



Algae-based models to configure consumptive flows for  
ecological benefit in the highly regulated MacKenzie  
River, south-east Australia

by

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## **Abstract**

Many river ecosystems, especially those in arid and semi-arid, are experiencing severe stress due to the increasing demands on the ecosystem services they provide, coupled with anthropogenic catchment impacts and factors associated with climate change and weather extremes. The flow regime of the Mackenzie River was substantially modified since the construction of a water supply reservoir on its upper reach in 1887. Water is now regulated at several locations downstream of the reservoir, creating a substantially modified flow regime, impacting key environmental values of the river. The river receives an environmental flow allocation and the river channel is used to transfer water dedicated for consumptive use. Water Quality and algal monitoring formed the basis of models that were developed evaluate the ecological condition of this working river under base flow and before, during and after freshes that deliver water to users.

Samples of diatoms, soft algae and measurements of water quality were analysed at ten sampling sites for three years (between February 2012 and November 2014) along the MacKenzie River in different seasons and under different flow regimes to understand the spatial and temporal variation in the relationship between algal communities and water quality, and so stream condition. Baseline information on algal communities and water quality was collected during base flow conditions, while experiments on the effect of water releases on algal communities were based on flow regime variations (manipulated flow regimes), specifically on the algae community structure, water quality and ecosystem function. These comprised cease to flow (0 ML/day), low flows (10-15 ML/day), freshes (35-40 ML/day) and high flow (55ML/day) conditions. Physical and chemical characteristics of water, including pH, temperature, turbidity, electrical conductivity, dissolved oxygen, total nitrogen, phosphorus and cations and anions were measured. Biological properties of the algal periphyton communities, including dry mass, ash-free dry mass, chlorophyll-*a* concentration and species composition, were also measured. Furthermore, the DSIAR (Diatom Species Index for Australian Rivers) score was calculated to classify the condition of the waterway.

The results showed the algal species composition changed under different flow regimes along the river. The sensitivity of diatoms to changes in water quality and flow rates deemed them useful indicators of river condition. The results indicated that flows tended to improve DSIAR scores and diatoms versus green algae and cyanobacteria

biomass measures in the mid and lower reaches. The biological properties of the algal periphyton communities, and the species composition, varied between sites under different flow regimes. The accumulation of dry mass (not ash-free) decreased downstream during freshes, however the accumulation of AFDM (ash-free dry mass) gradually increased downstream. The results showed that the concentration of chlorophyll-*a* decreased downstream under water release events.

The Pearson's correlation matrix revealed flow regimes had a significant influence on the water chemistry characteristics and biological properties. The principal component analysis (PCA) illustrated that upstream species of algae were associated with low pH and temperature and higher DO. In contrast downstream species were associated with higher turbidity, TSS, conductivity, TN, and TDS. The correspondence analysis (CA) and detrended correspondence analysis (DCA) showed a split between algal assemblages during water release events in comparison with before and after water release. The canonical correspondence analysis (CCA) identified five significant environmental variables including pH, TSS, Turbidity, TN and TP explaining algal assemblage and structure along the river.

The collected data were used to develop ecological response models based on algae communities living under different flow regimes in the MacKenzie River. The algae-based models across a hydraulic gradient may be useful in water management efforts to find sustainable solutions in the river by balancing environmental and human values. The empirical data and models showed the lower reaches of the river to be in poor condition under low flows, but this condition improved under flows of 35 ML/day, as indicated by the reduction in green algae and cyanobacteria and improvement in DSIAR scores. The results are presented to tailor discharge and duration of the river flows by amalgamation of consumptive and environmental flows to improve the condition of the stream thereby supplementing the flows dedicated to environmental outcomes. Ultimately the findings can be used by management to configure consumptive flows to enhance the for ecological condition of the MacKenzie River.

## **Declaration**

This thesis describes the original work of the author, and has not been submitted previously for a degree or diploma from any university. It contains no material previously published or written by another person except where due reference is made. The thesis is less than 100,000 words in length. Some material from this thesis has been published or presented.



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## **Chapter 1: Introduction**

### **1.1 River management and ecological health**

Traditionally, the management of rivers and streams has focused on extracting water for consumptive use for agriculture, industry and urban water supply (Acreman and Dunbar 2004). The main concern has been the amount of water available and the quality of water with respect to its suitability for anthropogenic use (Norris and Thoms 1999). More recently, water managers have realised that the protection of natural ecological processes in rivers and streams also helps to protect some of their utilisation value, as well as the ecosystem services they provide (Arthington and Pusey 2003, Richter et al. 2003, Gordon et al. 2004, Kondolf et al. 2006).

There is an increasing requirement to conserve and restore the ecological and biological health of rivers and their associated aquatic ecosystems which supported by international, national and regional legislation (Acreman and Dunbar 2004, Millennium Ecosystem Assessment 2005, Arthington 2012). To enable this, many organisations at the international (e.g. International Union for Conservation of Nature) and national level (e.g. Australian Land and Water Resources, Murray-Darling Basin Authority, Commonwealth Scientific and Industrial Research Organization (CSIRO), Australian Rivers Institute, The Murray-Darling Freshwater Research Centre, eWater Cooperative Research Centre) have developed methods for determining environmental flow requirements. Typically, these flow requirements specify a regime directed to support the structure and function of aquatic ecosystems within rivers. Current scientific understanding of hydrologic controls on riverine ecosystems, and evidence obtained from river studies, support the development of environmental flow standards at regional scales (Arthington et al. 2006, Poff et al. 2010, Pahl-Wostl et al. 2013).



Anthropogenic modification of waterways and human demands for freshwater are changing the condition of inland aquatic ecosystems worldwide. However, there are many appropriate measures available within water allocation (water abstraction) protocols that may act to minimise environmental impacts and accommodate the flow requirements of key organisms and ecosystems (Poff et al. 2010, Arthington 2012). This approach requires water managers to optimise water availability between consumptive users and the environment. In fact, optimising water allocation procedures between consumptive users and the environment section increases the value of the water by enabling benefits to accrue to both sides of the usual contest for volume. In some cases, the water used for hydropower generation or cooling in an industrial plant can be returned to the river to enhance ecological condition (Acreman and Dunbar 2004). However, while the water quality is high enough to allow the water to be returned to the river without detrimental effects, some physical characteristics may have changed through the industrial process which may limit environmental benefit. Nevertheless, careful treatment of returned consumptive water can help serve the flow needs of stream ecosystems particularly where climate variability and resource demand have affected the natural flows.

The challenge of maintaining and restoring healthy rivers lies in achieving a balance between environmental requirements and the broader social and economic elements which sustain productive industries and communities (Baron et al. 2003, Arthington et al. 2006, Arthington 2012, Boulton et al. 2014, Bunn 2016). There is no better contemporary example of the difficulty of this challenge than Murray-Darling Basin Plan (MDBA 2014a). The uptake of flow management recommendations by agencies and water managers, and their acceptance by regional communities, is

dependent on an understanding of the interdependencies among management actions, ecosystem health and community prosperity and well-being (Ryder et al. 2010).

In Australia, the delivery of “environmental” water has been assisted by legally defining a share of the available water resource for the environment through State and Commonwealth legislation and associated legal instruments (DAWR 2015, DELWP 2015). This has mostly resulted in recognised entitlements of water for the environment. However, environmental entitlements only form part of the overall water balance for most water supply systems and there remains other ways to improve and maintain aquatic ecosystem health.

## **1.2 Environmental flows concept**

The concept of environmental flows was introduced more than a century ago by river scientists and water managers with the intent of supporting healthy river ecosystems (Tharme 2003, Acreman and Dunbar 2004, Poff et al. 2010). The original concept of environmental flows focused only on the level of water in rivers and streams (Acreman and Dunbar 2004). The International Union for Conservation of Nature (IUCN) state that:

*“An environmental flow is the water regime provided within a river, wetland or coastal zone to maintain ecosystems and their benefits where there are competing water uses and where flows are regulated. Environmental flows provide critical contributions to river health, economic development and poverty alleviation, they ensure the continued availability of the many benefits that healthy river and groundwater systems bring to society” (Dyson et al. 2003).*

The concept is often discussed using a variety of terms, including: environmental flows (regime), in-stream flow, environmental allocation or ecological flow requirement (Gustard et al. 1987, Acreman and Dunbar 2004). An environmental flow describes a deliberate water release or naturally occurring flow that is intended to cover all environmental requirements so that, in this process, the quality of water is as important as the quantity (Boulton and Brock 1999, Arthington 2012). There has been considerable international focus (e.g. The Earth Summit in Rio de Janeiro in 1992, the second World Water Forum in The Hague in 2000, and the Johannesburg World Summit on Sustainable Development in 2002) on ecological conservation, including conservation of aquatic ecosystems (Acreman and Dunbar 2004). An environmental flow is considered to be a flow that is of adequate magnitude to meet ecological requirements and management objectives for a river (Acreman and Dunbar 2004, Poff et al. 2010, Arthington 2012, Pahl-Wostl et al. 2013). According to The Brisbane Declaration (2007), “*Environmental flows describe the quantity, timing and quality of water flows required to sustain freshwater and estuarine ecosystems and human livelihoods and well-being that depend upon these ecosystems*”.

The Brisbane Declaration (2007) proposed improvements to the environmental legislation directed to ecosystem conservation and natural resource management and extended these agreements to the allocation of water to an ecosystem, alongside the rights demanded by people. International (e.g. IUCN) and national organisations (such as the Australian Land and Water Resources Audit) advocate for environmental flows in aquatic ecosystems as an important element in water resource management (Richter et al. 2003, Arthington et al. 2006). Overall, the stakeholders, water managers and river scientists try to improve the environmental flow standards at international by updating and applying new evidence.

