cole ***et al.*** 2011) and they may be altered by DOC deliver in the Namoi River. There is a need to manage lowland river systems more holistically to increase ecological outcomes. Particular emphasis needs to be focused on small and medium sized flow events that have been dampened by flow regulation (Figure 6). Restoring these should provide regular pulses of terrestrial organic carbon to the river ecosystem, provided adequate extant riparian vegetation exists. Strategies for storage releases that coincide with rainfall events in unregulated tributaries lower in the catchment as well as protection from extraction should in part be able to restore these ecologically important flow events. This will help to deliver more regular pulses of allochthonous DOC into the river with the associated benefits to river functioning and ecology.

Chapter 4. Diel variation of dissolved organic carbon during large flow events in the lower Namoi River.

4.1. Introduction

Total organic carbon is usually characterised into dissolved organic carbon (DOC) and particulate organic carbon (POC) with the distinction between the two generally made based on whether or not it passes through a 0.2-0.45 µm filter (Hope ***et al***. 1994; APHA 1998; Raymond & Bauer 2001). Carbon budgets for aquatic ecosystems have revealed that the DOC fraction often represents the major pool or flux of organic carbon being transported through the system (Thomas 1997; McKnight ***et al***. 2003). Fulvic acids are the largest component of the dissolved fraction (45-65%) in a typical riverine sample (McKnight ***et al***. 2003) and together with the humic acid fraction (responsible for much of the colour seen in many rivers) can represent 75% of the total DOC fraction (Hope ***et al***. 1994; McKnight ***et al***. 2003). Both these acids fall within the general colloidal range of dissolved organic matter and carbon compounds in natural waters (Hope ***et al***. 1994).

Diel variations in DOC have been reported in both marine (Sieracki and Sieburth, 1986; Gasol ***et al.,*** 1998) and freshwater systems including lakes (Geller, 1986; Lindell ***et al.,*** 1996), wetlands (Ziegler and Fogel, 2003) and rivers (Manny and Wetzel, 1973; Harrison ***et al.,*** 2005; Spencer ***et al.,*** 2007; Parker ***et al.,*** 2010). These studies have shown that bulk DOC concentrations are often significantly elevated during day-light hours (particularly the afternoon) compared with concentrations detected at night (Sieracki and Sieburth, 1986; Kaplan and Bott, 1989; Parker ***et al.,*** 2010). This may be due to an increase in photosynthetic production with the release of photosynthates during daylight hours (Parker ***et al.,*** 2010) and also the phototransformation of recalcitrant DOM into more available forms (Bushaw ***et al.,*** 1996; Lindell ***et al.,*** 1996). The direct negative effects of sunlight on bacteria can result in decreased metabolic rates during the day and conversely increased heterotrophic consumption at night (Lindell ***et al.,*** 1996). The over-arching factor in determining the relative importance of these two counteractive processes is the time spent by bacteria and DOM in light exposed zones during mixing (Lindell ***et al.,*** 1996). Further to this, in-stream biotic processes can influence DOC and also dissolved inorganic carbon concentrations, and are dependent on diurnal factors such as light and temperature (Dawson ***et al.*** 2001).

Diel patterns in DOC do not always follow this elevated daytime pattern. Some studies have found minimal diurnal variation in a small headwater stream in the USA (Manny and Wetzel, 1973). Parker ***et al.*** (2010) examined diel variability in DOC in the Big Hole River (Montana, USA) and showed a marked increase in night-time DOC coinciding with an increase in flow and pH, suggesting that other factors can influence diel variability. Worrall and Burt (2004) examined long-term inter-annual river DOC records in the UK and showed that trends in DOC concentrations were not readily explained by trends in flow, or water quality parameters but coincided more with increases in temperature. At a smaller scale, Dawson ***et al.*** (2001) measured potential diurnal changes in DOC every 2-h over a 24 h period in two acidic headwater streams in Scotland and found no significant difference between DOC concentrations during daylight hours and at night. Even if changes in DOC are not detected, the composition of DOC may change as measured by light absorbance (Spencer ***et al***., 2007), along with its bioavailability (Findlay ***et al***., 2003). Spencer ***et al.*** (2007) suggest that this inconsistency in diel variation may be a reflection of the relatively small diel changes in DOC concentration that can exist (influenced by the concentration of the total DOC pool) and the fact that these small changes cannot be measured within the analytical precision of conventional DOC analysers. To our knowledge no studies have looked at DOC diel variability in lowland rivers during high flow events. Increasing our awareness in this area should help us determine the mechanisms and processes (e.g., biological & photochemical) that drive DOC diel variability in lowland rivers, which in turn will help us understand the ecological importance of maintaining aquatic-terrestrial linkages.

The purpose of this study was to determine if patterns in diel variation of DOC existed within the Namoi River during periods of increased flow. Floods have the potential to inundate the surrounding landscape (e.g., benches and flood-runners), which in turn can mobilise large quantities of carbon, often increasing DOC by several orders of magnitude (Hinton ***et al.,*** 1997; Robertson ***et al.,*** 1999; Dawson ***et al***., 2001; Aitkenhead-Peterson ***et al.,*** 2003).

4.2. Materials and Methods

4.2.1. Catchment and sites

The Namoi catchment is located in central north New South Wales (NSW), a semi-arid region covering an approximate area of 43 000 km^2 and forms part of the Murray-Darling Basin Drainage System. Flows are regulated by Keepit Dam (storage capacity of $425x10^6$ m^3) on the Namoi River and from Chaffey Dam (capacity $62x10^6$ m^3) on the Peel River. Two

sites were selected along the Namoi River within the lower landform zone (riverine plains) of the Namoi Catchment (Figure 7). The slope of the land is less than 3 degrees with an elevation of approximately 150-m above sea level, and an average annual rainfall of 400-600 mm (DLWC 2000). The dominant tree species at each site were River red gums (***Eucalyptus camaldulensis***) and Weeping willows (***Salix babylonica***) at Bugilbone and River red gums and wattle (***Acacia stenophylla***) at Walgett. River sediments were comprised of a mixture of approximately 60% sand, 30% clay and 10% silt (unpublished data). A water quality report for the Namoi catchment covering the years 2002 – 2007 (Mawhinney 2011), revealed that turbidity readings often exceed the acceptable water quality limits (50 nephelometric turbidity units: NTU) for aquatic ecosystems in lowland streams. Turbidity generally increases downstream, with large runoff events occasionally increasing turbidity levels to over 1000 NTU. Total phosphorus and total nitrogen concentrations also often exceed the acceptable water quality limits (50 µg L^{-1} and 600 µg L^{-1} respectively; ANZECC, 2000) for aquatic ecosystems in lowland streams (Mawhinney 2011) and algal blooms are a major problem in the water storages of the Namoi catchment (DLWC 2000), whilst pH values were stable ranging between 7.5 – 8.5. Electrical conductivity also often exceeded the ANZECC (2000) guidelines of 300 µS cm^{-1} (Mawhinney 2011). During periods of low flow mean DOC concentrations within the lower Namoi River approximate 7 mg L^{-1} (Westhorpe ***et al***. 2010). The Namoi River feeds into the Barwon-Darling River, the only major river system of this large, semi-arid region.

4.2.2. DOC sampling

To determine diel variation in DOC, samples were taken 4 hourly (Table 4). Manual sampling was undertaken during day light hours (0700 – 1800 hr) and a programmable Manning auto-sampler (Model 4901) was used during night-time sampling (1900 – 0600 hr). The auto-sampler was cooled with ice each night and the temperature was consistently below 10°C the following morning when samples were removed. Due to lack of resources complete sampling across each flood event was not possible.

Samples for DOC analysis were collected near midstream from 20 cm below the water surface and methods were as described in Chapter 3.

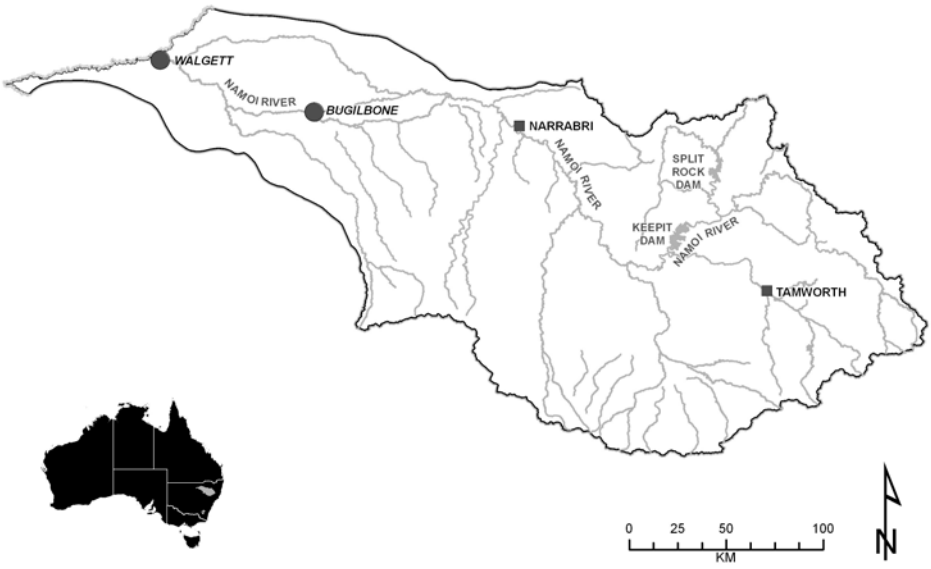

Figure 7: Map of the Namoi catchment in northern NSW, Australia, showing the two study sites, major towns and reservoirs.

4.2.3. Flow and water temperature data

Hydrographic data for the two sites on the Namoi River were obtained from gauging stations operated by the NSW Office of Water (NOW). Stage was recorded every 15 minutes and converted to mean daily flow. Hourly stream water temperature data for the Bugilbone-2008 flood event were obtained from the NOW gauging station located near the site. There was no temperature data available for the Walgett flood event in 2004.

4.2.4. Data analysis

Significant changes in DOC concentrations between day and night samples were determined using a two-way factorial analysis of variance (ANOVA) after Levene's test for homogeneity of variances. The interaction term 'day/night x location' was used to determine if diel variability in DOC concentrations from both flow events was being influenced by 'location' within the catchment. If 'location' was not an influencing factor then DOC concentrations should respond in the same way. Temperature data was analysed using the t-test for dependent samples.

4.3. Results

4.3.1. Diel variations in DOC

Hydrographs of the 2 sampling events and measured DOC concentrations are presented in Figure 2. The mean DOC concentrations (± standard deviation) detected across each sampling event was 21.1 ± 7.2 mg L^{-1} during the flood at Walgett (Fig 8a) and 12.5 ± 1.5 mg L^{-1} during the flood at Bugilbone (Fig 8b). A DOC relationship with flow was also evident for the high flow event at Walgett, with diel DOC variation declining in line with general DOC concentrations (which peaked in concentration at the beginning of the hydrograph), from the rising limb through to the crest and the falling limb (Fig 8a).

DOC samples obtained from both events examined over a diel range were split up into 'day' and 'night' samples. The mean diel differences in river water DOC concentrations indicated a general trend for higher night-time concentrations, with the magnitude of these differences quite variable, from less than 1.0 mg L^{-1} to 8.5 mg L^{-1} (Table 4). The ANOVA revealed that DOC concentrations were significantly higher during the night (***P***<0.05; ***F-value*** 4.23). Location (Bugilbone vs Walgett) was also significantly different (***P<***0.001; ***F-value*** 50.1). However, the interaction 'day/night x location' was not significant (***P>***0.05; ***F-value*** 2.07) suggesting that DOC concentrations at both locations follow the same pattern of higher concentrations at night.

Table 4: Sampling details with mean and range of DOC concentrations obtained from each flow event; # rising limb only.

Flow event	No. of samples	Sample period	Hydrograph position	Mean DOC (±S.D.) mg L^{-1}		Minima / maxima DOC concentrations mg L^{-1} (24hr)	
				day	night	day	Night
Walgett	59	13th – 23rd December 2004	Rising limb, crest & part of falling limb	18.9 (6.5) 20 (8.1)#	23.4 (7.4) 28.5 (7.8)#	7.0 (1130) & 34 (1530)	14 (0330) & 44 (1930)
Bugilbone	36	06th – 12th December 2008	Later stages of rising limb, crest & part of falling limb	12.1(1.3)	12.9 (1.6)	8.9 (1130) & 14 (1700)	10 (2100; 0100) & 18 (0515)

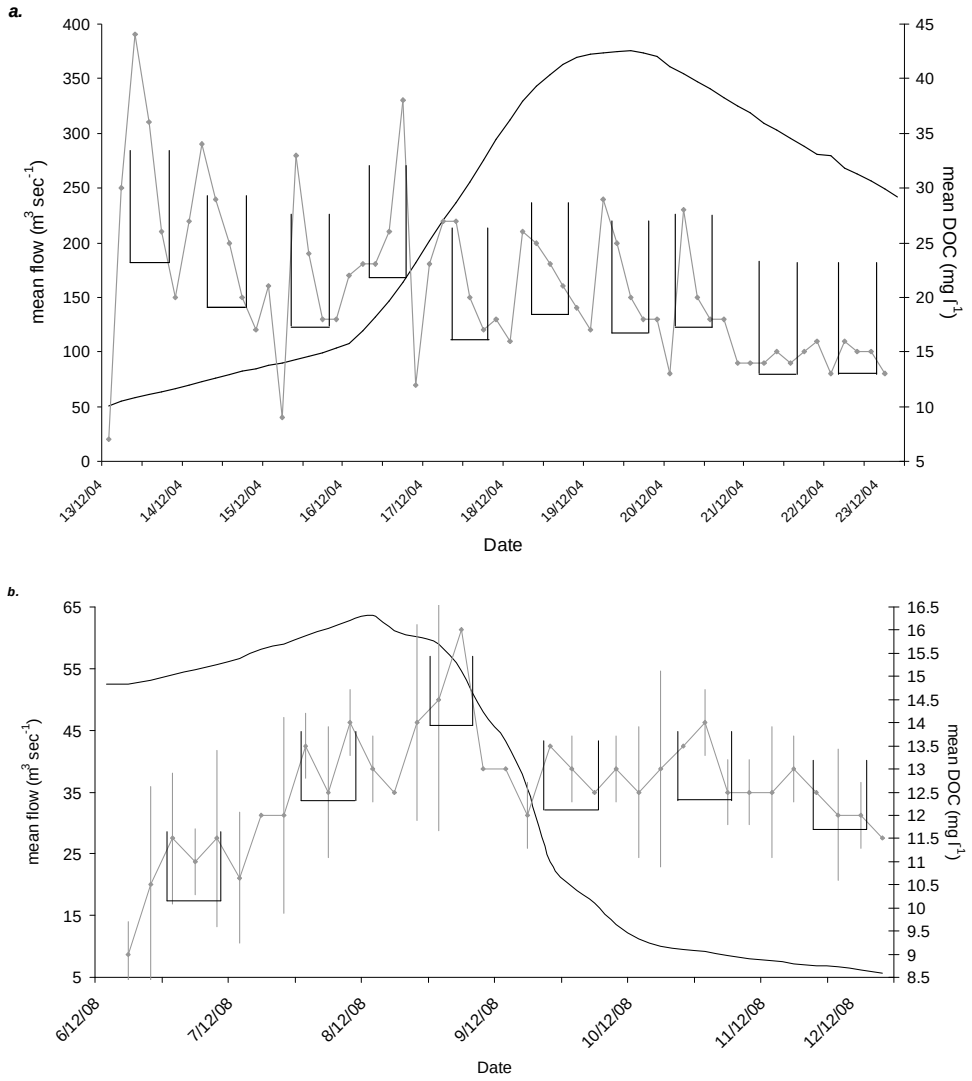

Figure 8: Mean flow (m^3 sec^{-1}) and DOC concentration (mg l^{-1}) measured 4-hourly in the Namoi River at: ***a***. Walgett during a large flood in December 2004; and ***b.*** Bugilbone during minor flooding in December 2008. Graphs: ***a***, ***n***=1; ***d***, ***n***=2 ± s.d. night time samples lie within vertical bars**.**

4.3.2. Day and night DOC minima and maxima concentrations

Examination of DOC fluctuation within 'day' hours only and then 'night' hours only revealed that DOC concentrations during daylight hours showed a distinct pattern increasing from the morning to the afternoon across the two flow events with minima detected around mid-morning (e.g., 7.0 mg l^{-1} at 11:30 hours) and maxima (e.g., 34 mg l^{-1} at 15:30 hours) detected mid to late afternoon (Table 4). No distinct patterns were observed for the night

samples with both minimum and maximum concentrations occurring at different times (Table 4).

4.3.3. Diel variations in temperature

A variation in the diel mean temperature between 'day' and 'night' existed during the Bugilbone flood event in December 2008 with the mean day-time temperature (22.2°C: ± 0.3) significantly higher (***P***<0.05; t=1516, ***n***=96) than the mean night-time temperature (22.05°C: ± 0.25).

4.4. Discussion

4.4.1. Diel variations in DOC

Several studies have shown that diel variability of DOC concentration exists and that DOC can be higher during daylight hours (in particular the afternoon) than at night (e.g., Rutherford and Hynes, 1987; Kaplan and Bott, 1989; Parker ***et al.,*** 2010). Our findings within the lower Namoi River based on two separate sampling occasions across a moderate and a high flow event (Figure 2) showed night-time DOC concentrations were significantly greater (***P***<0.05) than those detected during the day-time. Parker ***et al.*** (2010) also found diel changes in flow to be an influencing factor, with increased flows at night resulting in a DOC maximum concentration at night. The streams in the study by Parker ***et al.*** (2010) were a headwater and second order stream respectively with elevations greater than 1300-m above sea level. In contrast both our sampling sites were in a meandering lowland river (slope <3°) and the hydrographs (Figure 2) were rounded with flow events spread over 6 - 10 days. As such, it is unlikely that there were rapid diel variations in flows that would influence DOC concentrations as reported by Parker ***et al***. (2010).

We cannot unequivocally identify the underlying factors contributing to the DOC diel variations detected in the Namoi River and the purpose of this paper is to report this pattern. However, transformation of recalcitrant DOC during day-light hours into more labile forms by photo-degradation could be one possibility, with a rapid assimilation of this DOC by heterotrophic bacteria and comparably reduced concentrations to those at night. Based on much of the available literature previously cited, it would appear that natural sunlight plays a key role in photochemically altering DOC, in particular the conversion of recalcitrant DOC into assimilable forms (Lindell ***et al.,,*** 1995). This is particularly so for recalcitrant DOM

derived from allochthonous sources such as riparian vegetation (Wetzel ***et al.,*** 1995; Moran and Covert, 2003), which is readily assimilable by heterotrophic bacteria within a relatively short time frame (e.g., <4-h with light resembling the UV of natural sunlight) during day-light hours. At night the DOC is predominantly recalcitrant and unavailable to bacteria, remaining in the water column (Lindell ***et al.,*** 1995). Howitt ***et al.*** (2008) have shown that both bacterial abundance and CO_2 production increased in response to the increased bioavailability of the DOM after exposure to sunlight.