### **1.2.1 A new opportunity for improving environmental flows**

Water resource managers have identified that the provision of an environmental flow is critical for the maintenance of a healthy working river and to the conservation of the ecological values of a river (Tharme 2003, Arthington 2012). The deliberate release of water into rivers has two components: the consumptive flow and the environmental flow.

Consumptive water is provided for urban use, agriculture, fisheries, industry, commercial and recreational use and is usually allocated with little attention to the stream's environmental needs. Rivers and streams are often used to deliver, or transfer, water from storages to water users or to downstream storages. It is possible to alter the timing and route of this transfer to provide environmental benefit without adversely impacting water users (VEWH 2012).

Although much attention has been directed towards the possibility of improving the environmental flows in some parts of the world (Acreman and Dunbar 2004, Arthington et al. 2006, Poff et al. 2010, Arthington 2012, Pahl-Wostl et al. 2013), research into understanding the structure and function of aquatic ecosystems for optimising and configuring consumptive flow has largely been neglected, as quantifying consumptive water needs is quite well advanced. Indeed, consumptive flows can provide an opportunity to improve and support environmental flows in regulated riverine ecosystems. The exploitation and extraction of water for consumptive users, combined with the anthropogenic alteration of natural flows, have influenced ecosystem function and processes, and so have affected many Australian rivers (Ryder and Boulton 2005, Davis et al. 2010, Powell et al. 2013) and also most of the rivers of the world (Richter et al. 2003, Poff et al. 2010, Poff and Zimmerman 2010, Brown and King 2012).

### **1.2.2 Challenges for healthy river**

The main challenge for river scientists and water managers is to keep rivers healthy whilst sustaining productive industries and communities. To achieve this, the in-stream flow recommendations used by water management agencies, and their acceptance by regional communities, is related to an understanding of the interdependencies of management actions and ecosystem health (Ryder et al. 2010, Bunn 2016). The environmental flow regime relates not only to the volume of flow through a river system, but also the pattern of those flows (e.g. water frequency, water speed and depth of water) (Arthington 2012). The concept of the environmental flow regime has been accompanied by an expectation, and prediction, that ecologists can provide environmental flows prescriptions that sustain and improve the condition of riverine ecosystems (Arthington et al. 2006).

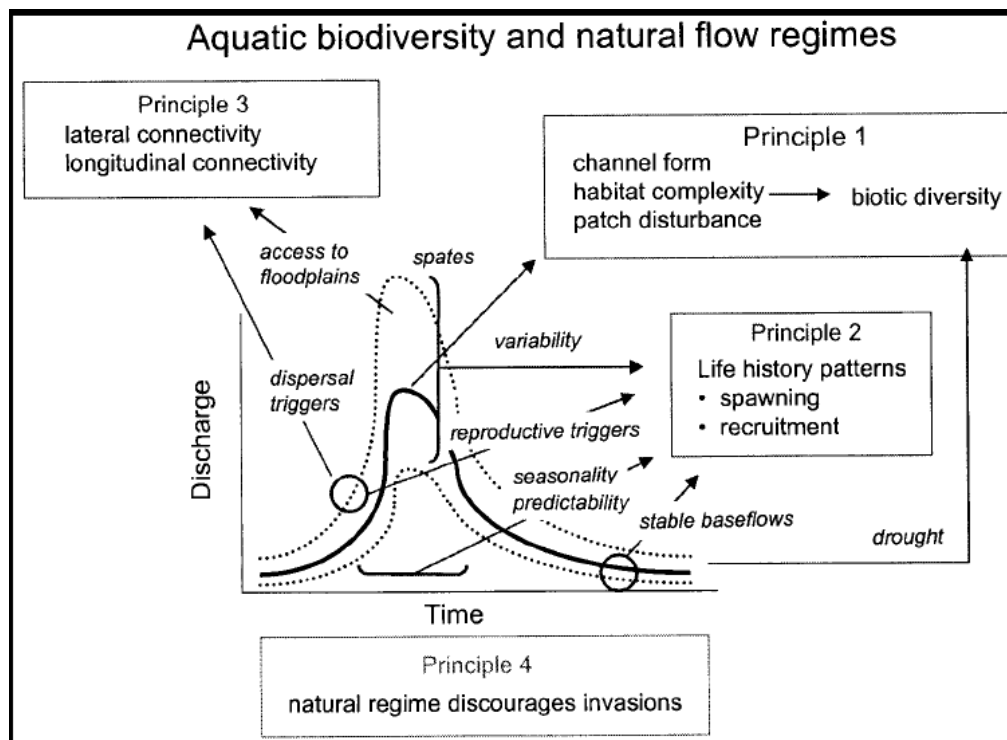
There is the potential for a range of environmental benefits to be obtained during large and small pulsed flows; the general form of consumptive water transfers is via a pulse flow (Watts et al. 2009b). Pulsed flows are water releases from structures, such as dams, reservoir and weirs, to affect water transfers between storages to meet certain demands and water supply requirements (Watts et al. 2009a).

In order to optimise pulsed flows it is essential to monitor the response of the river's condition (river health). Although there are many studies based on modelling, there is a lack of information about the benefits and advantages of the use of pulsed flows for environmental benefit. Indeed, many models have been developed across the world for the riverine ecosystems to address environmental flows (Krysanova and Arnold 2008, Powell et al. 2008, Watts et al. 2009a, Poff et al. 2010, Watts 2010, Hipsey et al. 2011, Yang 2011, Arthington 2012, de Little et al. 2012, Marsh et al. 2012,

Webb et al. 2012, Klaar et al. 2014, King et al. 2015, Horne et al. 2017) but the modelling of consumptive flows to making policy to return water from consumptive flows to the environmental allocation, without compromising the consumptive users values, is a new challenge for river scientists.

### **1.3 Hydro-ecological principles and aquatic biodiversity**

Development of hydro-ecological principles and models help to better understand the ecological requirements (environmental flows) of riverine ecosystems. Bunn and Arthington (2002) developed a conceptual model with four hydro-ecological principles including a) flow regime is master variable in freshwater ecosystems which affects channel form, habitat, distribution and abundance of species, and diversity and species composition of aquatic communities, b) flow regime has major impacts on life history patterns such as spawning and recruitment of the aquatic species, c) flow regime governs and maintains the lateral and longitudinal connectivity in channels which is very important for aquatic species, d) natural flow regime prevents of appearance of the exotic species in the system. In other words, flow regulation introduces exotic and invasive species to the system (Figure 1.1).



**Figure 1.1:** Conceptual model of four hydrological principles indicating how natural flow regime facets maintain aquatic biodiversity in riverine ecosystems. Source: Bunn and Arthington (2002).

### 1.3.1 Climate change, anthropogenic modifications and ecosystem responses

Anthropogenic caused climate variability and climate change are increasingly placing water resources and aquatic ecosystems under stress (Millennium Ecosystem Assessment 2005). This is particularly acute in regions where water season rainfall and runoff are likely to decline (e.g. mid-latitude and Mediterranean climate zones). These are regions of high anthropogenic water use so anthropogenic climate change is likely to increase the stress on water resources and elevate the necessity to carefully manage the quality and volume of water that is available at any point in time (Bond et al. 2008, Dube et al. 2014).

The fundamental challenge of maintaining a healthy river, whilst sustaining productive catchment industries and communities through adaptive management techniques (Hillman and Brierley 2005), can only be addressed if ecosystem health and the potential impacts of management actions are understood prior to making recommendations to water agencies and other end users (Prato 2003, Webb et al. 2014).

Geomorphological characteristics, ecological functions and biological processes of the aquatic ecosystems have been altered, largely in response to flow alterations, which is particularly obvious in downstream river reaches (e.g. introduction of exotic and invasive species, reduction of low tolerance endemic species, synchronisation of reproduction and life cycle, reduction of ecological habitats, and changes in physical shape of the rivers and floodplains) (Bunn and Arthington 2002, Dudgeon et al. 2006, Richter and Thomas 2007, Arthington 2012); such alterations and regulations of flow have had significant impacts on the natural flow regimes of riverine ecosystems (Petts 1984, Poff et al. 1997, Murchie et al. 2008). The alteration and regulation of flow from reservoirs and dams, coupled with catchment water abstraction, causes severe stress in river ecosystems (Shafroth et al. 2010) and, as a result, can have a negative impact on water quality (reduced oxygen levels, and increases in temperature, suspended solid, organic matter and nutrients), biotic structure and function and the metabolism of rivers and streams (Bunn and Arthington 2002, Gordon et al. 2004).

#### **1.4 Justification of the study**

This thesis is focused on the influence of flow regimes on algal periphyton community (biofilm assemblages), water quality and ecosystem function. Such assessments are often neglected, but are helpful to the development of an operational framework to optimise the delivery of water, for both environmental and consumptive users, to



improve overall stream condition. The identification of key indicator taxa can provide evidence for the mechanisms underlying biophysical changes following water release events and allow for the design of an efficient, long-term monitoring program.

Algal communities (including diatoms and soft algae) are ideal indicators for the purpose of understanding the impacts of environmental flows on riverine ecosystems because they reflect directly any physical, chemical and biological changes in a river and reveal changes associated with any flow conditions that result in changes in nutrient concentration, salinity and alkalinity (Prygiel and Coste 1993, Gell 1995, Kelly and Whitton 1998, Hill et al. 2000, Stevenson et al. 2010). Furthermore, the collection of algae is simple, inexpensive and environmentally friendly in comparison with the sampling of other organisms such as macro-invertebrates including snails and mussels, and vertebrates including fish, platypus, frogs, and so constitutes an easily repeated means of assessment (Lowe and Pan 1996, Hill et al. 2000, Stevenson 2014).