During flooding in the Lower Namoi River, surrounding floodplains are inundated and allochthonous sources of carbon dominate (Westhorpe ***et al.,*** 2010). Increasing landscape connectivity mobilises allochthonous carbon in various forms (e.g., DOC, particulate organic matter and large-woody debris). This was reflected in the increased concentrations of DOC detected during sampling of the two floods. Much of this allochthonous material remains recalcitrant until it has undergone photochemical transformation via solar radiation. Once transformed, various labile forms of DOM are rapidly utilised by the heterotrophic bacteria. Rapid uptake of this transformed DOC by heterotrophic bacteria could result in diel variation in DOC concentrations (cf. Harrison ***et al.,*** 2005; Parker ***et al.,*** 2010). However, this suggests the greater diel variability in DOC concentration seen at the Walgett site (Table 1; Figure 2) may be due to higher DOC concentrations measured. If this is the case, the diel variability witnessed may be dependent on flow regime, and only occur with increased flows and flood events.

Several studies have shown DOC concentrations and fluxes to be controlled by stream flow (e.g., Clark ***et al.***, 2007; Eimers ***et al.***, 2008). The smaller flow event at Bugilbone (Table 1; Figure 2) with less well pronounced diel variability may be due to the counter-active biotic factors (e.g., microbial utilisation of DOC) playing a larger role in determining the DOC concentrations, whilst these biotic processes may not have been as evident during the larger flow event at Walgett, as substantially greater concentrations of DOC were mobilised (see Eimers ***et al.***, 2008).

Turbidity may also be another underlying factor in the diel DOC variation we measured. In highly coloured waters the rapid attenuation of sunlight dictates that the production of most biologically available DOM photoproducts is constrained to the photic zone (top metre or shallower) of the water column. An increase in turbidity of lowland rivers is often associated with an increase in flow (from dam releases, rainfall events and runoff) that leads to the

scouring of the geomorphic features of the river and subsequent re-suspension of sediments. Recently published work (Riggsbee ***et al.***, 2008) has shown that exposure of re-suspended sediments to solar radiation leads to the photo-chemical transformation and release of this previously sorbed organic matter into DOC and other organic compounds such as nitrogen back into the water column (with most of the desorption activity taking place in the first 2 h of exposure). A study by Southwell ***et al***. (2010) in tidal systems supports the findings of Riggsbee ***et al***. (2008) in that the photo-production of dissolved nutrients from re-suspended sediments can contribute to the overall water column concentration, particularly during episodic events. The humic acid fraction of the DOM, along with suspended particulate material also intercepts sunlight and can contribute substantially to light attenuation in highly turbid environments (Moran and Zepp, 1997; Oliver et al., 2010). The Namoi River during our study period had high turbidity often above 1000 NTU.

Another factor that can influence bacterial utilisation of DOC is temperature, with declining temperatures at night potentially constraining heterotrophic uptake / activity (Kaplan and Bott, 1989). Our two sampling occasions took place in austral summer and the average (2001 - 2009) minimum and maximum air-temperature range was 30.4 – 36.3°C for these months (Australian Bureau of Meteorology, 2009). The diel variability in surface water temperature at Bugilbone in 2008, although significant ($P<0.05$), was considered minimal ranging from 22.2°C: (± 0.3) - 22.05°C: (± 0.25). The temperature range presented does not appear to lie out of the suitable growth range of natural bacterial populations in south-eastern Australia (e.g., Boon, 1991).

4.4.2. Day-time DOC maxima and minima

During day-time readings, consistent morning minima and afternoon maxima concentrations of DOC were detected across both sampling periods suggesting the rate of release / production due to photosynthesis by autotrophs producing DOC is substantially increased, likely in the form of carbohydrates (Sieracki and Sieburth, 1986; Rutherford and Hynes, 1987; Kaplan and Bott, 1989; Rier and Stevenson, 2002). Measurements of carbohydrates in both marine and freshwater planktonic habitats have revealed distinct pre-dawn minima and mid to late afternoon maxima (Sieracki and Sieburth, 1986; Kaplan and Bott, 1989). There is also general agreement that diel patterns of carbohydrate concentrations in these planktonic systems are biologically driven and light-dependent, showing strong correlations with primary production (Sieracki and Sieburth, 1986; Kaplan and Bott, 1989; McKnight ***et***

al., 2003); and concomitantly bacterial uptake (Bertilsson and Jones, 2003). This is likely to be the case during periods of low flow in the lower Namoi River. However during high flow events allochthonous material dominates due to increased wetting of terrestrial organic matter on lateral landscapes (Westhorpe ***et al.,*** 2010) with most of this DOM derived from soils with origins from vascular plants (Benner, 2003). The light climate is also greatly reduced due to increased turbidity (potentially derived in part from an increase in humic acids) and this would be expected to reduce phytoplankton biomass (Oliver ***et al.,*** 2010). Despite this, a pattern of increased DOC in the late afternoon was apparent. This has been observed in other rivers (e.g., Kaplan and Bott, 1989; Parker ***et al.,*** 2010), with in-stream sources (e.g., benthic algae and phytoplankton) giving rise to significant diurnal fluctuations in DOC and POC concentrations (Hope ***et al***., 1994). Both Kieber et al. (2006) and Riggsbee et al. (2008) in estuarine and freshwater systems respectively, have shown that solar mediated reactions in systems with re-suspended sediments can release substantial amounts of sorbed DOC back into the water column. Therefore, autochthonous (in-channel) re-suspended sources may provide significant amounts of organic material to the river during high flow events (Riggsbee ***et al.***, 2008).

As bacterial samples were not collected during the course of this study there is no way of predicting the behaviour of the heterotrophic bacterial population. However, manipulation trials on hyporheic bacterial communities with natural stream water DOM revealed significant changes in metabolic activity, specifically bacterial growth and dissolved oxygen (Findlay ***et al.***, 2003). A manipulation study by Westhorpe ***et al.***, (2010) in the lower Namoi River showed a significant response by heterotrophic bacterioplankton (within 1 – 2 days) to additions of DOC (within the temporal range of DOC detected in the river). Responses included a drop in dissolved oxygen concentration (primarily due to an increase in bacterial respiration), a decrease in phytoplankton productivity (due to bacteria outcompeting phytoplankton for essential inorganic nutrients) and an increase in bacterial growth. These findings suggest that a variation in DOM supply can alter compositional shifts in plankton communities within relatively short time periods (Findlay ***et al.***, 2003).

Sieracki and Sieburth (1986) suggest that in marine environments a build-up of DOC (in the form of polysaccharides) during the afternoon was concurrent with a daytime decrease in bacterial abundance. A possible explanation was sunlight-induced inhibition of bacteria (Sieracki and Sieburth, 1986) as aquatic bacteria exposed to light often have decreased

metabolic activity (Lindell ***et al.,*** 1996). This appears to be one of the controlling factors in diel cycling of OM which allows DOM to build up during the light period by temporally decoupling the link between photosynthesis production and bacterial utilization (Sieracki and Sieburth, 1986). The impact of sunlight may be diminished in the lower-Namoi (during flow events) due to high turbidity levels and mixing of the water column.

4.4.3. Night-time DOC maxima and minima

The unpredictable behaviour in night-time DOC minima and maxima concentrations may be due to small scale changes in microbial habitat altering rates of respiration, or potentially the decoupling of bacterial activity due to prior sunlight exposure, as DOC concentrations do not appear to follow any clear temporal patterns of utilisation. Sieracki and Sieburth (1986) ran bioassays to compare the effects of sunlight on the growth of bacteria. The filtered-light bottles (sunlight exposed) had a significant delay in exponential growth of bacteria compared to the filtered-dark (control) bottles. The lag period in the light bottles being consistently longer than the dark bottles, with means of 26-h and 19-h, respectively. It is therefore possible that this lag effect may result in inconsistent DOC utilisation as 'surface' bacterial communities that had been previously exposed to solar radiation are mixed and diluted with bacterial communities that may have been in deeper water or shaded habitats throughout the day.

4.4.4. Improving our ability to assess diel variation

Although bulk DOC readings were able to pick up significant diel differences in our study, more sensitive measures of DOC may be useful to detect subtle changes. Techniques such as the deployment of ***in situ*** optical instrumentation designed to reveal changes in DOM concentration that are not always evident from bulk DOC measurement have been successful (Spencer ***et al.,*** 2007). The changes in the isotopic composition of amino acids from DOM fractions may also provide greater resolution (Ziegler and Fogel, 2003). We concur with Parker ***et al.*** (2010) with regard to the importance of better understanding the dynamic nature of diel changes in DOC concentration in rivers, as it is evident that patterns in DOC concentration exist at a range of temporal scales. Therefore a sampling regime such as employed in our study, or more frequent samples (e.g., Dawson ***et al.*** 2001) will help develop a better understanding of DOC variability within lotic systems over a range of flow conditions. An understanding of the specific drivers of DOC production, solubility and transport within a catchment also needs to be considered. Clark ***et al.*** (2010) suggest that

when examining smaller temporal scales, such as days, the key drivers of land management (e.g., drainage & soil type) and climate (e.g., precipitation) play a major role in determining DOC dynamics within stream waters. DOC fluxes have been shown to increase with rainfall and runoff (Clark et al., 2010). Del Giorgio and Davis (2003) offer a number of examples where seasonal floods have mobilised large quantities of labile DOC to streams that had accumulated in the upper soil horizons and groundwater. Floodplain inundation during the Walgett flood and the wetting of soil substrate and associated debris resulted in large volumes of carbon being processed and mobilised. However, we are unsure as to which factors are contributing to the diel DOC variability.

This chapter shows temporal variations in the concentration of DOC from two flow events in a semi-arid lowland river in New South Wales, Australia. There is evidence of distinct diel DOC variation in the lower Namoi River during periods when flows inundate adjacent river banks, benches and surrounding floodplain landscapes, with increased DOC concentrations at night compared with concentrations during daylight hours. This was more apparent during the higher flow event at Walgett where DOC concentrations were increased compared with Bugilbone flows. However, the ANOVA results indicated that DOC concentrations responded in the same way on both occasions, increasing at night. This pattern is unusual as it differs from the typically reported autochthonously driven afternoon maxima found in many rivers.

Chapter 5. Limitation of lowland riverine bacterioplankton by dissolved organic carbon and inorganic nutrients

5.1. Introduction

Dissolved organic carbon (DOC) in aquatic systems derives from two distinct sources; autochthonous primary production within the system, or allochthonous terrestrial organic matter washed into the system from the water-shed (Cole ***et al.*** 2002). Both sources of DOC may influence the heterotrophic plankton community (Meyer & Tate, 1983; Aitkenhead-Peterson ***et al.*** 2003). Although a considerable fraction of allochthonous DOC present in rivers is refractory (Kaplan & Newbold, 1993) the smaller fractions of labile carbon are rapidly utilised in heterotrophic energy pathways (Wilcox ***et al.*** 2005). Therefore when allochthonous labile DOC is available, bacterioplankton production can be many times higher than production from, and coupled to, autochthonous DOC release (Jansson ***et al.*** 2000). In shallow lakes, the bottom of the food chain can be altered by DOC availability, and bacterioplankton biomass has been shown to increase at the expense of the phytoplankton (Blomqvist ***et al.*** 2001). Heterotrophic bacterioplankton production can out-compete and be dominant over phytoplankton production when DOC supply is not limiting, due to greater surface area to volume ratios (Drakare ***et al.*** 2002).

Concentrations of DOC can increase in rivers during high flow events, often by several orders of magnitude (Hinton ***et al.*** 1997; Aitkenhead-Peterson ***et al.*** 2003). The periodicity and magnitude of flooding is a major regulator of the amount of DOC (Robertson ***et al.*** 1999) and nutrients (Westhorpe ***et al.*** 2008) delivered to a river. River regulation decreases both flood frequency and intensity (Richter ***et al.*** 2003; Page ***et al.*** 2005). The relative proportions of allochthonous and autochthonous sources of carbon vary between water bodies and systems (Cole ***et al.*** 2002) and these have likely changed within river systems because of flow regulation, with the amount of allochthonous carbon reduced (Vink ***et al.*** 2005; Oliver & Merrick, 2006). This may lead to a shift of carbon pools dominated by in-channel algal production (Robertson ***et al.*** 1999; Barmuta, 2003).

Bacterioplankton growth is also dependent upon the availability of inorganic nutrients, with changing nutrient concentrations likely to have both direct and indirect effects on bacterioplankton growth (Pinhassi ***et al.*** 2006). The importance of the inorganic nutrients phosphorus (P) and nitrogen (N) in freshwater aquatic ecosystems is well documented (e.g., Rier & Stevenson, 2002; Baldwin ***et al.*** 2010), particularly as a key factor in controlling phytoplankton growth, with P often regarded as the most common limiting nutrient (Kobayashi & Church, 2003). However, when P and N are abundant in aquatic systems heterotrophic bacterial growth is often increased by the addition of DOC (Buffam ***et al.*** 2001), often out competing phytoplankton (Findlay, 2003).

The Namoi River, New South Wales (NSW), Australia, is highly regulated with river water used heavily for irrigation. Flows are greatly modified through the presence of two headwater dams and four main channel weirs. A consequence of regulation and diversion is a decline in total river flows, particularly in the mid to lower parts of the system. The frequency of flooding has also declined. The seasonal flow patterns have also been altered, with a decrease in winter-spring discharge and an increase in summer-autumn discharge (Chessman, 2003; Page ***et al.*** 2005). These conditions are likely to have reduced loads of allochthonous DOC entering the river (Burford ***et al.*** 2008), potentially switching the river to an autotrophically dominant system. Environmental flows aiming to restore a proportion of the natural flows to the Namoi River have been allocated (Chessman, 2003) and some of these are likely to increase DOC concentrations in the river through greater wetting of benches, flood runners and the floodplain (Westhorpe ***et al.*** 2008). Malanson (1993) and more recently, Kobayashi ***et al***. (2011) suggest that the floodplain is often the primary area of carbon storage and primary source of carbon input to lotic ecosystems.

The changes in aquatic ecosystem function when allochthonous sources of DOC enter a lowland river are not well-understood (Rees ***et al.*** 2005). Metabolism in many aquatic environments is significantly subsidised by terrestrial sources of carbon, yet few studies have examined the fate of allochthonous carbon (Kritzberg ***et al.*** 2006). In a study in North

Carolina, USA, researchers added carbon (as dextrose) to a heterotrophic headwater stream to examine the responses of food web processes to increased labile carbon. They found significant microbial and invertebrate responses (abundance and biomass) implying that added labile carbon can stimulate food web processes even when a system is abundant with organic matter (Wilcox ***et al.*** 2005). Studies in inland rivers are limited and to our knowledge the impact of large reductions in flow due to river regulation on food web processes have not been tested. In Australia, a study by Vink ***et al.*** (2005) quantifying ecosystem metabolism in the middle reaches of the Murrumbidgee River showed that phytoplankton production dominated ecosystem production despite potentially being phosphate limited. However, the authors speculated that the high contribution of phytoplankton in the Murrumbidgee system could be a consequence of flow regulation and resultant loss of riverine connectivity with adjacent floodplains (Vink ***et al.*** 2005). Floodplain riparian areas in lowland rivers provide significant allochthonous energy sources such as leaf litter, with the quality and quantity of leaf litter input (e.g., DOC) forming the first step in many of the river's trophic relationships (Schulze 1995). The hypothesis that we tested in the lower Namoi River was that lowland riverine bacterioplankton are DOC limited when low to medium flow events are greatly reduced or removed, as predicted by Vink ***et al.*** (2005), and that simulating an increase in assimilable DOC similar to that expected during an environmental flow event will lead to heterotrophic dominance. We used microcosms (bioassays) to test this hypothesis by examining both DOC and inorganic nutrient limitation of the bacterioplankton and phytoplankton communities in the lower Namoi River. Responses to additions of DOC added as either glucose or leaf leachate of two common riparian tree species, with and without nitrogen and phosphorus; and nitrogen and phosphorus alone, were tested.

5.2. Materials and Methods

5.2.1. Study area and study site

The Namoi catchment is located in central north NSW, a semi-arid region covering an approximate area of 43 000 km^2 and forms part of the Murray-Darling Basin Drainage

System. Flows are regulated by Keepit Dam (storage capacity of 425 000 ML) on the Namoi River and from Chaffey Dam (capacity 62 000 ML) on the Peel River. A single study site was selected at Boggabri along the Namoi River within the mid to lower landform zones of the Namoi Catchment (Figure 9) with an elevation of ~250 m and average annual rainfall of 500-600 mm (DLWC, 2000). The dominant riparian tree species in the lower catchment were river red gum (***Eucalyptus camaldulensis***) and weeping willow (***Salix babylonica***). River sediments were comprised of a mixture of approximately 60% sand, 30% clay and 10% silt. Turbidity ranges between 5-50 nephelometric turbidity units (NTU) and large runoff events can increase turbidity levels one hundred fold. Phosphorus concentrations often exceed the acceptable water quality limits (50µg L^{-1}: ANZECC 2000) for aquatic ecosystems and algal blooms are a major problem in the water storages of the Namoi catchment (DLWC, 2000). The Namoi River feeds into the Barwon-Darling River, the only major river system of this large, semi-arid region. Hydrographic data for the Namoi River at Boggabri were obtained from a gauging station operated by the New South Wales Office of Water (NOW). Stage was recorded every 15 minutes and converted to mean daily flow (ML).

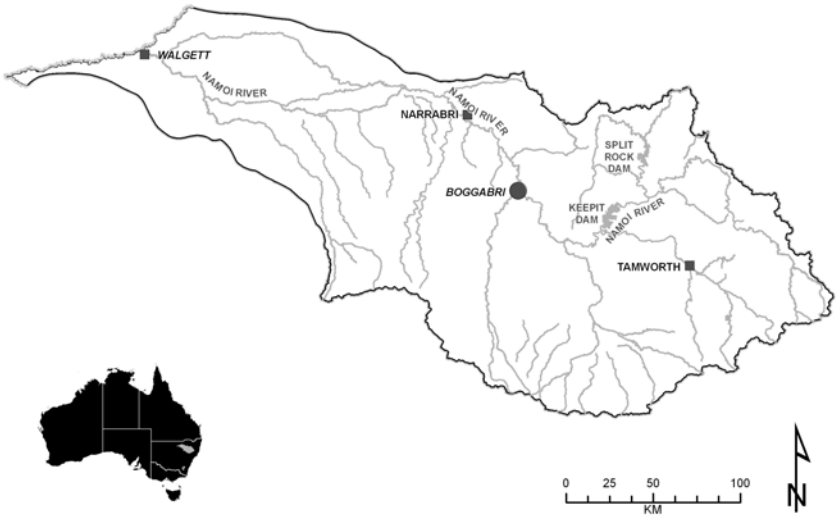

Figure 9: Namoi River catchment and location (●) of the study site at Boggabri, and major towns (■).