Algal periphyton communities (micro-floral communities living attached to the surfaces of submerged substrates) are valuable indicators of ecological disturbance and response, and provide important complementary evidence of river health and water quality over a range of temporal scales. The term ‘periphyton or biofilms’ in limnology refers to microflora communities living attached to the surfaces of submerged substrates in rivers and lakes; it includes algae, bacteria, fungi and protozoa within a mucopolysaccharide matrix.

This research project is significant at both regional and national levels. Outputs from this research are directed towards providing waterway managers with evidence for the configuration of consumptive flows for environment benefits and further toward achieving a healthy working river. This information has the capacity to generate functional and informative models that can be used by water resource decision makers

to improve water transfer for environmental benefit in the MacKenzie River. The outcomes from this research can be applied to other rivers within the region, and in similar contexts across Australia and globally.

At a regional level, this project is intended to generate operational guidelines for the local water agency, GWMWater, to optimise and configure consumptive flows through the MacKenzie River system for the purposes of augmenting environmental benefits. Further, it is anticipated that the outcomes from this project provide evidence of the response of algal communities to stream flow events generally and thereby inform the management of the broader Wimmera and Glenelg River systems, and aquatic systems across south-east Australia.

Nationally, this project is important in the way it examines the response of stream condition and aquatic biota to water release events (i.e. consumptive transfers), with the intent of determining how much water, and in what configuration, needs to be delivered to the environment to sustain a healthy working river. Further, the outcomes of this project form the evidence base for the development of ecological response models that will inform decision making processes directed at configuring flows in other rivers and streams in Australia, and internationally.

### **1.5 Aims and research questions**

The main goal of this research is to develop ecological response models using freshwater algal assemblages to underpin a ‘healthy working river’ and to demonstrate the capacity for multiple benefits to accrue from allocations of scarce water resources. This constitutes using the whole water balance (all water releases) to benefit the environment when possible, and not just relying on the environmental entitlement to achieve positive environmental outcomes. In principle, this research is aimed at

developing a new approach to the allocation of water, from a contest over volume towards a cooperative approach providing multiple social, economic and environmental benefits from its allocation.

The MacKenzie River in western Victoria has been chosen as the case study because this river has been substantially modified for an extended period, and has been shown to be degraded as a consequence of catchment and water resource development (Alluvium 2013). The flow regime of the river is tightly controlled by Grampians Wimmera Mallee Water as the water corporation who owns and operates the dams, weirs and other assets associated with the water supply system. There is great potential to use this system as a natural laboratory to explore the benefits of manipulating consumptive water releases.

This research will address the following objectives in order to improve “operational” flows in this river system:

- (a) To investigate the current hydrological, limnological and environmental values (stream structure and function) related to the MacKenzie River;
- (b) To determine the impact of water release events/transfers on in-stream biological structure (algal periphyton, biological index);
- (c) to biomonitor the short-term and long-term responses to water release events using algal periphyton communities to understand ecosystem response to hydrologic disturbances;
- (d) to develop an ecosystem response model (algal-based model) to inform operational managers in order to provide a means to maximise the ecological benefit (diversity, productivity, stability) from water transfers;

- (e) to understand the stream water quality and hydrological characteristics that influence the algal communities in the system; and
- (f) to use the results from this study, including the experimental programme and ecosystem response model, to develop operational guidelines that will enable the operator to enhance ecological benefit from its water transfers.

*To understand how algal assemblages respond to water releases in the MacKenzie River and how this response can be transferred into models to tailor consumptive water delivery to provide ecological benefits.*

This will be achieved by answering the following questions:

- (1) How does flow, or change in flow regime, affects the aquatic ecosystem in this river?
- (2) What are the flow regime configurations that enhance stream ecosystem condition?
- (3) How can operators improve discharge and duration of consumptive water transfer regimes to maximise environment benefits to this river?

## 1.6 Thesis outline

This thesis contains three main sections: the first section of the thesis is focused on a review of the literature on regulated riverine ecosystems and river flow assessments (**Chapter 2**). An overview of the ecology, geomorphology, hydrology, climatology, vegetation and other natural values in the Grampians region with emphasis on the MacKenzie River, a tributary of the Wimmera River, south-east Australia, (**Chapter 3**), is then provided. This is followed by a detailed presentation of all methods and materials employed in this survey which include the strategy of sampling site selection, sample collection (water, soft algae and diatoms), and analysis of all samples under different flow regimes (**Chapter 4**).

The second section of thesis presents the results of these field surveys to document evidence for the influence of base flows and manipulated flows (freshes and high flows) on algal community structure along the MacKenzie River. This is followed by development of algae-based models intended to configure consumptive flows for ecological benefit in the MacKenzie River (**Chapter 5**).

In the third section of the thesis, the findings of this survey, and its comparison with other published records and operation protocols in configuring consumptive flows for ecological benefit (strategic rules, operation rules and specific rules) in the MacKenzie River, and their implementation in river management elsewhere, are discussed in **Chapter 6**. The final chapter of the thesis focuses on conclusions, recommendations and future research challenges (**Chapter 7**) which highlight the contribution and innovation of this study in river science and management. The recommendations of this work are directed to the recognition of knowledge gaps in terms of consumptive flow configuration for ecological benefits in riverine ecosystems.

## **Chapter 2: Regulated riverine ecosystems, river flow assessments and ecohydrology models: A review**

### **Chapter outline**

In this chapter, regulated riverine ecosystems and the various types of flow in such systems are reviewed. The review also focuses on the assessment of river condition using indicators species (such as algae) and the development of eco-hydrological models across Australia and globally. Finally, the opportunities and constraints of water resource development in the MacKenzie River, as a tributary of Wimmera River, are described.

### **2.1 Regulated riverine ecosystems**

Rivers, streams, wetlands and floodplains are among the systems most heavily impacted by river regulation that has been imposed to ensure reliable provision of water resources for anthropogenic and environmental uses. As a result, most riverine ecosystems are heavily impacted by human activities without even considering the artificial control of waterways and their flows (Arthington 2012). The regulation of a river system is often related to the control of water volume in order to enhance water supply for consumptive users (Millennium Ecosystem Assessment 2005). Such control of flow is used to support human requirements such as water supply for urban usage, industry, agriculture, recreation, flood mitigation and hydro-power generation. Structures such as dams, weirs and channels have been built on rivers to ensure supply for water harvesting and to control water flow (WCD 2000, Lahiri-Dutt 2003). This regulation of river systems has played a central role in the intensification of resource use by modern human society by ensuring the supply of water to cities and industries, controlling the risk of flooding,

allowing for the expansion of food provision through irrigated agriculture, providing hydro-electrical energy and enhancing access to fisheries (WCD 2000, Anderson et al. 2006). However, the impact of these structures and the intensive use of water by people has negative consequences on the condition of riverine ecosystems globally, exacerbating issues associated with eutrophication, increased sediment transport, flow regime alteration and the proliferation of exotic species (Dudgeon et al. 2006, Palau 2006). This physical alteration of rivers has increased globally through the ‘Great Intensification’ of the 20<sup>th</sup> century to service human demands in developed and developing countries (Murchie et al. 2008, González et al. 2010), and its impacts persist today.

Over the last fifty years, thousands of dams have been constructed around the world (e.g. United States, Canada, Europe, Asia and Australia) to supply hydroelectricity for human use (Dynesius and Nilsson 1994, Anderson et al. 2006) that are documented. There are approximately 57,000 large dams (dam wall higher than 15 m), more than 800,000 small dams and countless weirs in over 140 countries across the world (Tharme 2003, Watts et al. 2009a, Rivers 2015). In Australia, it is estimated that there are more than 500 large dams and thousands of smaller structures (e.g. weir and lock) (ANCOLD 2008, Watts et al. 2009a).

The relationship between the physical and chemical and biological changes along rivers is known as the River Continuum Concept (Vannote et al. 1980). The in-stream consequences of river regulation are diverse and include decreased flow regime variation, decreased annual flow amplitude, and changes to the physical patterns of the stream, temperature and other physical and chemical characteristics of the water (Stanford et al. 1996, Bunn and Arthington 2002). Further, structures impede the movement of organisms reducing the ecological connectivity between upstream and



downstream reaches of the river (longitudinal connectivity) and so the biological pattern of the river changes and the native biodiversity decreases as exotic species replace endemic species (Stanford et al. 1996, Bunn and Arthington 2002, Palau 2006). While catchment development increases the run-off rate in the system through the increase in the area of impervious surfaces, river impoundments tend to modify flow patterns that lead to reduction in river discharge (Murchie et al. 2008). This reduction in floods reduces the connectivity between the river and its floodplain (lateral connectivity).

Overall, many riverine ecosystems are under severe stress due to the increasing demands on the ecosystem goods and services they provide (e.g. Colorado River (USA), Nile River (north-eastern Africa), Tigris-Euphrates River (Middle East), Indus River (south Asia), Yellow River (China) and Murray-Darling Basin (Australia)) coupled with anthropogenic catchment impacts and factors associated with climate change and increasing frequencies and magnitudes of weather extremes (Millennium Ecosystem Assessment 2005, Lake and Bond 2007). Due to increasing demands and uncertainty of supply, an increasing number of riverine systems, globally, are becoming more heavily regulated (Calow and Petts 1994, Lanza 1997, Downes et al. 2002, Arthington 2012).

## **2.2 Flow components of riverine ecosystems**

Flow components are defined based on hydrological and ecological process with emphasis on the influence of the different parts of the flow on the ecosystem (DEPI 2013). The components of flow are considered in association with key ecological characteristics of natural flow regimes including variation, duration, magnitude, frequency, timing and the rate of change (Richter et al. 1996, Poff et al. 1997, Arthington 2012). Each type of flow regime has its own ecological function and

hydrological conditions. There are six types of flow that constitute the full flow regime of a river (VEWH 2012), and these are discussed below.

### **2.2.1 Cease to flow**

Cease to flow is when there is no measurable surface flow in the river or stream. This type of flow is a common, natural phenomenon in arid and semi-arid zones, as found in much of inland Australia (Boulton et al. 2000, Boulton et al. 2014). In mid-latitudes it usually occurs in summer and autumn when there is less effective rainfall. Cease to flow may also arise due to regulation, water abstraction and modification in rivers. Flow cessation, particularly in the lower parts of a river, is one the consequences of engineering activities in the upstream reaches (Manariotis and Yannopoulos 2004). During cease to flow, surface water in a river may be limited to isolated pools along the channel, impacting ecological function and hydrological processes and causing some aquatic and riparian biota to experience stress, and limiting connectivity, which can result in local species extinctions (Lloyd et al. 2012, DEPI 2013). Sub-surface flow may continue within the river channel and so hyporheic communities may be less affected.

### **2.2.2 Low flows**

Within the context of the VEWB (2012) classification, low flows are base flows in a river which persists through the dry season of the year (Smakhtin 2001) or during prolonged dry weather (e.g. drought) (Bond et al. 2008, WMO 2015). Whilst water levels are very low, the water flowing through the channel allows pools that have developed to remain connected, allowing the retention of ecological function and hydrological processes, and the maintenance of habitat, aquatic biota and riparian vegetation (Arthington et al. 2007, Robson et al. 2009, Lloyd et al. 2012). When artificially regulating flows, the maintenance of low flow is important for fish communities and their recruitment in the lower parts of rivers (Humphries 1995), as

well as playing a fundamental role in maintaining, preserving and structuring riverine ecosystems (Bunn and Arthington 2002, Thoms and Sheldon 2002, Poff et al. 2010).

While low flows are more frequent in arid and semi-arid zones where there are various degrees of natural intermittency, their persistence remains critical for ecological function which is important to maintain flows to such a level that connectivity is retained (Kennard et al. 2010, Marsh et al. 2012).

### **2.2.3 Freshes**

The stream condition and water quality during low flows may provide short duration peak flow events (Watts et al. 2009b). Pulsed flows or freshes are defined as water releases from structures such as dams, reservoir and weirs, to transfer volumes of water between storages to meet certain demands and water supply requirements (Watts et al. 2009a). However, freshes can occur naturally in rivers and stream. These freshes, organised by water authorities, may continue for several days, and their design can be influenced by ecological processes or by season (Lloyd et al. 2012, DEPI 2013). The freshes may vary according to purpose, for example whether the flow event is designed for the maintenance or for the improvement of water quality, river health or condition in dry seasons, or for the mitigation of flood risks from overspill of the reservoirs in wet seasons (Watts et al. 2009a, DEPI 2013). Freshwater ecologists believe that freshes play key roles in enhancing the variability of natural flow regimes and improving riverine health in aquatic ecosystems (Poff and Allan 1995, Puckridge et al. 1998, DEPI 2013).

### **2.2.4 High flows**

High flows are defined as those which result from intensive rainfall events, particularly during wet seasons when absorption capacity in the surrounding soils is low (DEPI 2013). From a hydrological point of view, high flows can be described as a specific peak

discharge event with a particular frequency and duration (Gordon et al. 2004, DEPI 2013). In mid-latitude zones the probability of peak flows is high during winter and spring seasons but may arise from particularly intensive rainfall events in summer. The high flow plays a significant role in hydrological processes and ecological function along the river by maintaining river habitats and creating new habitats for aquatic biota (Brizga and Finlayson 1999, Arthington 2012). Furthermore, a link has been acknowledged between high flows and fish breeding in rivers, as high flows play a key ecological role in enabling fish migration through the catchment (Humphries 1995).

#### **2.2.5 Bankfull flows**

Bankfull flows are flows that reach the top of the channel bank with little or no overflow to the floodplain. They are larger than high flow and they mostly occur during the wet seasons especially, in mid-latitude zones, during winter and spring. During bankfull conditions all river benches are inundated. From a geomorphic perspective, bankfull flows are important in shaping all branches and channels within a river catchment. These flows also play a key role in maintaining and preserving in-stream habitats (Stromberg et al. 2007, DEPI 2013). In particular they enable the formation of a more diverse array of in-stream habitats, which enhances the diversity of aquatic flora and fauna including macrophytes, algae, macroinvertebrates, fish and maintaining key species such as the platypus (WCMA 2015). In addition to this, a bankfull flow aids improvement of riparian vegetation condition because the major stream branches inundate (Robertson et al. 2001).

#### **2.2.6 Overbank flows**

Flows with greater discharge than bankfull flows breach banks and create overbank flows. These flows usually occur in wet seasons, and impact whole catchments,

particularly in downstream reaches and across the floodplain (Lloyd et al. 2012, DEPI 2013). From an ecological point of view, overbank flows are important to improve stream condition, water quality and increasing the diversity of habitats for aquatic biota. These flows are also important for returning carbon to the stream ecosystem (Robertson et al. 2001). Overbank flows have an array of ecological benefits (e.g. the watering of floodplain vegetation (Nielsen et al. 2000)) enabling the colonisation of macroinvertebrates (Mitchell and Richards 1992, Rosenberg and Resh 1993, Quinn et al. 2000) and lateral connections of river-floodplain systems (Thoms 2003)). It has been suggested overbank flows are critical factor for stream ecosystem function under the Flood Pulse Concept which identifies the supply nutrients to floodplain areas and the provision of carbon from the floodplain to the stream as critical for its metabolism and ecosystem function (Junk 1999).

### **2.3 Environmental flow regimes**

Many terms such as environmental water allocations (EWAs), environmental flows (E-flows), ecological and environmental water requirements (EEWRs), ecological water demands, in-stream flow and environmental water consumption are widely used by aquatic ecologists to refer to the flows that maintain and preserve ecological and biophysical characteristics of the rivers (Acreman and Dunbar 2004). Such a dynamic extends from headstream to downstream reaches, across main channels and branches, and into groundwater, across floodplains, and includes estuaries and coastal zones (Arthington 2012, Lloyd et al. 2012). The flow regime is a significant feature that directly influences the physical river environment (Gordon et al. 2004). Furthermore, any physical and chemical changes in streams owing to flow regimes will affect riverine ecosystems. Any deviation from the natural flow regime must be considered when

trying to understand water quality and the allocation of water for environmental benefits (Poff et al. 1997, Norris and Thoms 1999, Kennard et al. 2010).

Understanding the influence of environmental flows through the measurement of the responses of aquatic flora and fauna is central to river management as many aquatic organisms (e.g. macro-invertebrates and algae) are sensitive to any changes to the ecosystem flow (Norris and Thoms 1999). Therefore, equitable and effective sharing of water, between consumptive users and the environment, is important. The main challenge for river scientists is to inform decision makers to balance the demands that arise from the socioeconomic and environmental values of the system (Poff et al. 2003, Arthington 2012, Bunn et al. 2014). For example, the necessity of environmental flows has increased significantly because of the incidence of drought, salinisation and the increased scarcity of water in southern Australia (Bond et al. 2008, Barton et al. 2011). Therefore, operational plans are essential for Australian rivers, and these seek a balance between water for consumptive and environmental uses (Arthington and Pusey 2003).

The natural flow regimes in Australia have been classified to reflect environmental flows (Kennard et al. 2010). In Victoria (Australia), the Department of Environment, Land, Water and Planning (DELWP) has the responsibility of developing environmental flows policies across the Victorian catchments. The water resources are managed through a ‘whole of system’ approach with an entitlement and allocation framework that encompasses resources for both consumptive and environmental purposes. Entitlements are provided based on ‘resource sharing’ principles among all entitlement holders using an agreed set of rules (DELWP 2015).

### **2.3.1 Environmental flow assessment**

Dams and reservoirs to modify the main facets of stream flow regimes including flow magnitude, duration, timing, frequency and natural flow rate. These structures have had considerable impacts to stream chemical and physical conditions including water temperature (Todd et al. 2005), sediment transport (Vörösmarty et al. 2003), nutrient flux (Turner et al. 2003) and salinity patterns (Rood and Mahoney 1990, Richter and Thomas 2007). As a result the biological processes, ecological functions and geomorphological characteristics of river ecosystems have changed greatly (Bunn and Arthington 2002, Richter and Thomas 2007, Arthington 2012). Many studies have shown that alterations in the riverine ecosystems across the world can affect ecological processes. For example evidence has been presented for America (Sparks 1992, Richter et al. 1997, Richter et al. 2003, Poff et al. 2010); Europe (Dynesius and Nilsson 1994); Asia (Chen 1992, Postel 1995) and Australia (Walker et al. 1995, Arthington 2012, MDBA 2014b).

Richter et al. (1997) introduced a new approach entitled “Range of Variability Approach” to define how much water is needed for a river. The Range of Variability Approach (RVA) employs aquatic ecology theory and river flow regime facets (magnitude, duration, frequency, timing and rate of change) to promote sustainable aquatic ecosystems (Richter et al. 1997). In this way ecosystem structure and processes can be changed by flow regulation and alteration (Ryder and Boulton 2005, Boulton et al. 2014). The ecological impact of flow regulation during the last few decades showed the important role of water allocation in conserving riverine ecosystems (Petts 2009).