5.2.2. Experimental design and set-up

Three seasonal (spring and summer 2005 and autumn 2006) ***in situ*** microcosm experiments were undertaken. Each microcosm experiment was performed using 1.25 L transparent polyethylene terephthalate (PET) bottles and 15 L transparent PET carbuoys to determine the effect of microbial community responses to additions of DOC (both container types), and the inorganic nutrients N and P with and without DOC to the bottles only. The use of bioassays can provide direct evidence of nutrient limitation in natural plankton communities in which the planktonic growth response is measured by the addition of one or more nutrients (Kobayashi & Church, 2003). Another advantage of ***in situ*** microcosms (bioassays) in determining DOC limitation as opposed to open water experiments, is that it is a relatively simple protocol that is easily repeatable and allows a direct comparison between treatment types and are deemed to give a reasonable estimate of potential water-column activity (Buffam ***et al.*** 2001). The larger volume (15 L) carbuoy experiments were included as the labile DOC fraction is less likely to be quickly depleted and therefore more resembles in situ experiments (del Giorgio and Davis, 2003). The slightly longer period (additional 24-48 hr) in the carbuoy experiments was also required to allow phytoplankton responses to be measured (Vrede 1996) which may not have been achieved over the shorter periods of the bottle experiments.

Water was collected from midstream at a depth of approximately 0.25 m, filtered using a 63-µm mesh size nylon membrane plankton net to exclude zooplankton and then decanted into either the bottles or carbuoys. It is recognised that smaller organisms, in particular protists can also be important consumers of bacteria in lotic systems; however, abundances of phagotrophic protists are often greater in sediments attached to substrate and in organically enriched sites than in the water column (Ward & Johnson, 1996) and were therefore deemed not to be dominant consumers of bacterioplankton during the experiments. Bottles and carbuoys were randomly placed along anchored lines and submerged, just beneath the water surface with each container type at the same depth. Due to the different size of the two container types, the mean photosynthetically active radiation received by the carbuoys

was less than that for the bottles. The bottles were placed at approximately 50-75%, and carbuoys 10-30% surface irradiance, depending on the turbidity.

5.2.3. Treatment amendments

A 20 g L^{-1} solution of carbon as glucose was prepared with distilled water using Sigma® chemicals. Two other DOC stock solutions of leachate were prepared by soaking approximately 500 g of either fresh red gum or weeping willow leaves in approximately 1 L of distilled water for 72 hr at less than 5°C in a dark environment, thus minimising microbial utilisation of easily assimilable DOC (Ward & Johnson, 1996). Leachate solutions were then filtered using a 0.2 µm pore size (polycarbonate membrane) filter to remove bacteria and particles (Ward & Johnson, 1996). The DOC concentration of these solutions was determined (see procedure in section 5.2.4) to allow appropriate dilution for experiments.

Treatment amendments are detailed in Table 5 and were all performed in triplicate. DOC was added as glucose, red-gum leachate and willow leachate. Amended DOC concentrations varied arbitrarily across seasons, ranging from 1.0 – 19.5 mg L^{-1} above ambient concentrations (Table 5) falling within the range of DOC concentrations reported during flow events within the mid-lower Namoi River (Westhorpe ***et al.*** 2008). Glucose and other sugars have been used as a source of DOC in similar experiments (Bell ***et al.*** 1993; Blomqvist ***et al.*** 2001; Farjalla ***et al.*** 2002; Wilcox 2005), with glucose generally assimilated more rapidly by heterotrophic bacterioplankton than other sugars with a turnover rate fast enough to support a large proportion of bacterial growth (Kirchman, 2003). The inclusion of glucose in these experiments represented a ‘pseudo-control’ as the literature previously cited states that this form of carbon is rapidly assimilated, thus allowing us to compare the potential uptake of the two leaf leachate treatments with that of the glucose over the incubation periods chosen. The use of a natural leachate as a source of DOC was deemed important as both leachates were derived from the dominant riparian tree species within the lower Namoi River, and potentially provides a better estimate of microbial response to dissolved organic matter than would glucose (Ward & Johnson, 1996). Nitrogen was added

as KNO_3 (approximately 0.5 mg L^{-1}) and phosphorus as KH_2PO_4 (approximately 0.3 mg L^{-1}) to the bottle experiments only. The experiments lasted for 3 to 4 days (bottle experiments) and 4 to 6 days (carbuoy experiments).

Table 5: Ambient and amended DOC and inorganic nutrient concentrations for the bottle and carbuoy experiments.

Season	DOC (mg L^{-1})				Filterable oxidised nitrogen (mg L^{-1})		Filterable reactive phosphorus (mg L^{-1})	
	Ambient	Amended as Glucose	Amended as red-gum leachate)	Amended as willow leachate)	Ambient	Amended	Ambient	Amended
				Bottles				
Spring	4.5±0.18	10.1±0.87	16.7±0.33	10.7±0.33	<0.0025	0.59±0.01	<0.0025	0.33±0.03
Summer	6.2±0.2	23.0±0.11	17.0±0.20	14.7±0.3	<0.0025	0.52±0.01	0.028±0.002	0.50±0.01
Autumn	6.7±0.1	12.0±0.1	nt	nt	<0.0025	0.37±0.01	<0.0025	0.012±0.001
				Carbuoys				
Spring	4.5±0.18	5.5±0.42	10.7±0.33	8.9±0.09	<0.0025	nt	<0.0025	nt
Summer	6.2±0.2	25.7±1.4	11.8±1.69	11.3±1.2	<0.0025	nt	0.028±0.002	nt
Autumn	6.7±0.07	nt	9.9±1.12	10.3±0.7	<0.0025	nt	<0.0025	nt

Amended is the final concentration after additions. Mean ± standard error (***n*** = 3). ***nt*** Not tested.

5.2.4. Sampling procedures

Samples for DOC, nutrients and chlorophyll ***a*** were taken at the start of the experiment from additional bottles to determine the amended concentrations. For the bottle experiments, initially and on days two and four, dissolved oxygen was first measured and then after homogenisation by rotation, samples for bacteria (5 ml) were taken over the course of the experiments. Chlorophyll a (200 ml) and nutrient (20 ml) samples were taken from the final populations. For the carbuoys, initially and on days two and five (spring experiment) and days two, four and six (summer and autumn experiments), dissolved oxygen was first measured (before mixing). After homogenisation by rotation, samples for bacteria, chlorophyll ***a*** and nutrients were taken over the course of the experiments with the same volumes as stated above.

Dissolved Oxygen (DO) was measured ***in-situ*** using a calibrated thin-probed WTW dissolved oxygen meter before homogenisation by placing the probe through the mouth of the bottle or carbuoy. DOC samples were pre-filtered in the field (0.45 µm pore size), acidified with hydrochloric acid and refrigerated at 4°C. Samples were analysed in the laboratory by the High Temperature Combustion Method (APHA, 1998). Nutrient samples were taken after filtration through 0.45 µm pore size syringe filters in pre-washed and sample rinsed PET bottles. Samples were analysed for filterable reactive phosphorus (FRP) and oxidised nitrogen (NO_x-N). The FRP sub-samples were analysed using the ascorbic acid method and the NO_x-N sub-samples were analysed using an automated cadmium reduction method (APHA, 1998). Samples for chlorophyll ***a*** determination were filtered onto GF/C filters then frozen until determination by Standard Methods (APHA, 1998). Water temperature was measured daily ***in situ*** using a calibrated WTW temperature/dissolved oxygen meter. Conductivity and pH were recorded using a TPS Pty Ltd digital conductivity meter and pH meter, respectively.

5.2.5. Determination of bacterioplankton response

Bacterioplankton samples were collected in clean and sterile centrifuge tubes and fixed with 0.2 ml of concentrated 0.2 µm pore size filtered formalin. In the laboratory, 2 ml of each sample was stained with DAPI (4'6-diamindion-2-phenylindole) for 15 minutes, and filtered through a polycarbonate black 0.2 µm pore-sized filter (Porter and Feig 1980). Polycarbonate filters were mounted onto microscope slides and non-fluorescence immersion oil used. Slides were examined at 1000× using a fluorescence-equipped Leitz Diaplan compound microscope. For each slide three to four pictures of random views were captured using Leica IM Version 4.0 software. Pictures were then analysed using Image-Pro Plus imagery software and the surface area of bacteria calculated per image. This method was used as large differences were found between the size of individual cells and this facilitated faster sample analysis (cf. Sieracki ***et al.*** 1985). For each replicate, a mean bacterial surface area per view was calculated and represented as relative surface area (RSA).

5.2.6. Data analyses

Data were strongly skewed despite repeated transformations, so analyses were conducted with the non-parametric PERMANOVA (permutational multivariate analysis of variance) routines developed by Anderson (2001a, 2001b) and Anderson and ter Braak (2003). Analyses were undertaken using the software package PERMANOVA+ for PRIMER (Anderson ***et al.*** 2008).

The PERMANOVA routine creates a non-parametric, permutational analogue of ANOVA when applied to univariate data, generating a permuted test statistic called a pseudo f. A permutation procedure is used to obtain an appropriate distribution for the pseudo f statistic. Manly (2006) and Anderson (2008) recommend at least 999 permutations be performed in order to draw precise inferences from tests at a significance level of 0.05. The number of samples determines the number of available permutations. There were some instances where post-hoc tests in particular were well below 999 permutations. Rather than reject significant tests with low permutations outright, each one was assessed on a case-by-case basis.

Post-hoc pairwise comparisons made with t-tests utilise a permuted distribution (Anderson 2001b). Repeated measures PERMANOVA models were employed with sampling date as the repeated factor, season and treatment as fixed factors (see Anderson ***et al.*** 2008 for full methodology). Bottle and carbuoy experimental data were analysed separately. The assumption of exchangeability of repeated measures samples was tested with the PERMDISP routine (Anderson ***et al.*** 2008) which provides a non-parametric equivalent to Levene's (1960) test for homogeneity of variances, comparing the dispersal of measurements in each level of the repeated measure. In some cases, the data (i.e., bacterial counts) were graphed and analysed after log transformation, not to normalise data, but to reduce the influence of outliers on analyses. All means and standard deviations discussed hereafter refer to untransformed values.

5.3. Results

5.3.1. Flow and temperature

The study period (October 2005 to March 2006) and preceding six months were characterised by low flows (median of 231 ML d^{-1}) (Figure 10). The summer (December 2005) and autumn (March 2006) experiments were performed after small rises in flow and increased DOC concentration in the river. These small rises in flow are well below those required to significantly wet organic matter in terrestrial habitats, with the ambient mean DOC concentrations (Table 5) similar to longer-term low flow mean DOC concentrations of 6.8 ± 0.4 mg L^{-1} (n=71) from 2003 to 2005. Mean daily water temperatures for the spring, summer and autumn experiments were 20.6, 25.3 and 22.9 ºC, respectively.

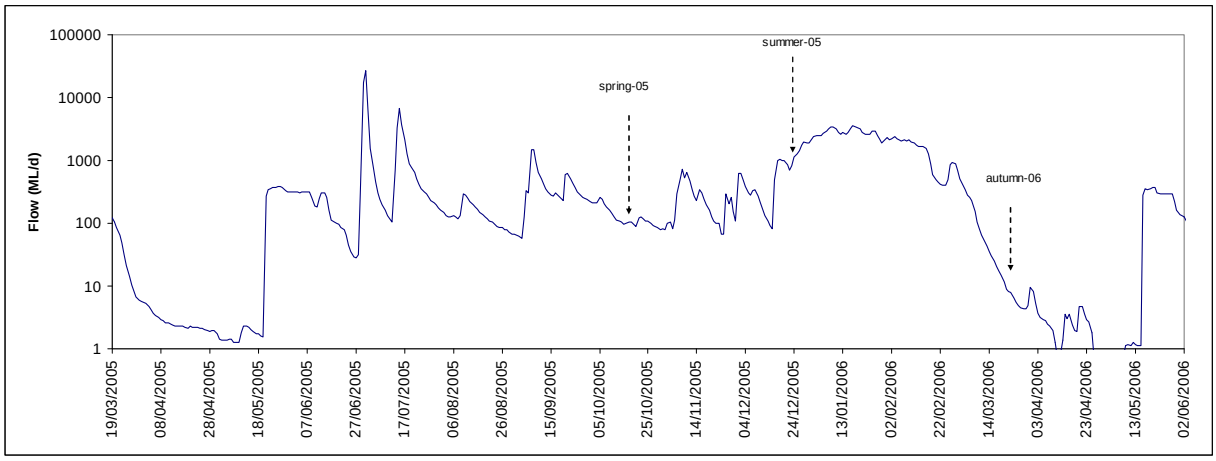

Figure 10: Mean monthly flow prior to and during the experiments at the gauging station Namoi River at Boggabri from January 2005 to June 2006.

5.3.2. Homogeneity of dispersions

Prior to running PERMANOVA, the dissolved oxygen (DO) and bacteria (within a season) of treatment types were tested for homogeneity of dispersions using PERMDISP. There were no significant differences between sample dispersals in all cases except for the Autumn DO which differed significantly in both bottle and carbuoy trials (pseudo f = 6.735, ***p***= 0.011; pseudo f = 7.889, ***P***= 0.003) respectively. The chlorophyll ***a*** samples were not tested due to the lack of temporal replication.

5.3.3. Dissolved oxygen responses

The PERMANOVA's for the average DO concentration differed significantly for both the carbuoy (***P***= 0.034; 0.001; & 0.001) and bottle (***P***= 0.013; 0.001; & 0.006) experiments between treatments for all three seasonal experiments (spring, summer & autumn) respectively (Tables 6 & 8). Significant differences existed for DO concentrations between days (***P***=0.008) during summer for the carbuoy experiments, also for the day*treatment interaction (***P***=0.03) during autumn only (Table 8). For the bottle experiments, significant differences only existed (***P***= 0.003; & 0.022) between the day*treatment interaction for summer and autumn respectively (Table 6).

Pairwise tests for the carbuoy experiments revealed that the DOC treatments were significantly lower compared to that of the control (***P***=0.024; 0.001; & 0.001) for spring, summer and autumn respectively (Table 8). On the final day of each experiment all DOC treatments had reduced DO concentrations compared with that of the control (Figures 12a-c), supporting the patterns found in the bottle experiments.

Table 6: PERMANOVA Results, 1.25-L bottle experiments.

Season	Between treatments				Between days				Treatment / day interaction			
	Pseudo f	df	***p***	perms	Pseudo f	df	***p***	perms	Pseudo f	df	***p***	Perms
Dissolved oxygen												
Spring 2005	5.263	2	**0.013**	999	0.417	1	0.523	999	0.8	2	0.464	999
Summer 2005	32.866	3	**0.001**	999	0.592	1	0.459	999	6.1	3	**0.003**	999
Autumn 2006	18.096	3	**0.006**	999	0.229	1	0.651	999	4.159	3	**0.022**	999
Bacteria												
Spring 2005	6.058	2	**0.001**	999	1.985	1	0.171	999	2.346	2	0.112	999
Summer 2005	14.317	3	**0.001**	997	8.254	1	**0.011**	**997**	0.211	3	0.889	999
Autumn 2006	47.965	3	**0.001**	999	0.385	1	0.545	998	0.863	3	0.493	999
Chlorophyll ***a***												
Spring 2005	13.595	2	**0.004**	999	nt	nt	nt	nt	nt	nt	nt	nt
Summer 2005	17.079	3	**0.001**	999	nt	nt	nt	nt	nt	nt	nt	nt
Autumn 2006	18.096	3	**0.006**	999	nt	nt	nt	nt	nt	nt	nt	nt

P values in bold font are significant. ***nt-***Not tested

For the bottle experiments, the addition of DOC alone in the spring and summer (as glucose solution, red gum or willow leachate) resulted in a marked reduction in DO concentrations over time with all treatments being significantly different (***P***<0.05) to the control by day 4 (Figures 11a, b; Table 7). The addition of DOC also led to a reduction in DO concentrations in treatments from the autumn experiment, although not significantly (Figure 11c; Table 7). The greatest drop in DO concentration occurred during summer in both the carbuoy and bottle experiments, which had the highest mean water temperature (25.3ºC). The addition of nutrients alone in the bottle experiments was always significantly different to that of the control and other treatments, including the DOC+nutrients treatment, with increased DO concentrations during the summer and autumn experiments (Table 7; Figures. 11b, c) due to increased phytoplankton production. The DOC + nutrients treatment in summer and autumn was also significantly different to that of the control but not to DOC alone, with both treatments having reduced DO concentrations (Table 7; Figures 11b, c). This was not the case during the spring experiment with no significant difference between the control and DOC+nutrients treatment (Table 7; Figure 11a).

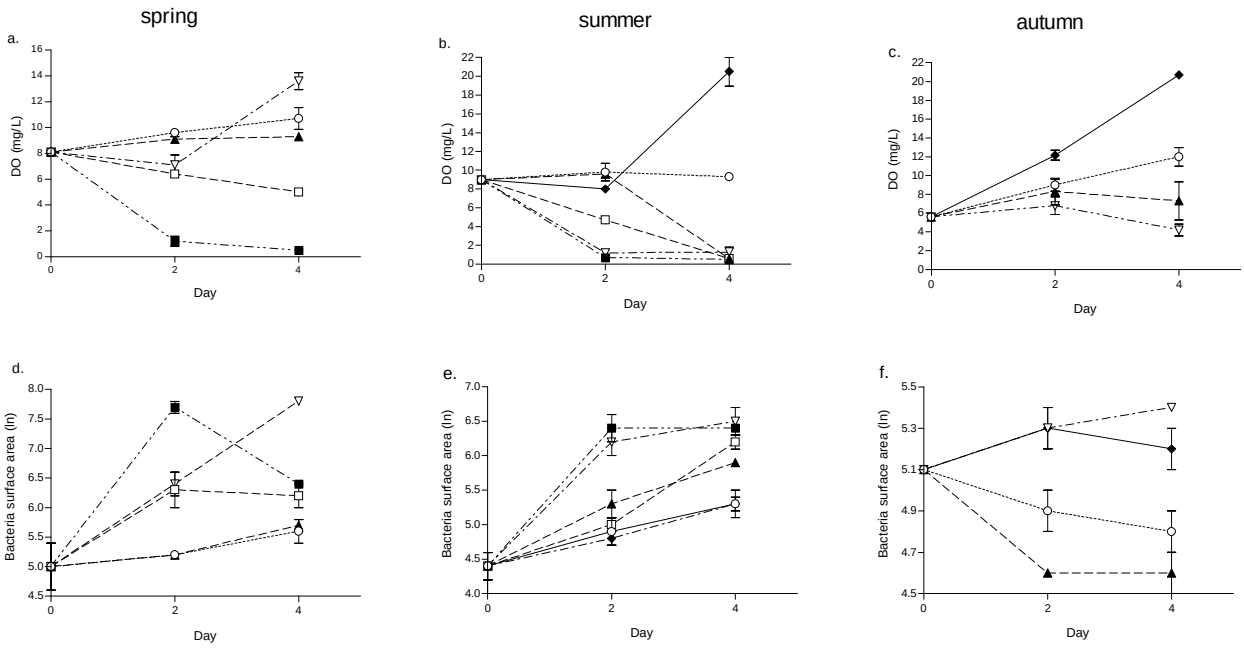

Figure 11: Mean seasonal concentrations (n=3; ± s.e.) of: dissolved oxygen (a, b, c); ln transformed bacterial surface area (d, e, f) from 1.25 L bottle experiments. Seasons from left – right: spring 05, summer 05 and autumn 06. Diagrams: a – f: control. O; glucose. ▲; glucose + nutrients. ▽; nutrients. ♦; willow leachate. ■; red-gum leachate. □. Diagram 'd' no day 0 data available, values based on the combined mean summer and autumn bacteria data (n=6).

Table 7: Pairwise comparison results, 1.25-L bottle experiments.

	Control vs. DOC			Control vs. DOC+nutrients			Control vs. nutrients			DOC vs. DOC+nutrients			DOC+nutrients vs. nutrients			DOC vs. nutrients		
	t	p	perms	t	p	perms	t	p	perms	t	p	perms	t	p	perms	t	p	perms
Dissolved oxygen																		
Spring 2005	2.395	**0.02**	996	0.451	0.651	981	nt	nt	nt	1.883	0.065	997	nt	nt	nt	nt	nt	nt
Summer 2005	5.573	**0.001**	991	12.134	**0.003**	987	2.742	**0.047**	983	0.947	0.35	997	10.619	**0.002**	980	6.979	**0.001**	996
Autumn 2006	1.744	0.124	981	5.762	**0.001**	979	7.23	**0.003**	977	1.499	0.186	985	4.412	**0.001**	971	10.745	**0.001**	966
Bacteria																		
Spring 2005	2.00	**0.057**	997	9.002	**0.001**	966	nt	nt	nt	1.883	0.065	997	nt	nt	nt	nt	nt	nt
Summer 2005	3.4464	**0.002**	995	7.725	**0.002**	988	0.4002	0.682	975	1.225	0.242	998	3.509	**0.013**	981	3.201	**0.005**	997
Autumn 2006	3.051	**0.019**	985	6.4516	**0.003**	982	6.2808	**0.002**	990	9.8146	**0.002**	986	1.5601	0.173	992	10.882	**0.001**	987
Chlorophyll ***a***																		
Spring	***0.588***	***0.54***	***173***	***4.55***	***0.088***	***10***	nt	nt	nt	***5.179***	***0.002***	***218***	nt	nt	nt	nt	nt	nt

2005																	
Summer																	
2005	***0.848***	***0.577***	***138***	***1.519***	***0.207***	***10***	***4.545***	***0.11***	***10***	***2.2***	***0.07***	***156***	***12.41***	***0.09****	***10***	***8.09***	***0.009***	***174***
Autumn																	
2006	***2.371***	***0.194***	***10***	***0.284***	***0.911***	***10***	***3.249***	***0.102***	***7***	***5.581***	***0.103***	***10***	***12.441***	***0.099****	***7***	***12.278***	***0.112***	***7***

P values in ***bold font*** are significant. A value in ***italics*** represents a low number of permutations and considered unreliable. ***nt*** Not tested

Table 8: PERMANOVA & Pairwise results for 15-L carbuoy experiments.

Season	Between treatments				Between days				Treatment / day interaction				Pairwise tests		
	Pseudo f	df	***p***	perms	Pseudo f	df	***p***	perms	Pseudo f	df	***p***	perms	t	***p***	perms
Dissolved oxygen															
Spring 2005	5.936	1	**0.034**	999	0.197	1	0.664	999	0.829	1	0.376	999	2.436	**0.024**	997
Summer 2005	298.68	1	**0.001**	999	7.218	1	**0.008**	999	3.347	1	0.055	999	17.282	**0.001**	997
Autumn 2006	45.874	1	**0.001**	999	1.703	1	0.214	999	5.324	1	**0.03**	999	6.773	**0.001**	997
Bacteria															
Spring 2005	13.092	1	**0.001**	995	0.9352	1	0.344	997	0.078	1	0.774	999	3.621	**0.004**	999
Summer 2005	6.272	1	**0.035**	999	0.115	1	0.754	999	0.075	1	0.975	999	2.504	**0.017**	998
Autumn 2006	7.809	1	**0.015**	999	0.573	1	0.539	999	0.117	1	0.877	999	2.795	**0.009**	998
Chlorophyll ***a***															
Spring 2005	4.956	1	**0.039**	999	nt	1	nt	nt	nt	1	nt	nt	***2.226***	***0.051***	***95***
Summer 2005	3.618	1	**0.001**	999	nt	1	nt	nt	nt	1	nt	nt	***1.902***	***0.083***	***175***
Autumn 2006	7.696	1	**0.018**	999	nt	1	nt	nt	nt	1	nt	nt	***2.774***	***0.032***	***84***

P values in ***bold font*** are significant. A value in ***italics*** represents a low number of permutations and considered unreliable. Pairwise comparisons: control versus. combined DOC treatments. ***nt*** Not tested

5.3.4. Bacterioplankton responses

Similar to the DO results, bacterioplankton responses, measured as a change in relative surface area (RSA), differed significantly (***P*<**0.05) for both container types, between the treatments for all three seasons respectively and between days (***P***=0.011) for the summer only bottle experiment (Tables 8 & 6). The day*treatment interactions for all experiments across seasons were not significant (***P***>0.05; Tables 6 & 8).

Pairwise tests on data from the carbuoy experiments comparing changes in bacterial RSA showed that the DOC treatments were significantly different to that of the control (***p***=0.004; 0.017; & 0.009) for spring, summer and autumn respectively (Table 8). On the final day of the experiment, all DOC treatments showed distinct increases in bacterial RSA compared with the control (Figures 12d, e, f). Pairwise tests on data of the bottle experiments across the three seasons for the DOC and DOC + nutrients treatments were significantly greater than that of the control (Table 7, Figures 11d, e, f.) thus supporting the findings of the 15 L carbuoy experiments. In all cases, the combination treatment of DOC+nutrients resulted in the greatest measured increase in bacterial RSA (Figures 11. d, e, f).

5.3.5. Chlorophyll *a* responses

There was consistency between container type (1.25 L bottles & 15 L carbuoys) experiments at the between treatments level (measured as a change in chlorophyll ***a*** concentration), with significant differences (***p*<**0.05) for all three seasons respectively. Inspection of the means on the final day for the carbuoy experiments from any one season indicated that chlorophyll ***a*** production (photosynthesis) was limited with the addition of DOC. For example, in autumn the mean chlorophyll ***a*** concentrations for the control and combined DOC treatments were 4.97 ± 0.44 µg L^{-1} and 3.18 ± 0.31 µg L^{-1}, respectively (Figure 13f). Both experiments were similar in that there was insufficient sampling across time to assess effects between days and the day*treatment interaction. The low number of available permutations across all seasons greatly reduces the reliability of pairwise tests (Tables 7 & 8).

Mean chlorophyll ***a*** concentrations for the bottle experiment in summer indicated that the nutrients alone treatment was at least 5 times higher than that of other treatments with a mean of 123.33 ± 5.09 µg L^{-1}, compared to a mean of 26.0 ± 2.65 µg L^{-1}, 15.24 ± 3.97 µg L^{-1} and 14.23 ± 4.35 µg L^{-1} for the DOC+nutrients, DOC, and control respectively. In autumn the nutrients treatment had the highest mean chlorophyll ***a*** of 72.33 ± 2.33 µg L^{-1} compared to 19.0 ± 5.12 µg L^{-1}, 18.33 ± 2.03µg L^{-1} and 4.37 ± 1.22 µg L^{-1} for the control, DOC+nutrients and DOC, respectively. The addition of inorganic nutrients increased the average concentration of chlorophyll ***a*** substantially in all experiments (Figures 13a, b, c), suggesting inorganic nutrient limitation for the phytoplankton.

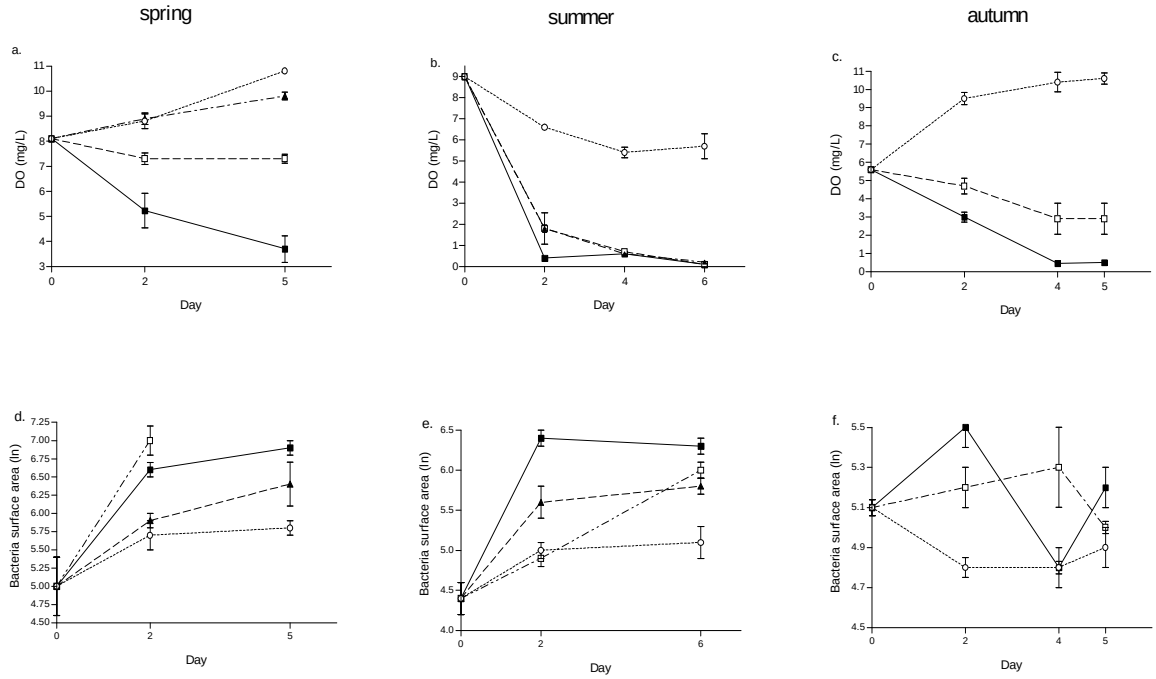

Figure 12: Mean seasonal concentrations (n=3; ± s.e.) of: dissolved oxygen (a, b, c); ln transformed bacterial surface area (d, e, f) from 15 L carbuoy experiments. Seasons from left – right: spring 05, summer 05 and autumn 06. Diagrams: a – f: control. O; glucose. ▲; willow leachate. ■; red-gum leachate. □.

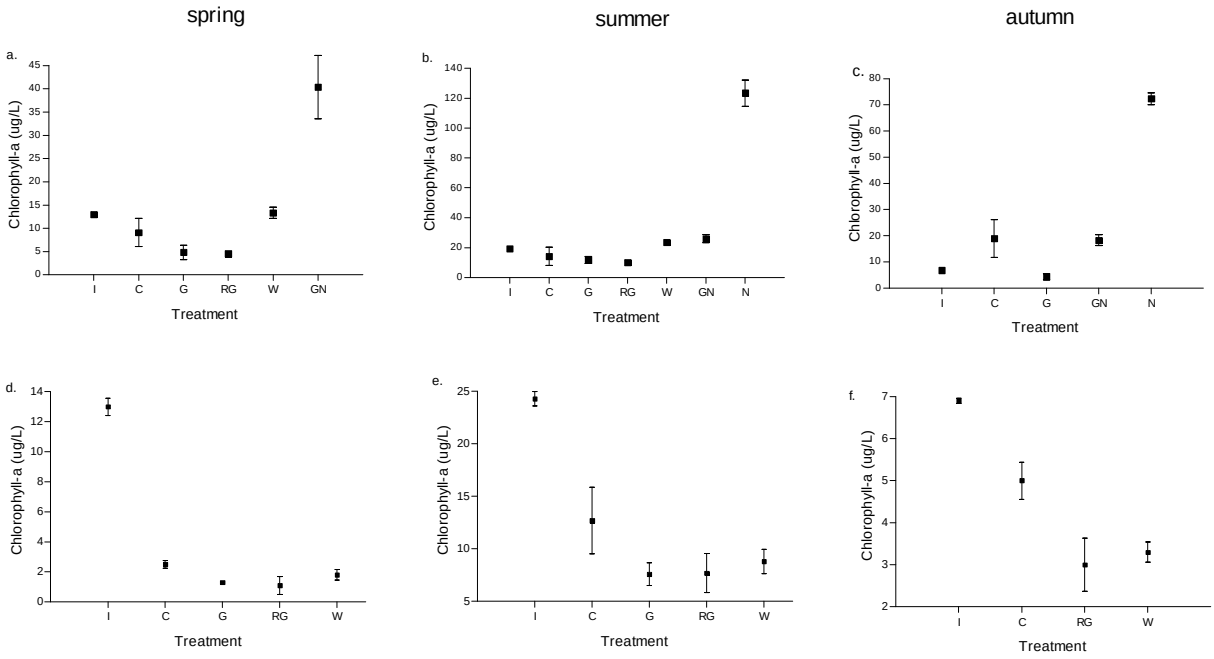

Figure 13: Mean seasonal concentrations (n=3; ± s.e.) of chlorophyll-a from 1.25 L bottle (a, b, c); 15 L carbuoy (d, e, f) experiments. Seasons from left – right: spring 05, summer 05 and autumn 06. I = initial, C = control, G = glucose; RG = red gum; W = willow; GN = gluc + nutrients, N = nutrients; Initial is at the start of the experiment and all other values relate to the final day for each experiment.

5.4. Discussion

5.4.1. Bacterioplankton responses to DOC and inorganic nutrients

Differences in the amended DOC concentrations between the three seasons examined make comparisons between seasons more difficult. However, it is evident that the results from both sizes of bioassay experiments showed that additions of assimilable DOC as glucose or leaf leachate (with and without nutrients) led to a significant increase in bacterial RSA above that of the control (Tables 7 & 8). A reduction in dissolved oxygen concentrations through heterotrophic respiration for all DOC treatment types over the three seasons was also evident (Figures 11 & 12), although not always significantly (Tables 7 & 8). del Giorgio & Davis (2003) looked at the reliability of bioassays in determining metabolic estimates of DOM consumption. The authors looked at a number of bioassays with incubation periods of <1.5 days. When these rates were compared with the range of total bacterial DOC consumption (sum of bacterial respiration (BR) & bacterial production (BP) based on 478 pairs of observations) the authors found that there was good agreement

(within a factor of 2) between the median initial rates of consumption in the bioassays and the median rates of consumption "***in situ***" calculated from BR & BP measurements. This suggests that in most cases short-term bioassay experiments may capture the essence of the bacteria-DOM interaction that occurs in natural waters (del Giorgio & Davis 2003). The graphical representation of our 1.25 L bottle experiments clearly supports this assumption (Figure 11). The results suggest that the ambient DOC levels in the Namoi River were limiting the heterotrophic community at the time of each experiment. A switch from autotrophic dominance to heterotrophic dominance occurred after amendment with DOC for the three seasons examined.

Our findings also suggest that the bacterial communities within the lower Namoi River can readily utilise various DOC sources. A study by Judd ***et al.*** (2004) determined that aquatic heterotrophic bacterial communities contain a nearly full complement of carbon processing functional groups, thus having the ability to utilise different carbon sources rapidly. Either movement of leaf types into the river is by direct fall, wind, storm events or in response to increased river discharge can result in uptake and assimilation by heterotrophic bacteria. In the Namoi River willows, being deciduous, have a more concentrated leaf fall in autumn whilst red gums have a more continuous pattern of leaf fall. Further to this, willow leaves breakdown more rapidly (half-life, t50=14-26 days) than do red gum leaves (t50=27-50 days). Red gum leaves may provide a constant low-level input of nutrients to the river (Schulze 1995). However, large increases in DOC to the river occur only after increased wetting of terrestrial areas due to increased discharge and/or rainfall (Westhorpe ***et al.*** 2008).

In other lotic systems, DOC limitation of the heterotrophic community has been reported for the Amazon River during periods of low flow (Benner, 1995; Anesio ***et al.*** 1997) and a freshwater tidal estuary (Findlay, 1992). In lentic systems, Bergström & Jansson (2000) and Drakare ***et al***. (2002) found that bacterioplankton production was regulated by DOC input to a lake from the catchment following high flow events. The high flow events apparently

stimulated bacterial production by supplying large amounts of relatively bioavailable DOC and the growth of bacterioplankton was independent of DOC produced by phytoplankton. Similarly, Lind & Barcena (2003) found bacterial biomass more than doubled 13 days after a flow pulse into a reservoir.

Although bacterioplankton often use autochthonously produced DOC preferentially over allochthonous sources (Kritzberg ***et al.*** 2006), the watershed can supply large amounts of allochthonous assimilable organic carbon, contributing to significant increases in bacterial production. In freshwater lakes, allochthonous carbon has been reported to support approximately 43 to 75% of bacterial growth (Kritzberg ***et al.*** 2006). When allochthonous DOC is available, bacterioplankton production can be many times higher than the production based on autochthonous DOC release alone (Jansson ***et al.*** 2000). Bacterial production in a lake in Sweden relied on the utilisation of allochthonous DOC sources and was independent of DOC generated by phytoplankton (Jansson ***et al.*** 1999). It is likely that allochthonous DOC availability through flows in the highly regulated lowland Namoi River increases bacterioplankton production compared to that available from the autotrophic pool.

Additions of DOC and inorganic nutrients together led to significant increases in bacterial abundance above that of DOC added alone across the bottle experiments (Table 7 & Figure 11). This may be due to inorganic nutrients becoming a limiting factor after DOC limitation was removed. In other river systems, when there are large inputs of land derived DOC during high flow periods, heterotrophic production has been limited by inorganic nutrients such as phosphorus (Farjalla ***et al.*** 2002). In lake experiments, Bell ***et al.*** (1993) found that bacterioplankton were only stimulated by DOC (as glucose) in combination with phosphorus and nitrogen. Elser ***et al.*** (1995) found that abundance was in some years limited by nitrogen and phosphorus alone, while Vrede (1996) found DOC limited growth. The most dramatic responses in bacterial respiration (measured as DO consumption) occurred in summer (Figures 11 & 12), when ambient water temperature was highest (25.3 ºC). A study in the Mediterranean Sea showed bacterial composition changed seasonally (Pinhassi ***et al.***

2006); whilst an earlier study by Cotner ***et al***. (2000) found bacterial production and biomass accumulation in response to nutrient additions was greatest during summer. Seasonal influences (e.g., ambient temperature) can therefore play an important role in determining the rate responses of heterotrophic bacterioplankton to the delivery of organic and inorganic nutrients into aquatic environments.

5.4.2. Phytoplankton responses to DOC and inorganic nutrients

Addition of glucose led to a decrease in chlorophyll ***a*** (a measure of phytoplankton abundance) compared to that of the control in the autumn bottle experiment (Figure 13c). However, this was not the case for all the bottle experiments with reduced time being the limiting factor in phytoplankton response (Vrede 1996). The three respective DOC treatments (glucose, red gum and willow) added to the carbuoys led to a significant decrease in chlorophyll ***a*** compared to that of the control (Figures 13d, e, f). This is likely due to planktonic bacteria being able to out-compete phytoplankton for available inorganic nutrients when DOC was added (and no longer limiting), due to the higher surface area to volume ratio of bacteria (Blomqvist ***et al.*** 2001; Drakare ***et al.*** 2002; Findlay, 2003), and increased experiment time no longer being a limiting factor (Vrede 1996). This supports the contention that when DOC is not limiting, such as during high flow events, planktonic heterotrophic processes may dominate. Drakare ***et al.*** (2002) found that high flows carrying DOC into a lake led to low primary production despite high solar insolation, high inorganic nutrient concentrations and season, being early summer. In their study, primary production never exceeded bacterial production for approximately 20 days after a flow episode had replenished DOC concentrations in the lake.