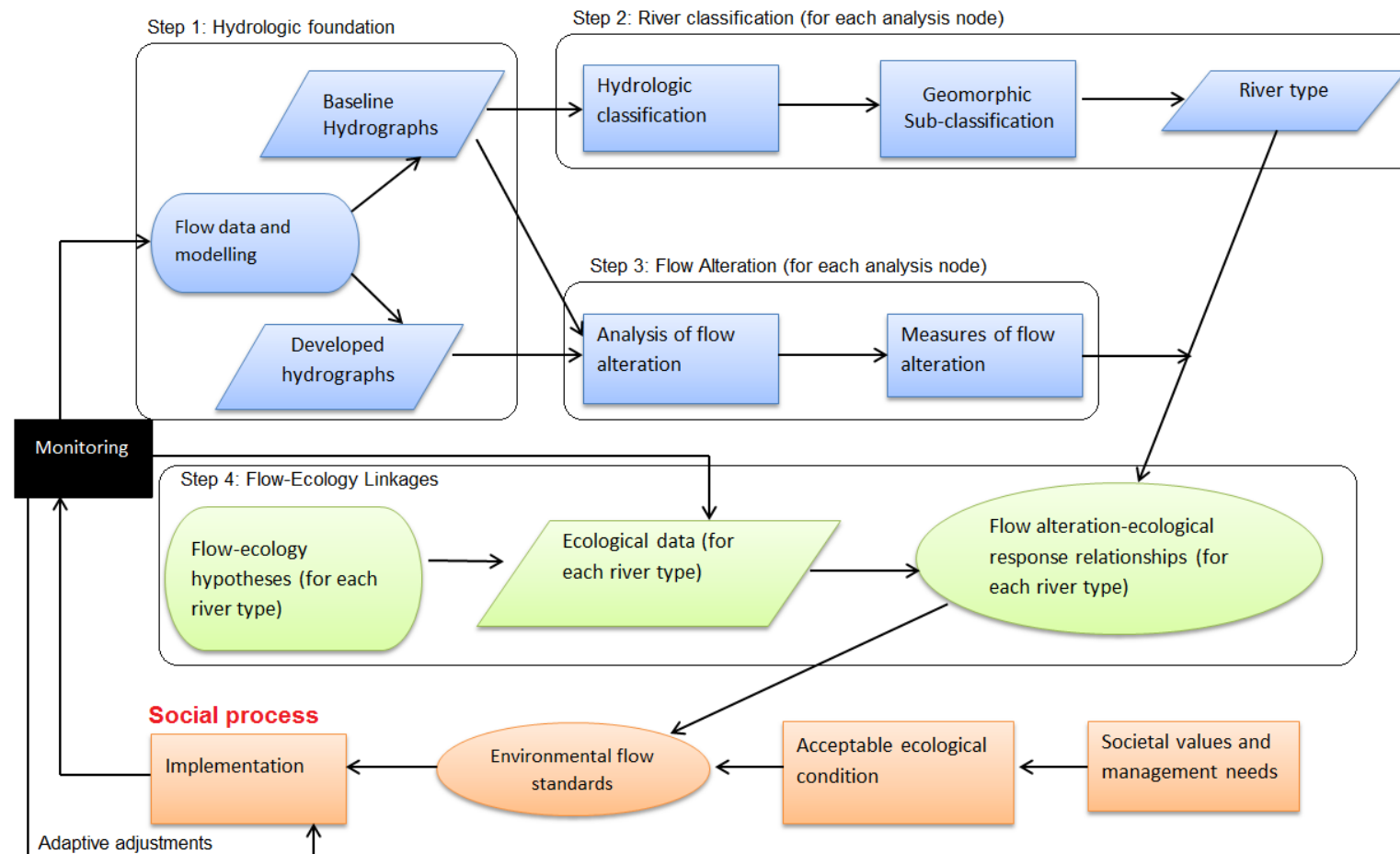
Arranging appropriate monitoring approaches and the selection of ecological indicators is a critical stage in environmental flow assessment and implementation (Arthington 2012, Lloyd et al. 2012). Until now, many models and frameworks have been suggested for environmental flow assessment worldwide. Recently, the

international freshwater science community has reached a consensus regarding this and, collectively, they advocate for the use of the approach of ecological limits of hydrologic alteration (ELOHA) (Poff et al. 2010), which is a monitoring approach within the ecosystem, ecological community, and organisms identified at family and species levels. The ELOHA framework helps decision makers and stakeholders to identify ecological and hydrological alterations during flow assessment. The authors advocate that two aims should be achieved during monitoring. First, an accepted monitoring method should assess whether the recommended environmental flow rules are addressed during the monitoring process. Second, water management regulations and environmental flow rules should be optimised, in appropriate ways and methods, to obtain desired ecological and biogeochemical outcomes (Arthington 2012).

These investigations aimed to support and improve the ecological condition of a system by applying environmental flows in riverine ecosystems. The ecological limitation of hydrologic alteration (ELOHA) has been designed by Poff et al. (2010) to develop regional environmental flows. The ELOHA framework comprises both scientific and social process towards river management strategies which can be addressed by hydrological and ecological models simultaneously (Figure 2.1). The ELOHA framework emphasises hydrologic modelling, ecological characteristics, current condition deviation, flow alteration, ecological response, uncertainty and adaptive management for each river type (Poff et al. 2010). The ELOHA framework is a good roadmap for ecologist and hydrologists to develop hydro-ecological models for updating and improving environmental flows (Poff et al. 2010, Klaar et al. 2014).



## Scientific process



**Figure 2.1:** The scientific and social processes in ecological limits of hydrologic alteration (ELOHA) (redrawn from Poff et al. 2010).

## **2.4 Consumptive flows**

Consumptive water is water withdrawn from a river or reservoir for human purposes including urban usage, industrial and commercial needs, irrigation, agriculture (livestock), fisheries and recreation. The quantity of a river's water volume that is evaporated, transpired, lost to leakage or in any other way removed from the river, is included under consumptive flows for the purposes of consumption (Hutson et al. 2004).

Human demands for water are increasing worldwide due to population growth, while in some areas surface and groundwater use is an increasingly critical source of water owing to declining rainfall (Vörösmarty et al. 2000, Konikow and Kendy 2005). It is increasingly obvious, in catchments such as the Murray Darling Basin, that there is not sufficient water to supply all consumptive user demands and environmental requirements (MDBA 2014b) and, tensions between allocations to the environment and consumptive users are increasing. In order to cope with such a water demand crisis, appropriate guidelines are required to direct water allocations from freshwater river systems in the future (Mercer et al. 2007, MDBA 2014b). These guidelines need to be based on evidence provided by river scientists that assist water managers, policymakers and stakeholders in their decision making processes. There are various methods, such as 'best available science' (Ryder et al. 2010), evidence-based practice (Webb et al. 2010a), scenario-based (Acreman and Dunbar 2004) and objective-based approaches (Tharme 2003), to help water managers make water allocation decisions between consumptive users and environmental sectors. However, optimisation of water allocation amongst consumptive users remains a controversial topic as the driving factor that determines river ecosystem health and sustainability is the availability of water, and, as such, sustainable water management needs co-operation and balance between the social, economic and environmental values (Powell et al. 2013).

Returning water to the environment by optimising the balance between consumptive users and environment requirements in order to gain ecological benefits, without unduly impacting the consumptive supply, is a new challenge for river scientists and water managers (Nichols et al. 2013). Although some efforts have been directed towards delivering consumptive flows to enhance environmental flows, the ecological benefits that may accrue from consumptive flows *per se* have largely been neglected (Arthington et al. 2006, Nichols et al. 2013, Powell et al. 2013). In fact, the neglect of this portion of the water holding poses a new challenge for river ecologists and engineers to develop eco-hydrological models which provide a precise rationale that covers benefits for both consumptive users and the environment (Powell et al. 2013). The new method should find appropriate ways to effectively ‘borrow or return’ some of the consumptive flow asset, without compromising the consumptive user’s values, to assist in efforts to improve the state of the river’s environment.

## **2.5 Assessment of river and stream condition**

A large number of indices have been used for the evaluation of river condition. These indices generally classify river condition qualitatively (e.g. in categories ranging from poor to excellent) (Ladson et al. 1999, Hill et al. 2003, Gordon et al. 2004, Acreman and Ferguson 2010). In South Africa, in order to assess the river health and stream condition the Index of Stream Geomorphology has been developed by South African Government’s River Health Program where geomorphic variables are the main elements measured under the assumption that the morphology of a channel provides the physical frame for all aquatic biota in the river (Rowntree and Wadeson 1998, Gordon et al. 2004). In Europe and North America the River Habitat Quality survey is used for assessments of river health and condition (Raven 1998). River geomorphological and

hydrological characteristics are often used for the evaluation of river health (Gordon et al. 2004)

The Index of Stream Condition (ISC) was developed and tested in some parts of Australia (Ladson et al. 1999). The ISC uses a subjective ranking system based on a comparison between the current condition of a river and its known or modelled pristine condition and includes measured physical characteristics of the river. Indeed, in this index there are five main sub-indices that are evaluated by their own indicators: hydrology, physical form, streamside zone, water quality and aquatic life. These individual components are rated, summed and scaled so that each sub-index value is between 0 and 10, providing a basis for reporting the environmental condition of rivers to the community and government (Ladson et al. 1999, Gordon et al. 2004). The ISC is a valuable index that provides a measure of the condition of river reaches that is comparable between rivers, and between reaches, and unlike some rapid assessment methods (e.g. biotic indices), it includes basic hydrological, water quality and macro-benthos data (Ladson et al. 1999, Gordon et al. 2004).

### **2.5.1 Biological monitoring**

Many biological monitoring systems have been developed based on fish and benthic macro-invertebrates, macrophytes, riparian vegetation and algae (Rosenberg and Resh 1993, Kelly and Whitton 1995, Norris and Morris 1995, Whitton and Kelly 1995, Harris and Silveira 1999, Munné et al. 2003, Gordon et al. 2004). In-stream biomonitoring can be undertaken using all these macro- and micro-organisms as biological indicators. However, benthic macro-invertebrates are used by a large number of scientists because they are sensitive to water degradation and river health and are easy to collect and identify (Rosenberg and Resh 1993, Reynoldson et al. 1997, Metzeling et al. 2006). Although benthic macro-invertebrates have many advantages in water quality

biomonitoring (e.g. easy to collect and identify) there are some issues in water bodies (e.g. eutrophication) where they show limited reaction or response (Kelly and Whitton 1998, Atazadeh et al. 2007). Algae react directly to changes in nutrients whereas invertebrates generally respond indirectly – mostly through the influence of water quality on habitat. For this reason, some approaches to understanding river condition have been based on algal biofilm/diatom communities since they are sensitive to many physical, chemical and biological changes (Hill et al. 2000, Chessman et al. 2007, Stevenson et al. 2010).