Additions of inorganic nutrients alone resulted in substantial increases in chlorophyll ***a*** in the bottles, indicating limitation of the phytoplankton population (Figures 13b, c), despite the absence of supporting statistical tests due to the reduced number of permutations (Table 7). Oviatt ***et al.*** (1986) similarly found that increased allochthonous inputs of inorganic nutrients without concurrent DOC tended to lead to autotrophic dominance. The chlorophyll ***a*** results

for the glucose + inorganic nutrient addition in the spring bottle experiment showed increased concentration compared to that of the control as well as increased bacterioplankton growth (Figures 13a & 3d). This suggests that both phytoplankton and bacteria were stimulated by the addition. A similar pattern was found in an estuary with the phytoplankton response to inorganic nutrients mediated by bacterioplankton growth associated with DOC (Hitchcock ***et al.*** 2010).

Any benefit of increased inorganic nutrients to phytoplankton may be negated by increased turbidity associated with high flow events in the Namoi River (Westhorpe ***et al.*** 2008). Light penetration is generally substantially reduced with increased flows, rendering the phytoplankton more susceptible to light limitation (Jassby ***et al.*** 1993; Cole & Cloern, 1994; Oliver ***et al.*** 2010). This could further augment bacterial growth compared to that of phytoplankton as relatively more nutrients become available to the heterotrophic bacteria.

5.4.3. Implications for flow management

As part of the Australian water reform process that began in the mid 1990s, the introduction of environmental flows to the Namoi River system was aimed at improving ecosystem processes and populations of native river-dependent species (Shields & Good, 2002). Environmental flows for the Namoi River include the setting of flow thresholds that restrict the amount of water extracted during high flow periods. This ensures that downstream river reaches receive water that can inundate benches, bars, flood runners and floodplains and thereby increase DOC concentrations in the river (Chessman, 2003). Both DOC and inorganic nutrients tend to increase in the Namoi River with flow (Westhorpe ***et al.*** 2008). The benefits of increased river discharge to regulated inland rivers in Australia are two-fold. Firstly, in disrupting conditions conducive to toxic and nuisance phytoplankton blooms such as persistent thermal stratification, particularly in weir pools (Mitrovic ***et al.*** 2003). Secondly, larger flows increase the lateral connectivity of the river with adjacent riparian landscapes, thereby increasing inputs of terrestrial organic matter (TOM) in various forms that can be utilised by heterotrophic bacteria. The impact of river regulation with regard to switching

rivers to autotrophic rather than heterotrophic dominance has been reported. Oliver & Merrick (2006) suggested that the autochthonous organic carbon fuelling the riverine metabolism of the lower Murray River is driven by reductions in flow (flow regulation) and a subsequent reduction in the supply of TOM.

The ***in situ*** microcosm experiments used in this study aimed to simulate conditions of carbon and nutrient enrichment that occur during flow events in the river (Westhorpe ***et al.*** 2008). The results of this study suggest that bacterioplankton growth will likely increase when DOC concentration is increased, as they are DOC limited at times when DOC rich inflows are absent. This may be particularly pertinent during the summer months when bacterial growth response rates appear to be at their highest. At the time of this study, the ecological benefits of environmental flows were particularly relevant as eastern Australia was experiencing one of the worst droughts on record (Bond ***et al.*** 2008). A bioassay study conducted in southeastern Australia in three rivers within distinctly different geographical regions during times of low flow (December 2006) had similar findings to the present study, in that heterotrophic respiration was limited by organic carbon, irrespective the ambient nutrient and DOC concentrations (Hadwen ***et al.*** 2009). Increased concentrations of inorganic nutrients may also alleviate any subsequent nitrogen or phosphorus limitation of bacterioplankton. They are also likely to promote phytoplankton growth, as long as factors such as turbidity do not play a limiting role.

Some studies in lakes indicate a shift from net autotrophy to net heterotrophy at DOC concentrations of ≥5 mg L^{-1} (Jansson ***et al.*** 2000). In the Namoi River, refractory DOC levels during very low flow conditions (<100 ML d^{-1}) are around 4.5 to 5.5 mg L^{-1} and inflows can increase DOC levels to over 20 mg L^{-1} (Westhorpe ***et al.*** 2008). The switch to net heterotrophy may occur at only a few mg/L above the refractory DOC levels. The addition of just 1 mg L^{-1} of glucose in the spring carbuoy experiment resulted in decreased dissolved oxygen (final day: 10.8 ± 0.07 & 9.8 ± 0.15 mg L^{-1} for control and glucose respectively) and increased bacterial response (final day: 696 ± 65 & 1251 ± 195 RSA for the control and

glucose respectively), although these changes were not statistically significant (Figures 12a, d).

In net autotrophic systems, phytoplankton account for the limited mobilisation of carbon that can become available to heterotrophs via processes such as exudation and lysis and there is a strong dependence or coupling between bacterioplankton and phytoplankton derived organic carbon (Blomqvist ***et al.*** 2001). When bacterioplankton have access to an allochthonous source of DOC, they are no longer bound to the carbon produced by autotrophs (Jansson ***et al.*** 1999). Allochthonous DOC availability can have implications for the overall productivity of pelagic systems, as well as altering the food chain to one based on heterotrophs instead of phytoplankton (Jansson ***et al.*** 1999, 2000).

Moderate and regular influx of allochthonous DOC may be important for switching the system to a bacterioplankton-based food web. With decreased DOC concentrations in regulated rivers, the food chain may be based primarily on phytoplankton as heterotrophic production is limited by the smaller autotrophic produced DOC pool (Jansson, 2003). In regulated lowland rivers, flow regulation has reduced the amount of allochthonous DOC entering main channels through less frequent wetting of benches, flood runners and floodplains.

In summary, our ***in-situ*** bioassay study has provided evidence of a relationship between heterotrophic bacterial growth and DOC. However as Rees ***et al.*** (2005) suggests this does not lessen the potential role of other carbon fractions (e.g., coarse particulate organic matter) in driving bacterial production. Based on our findings we predict that increased bacterial production will occur with increased DOC concentration and this has the potential to play an important part in the transfer of carbon to higher trophic levels. Hall ***et al.*** (2004) suggest that heterotrophic bacteria are an important food source of zooplankton grazing communities. Further research may show the importance of including heterotrophic bacteria in lotic food web studies. The ability of heterotrophic bacterial communities to rapidly utilise different carbon sources (Judd ***et al.*** 2004) when available, and out-competing phytoplankton

for inorganic nutrients (Drakare ***et al.*** 2002) also has positive benefits for water quality by reducing the potential for the development of toxic and nuisance algal blooms. Environmental flows should increase the duration of allochthonously driven heterotrophic dominance in the lower Namoi River, if delivered at the appropriate time. This is particularly so during the summer months with warmer temperatures and when red gum leaf fall is at its greatest, thus rendering the river more like natural (pre-regulated) conditions for longer periods.

Chapter 6. Potential food web changes with allochthonous dissolved organic carbon delivery to the lower Namoi River

6.1. Introduction

Dissolved organic carbon (DOC) in aquatic systems derives from two distinct sources; autochthonous primary production within the system, or allochthonous organic carbon washed into the system from the water shed (Cole ***et al.*** 2002). When allochthonous sources of DOC enter aquatic systems, bacterioplankton production may be de-coupled and can greatly exceed that based solely on autochthonous DOC production (Jansson ***et al.*** 2000; Drakare ***et al.*** 2002) despite a smaller proportion of the DOC being available (Kaplan & Newbold, 1993; Wilcox ***et al.*** 2005). These external sources of allochthonous DOC may support the production of consumers in the receiving waters such as lakes (Carpenter ***et al.*** 2005; Cole ***et al.*** 2006). In rivers, allochthonous DOC can be mobilised during flow events when linkages with terrestrial environments are increased (Hinton ***et al.*** 1997; Aitkenhead-Peterson ***et al.*** 2003). This is particularly evident in lowland floodplain rivers which have large linkages with terrestrial environments at high flow (Robertson ***et al.*** 1999). The floodplain is often the primary area of carbon storage and primary source of carbon input to lotic ecosystems (Malanson 1993).

The regulation of rivers through water capture in large dams, as well as direct extraction of water, decreases both flood frequency and intensity and can alter the timing of events (Richter ***et al.*** 2003; Page ***et al.*** 2005). With climate change, this will likely be exacerbated in regions that become more arid, with decreased inflow magnitudes and increased period between inflow events (Russell ***et al.*** 2006). River regulation is likely to reduce mobilisation of allochthonous DOC to rivers due to less frequent and extensive wetting of benches, flood runners and the floodplain. Such changes can affect system-level characteristics, such as the loading of DOC and nutrients (see Chapter 3) and may influence riverine ecosystem structure and functioning, including energy transfer to higher trophic levels (Carpenter ***et al.*** 2005).

The importance of allochthonous DOC in subsidizing aquatic food webs is becoming clearer in some lakes (Carpenter ***et al.*** 2005; Cole ***et al.*** 2006) and has been suggested to be as much as 70% (Cole ***et al.*** 2011). However, the role of allochthonous sources of DOC in ecosystem functioning and the structuring of planktonic food webs in lowland rivers needs elucidation (Rees ***et al.*** 2005; Westhorpe ***et al.*** 2010). Zooplankton are particularly important, as they are a key organism for the transfer of carbon to higher trophic levels (Grey ***et al.*** 2000; Karlson ***et al.*** 2003). Microzooplankton such as protozoans, rotifers and juvenile copepods are the major planktonic consumers throughout the year in freshwater rivers (Kobayashi ***et al.*** 1998; Lair 2006) and many are consumers of bacteria, algae or both (Kobayashi ***et al.*** 1996).

The Namoi River, New South Wales (NSW), Australia, is a highly regulated river with two large headwater dams and four main channel weirs and heavy irrigation water demands. Since regulation, the frequency of flooding has declined and there has been a large decline in mean annual total river discharge, particularly in the mid to lower parts of the system. These conditions are likely to have reduced loads of allochthonous DOC entering the river (Burford ***et al.*** 2008) potentially switching the river to an autotrophically dominant system (Westhorpe ***et al.*** 2010). With the objective of improving riverine environments, increased flows that restore a greater proportion of the natural flows to the Namoi River have been allocated (Chessman, 2003). These are likely to increase DOC concentrations in the river through greater wetting of benches, flood runners and the floodplain (see Chapter 3). How the riverine food web will respond to the increased concentrations of DOC is currently unknown. The influence of the inorganic nutrients phosphorus and nitrogen which are likely to be carried in flow events also needs to be understood.

This study investigated the responses of the planktonic food web (up to zooplankton) to increased DOC concentrations of two different types. This simulated typical allochthonous DOC concentrations that would occur with larger inflow events. We hypothesised that increased concentration of allochthonous DOC would increase heterotrophic activity and

would alter the zooplankton community. This was tested using 70 L mesocosms where the ambient planktonic community was amended with either DOC as glucose or with a leachate of a common riparian tree (red gum). Treatments with the DOC sources plus inorganic nutrient addition were also examined.

6.2. Materials and Methods

6.2.1. Study area and study site

The study was performed at Boggabri on the Namoi River (Figure 9) and the site and location has been described in previous chapters.

6.2.2. Flow rates and water temperature in the Namoi River

Hydrographic data for the Namoi River at Boggabri were obtained from a gauging station operated by the NSW Office of Water (NOW). Stage was recorded every 15 minutes and converted to mean daily flow. Temperature was recorded with a Hydrolab Surveyor and MS5 sonde probe.

6.2.3. Experimental design

The mesocosm experiment was performed between the 17th and 25th of April 2007 at Boggabri on the Namoi River, NSW Australia (30 40′ 05″S; 150 03′ 21″E). The experiment was performed using twenty 70 L plastic mescosms (height 1 m, diameter 0.5 m). About 2000 L of experimental river water was collected at Boggabri (Figure 14) from a deep pool at a depth of about 0.3 m using a pump, stored in a large container, and transported to the experimental facility near the river within 1 hour of collection. The experimental water was added to each 70 L mesocosm by hose pipe. Mesocosms were rinsed three times with river water prior to filling. The water in the tank was continuously mixed gently to ensure the random distributions of plankton populations. After filling all twenty mesocosms, they were placed in a roofed, semi-sun area and kept uncovered to ensure free gas exchange from the surface. The physico-chemical conditions and community structure of plankton in each mesocosm were assumed to be similar to those in the river at the commencement of the experiment.

The mesocosms were filled in the morning of the 17th April (hereafter referred to as Day 0). Experiments ran over 8 days with each of the four treatments performed in quadruplicate and randomly assigned to mesocosms. Treatments were a control, amendment with glucose, amendment with glucose and the inorganic nutrients phosphorus and nitrogen (called nutrients from here on), amendment with red gum leachate and amendment with red gum leachate and nutrients. A 20 g L^{-1} solution of carbon as glucose was prepared with distilled water using Sigma® chemicals. A DOC stock solution of red gum leachate was prepared by soaking approximately 500 g of fresh ***E. camaldulensis*** leaves in approximately 1 L of distilled water for 72 hr at less than 5°C in a dark environment, thus minimising microbial utilisation of easily assimilable DOC (Ward & Johnson, 1996). Leachate solutions were then filtered using a 0.2 µm (polycarbonate membrane) filter to remove bacteria and particles (Ward & Johnson, 1996). The DOC concentration of these solutions was determined before experiments to allow appropriate dilution for experiments. Glucose and red gum amendments were added to increase DOC levels to approximately 20 mg L^{-1}. This falls within the range of DOC concentrations reported during flow events within the mid-lower Namoi River. Nitrogen was added as KNO_3 (approximately 0.5 mg L^{-1}) and phosphorus as KH_2PO_4 (approximately 0.2 mg L^{-1}).

6.2.4. Measurements of physico-chemical conditions

Dissolved oxygen was measured daily using a Hydrolab Surveyor and MS5 sonde probe by placing the sonde 10 cm below the surface of each mesocosm prior to any homogenisation. Water temperature, conductivity and pH were recorded on each occasion. The probe was rinsed with distilled water between readings to avoid any contamination between mesocosms. After homogenisation through stirring with clean paddles, grab samples were taken to determine relative bacterial biomass (5 ml) on days 0, 1, 3, 6 and 8. Grab samples for chlorophyll ***a*** (200 ml), DOC (100 ml) and nutrients (20 ml) determinations were taken on days 0, 3, 6 and 8.

Nutrient samples were taken after filtration through 0.45 µm pore size syringe filters in pre-washed and sample rinsed PET bottles. Samples were treated and analysed as described in Chapter 5.

6.2.5. Sampling and enumeration of bacterioplankton

Bacterioplankton samples (10 ml) were collected and treated as described in Chapter 5. Slides were examined at ×1000 using a fluorescence-equipped Olympus BX41 compound microscope. For each slide, ten pictures of random views (≥500 total cells) were captured using an Olympus DP72 camera and cellSens Standard software (version 1.3). Images were analysed for cell abundance and volume using CellC software (Selinummi ***et al.*** 2005). Bacterial biomass was calculated using a conversion factor of 0.28 pg C μm^{-3} (Simon and Azam, 1989).

6.2.6. Sampling, enumeration and identification of zooplankton

Samples for zooplankton identification and enumeration were taken with a Haney-type trap (4.2 L), similar to that described by Gawler and Chappuis (1987) on days 0, 5 and 8. Samples were collected from each mesocosm on each day, except for day 0 where 4 random mesocosms were sampled to determine the initial zooplankton community structure. Zooplankton were retrieved by filtering through a 35-µm-mesh nylon netting (Likens and Gilbert, 1970) glued to the bottom of a Perspex cylinder, and preserved with a 70%v/v ethanol solution. A 1-ml Eppendorf automatic pipette and a Sedgewick-Rafter counting chamber were used for sub-sampling and counting of zooplankton. The disposable tip of the pipette was cut to make a 4 mm diameter opening so that large crustacean zooplankton would not be under-subsampled (Edmondson and Winberg 1971). Each sample bottle was stirred thoroughly in order to ensure the random distribution of the specimens within the sample bottle. A 1-ml sub-sample was taken with the automatic pipette and placed in the counting chamber and the zooplankton counted under a Leica DM2500 compound microscope at a magnification of x50. Preliminary counting of five replicate samples established that the coefficient of variation was reduced to ~10% when the mean number of

specimens counted exceeded 100. Therefore, subsampling and counting were repeated until a minimum of 100 specimens of the most abundant taxon were counted. Zooplankton, except copepods were identified to the genus level using the taxonomic keys and descriptions of Shiel (1995) and Kobayashi ***et al.*** (2009). Copepods were identified as nauplii, calanoid copepodites (calanoids hereafter) or cyclopoid copepodites (cyclopoids hereafter) as they were all juveniles in this study. Ciliates were grouped together except for the genus ***Vorticella*** which was quantified separately.

6.2.7. Data Analysis

Repeated measures analysis of variance (ANOVA), with treatments and time as factors, were used to assess the dissolved oxygen, bacteria, chlorophyll ***a***, and DOC data. Before analysis data were transformed to reduce skewness and to homogenise variances. The data were analysed with the ***Statistica*** Version 7 (2004) and Graph Pad Prism packages. When the ANOVA main test provided significant results, specific pair-wise differences were determined using the Bonferoni post-hoc test (Zar 1984).

Non-parametric permutational multivariate analysis of variance (PERMANOVA, Anderson 2001a, 2001b) was used to assess zooplankton responses. The PERMANOVA main test examined the differences between treatments and days. Specific contrasts were also examined between the following groupings: control / amended; glucose / red gum and glucose / red gum with and without nutrients. PERMANOVA provides a distinct advantage when testing multiple contrasts because frequency distributions are freshly generated for each contrast: this avoids the problem of accumulated type I errors that is commonly encountered with parametric ***post-hoc*** tests. The PERMANOVA test was treated as an orthogonal design with days 5 and 8 as time factors: the ***a priori*** decision was made that if a significant interaction was found between treatments and time, then contrasts would be examined separately for days 5 and 8. If the interaction term was not significant, then contrasts were examined across the pooled days.

Multivariate PERMANOVA tests were performed on the zooplankton community data and followed by similarity percentages (SIMPER) analyses to establish which individual taxa were most influential in driving the differences between factors. Univariate PERMANOVA tests were performed using the same test design that was applied to the zooplankton community, on total zooplankton abundance and each individual taxon that had been identified as influential by the SIMPER analyses. Nauplii were included in the total zooplankton abundance counts, but excluded from the community analyses because it was possible that calanoid and cyclopoids were included, in unknown numbers, in the nauplii counts. Non-metric multidimensional scaling (nMDS) was used to visually represent the trajectory of change in zooplankton communities across all treatment and time factors. All zooplankton abundances were log10(x+1) transformed prior to analysis. The data were analysed using the ***PRIMER*** V6.1.13 and ***PERMANOVA+*** V1.0.3 package.

In order to examine the effect of carbon and nutrient treatments on zooplankton diversity, Simpson's ***D*** (Simpson 1949) and Shannon-Wiener's ***H*** (Shannon 1948) species diversity indices were calculated from the taxon and density (individuals L^{-1}) data for each treatment on Day 10. Simpson's ***D*** is given as $D = 1 - \sum_{i=1}^{K} p_i^2$ and Shannon-Wiener's ***H*** index is given as $H = -\sum_{i=1}^{K} p_i \log_2 p_i$ where ***K*** is the total number of zooplankton taxa, and p_i is the proportion of zooplankton taxon ***i*** in the total sample count over all ***K*** taxa. Simpson's ***D*** gives the probability of picking two individuals at random that are from different taxa (Krebs 1985). Shannon-Wiener's ***H*** derives from information theory, and the $\log_2$ usage provides information units in 'bits' (binary digits) (Krebs 1985).

6.3. Results

6.3.1. Bacterioplankton responses

At the time of the experiment, flows in the river were low (<0.06 m^3 sec^{-1}) and had been for the preceding 14 days (Figure 14). Prior to that only minor flow events had occurred in the

river, not exceeding 40 m^3 sec^{-1}. Water temperatures in the mesocosms ranged from 18 – 22.5°C with a mean temperature of 20.2°C over the course of the experiment. Heterotrophic respiration, measured as dissolved oxygen consumption (not corrected for O_2 production and diffusion), differed significantly ($P<0.001$) between treatments, times and the interaction (Figure 15). By day 2, dissolved oxygen levels in DOC treatments with nutrients had dropped significantly ($P<0.05$) below the control and remained significantly different until day 6. The glucose + nutrients and the red gum + nutrients treatments had lower dissolved oxygen levels on these days than the glucose alone and red gum alone treatments. After day 3, all amended treatments were similar and remained below that of the control until the end of the experiment, although levels became closer to that of the control on days 6, 7 and 8.

Bacterial biomass differed significantly between treatments (***P***<0.01), times and the interaction (***P***<0.001) (Figure 15). When DOC was added as glucose, with or without nutrients, bacterial biomass peaked at day 6 and was significantly higher than that of the control (***P***<0.001). At day 8 both glucose treatments decreased in bacterial biomass and only the glucose alone had a significantly higher biomass than that of the control (***P***<0.001). The treatment with DOC added as red gum showed increasing bacterial biomass across the experiments and was significantly higher than that of the control (***P***<0.05) at days 3, 6 and 8. The red gum + nutrients treatment peaked at day 3 and was significantly higher than that of the control (***P***<0.001), before declining in biomass at days 6 and 8.

DOC concentrations throughout the experiment are shown in Figure 15. The amendments with glucose and the red gum leachate at the start of the experiment (day 0) brought all levels up to approximately 20 mg L^{-1} (Figure 15). The ambient DOC level in the control was 4.7 ±0.04 mg L^{-1} and this did not vary greatly over the experiment. At day 3, DOC utilisation showed similar patterns to the dissolved oxygen results, with the treatments amended with inorganic nutrients consuming DOC at a significantly ($P<0.001$) greater rate than those without. By days 6 and 8, the glucose amendments had significantly lower DOC

concentrations than the red gum treatments (P<0.001) although they remained higher than that of the control. The red gum + nutrients treatment had significantly lower DOC concentrations than red gum alone (***P***<0.001). Chlorophyll ***a*** concentrations decreased in all treatments across the 8 days (Figure 15). Results differed significantly with time, treatment and the interaction (***P***<0.05). The glucose treatment had significantly lower chlorophyll ***a*** concentrations at day 6 (***P***<0.01). The glucose + nutrients treatment demonstrated no significantly different concentrations at all days. When DOC was added as red gum leachate, chlorophyll ***a*** concentrations were significantly lower than that of the control at days 6 and 8 (***P***<0.01) with nutrients at days 3, 6, and 8 (***P***<0.05).

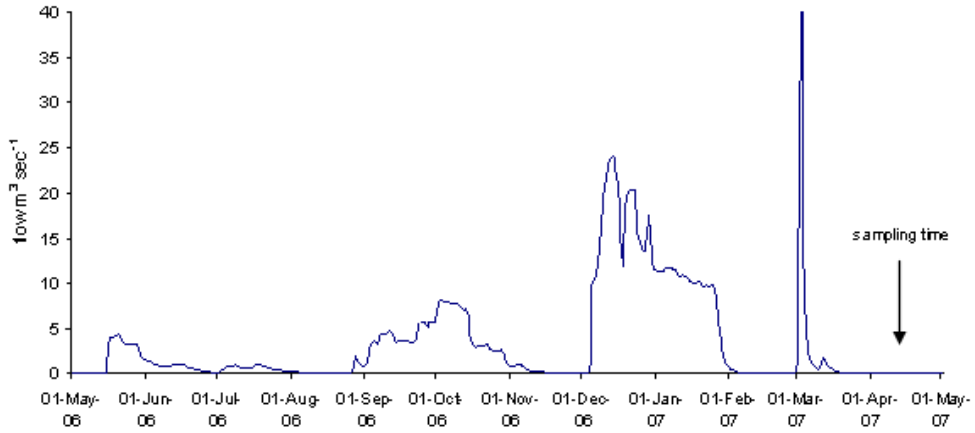

Figure 14: Mean monthly flow prior to and during the experiment at the gauging station Namoi River at Boggabri.

6.3.2. Zooplankton responses

A total of 19 taxonomic groups of zooplankton were identified. Mean concentrations of the dominant taxa are shown in Table 9. Total zooplankton abundance increased in all treatments from day 0 to day 5, with the largest increase (approximately fourfold) occurring in the glucose and red gum treatments (Figure 16). Abundances across all treatments declined in roughly the same proportions between day 5 and day 8. PERMANOVA tests identified strongly significant differences between treatments and days (Table 10; ***P***<0.005 in both tests), but there was no significant interaction between the two. The control/amended

and glucose/red gum contrasts were strongly significant (***P***<0.005 in both tests), but no significant difference was found in the nutrient/no nutrient contrast (***P*** = 0.27).

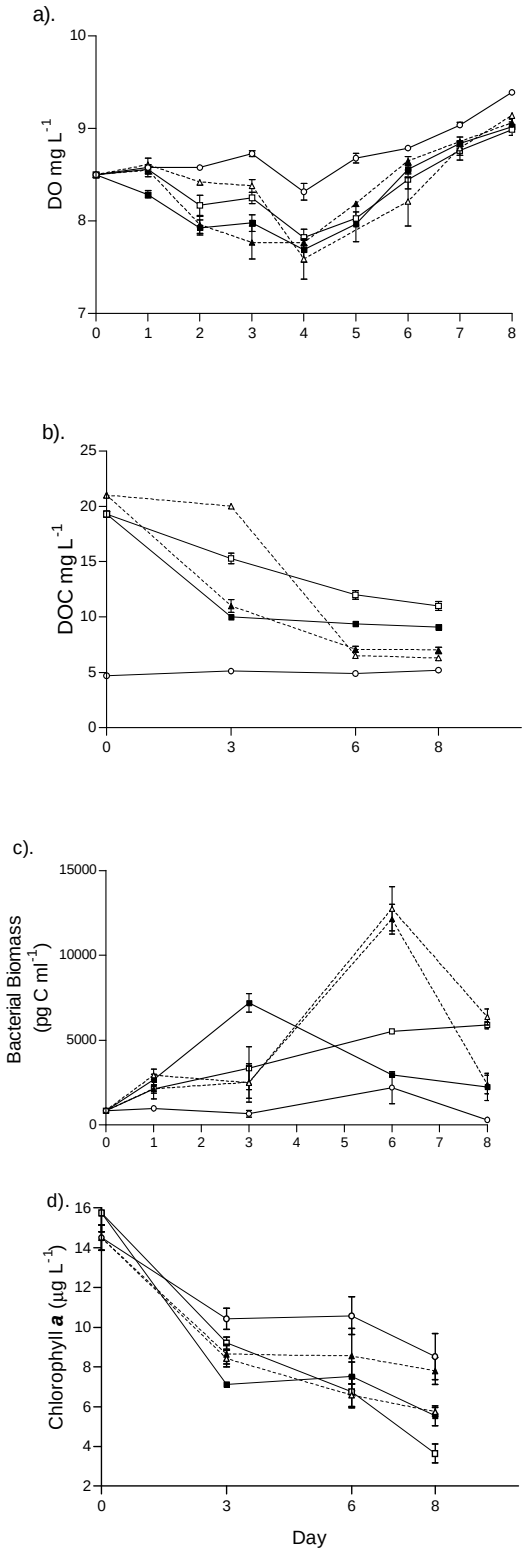

Figure 15: Changes in a). dissolved oxygen; b). DOC; c). bacterial biomass; and d). chlorophyll-***a*** concentrations over the course of the experiment (***n***=4; ± S.E. for all except bacterial biomass ***n***=2; ± S.E.). ***open circle*** control; ***filled triangle*** glucose; ***open triangle*** glucose + nutrients; ***filled square*** red gum; ***open square*** red gum + nutrients

Multivariate PERMANOVA tests of zooplankton community structure identified significant differences between treatments and days, and a significant interaction between both factors (Table 10; ***P***<0.005 in all three tests). Subsequently the three contrasts were tested separately across days 5 and 8, and all six of these identified significant differences (Table 11). Figure 15 shows clearly that the trajectories of the glucose, red gum and red gum + nutrients zooplankton community are very similar at day 5. By day 8, the two red gum amended treatments continued to respond similarly, whilst the two glucose treatments were more similar than at day 8. The zooplankton community in the control treatment was consistently very different from that of the other treatments.

SIMPER analyses identified eleven taxa as accounting for more than 90% of the differences between each treatment (Table 10). Given that several taxa were rare, those identified by the SIMPER analyses were the ones that occurred most regularly across the spread of samples and treatments (Table 9). PERMANOVA tests of these eleven taxa showed considerable variation in responses (Table 10). All taxa except ***Keratella*** spp. differed significantly between treatments, and all differed strongly between days (***P***<0.005 in all eleven tests). There were seven taxa with significant treatment/day interactions, displaying either declines after day 5 or little change until day 8 (Figure 18).

Responses to the control and amended treatments varied with different taxa. ***Proalides*** spp***,*** ***Keratella*** spp***,*** ***Hexarthra*** spp. and ***Brachionus*** spp. Densities showed no significant difference between the control and amended treatments (Table 10). Ciliate (excluding ***Vorticella)*** densities did not differ from that of the control until day 8, when the densities of control and glucose treatments increased markedly. However, no increase in ciliates occurred in the red gum and nutrient treatments (Table 9, Figure 18). ***Vorticella*** spp, ***Polyarthra*** spp, ***Asplanchna*** spp, ***Trichocerca*** spp, ***Anuraeopsis*** spp and cyclopoid copepods densities all differed significantly from that of the control on days 5 and 8, but with different trends: ***Vorticella*** spp.***, Anuraeopsis*** spp. and ***Trichocerca*** spp. declined after day 5, whilst ***Asplanchna,*** spp., ***Proalides*** spp. and cyclopoid copepods increased.

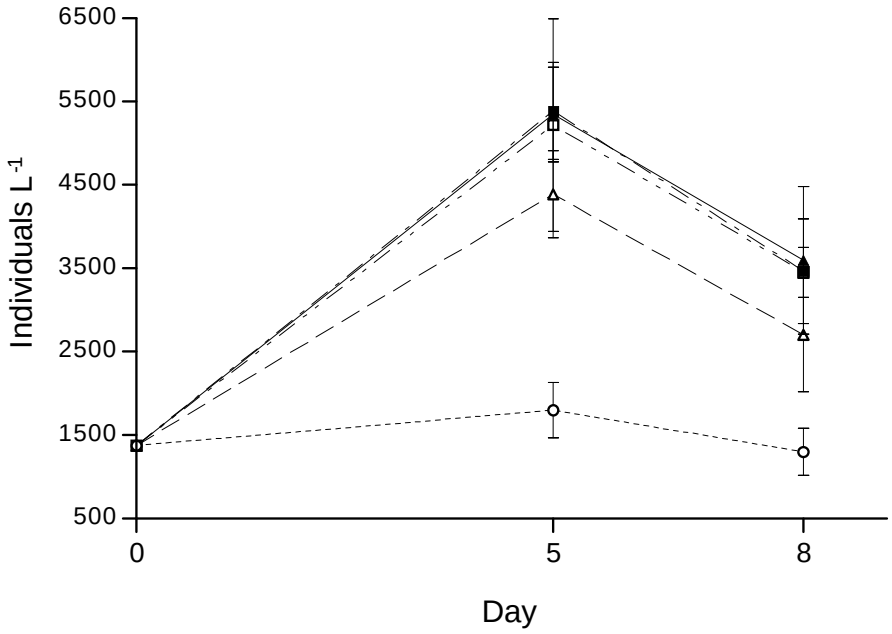

Figure 16: Changes in total zooplankton density over the course of the experiment (***n***=4; ± s.e.). ***open circle*** control; ***filled triangle*** glucose; ***open triangle*** glucose + nutrients; ***filled square*** red gum; ***open square*** red gum + nutrients.

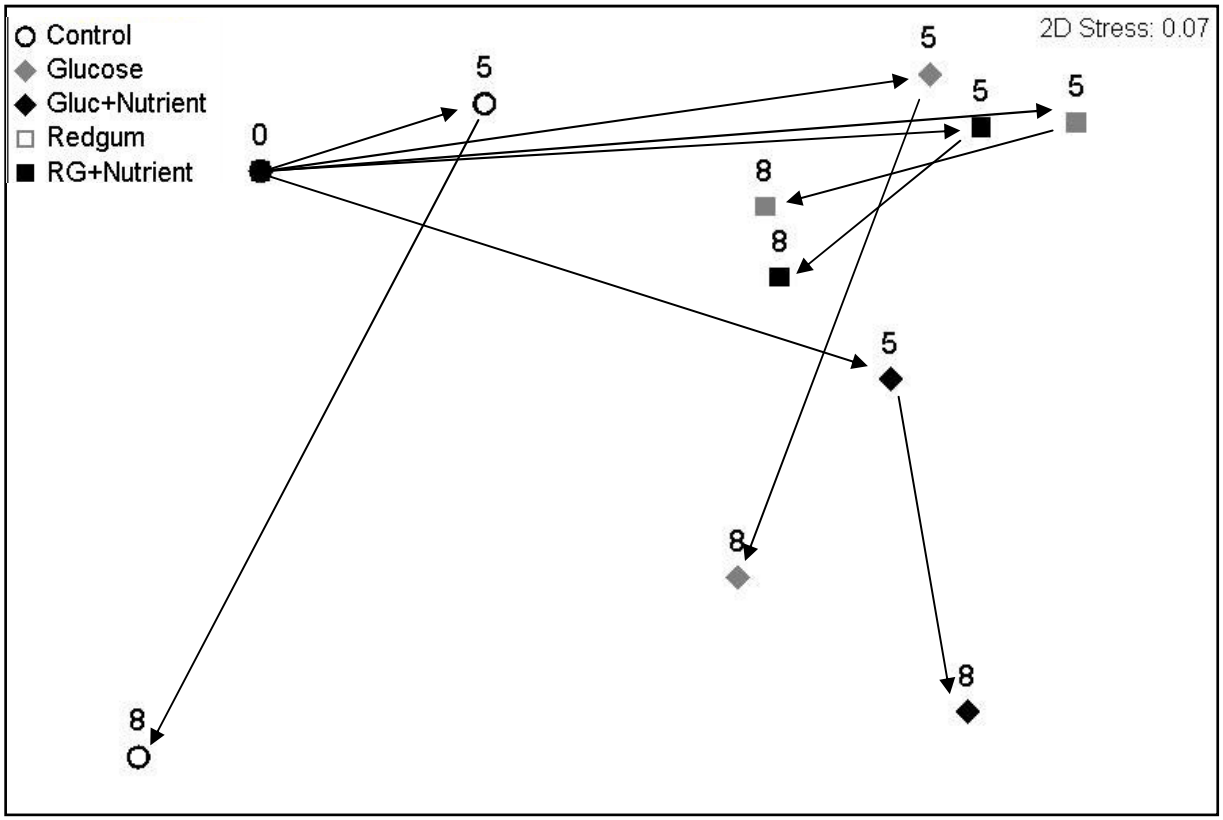

Figure 17: nMDS plot of all mean zooplankton genera values (Log10(x+1) transformed for each treatment type across 3-days (0, 5 & 8 days).

Table 9: Mean abundances of taxa per sample.

Phylum / Order	Taxon	Control			Glucose		Glucose+Nutrients		Red gum		Red gum+Nutrients	
		Day 1	Day 5	Day 8	Day 5	Day 8	Day 5	Day 8	Day 5	Day 8	Day 5	Day 8
Protozoa	***Vorticella spp.***	**<1**	9 (6)	8 (14)	1113 (750)	900 (812)	1616 (884)	1324 (907)	1388 (426)	503 (570)	1681 (1145)	848 (316)
Protozoa	Ciliates (excl. ***Vorticella***)	**7 (3)**	15 (10)	260 (99)	9 (2)	222 (211)	8 (3)	10 (8)	13 (7)	32 (32)	5 (4)	6 (2)
Rotifera	***Proalides spp.***	**147 (55)**	696 (358)	383 (261)	1596 (673)	424 (462)	568 (161)	20 (13)	1177 (693)	666 (547)	1229 (712)	850 (264)
Rotifera	***Keratella spp.***	**265 (42)**	525 (152)	484 (220)	1050 (235)	672 (237)	1067 (218)	459 (188)	1007 (140)	764 (398)	905 (508)	606 (131)
Rotifera	***Hexarthra spp.***	**82 (18)**	35 (32)	1 (1)	521 (344)	128 (103)	4 (2)	<1	455 (103)	415 (296)	63 (59)	59 (36)
Rotifera	***Brachionus spp.***	**316 (60)**	345 (77)	50 (41)	614 (206)	157 (141)	306 (129)	19 (15)	918 (174)	431 (341)	1082 (240)	369 (149)
Rotifera	***Asplanchna spp.***	**<1**	3 (4)	4 (4)	6 (7)	107 (131)	18 (19)	40 (31)	10 (3)	77 (22)	14 (11)	100 (66)
Rotifera	***Polyarthra spp.***	**429 (50)**	74 (67)	82 (69)	249 (148)	937 (658)	609 (254)	760 (524)	149 (43)	413 (89)	64 (59)	436 (122)
Rotifera	***Trichocerca spp.***	**79 (49)**	80 (63)	1 (2)	144 (76)	15 (16)	173 (87)	43 (23)	200 (31)	51 (27)	135 (88)	61 (28)
Rotifera	***Anuraeopsis spp.***	**1 (1)**	3 (2)	<1	20 (8)	<1	9 (3)	6 (4)	18 (8)	16 (8)	4 (2)	3 (2)
Rotifera	***Filinia spp.***	**<1**	<1	<1	9 (10)	1 (1)	<1	<1	22 (4)	9 (5)	6 (5)	5 (5)
Rotifera	***Lecane spp.***	**<1**	1 (1)	<1	2 (1)	1 (1)	1 (1)	1 (1)	4 (2)	2 (1)	1 (2)	3 (1)
Rotifera	***Euchlanis spp.***	**<1**	<1	<1	<1	13 (22)	<1	<1	<1	1 (1)	2 (1)	3 (3)
Rotifera	***Lepadella spp.***	**<1**	<1	<1	<1	<1	<1	<1	2 (1)	<1	<1	<1
Rotifera	***Colurella spp.***	**<1**	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Rotifera	***Plationus spp.***	**<1**	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Rotifera	Bdelloidea	**1 (1)**	3 (2)	<1	7 (3)	1 (1)	2 (2)	8 (5)	6 (5)	5 (8)	3 (0)	<1
Copepoda	Cyclopoida	**14 (6)**	1 (2)	2 (2)	1	4 (2)	1 (1)	5 (6)	6 (5)	16 (8)	5 (4)	16 (9)
Copepoda	Calanoida	**<1**	<1	1 (1)	<1	1 (1)	<1	<1	<1	<1	<1	<1

standard deviations in parentheses

Table 10: Two way PERMANOVA tests of log abundance for all taxa except those with very low abundances.