Biofilms are a major component of river food webs, and are central to stream nutrient and biogeochemical processes (Burns and Ryder 2001, Ryder et al. 2006, Stevenson 2014). Microalgae are the main food source for aquatic fauna (e.g. mayfly and snail) in freshwater ecosystems. Indeed, algae produce and synthesize organic matter (carbon) allow it to enter the food web from which it finally reaches higher trophic consumers (e.g. fish and waterbirds) (Bunn et al. 2006a, Guo et al. 2016a). In other words, algae are the essential part of food webs and biogeochemical cycling in freshwater ecosystems (Stevenson 2014). It has been reported that epiphytic algae are an appropriate food source for stream invertebrates because freshwater algae have high concentrations of polyunsaturated fatty acids (Torres-Ruiz et al. 2007, Guo et al. 2016a, Guo et al. 2016b). It has also been revealed that, among the algal groups, diatoms and cryptophytes provide higher quality food for aquatic invertebrates because of long chain Omega-3 polyunsaturated fatty acids (Brett and Muller-Navarra 1997, Guo et al. 2016b). However, the primary production of these **key indicator species** (indicators of river health) can be limited or decreased after release of water with high turbidity impacting on light availability (Bunn et al. 1999, Davies et al. 2008). In fact, turbidity,

shading, shear stress, and low nutrients are significant inhibitors for algal primary production within key indicator species in rivers.

Algae often respond to changes in environmental conditions, due to their sensitivity, before effects on higher organisms are detected (Kelly and Whitton 1995, Stevenson et al. 2010). Flow variation in rivers has been shown to affect biofilm structure (Ryder et al. 2006) and ecosystem processes (Ryder 2004, Ryder and Miller 2005). Within the biofilm, diatom assemblages are highly responsive to shifts in water quality (Reid et al. 1995), so their identification can reveal ecological responses to flow-driven changes in stream water quality.

### **2.5.2 Biological indices**

Several features of the algal periphyton community, including structure, diversity, similarity, evenness and dominance have been used in various biological indices (Ziglio et al. 2006). However, these biological indices have been criticised (Suter 1993) due to the reduction of data into a single value, which has affected the statistical behaviour of the indices. Nevertheless these indices are well developed and utilised in most countries (e.g. USA, UK, France, Spain, Australia and Canada). The legislation of most countries require that their agencies and companies (e.g. water companies and other associate companies) use biological indices for assessing water quality, stream condition and the impact of water abstraction in riverine ecosystems (Gordon et al. 2004). Biological monitoring is a valuable tool in water resources management, a current example being the European Water Framework Directive (established in 2000) to assess stream health across Europe. Therefore, biological indices are part of ecological assessment, useful in interpreting the results of monitoring by simplifying outcomes.

Biological indices can be used in conjunction with multivariate statistical analysis to understand the sensitivity of the aquatic biota and to determine what drivers

control their response (Downes et al. 2002, Gordon et al. 2004). There are a number of biological indices that are derived from multivariate analysis techniques such as RIVPACS (River Invertebrate Prediction And Classification Scheme) which was developed in the UK (Wright et al. 1993, Wright et al. 1998), and BEAST (Benthic Assessment of SedimentT) developed in Canada (Reynoldson et al. 1997, Reynoldson et al. 2000), AusRivAS (Australian River Assessment System) (Coysh et al. 2000), and SIGNAL (Stream Invertebrate Grade Number Average Level) developed in Australia (Chessman et al. 1997).

Multi-metric techniques (biotic integrity indices) are used as an alternative approach as they maintain an integrated balance in adaptive biological systems between elements (e.g. species, genus and assemblage) and processes (e.g. nutrient and energy dynamic, biotic interaction and meta-population process) in natural habitats (Karr 1996). The concept of biotic integrity has been developed for fish (Index of Biotic Integrity; IBI) in shallow rivers in the USA (Karr 1981). According to Gordon et al. (2004), within the biotic integrity index minimal disturbance to the system has negligible impact on the biological integrity of the system. The most well-known biological indices based on biotic integrity are the: IBI (noted above); BIBI (Benthic Index for Biotic Integrity) based on macro-invertebrates (Kerans and Karr 1994); PIBI (Periphyton Index for Biotic Integrity) employing algal periphyton (Hill et al. 2000) and DSIAR (Diatom Species Index for Australian Rivers) using diatoms (Chessman et al. 2007).

## **2.6 Role of algae in ecological assessment of rivers**

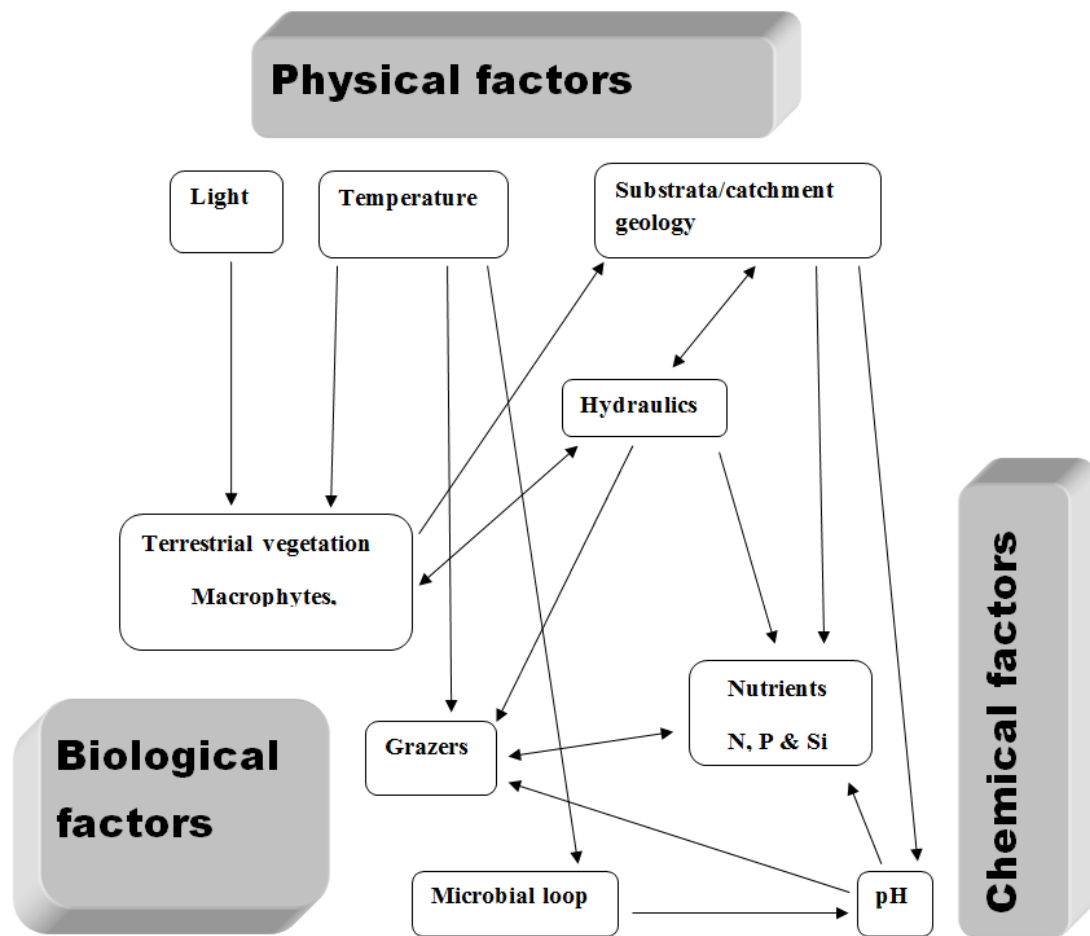
Algae play an important role in aquatic ecosystems as primary producers. They are also sensitive to changes in environmental conditions and respond to disturbance in riverine ecosystems (Dixit et al. 1992, McCormick and Cairns Jr 1994, Biggs et al. 1998, Smol and Cumming 2000, Potapova and Charles 2002, Ryder 2004, Stevenson et al. 2010,

Lacoursière et al. 2011, Stevenson 2014). They have proven to be ideal candidates for monitoring the environmental conditions in aquatic ecosystems across the world (Sládeček 1973, Van Dam et al. 1994, Whitton and Kelly 1995, Lowe and Pan 1996, Kelly and Whitton 1998, Bartleson et al. 2005, Stevenson et al. 2010, Stevenson 2014).

Algal community structure, biomass standing crop and species composition have been used to assess the ecological condition of rivers (Bothwell 1989, McCormick and Stevenson 1998). Furthermore algae are abundant and cosmopolitan in their distribution, can be sampled rapidly and have a wide range of structural (biomass, composition) and functional (metabolism) attributes (Burns and Ryder 2001, Victoria EPA 2003a). Flow variation in rivers has been shown to affect biofilm structure (Biggs and Hickey 1994, Biggs et al. 1998, Ryder et al. 2006) and ecosystem processes (Ryder and Miller 2005). Within the biofilm, diatom algal assemblages are highly responsive to shifts in water chemistry (Reid et al. 1995) and so their composition can reveal ecological responses to flow-driven changes in stream water quality. Using algae to assess ecological status can help to detect effects of human activities in riverine ecosystems (Stevenson 2014), and can be used to provide evidence for decision making in water resource management.