	Main Test			Treatment Contrasts		
Taxon	Treatments	Days 5 and 8	Treatment/Day interaction	Control/amended	Glucose/Red gum	Glucose / Red gum with & without Nutrients
All Taxa	**14.176** (9909)**	**19.878** (9941)**	**3.47** (9912)**	**Day 5: 9.91** (4228)** **Day 8: 13.408** (4232)**	**Day 5: 3.591* (5060)** **Day 8: 4.765** (5073)**	**Day 5: 6.433** (5027)** **Day 8: 3.010* (5050)**
Vorticella spp.	**49.428** (9947)**	**7.754** (9841)**	1.043 (9955)	**Pooled Days: 83.44** (9484)**	Pooled Days: 0.921 (9853)	Pooled Days: 3.594 (9816)
Ciliates excl. ***Vorticella***	**13.973** (9947)**	**29.219** (9844)**	**8.486** (9961)**	Day 5: 1.271 (508) **Day 8: 9.841** (2346)**	Day 5: 0.038 (276) Day 8: 2.506 (1870)	**Day 5: 4.67* (276)** **Day 8:11.603** (1869)**
Proalides spp.	**11.776** (9951)**	**51.283** (9954)**	**7.471** (9936)**	Day 5: 1.544 (4162) Day 8: 0.176 (3586)	Day 5: 0.129 (5030) **Day 8: 11.856** (3960)**	Day 5: 1.919 (5044) Day 8: 1.894 (3970)
Keratella spp.	2.397 (9945)	**63.019** (9949)**	1.358 (9936)	Day 5: ***no test*** Day 8: ***no test***	Day 5: ***no test*** Day 8: ***no test***	Day 5: ***no test*** Day 8: ***no test***
Hexarthra spp.	**34.874** (9956)**	**20.332** (9944)**	**10.664** (9942)**	Day 5: 1.015 (3565) Day 8: 4.283 (1094)	Day 5: 1.49 (4018) **Day 8: 8.093* (930)**	**Day 5: 39.883** (3973)** **Day 8: 7.609* (925)**
Brachionus spp.	**11.101** (9946)**	**41.398** (9944)**	**4.147** (9951)**	Day 5: 4.262 (4199) Day 8: 1.268 (3609)	**Day 5: 19.273** (5050)** **Day 8: 13.159** (5066)**	Day 5: 0.894 (5016) Day 8: 0.832 (5046)
Polyarthra spp.	**10.986** (9941)**	**17.793** (9952)**	**4.408** (9946)**	**Day 5: 5.001* (4218)** **Day 8: 21.453** (4156)**	**Day 5: 15.529** (5007)** Day 8: 0.883 (5094)	Day 5: 0.005 (5026) Day 8: 0.001 (5049)
Asplanchna spp.	**3.805* (9960)**	**48.837** (9951)**	1.458 (9941)	**Pooled Days: 10.569** (9836)**	**Pooled Days: 4.287* (9806)**	Pooled Days: 0.239 (9838)
Trichocerca spp.	**11.4** (9951)**	**47.74** (9959)**	**5.579** (9928)**	**Day 5: 8.213* (3595)** **Day 8: 34.61** (2565)**	Day 5: 0.043 (5056) **Day 8: 5.207* (3070)**	Day 5: 0.567 (5050) Day 8: 2.334 (3075)
Anuraeopsis spp.	**8.652** (9955)**	**35.713** (9949)**	**5.277** (9945)**	**Day 5: 10.17** (1437)** **Day 8: 5.366* (267)**	Day 5: 1.717 (1769) Day 8: 3.914 (493)	**Day 5: 18.589** (1778)** Day 8: 0.059 (492)
Cyclopoids	**5.168** (9955)**	**28.857** (9951)**	1.565 (9940)	**Pooled Days: 5.51** (9845)**	**Pooled Days: 15.567** (9846)**	Pooled Days: 0.120 (9818)
Total zooplankton abundance	**12.477** (9962)**	**14.294** (9821)**	0.112 (9951)	**Pooled Days: 51.993** (9827)**	**Pooled Days: 14.294** (9821)**	Pooled Days: 1.282 (9836)

Pseudo-f values are supplied, with the number of unique permutations in parentheses. Values in bold font are significant: * denotes p <0.05, ** denotes p < 0.005. Where the main test identified a significant interaction between treatments and days, treatment contrasts were conducted separately for each day. No contrasts were conducted for ***Keratella*** spp. because the main treatment test was not significant.

Vorticella spp. and ***Keratella*** spp. did not respond significantly to either the glucose/red gum or nutrients/no nutrients comparisons. ***Hexarthra*** spp. responded significantly to both of the comparisons, but the remaining eight taxa showed significant responses to only one comparison or the other (Table 10). ***Proalides, Polyarthra*** and ***Asplanchna*** had significantly higher densities in glucose than in red gum treatments, but ***Hexarthra, Brachionus, Trichocerca*** and cyclopoid copepods responded in the opposite manner. Ciliates (excluding ***Vorticella), Hexarthra*** and ***Anuraeopsis*** were significantly more abundant in treatments with no added nutrients, but no taxa were more abundant in nutrient enhanced treatments. .

Zooplankton diversity (mean ± SE, ***n***=4) as measured by the Simpson's ***D*** diversity index was high on Day 10 for treatments with red gum (0.80±0.04 for red gum and 0.81±0.02 for Red gum + nutrients), relative to other treatments (0.63±0.04 for Glucose and nutrients to 0.76±0.02 for Glucose) (Table 11). Zooplankton diversity for Controls (0.79 ± 0.01 on day 0) was 0.67±0.04 on day 10.

Table 11: Zooplankton diversity for different treatment on different days based on Simpson's ***D*** and Shannon-Wiener's ***H*** indices.

Treatment	Diversity index	Day 0	Day 5	Day 8
Control	Simpson's ***D***	0.79±0.01	0.71±0.01	0.67±0.04
	Shannon-Wiener's ***H***	1.75±0.03	1.48±0.04	1.33±0.11
Glucose	Simpson's ***D***		0.78±0.03	0.76±0.02
	Shannon-Wiener's ***H***		1.73±0.08	1.69±0.05
Glucose	Simpson's ***D***		0.75±0.02	0.63±0.04
+ nutrients	Shannon-Wiener's ***H***		1.60±0.04	1.26±0.07
River red gum	Simpson's ***D***		0.80±0.01	0.80±0.04
	Shannon-Wiener's ***H***		1.81±0.03	1.87±0.08
River red gum	Simpson's ***D***		0.76±0.01	0.81±0.02
+ nutrients	Shannon-Wiener's ***H***		1.58±0.03	1.87±0.02

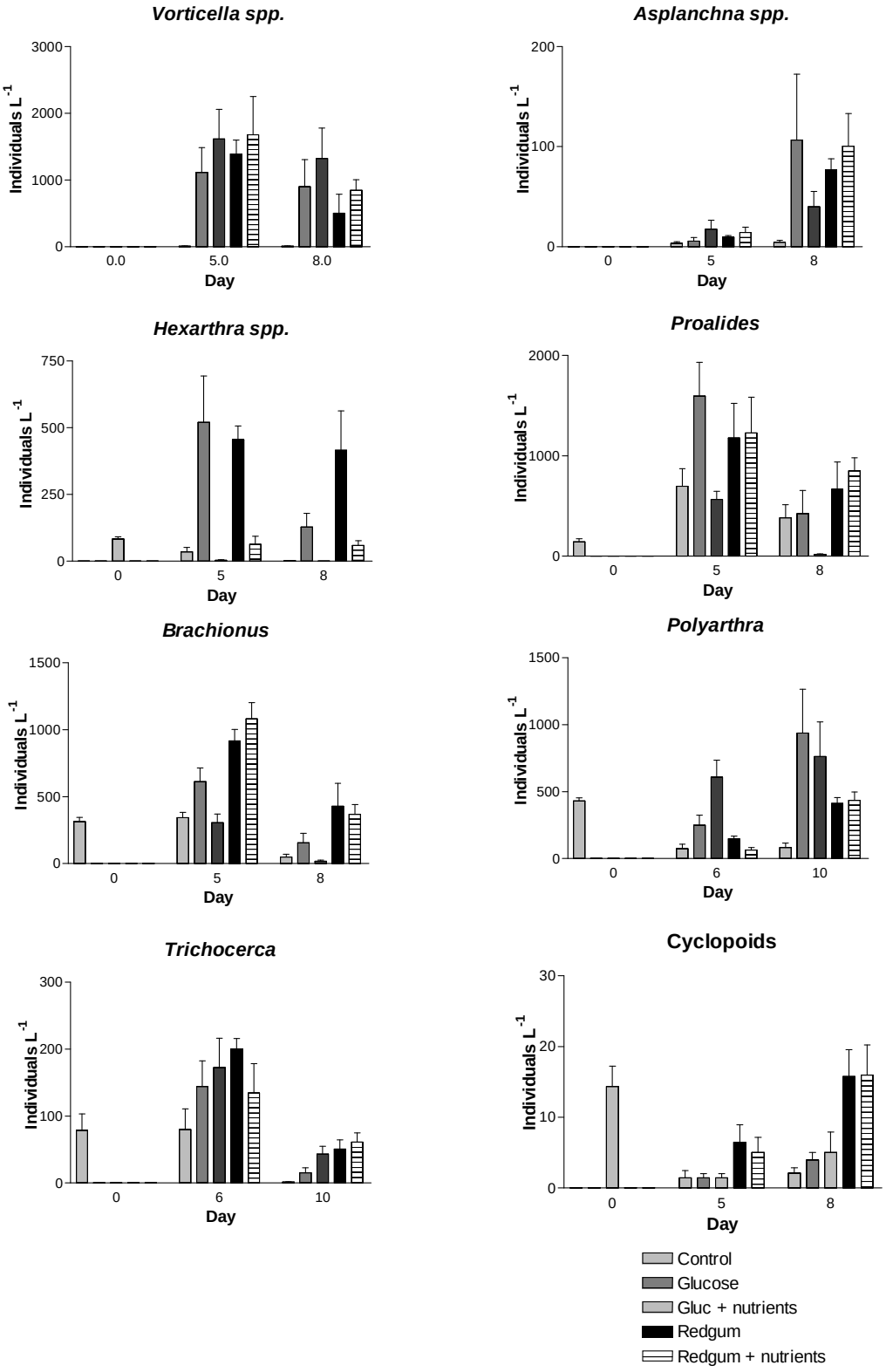

Figure 18: Changes in zooplankton densities for some dominant taxa over the course of the experiment (***n***=4; ± s.e.).

6.4. Discussion

6.4.1. Heterotrophic and autotrophic responses

At the ecosystem level, allochthonous DOC affects river metabolism by subsidizing ecosystem respiration relative to production (del Giorgio 1999.) As a result of DOC amendment, heterotrophic respiration of all treatments significantly reduced (***P***<0.05) dissolved oxygen on certain days of the experiment (mainly days 2-5) compared with the control (Figure 15). This suggests the heterotrophic bacterial community could utilise both DOC sources, probably as aquatic heterotrophic bacterial communities contain a nearly full complement of carbon processing functional groups (Judd ***et al.*** 2004). The bacterial community appeared to be DOC limited as previously seen in this river over three seasons in microcosm experiments (Westhorpe ***et al.*** 2010) and in other river systems during periods of low flow (Findlay, 1992; Benner, 1995; Anesio ***et al.*** 1997; Benner 2003). Dissolved oxygen was consumed faster during days 2 to 3 in the DOC treatments with added inorganic nutrients (Figure 15) suggesting some co-limitation of carbon with nitrogen and/or phosphorus. Supporting this, the bacterial biomass was significantly higher at day 3 in the red gum + nutrient treatment compared to that of the red gum only treatment.

DOC concentration data (Figure 15) supported the uptake of DOC by heterotrophs as DOC treatments with inorganic nutrients had lower DOC concentrations at day 3, a similar pattern to the dissolved oxygen data. By day 6 the difference was no longer apparent and the glucose treatments had lower DOC concentrations than that of the red gum, which coincides with bacterial biomass being significantly higher in the glucose treatments than that in the red gum treatments. DOC concentrations for the red gum treatments took longer to plateau and remained between approximately 10 and 12 mg L^{-1}, suggesting that this source of carbon took longer to be metabolised by bacteria. This may be due to the fact that glucose is considered low-molecular-weight and highly labile, whilst naturally occurring DOC is a diverse mix of different compounds of differing molecular weights and labilities (Berggren ***et***

al. 2010). If DOC was not being utilised by heterotrophs, this suggests less labile or more recalcitrant DOC than that found for the glucose additions.

Chlorophyll ***a*** declined in all treatments with time. Significantly lower chlorophyll ***a*** levels than the control (***P***<0.05) were found only at day 6 when glucose alone was added, at days 6 and 8 when red gum leachate alone was added, and at all days when red gum leachate was added with nutrients. This may have been due to planktonic bacteria being able to out-compete phytoplankton for available inorganic nutrients when DOC was added (and no longer limiting), due to a higher surface area to volume ratio of bacteria (Blomqvist ***et al.*** 2001; Drakare ***et al.*** 2002; Findlay, 2003). Drakare ***et al.*** (2002) found that high flows carrying DOC into a lake led to low primary production despite good conditions for phytoplankton growth. In their study, primary production never exceeded bacterial production for approximately 20 days after a flow episode had replenished DOC concentrations. Chlorophyll ***a*** may have also been reduced by grazing effects as the zooplankton communities changed with time and also treatment. Although not changed in these experiments, it should be considered that light penetration could be substantially reduced with increased flows, rendering the phytoplankton more susceptible to light limitation (Jassby ***et al.*** 1993; Cole & Cloern, 1994; Oliver ***et al.*** 2010). Increased turbidity to greater than 1000 NTU can occur with high flow events in the Namoi River (Westhorpe ***et al.*** 2008). This could further augment heterotrophic bacteria compared to phytoplankton as relatively more nutrients become available to the heterotrophic bacteria.

6.4.2. Zooplankton responses to DOC

Amendments with DOC increased densities of total zooplankton compared to that of the control (Figure 16). This was most pronounced for both red gum treatments and also for the glucose without inorganic nutrients treatment. For taxa such as ***Vorticella,*** DOC amendment was associated with increases of several orders of magnitude and there was no significant difference in response to either glucose or red gum treatments. The second protozoan group in this study, ciliates (excluding ***Vorticella)***, also showed no preference for one carbon

source or another. Protozoans constitute a high quality source for metazoans (Gifford 1991), as well as transferring carbon they also transfer minerals, vitamins and fatty acids to higher trophic levels (Gifford and Dagg 1991) and have been shown to be the preferred food source of metazoans (Gifford and Dagg 1988; Reaugh, Roman ***et al.*** 2007). It is likely that protozoans provided an important pathway for nutritional needs not present in glucose treatments. Amongst the Rotifera the response was less uniform, as some genera showed no detectable response at all to DOC amendments. The addition of inorganic nutrients did not increase the density of zooplankton in fact some taxa were more abundant in their absence.

Several taxa were found to have significant treatment/day interactions and upon examination, these reflected either peak densities at day 5 followed by slumps at day 8 (clearly illustrated by ***Vorticella, Proalides, Keratella*** and ***Brachionus***), or sharp increases between day 5 and day 8 (e.g., ***Asplanchna, Polyarthra*** and cyclopoids). This suggests at least two cascading stages of response, the first of which may be due to certain zooplankton taxa increasing rapidly possibly through consumption of bacterioplankton that had benefitted from DOC amendment as well as phytoplankton. The second stage, represented by taxa whose abundance increased after day 5, may have been zooplankton that benefited from increased predation opportunities on these first stage zooplankton as well as bacterioplankton and phytoplankton. This may help to explain why small sized taxa such as ***Vorticella*** increased very rapidly, while the larger predatory cyclopoids increased towards the end of the experiment.

In this experiment we performed treatments with glucose and also a red gum leaf leachate. The red gum treated mesocosms had significantly higher densities of copepods compared to that of the glucose treatments (Figure 18). It is well accepted that food quality, in particular phosphorus and fatty acid availability, can be limiting factors for the growth and reproduction of copepods (Gulati and DeMott 1997). Whilst glucose is a simple carbohydrate, the red gum leachate is quite complex (c.f. Baldwin 1999) and a recent study by Zander ***et al***. (2007)

has detected over individual DOC compounds comprised of a variety of semi-volatile and volatile organic compounds. Leachates may also contain high levels of phenolic acids such as tannin and lignin (Wetzel 2003). Whilst the different carbon additions may not have led to marked differences in coarse measurements such as bacterial biomass or densities of protozoans, there may have been more subtle effects on the species make-up of these communities and/or their nutritional value. If it is therefore possible that the use of a leachate which closely mimics naturally occurring DOC, could lead to a protozoan community of higher nutritional value than glucose additions.

Overall, it is clear that DOC amendment has a significant influence on zooplankton communities, which is detectable and that the responses of individual taxa are complex and varied. It is also clear that zooplankton have responded differently to carbon sources of different quality, with significantly different responses to glucose or red gum amendments in seven taxa (Table 10).

Additions of DOC tended to maintain the diversity of riverine zooplankton in this study. Diversity was higher when red gum was used as a carbon source regardless of whether nitrogen or phosphorus was added. Red gum is considered to be a more natural allochthonous source of carbon than glucose. In Australian lowland rivers red gums (***Eucalyptus camaldulensis***) often dominate the riparian vegetation communities. Diversity was not as high under glucose additions and was quite low with glucose and nutrient additions. Carpenter ***et al.*** (2005) found that inorganic nutrient enrichment reduced the importance of allochthonous carbon subsidies to zooplankton and fish considerably in lake manipulation experiments. The mid to lower Namoi River is relatively productive with high nutrient concentrations and algal blooms (Mitrovic ***et al.*** 2003; Westhorpe ***et al.*** 2010). Despite this, when nutrients were added with the glucose treatment, the zooplankton were seen to diminish in density and biodiversity.

6.4.3. Role of allochthonous carbon in food webs

Although bacterioplankton often use autochthonously produced DOC preferentially over allochthonous sources (Kritzberg ***et al.*** 2006), the watershed can supply large amounts of allochthonous assimilable organic carbon, contributing to significant increases in bacterial production (Wilcox ***et al.*** 2005). There is considerable debate concerning the degree of allochthonous organic matter subsidization to secondary productivity in aquatic environments (Carpenter ***et al.*** 2005; Cole ***et al.*** 2011). Some evidence has suggested bacterial carbon pools are a minor source for higher trophic levels (Cole ***et al.*** 2002; Sobczak ***et al.*** 2002; Sobczak ***et al.*** 2005; Van den Meersche ***et al.*** 2009; Brett ***et al.*** 2009). However, a growing number of studies are finding substantial subsidization of terrestrial organic carbon to zooplankton and fish (Grey ***et al.*** 2001; Carpenter ***et al.*** 2005; Cole ***et al.*** 2006). In freshwater lakes, allochthonous carbon has been reported to support approximately 43% to 75% of bacterial growth (Kritzberg ***et al.*** 2006) and a recent study by Cole ***et al.*** (2011) found that allochthony of some zooplankton taxa is greater than 20% and in some cases is up to 70% of the organism's diet. Wilcox ***et al.*** (2005) found significant microbial and invertebrate responses in a stream with labile carbon added which stimulated food web processes even in a system abundant with organic matter.

The majority of work on allochthonous carbon subsidies to metabolism and food webs has been focused on lake systems, with only a few examples available from lowland rivers (e.g., Thorp and Delong 2002; Bunn ***et al.*** 2003; Gawne ***et al.*** 2007; Westhorpe ***et al.*** 2010). Vink ***et al.*** (2005), in quantifying ecosystem metabolism in the middle reaches of the Murrumbidgee River, showed phytoplankton production dominated ecosystem production. However, the authors speculated that the high contribution of phytoplankton in the Murrumbidgee system could be a consequence of flow regulation and resultant loss of riverine connectivity with adjacent floodplains. Similar conclusions were suggested by Oliver and Merrick (2006) who found autochthonous organic carbon fuelled riverine metabolism of some regulated rivers, primarily by reductions in flow and subsequent reduction in organic matter. Hoffman ***et al.*** (2008) investigated the sources of organic matter supporting lower

food web production in the tidal freshwater portion of an estuary and found that the degree to which zooplankton were supported by autochthonous sources declined exponentially with discharge. Some other large rivers with reduced flows have been found to be carbon limited (Rees ***et al.*** 2005; Westhorpe ***et al.*** 2010; Hadwen ***et al.*** 2009) supporting these finding. Bunn ***et al.*** (2003) found autotrophs dominated the energy flow in the food web of pools in a lowland desert river however this was tested during a dry period and not after inflows to the pools. In this study fresh leaves were used as a source of DOC as opposed to 'naturally aged' leaves as we were after a relatively rapid response from the food web. Previous studies in Australia have used fresh river red gum leaves as a source of carbon in aquatic ecosystems (e.g., Baldwin 1999; O'Connell ***et al.*** 2000; Westhorpe ***et al***. 2010) with good responses. The advantages of using fresh leaves compared with aged leaves is that the organic matter is rapidly consumed by microbes, whilst organic matter (OM) obtained from aged leaves has been shown to be less bioavailable, with only 30% of the aged leaf OM utilised by the bacteria (Baldwin 1999).

6.4.4. Flows and allochthonous DOC supply

Delivery of allochthonous DOC to downstream locations is linked to flow regimes, with higher flows generally wetting greater terrestrial areas and inundating floodplains. Floodplain riparian areas in lowland rivers provide significant allochthonous energy sources such as leaf litter (Baldwin 1999; Glazebrook and Robertson 1999; Schulze 1995). Alteration of hydrology such as through regulation and climate change (where areas become more arid) will likely influence the linkages with terrestrial environments and reduce the amount of allochthonous DOC that is mobilised. In the Macintyre River, a 55% reduction in connection to a flood plain meant a decrease of up to 98% in the amount of dissolved organic carbon imported from some anabranch channels (Thoms ***et al.*** 2005).

The Namoi River has been highly regulated and the magnitude of small through to large flow events has been reduced. As DOC concentrations have been found to be positively related to flow, reductions in flow are likely to reduce the mobilisation of allochthonous DOC and

delivery to the river (see Chapter 3). This is likely to be an issue in many other lowland rivers world wide where flows have been considerably reduced. Whilst an increase in discharge can lead to an increase in DOC load (Chapter 3), flowing water environments have been shown to reduce zooplankton biomass, via advective transport; with increased discharge (and turbidity) generally favouring rotifers (small-bodied zooplankton) (Pace ***et al***. 1992). A recent study in a large floodplain river (Mississippi River) by Burdis and Hoxmeier (2011) also found that rotifers were favoured by advective environments (whilst cladocerans and copepods favoured backwaters) with short water residence times, as their high growth and reproduction rates offset downstream losses; they are also less susceptible to physical damage during transport (Pace ***et al***. 1992). Havel ***et al.*** (2009) also found rotifers to be the dominant zooplankton group in the warmer and more turbid lower channelised zone of the Missouri River (USA), particularly in summer with reproduction occurring within the river and not from upstream or lateral sources such as weirs and wetlands as tend to be the case for copepods and cladocerans. Rotifers were the dominant zooplankton group in the lower Namoi River and it is expected that rotifer densities would only be temporarily affected by large changes in discharge due to their rapid reproduction rates and positive response to phytoplankton densities (Havel ***et al.*** 2009; Burdis and Hoxmeier 2011).

Environmental flows have been allocated for many rivers across Australia with the aim of supporting ecosystem processes and populations of native river-dependent species (Shields and Good 2002). These flows are strongly oriented toward the partial restoration of the natural flow regime, a strategy advocated by many river ecologists (e.g. Power ***et al.*** 1996; Poff ***et al.*** 1997). Although environmental flows have been allocated to many rivers around the world, there is still not a good understanding of their ecological effects. This is partly due to the fact that large rivers are often most affected by flow regulation, and suitable control (or unregulated) rivers may not be available for comparison. This has led to a need for experimental and modelling studies to better elucidate the ecological changes that flow restoration may exert (Mitrovic ***et al.*** 2006).

In the Namoi River, rules that protect high flow events from extraction have been implemented when reservoirs spill or when high flows generated from unregulated tributaries occur. These environmental flows will inundate terrestrial environments such as floodplains, and have been found to increase DOC concentrations in the river with the most effective DOC delivery occurring when larger flow events are protected (see Chapter 3). Results from the present study indicate that DOC delivered to the river with the current environmental flows will increase heterotrophic production, diminish phytoplankton growth and will increase zooplankton abundance and diversity. This suggests that environmental flows will have a positive effect on the ecological functioning of the river system.

The current environmental flow rules in the Namoi do not greatly protect small to moderate size events. If these were protected, they would be more effective at increasing the frequency of allochthonous DOC delivery to the river, provided riparian habitats are maintained / improved. Partial restoration of the natural frequency of these events, and subsequent delivery of allochthonous DOC would resemble that occurring under natural conditions. From an ecological perspective frequent wetting may be important in ensuring continuity of terrestrial carbon supply and thus help maintain the heterotrophic food web. As the system is arid, a constant source of allochthonous DOC is not expected. Rather, flow pulses and inflows with greater periodicity and flow variability would more resemble flows before the development of irrigation for agricultural crops. These would still be interspersed with natural low flow periods, especially during drought periods. The timing of inflows should also be considered, as the timing of leaf fall and temperature can both determine the bioavailability and uptake of organic matter released into the system (Baldwin 1999; Boon 1991). It has been suggested that the seasonal fate of terrestrial carbon subsidies for plankton communities and higher trophic levels is still uncertain (Grey ***et al.*** 2001; Pace ***et al.*** 2004).

Elucidation of the different pathways for energy subsidization and its effects on the fundamental properties of food web dynamics and carbon cycling seems to be an expanding

frontier of ecological research. The impacts of cross-ecosystem subsidies depend on the characteristics of the imported material, the route of entry into the food web, the types of consumers present and the productivity of the recipient system (Cole ***et al.*** 2006). Here we have shown how a floodplain river may respond to inflows of allochthonous DOC using mesocosms. Increased DOC concentrations in the river have the potential to play an important role in the transfer of carbon to higher trophic levels. Amendment of DOC as leaf leachates of a common riparian tree (red gum) and glucose led to increased heterotrophic activity. Amendments with DOC, both with and without inorganic nutrients increased the densities of some bacterivorous protozoans, rotifers and cyclopoid copepods relative to that of the controls. Zooplankton also responded differently to differing carbon sources, with roughly equal proportions of taxa being more abundant in glucose treatments as were more abundant in red gum treatments. The nutrient amendment had negligible or a diminished effect on zooplankton densities. Zooplankton diversity also increased in the red gum treatment relative to that of the controls. Glucose increased the diversity of zooplankton when added alone but not when it was added with nutrients. These results support the contention that environmental flows which increase DOC delivery within the river will stimulate heterotrophic bacterioplankton and alter the food web. Furthermore, our findings support transfer of allochthonous DOC to higher trophic levels such as metazoan zooplankton. This contributes to the growing body of research that has found allochthonous carbon to be an important contributor to food webs of freshwater aquatic systems.

Chapter 7. Conclusion

7.1. Summary of findings

This study was aimed at assessing the influence of increased river flows on the delivery of allochthonous dissolved organic carbon (DOC) to the regulated lower Namoi River and how this increase in DOC drives and alters the aquatic food web. In particular, the study investigated a range of flow events to determine the quantities of allochthonous DOC being delivered and transported down the system. Comparative responses to increases in DOC (and in some cases inorganic nutrients) were then assessed between primary producers (phytoplankton) and primary consumers (heterotrophic bacterioplankton) through ***in situ*** microcosm experiments (1.25 & 15 L bottles); and a subsequent experiment using 70-L plastic mesocosms to determine whether increased heterotrophic bacterial activity was being transferred up the food web to secondary consumers such as zooplankton.

At the time of the study, the research was deemed highly important to the New South Wales Office of Water (NOW) as there was a need to undertake ecological monitoring and evaluation activities focused on specific clauses and performance indicators within the currently gazetted Water Sharing Plan (WSP) for the Lower Namoi Regulated River Water Source 2004 (NOW 2011). This research was therefore designed to help determine whether the WSP was meeting its environmental objectives with reference to environmental flow allocations, in particular maintaining or improving riverine ecosystem health.

DOC concentrations in the water column showed a positive relationship with flow. This was particularly evident during high flow events (e.g., > $50m^3$ sec^{-1}) that had the capacity to inundate associated low lying geomorphic features (e.g., benches & flood runners) of the riverine zone and floodplain, thereby mobilising large quantities of terrestrial organic matter. Periodic wetting and inundation of surrounding terrestrial landscapes is now widely acknowledged as a natural process within a healthy riverine ecosystem (Cottingham ***et al***., 2003). Modelling of the high flow event at Walgett in December 2004 gave a clear indication of the amount of DOC that could be mobilised and transported downstream with an average

daily load of DOC estimated to be 399.8 tonnes over the sampling period of the flood event. The increased delivery of allochthonous DOC measured in the river from controlled dam releases and tributary inflows should create conditions that resemble pre-regulated (natural) conditions, and thus stimulate riverine heterotrophic communities.

Williams and del Giorgio (2005) state that: all aquatic ecosystems require inputs of organic matter from adjacent ecosystems as ***in situ*** production alone does not always support river functioning and ecosystem processes. The findings of this current study in the lower Namoi River clearly support this statement, and has shown that at both the primary and secondary trophic levels of the aquatic food web, the addition of organic matter (as DOC) results in an increase in the density of bacterioplankton and also zooplankton abundance and diversity. This was also the case when DOC was added with inorganic nutrients (nitrogen and phosphorus) indicating that bacterioplankton can out-compete phytoplankton for nutrients when carbon is not limiting, with increased numbers of bacteria providing greater grazing opportunities for meiofaunal communities such as protozoans and zooplankton.

This study has shown that environmental flow allocations can increase DOC delivery within the lower Namoi River and in turn will stimulate heterotrophic bacterioplankton and alter the food web, as allochthonous DOC is transferred to higher trophic levels such as zooplankton. This flow – bacterioplankton relationship can also lead to general improvements in water quality, particularly during warmer months, as bacterioplankton out-compete phytoplankton for inorganic nutrients thus potentially suppressing harmful algal blooms.

7.2. Management implications

Before effective management decisions can be made it is necessary to acknowledge that human alteration of flow regimes has the most detrimental ecological impact on our rivers and associated floodplains (Poff ***et al***. 1997; Bunn and Arthington, 2002). Ecological monitoring is a vital tool in dealing with these impacts and in supporting the assessment of management activities and decisions, with such tools making it easier to defend a decision and also assess the efficacy of the action (Field ***et al.*** 2007). This study has shown that

DOC is positively related to flow and that partial restoration of natural flows can result in increased delivery of DOC to a river. We believe that this has the potential to increase the periods of heterotrophic dominance towards a more natural regime (however we know of no data that exists for natural regimes). The environmental flow requirements across river systems will vary and are related to the sensitivity of the ecosystem to reduced flows (Acreman and Dunbar, 2004). With this in mind, the following recommendations / suggestions based on this study are aimed at helping guide water planners and managers in making clear decisions on whether or not the WSP is meeting its objectives and if not how the rules can be modified to achieve the desired outcomes.

Findings and suggestions based on this study:

1. Results from this study support the maintenance of natural flow regimes – with delivery of large quantities of DOC to the river by the wetting of river benches, riparian zones and floodplains;
2. The current rules that limit the extraction of medium and high flows entering the lower Namoi River are currently more effective for the high flows due to greater connectivity and potentially residence time with the surrounding landscape;
3. From an ecological perspective it would be applicable to adjust environmental flow rules in favour of the protection of small to medium sized flows. Partial restoration of the natural frequency of these events, and subsequent delivery of allochthonous DOC would resemble that occurring under natural conditions. This may help maintain the balance between heterotrophic and autotrophic dominance. This is in line with the general physical character of the lower Namoi River as even small reductions in flow result in a relatively large reduction in wetted perimeter (Acreman and Dunbar, 2004);
4. Flow releases from storages that coincide with rainfall events in the unregulated tributaries lower in the catchment (i.e., ‘piggy backing’ of flows to enhance benefits)

as well as protection from extraction, should in part be able to restore these ecologically important flow events. This will help to deliver more regular pulses of allochthonous DOC into the river with the associated benefits to river functioning and ecology. It is worth considering that the frequency and duration of flow events will impact upon overall water quality and DOC concentrations (see Chapter 2: Figure 1 for potential changes in DOC concentration in relation to flow variability);

5. The volume of water required to achieve adequate connectivity with the surrounding landscape is dependent on river geomorphology (e.g., deeply incised river benches as seen at Boggabri will require greater flows to connect with the floodplain than shallower river banks at Bugilbone) with greater lateral connectivity achieved towards the end of the system with relatively smaller increases in flow;

6. When water is not entering the lower Namoi via River unregulated tributaries the timing of regulated environmental releases should coincide with environmental factors that will foster the best benefits from the release. For example leaf fall from native species (e.g., river red gums) is greatest in summer, however targeting releases in autumn rather than summer will ensure increased nutritional value is delivered to the river ecosystem as a result of a period of terrestrial ageing of the leaf litter prior to wetting (Watkins ***et al***. 2010);

7. Following on from the above point, the utilisation of supplementary 'environmental' flows would be most beneficial in river zones where extant native riparian exists. There would be little environmental benefit (from a food web stimulus perspective) in releasing a parcel of water that inundates a heavily cleared landscape. With this in mind management decisions that incorporate restoration and protection of native riparian vegetation would be highly beneficial. A part from providing a carbon source to the river the vegetation also reduces / maintains water temperatures, increases habitat complexity (e.g., snags) and reduces sediment inputs.

7.3. Future research

There are a number of difficulties that need to be taken into account when attempting to assess the impacts of river regulation in predicting and quantifying biotic responses to altered flow regimes. For example having the ability to distinguish the direct effects of a modified flow regime from impacts associated with land-use change that is often inherently related to water resource development (Bunn and Arthington, 2002). Funding is another major issue and Field ***et al.*** (2007) state that "***commitment needs to be sufficiently long-term to allow a change to be detected over and above the natural temporal fluctuations in the system in question.***" The authors suggest that there are few ecological variables likely to show significant change in less than 5 years, and that 10 years is a sensible minimum target for most ecological monitoring programs.

Ideally the following research / monitoring would enhance the findings of this current study and the understanding of the system:

- Routine (e.g., monthly) DOC sampling across existing water quality sites would help build up a long-term baseline data set that can be then related to spatial and temporal variations of flow and season. This can improve and calibrate the current DOC flow model;
- The capture of medium – high flow events longitudinally across the catchment will help strengthen the IQQM DOC flow scenarios in predicting DOC delivery under modelled and natural flow regimes as presented in Chapter 3. This will provide greater assurance that a particular flow can deliver associated environmental benefits;
- Ideally, other flow scenarios could be run with IQQM to compare DOC delivery under alternative flow management scenarios;
- A greater understanding of the linkages between the river and associated floodplain landscapes is essential. This may be achieved by the use of overlaying tools such

as ArcGIS to determine the extent of extant riparian vegetation (land-use) and also using other spatial analysis tools and techniques to determine river – floodplain geomorphology. This will help determine the magnitude of flow required to inundate a known percentage of adjacent floodplain;

- Ongoing routine and event based sampling (as stated previously) in conjunction with standard water quality sampling (e.g., nutrients and chlorophyll-***a***) will provide a more robust long term data set. This additional information will help determine improvements / changes in water quality that have occurred under the current WSP environmental flow rules (e.g., addressing issues such as algal management).

- Regular time series DOC sampling (at one or more sites, refer to Chapter 3) in relation to varying degrees of discharge (frequency, duration and volume) will help develop the conceptual model (Figure 1) outlined in Chapter 2. Thus providing a greater understanding of the hydrological patterns that best deliver allochthonously derived organic matter to the aquatic ecosystem.

- The findings of Chapters 5 and 6 suggest that the food web will respond under net heterotrophic conditions following an environmental flow (in particular an overbank flow) and associated delivery of terrestrial carbon inputs into the river. This prediction can be further tested by quantifying ecosystem metabolism within the lower Namoi River. For example, three sites within a defined reach (e.g., 100-km) would be monitored over a period of three months (e.g., January – March). The diel changes in dissolved oxygen concentration, temperature, pH, conductivity, depth and turbidity measured, along with the routine sampling of parameters such as chlorophyll-***a***, nutrients and DOC. The data gathered would provide estimates of gross community primary production and respiration; net primary production; and net daily metabolism. Methods have already been developed and implemented in Australian lowland rivers (c.f. Vink ***et al***. 2005; Oliver and Merrick 2006).

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