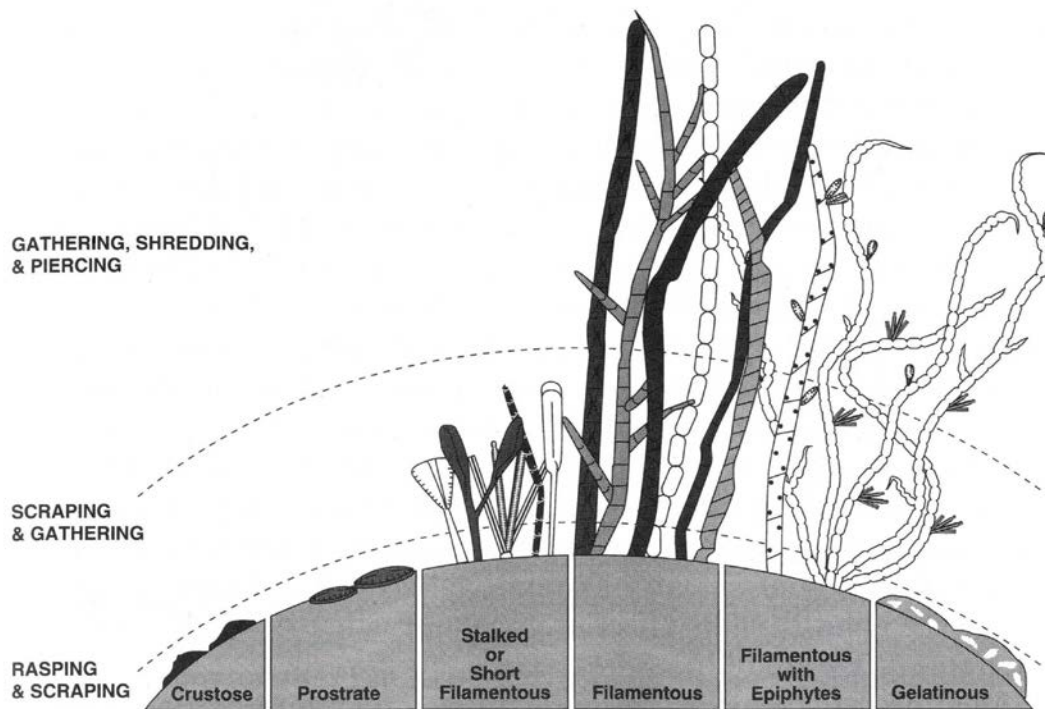
Algae are the main primary producers in lotic and lentic freshwater ecosystems (Round 1970, Vannote et al. 1980, Stevenson et al. 1996, Allan and Castillo 2007) and play an important role in the food web because they are the main source of energy for first order consumers such as small herbivores (e.g. snails, invertebrates). The growth of algae is mainly related to the concentration of nutrients (particularly nitrogen and phosphorus) in the water column and nutrients in the benthos (Stevenson et al. 1996, Rober et al. 2011), however several other factors affect algal growth such as hydrology and predation (Figure 2.2) (Biggs 1996, Stevenson et al. 1996, Law 2011).





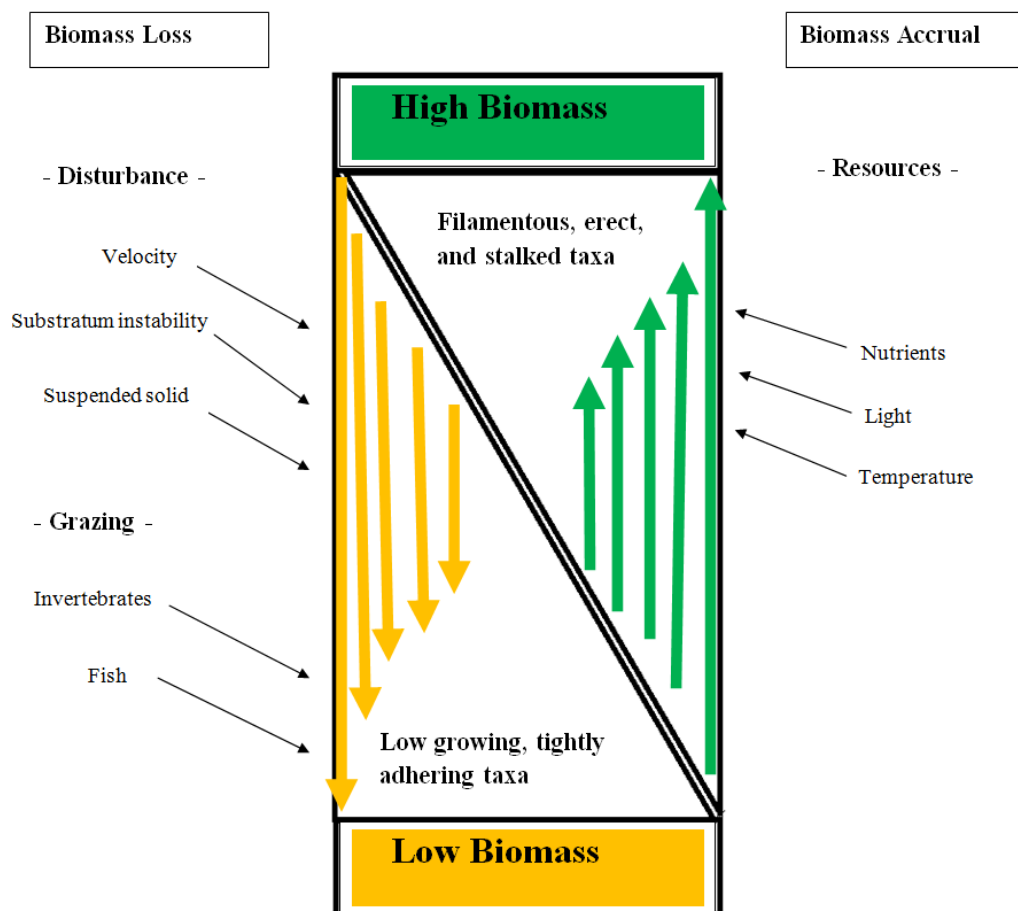
**Figure 2.2:** Major factors which affect benthic algal growth in riverine ecosystems (redrawn from Law 2011)

According to (Steinman 1996), benthic algae can assemble in different forms including filamentous, stalked or colonial aggregates or in unicellular states (Figure 2.3). Benthic algal biomass is a good indicator of water quality and, therefore, river health (Raschke and Schultz 1987) and analyses of algal biomass for the evaluation of river health and anthropogenic modifications in riverine ecosystems is often employed (including the analysis of chlorophyll-*a* concentration, dry mass, ash-free dry mass , bio-volume and peak biomass) (Stevenson et al. 1996).



**Figure 2.3:** Hypothetical representation of major growth forms of algal periphyton assemblages. Source: (Steinman 1996).

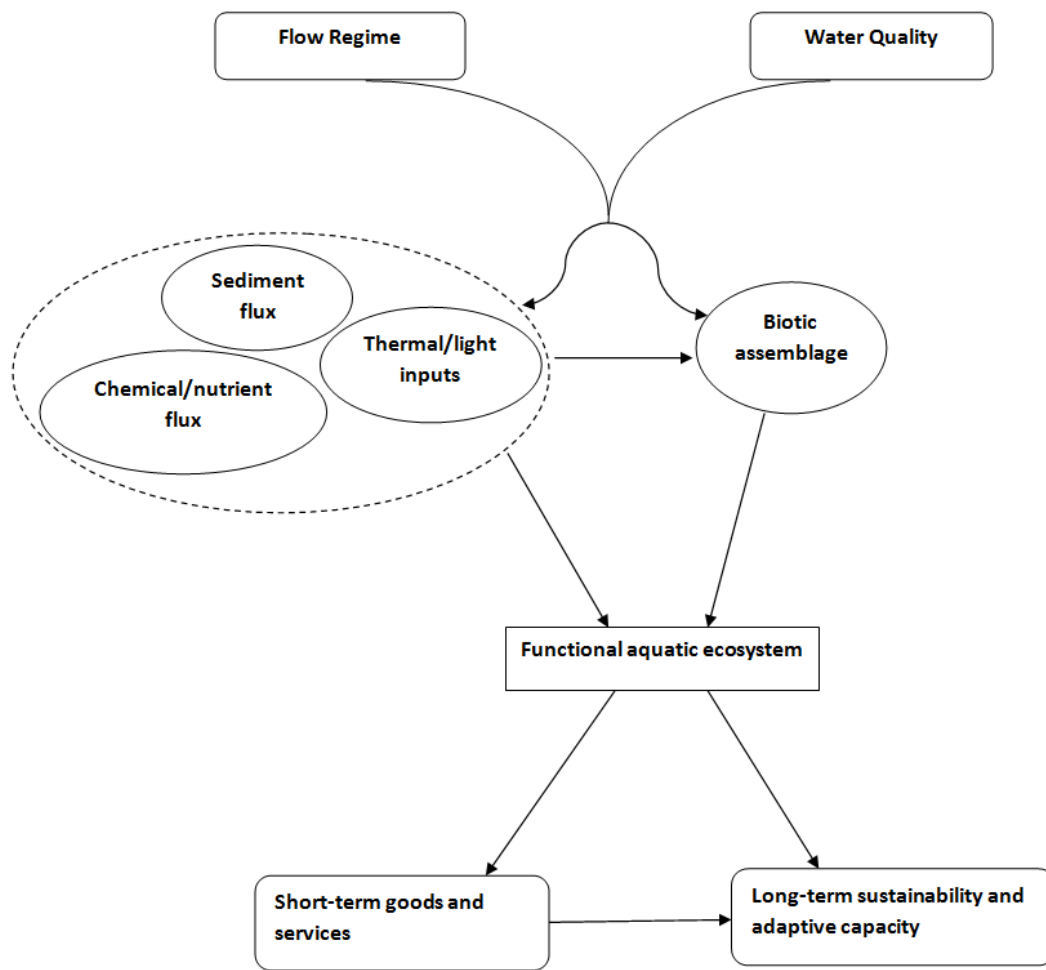
It has been suggested that flow regime, specifically stream velocity, correlates negatively with the concentration of chlorophyll-*a* (Biggs and Hickey 1994, Biggs 1996, Biggs et al. 1999). The concentration of chlorophyll-*a* has a tendency to increase downstream during constant flow. The influence of flow-related disturbance on biomass has also been identified by several researchers (Biggs and Hickey 1994, Biggs 1996, Biggs et al. 1999, Leland 2003, Riseng et al. 2004, Taylor et al. 2004). There are a number of factors that have been observed to decrease the algal biomass including: flow disturbance (velocity), substratum instability, suspended solids and grazers (e.g. invertebrates and fish); whilst nutrients, light and temperature are the main resources that promote algal biomass (Figure 2.4) (Biggs 1996).



**Figure 2.4:** Factors that control the biomass and physical structure of algal periphyton in streams (redrawn from Biggs 1996).

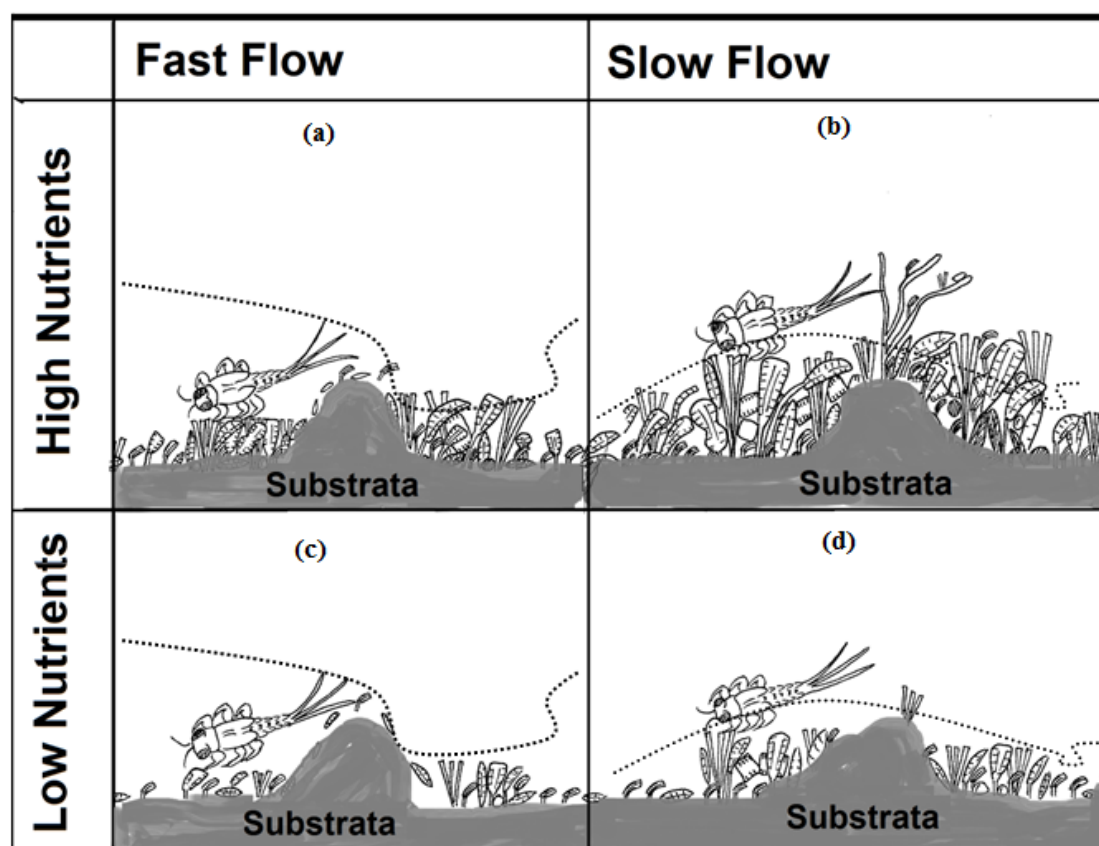
Algal growth and community structure can be influenced by nutrients, light and grazing pressure (Rosemond et al. 1993, Biggs and Lowe 1994). Nutrients and light are top-down controllers while grazers (e.g. fish and snails) are bottom-up controllers of algal biomass (McCormick 1996, Steinman 1996, Biggs et al. 1998). Furthermore the algal community structure may be controlled by flow disturbance (e.g. velocity and turbulence) in lotic systems (Biggs and Smith 2002, Allan and Castillo 2007, Cullis et al. 2013).

Other studies have shown algal colonisation and structure to be highly responsive to shifts in water quality and flow variation (Biggs and Hickey 1994, Robson 2000, Ryder et al. 2006, Allan and Castillo 2007, Robson et al. 2008, Chester and Robson 2014). Flow regime has a significant influence on water quality (oxygen level, temperature, suspended solid, organic matters and other nutrients), biotic structure and function and the metabolism of rivers or streams (Bunn and Arthington 2002, Gordon et al. 2004). According to Baron et al. (2002), the structure and function of aquatic ecosystems was affected by several environmental factors including flow regimes, water quality, sediment and organic materials, nutrients and other chemicals elements, temperature and light (Figure 2.5).



**Figure 2.5:** Conceptual model of the environmental drivers which affect structure and function of aquatic ecosystem (redrawn from Baron et al. 2002)

Law (2011) compiled and discussed the list of environmental factors, including light, temperature, pH, grazers, nutrients (N, P and Si) and hydraulics, which affect the structure and function of benthic algae in riverine ecosystems. Figure 2.6 shows Law's (2011) depiction of the influence of flow regimes and nutrients on algal community structure. Algal community structure, biomass standing crop and species composition decrease under fast flow and low nutrients. In contrast, algal biomass and community structure increase under slow flow and high concentration of nutrients.



**Figure 2.6:** The impact of multiple factors (flow regimes and nutrients) on the algal periphyton community in riverine ecosystems (redrawn from Law 2011).

### **2.6.1 Algae as indicators for assessing stream condition**

Algae are primary producers that power food webs and biogeochemical cycling in aquatic ecosystems (Stevenson 2014). Therefore, algae are an important and critical part of riverine ecosystems. Algae are present in almost every aquatic environment including fresh, brackish, marine and hypersaline water (Bold and Wynne 1984). The scientific study of algae started more than a century ago but the widespread use of these micro-organisms to assess the environmental condition in rivers and streams only began in the last century (Kolkwitz and Marsson 1908, Hustedt 1937, Sládecek 1973, Lowe 1974, Watanabe et al. 1986, Hill et al. 2000, Stevenson et al. 2010). While algae communities in rivers are often diverse and are rarely homogenous (Kelly et al. 1998, Kelly et al. 2001), some researchers have attempted to define discrete “epilithon” (on rock), “epidendron or epixylon” (on woody debris) “epiphyton” (on plants), “episammon” (on sand), “epipelon” (on mud) and “epizoon” (on animals) communities (Lowe and Laliberte 1996, Kelly et al. 2001).

A number of researchers have suggested that the floristic composition of algae in the benthos could be used for monitoring water quality, stream condition and eutrophication (Whitton and Kelly 1995, Kelly and Whitton 1998, Perona et al. 1998, Hill et al. 2000, Potapova and Charles 2007, Lebkuecher et al. 2015), however, some studies have put diatoms forward, largely because the diatom-based methods appear to be the most successful in bio-monitoring approaches (Kelly 1998, Stevenson et al. 2010). There are also practical problems in that it is usually more difficult to sample and make quantitative estimates of other algal groups than it is for diatoms, and there is a lack of identification keys for common river algae, especially the green algae (Kelly and Whitton 1995). Nevertheless, it is useful that other groups are tested, in case these can be shown to provide information not easily gained from diatom-based measures. There

are a number of studies that have shown that cyanobacterial and green algae biomass and diversity could be used to monitor eutrophication (Kelly and Whitton 1998, Perona et al. 1998, Codd 2000, Ferreira et al. 2011), especially as these groups can become a nuisance, so their monitoring can directly inform management efforts.

Diatoms not only have enormous ecological and environmental importance but they also play significant roles in biology, biotechnology, material science and engineering (Gordon et al. 2009). However, most emphasis is on their role in ecological assessment of aquatic systems related to water quality (Patrick 1973, Reid et al. 1995, Kwandrans et al. 1998, Atazadeh et al. 2007, Tan et al. 2014), eutrophication (Kelly and Whitton 1995, Potapova and Charles 2007), pollution (Wu and Kow 2002), urbanisation (Sonneman et al. 2001, Newall and Walsh 2005, John 2012), bioassessment (Barbour et al. 1999, John 2003, Chessman et al. 2007) and the general environmental condition of streams and rivers (Fore and Grafe 2002, Stevenson et al. 2010).

Diatoms respond directly to physical, chemical and biological changes in rivers and streams because they are sensitive to many changes in aquatic ecosystems (Hill et al. 2000). Many species reproduce rapidly and respond sensitively to water quality changes. Means of assessing the water quality of streams using diatoms have been developed in North America (Lowe and Pan 1996, Lavoie et al. 2006), Europe (Kelly and Whitton 1995, Kelly 1998, Prygiel et al. 2002), Asia (Watanabe et al. 1986, Tan et al. 2015), South America (Gómez and Licursi 2001), Africa (Bate et al. 2004, Taylor et al. 2007b) and Australia (Chessman et al. 1999, Abal et al. 2006, Chessman et al. 2007, Oeding and Taffs 2017).

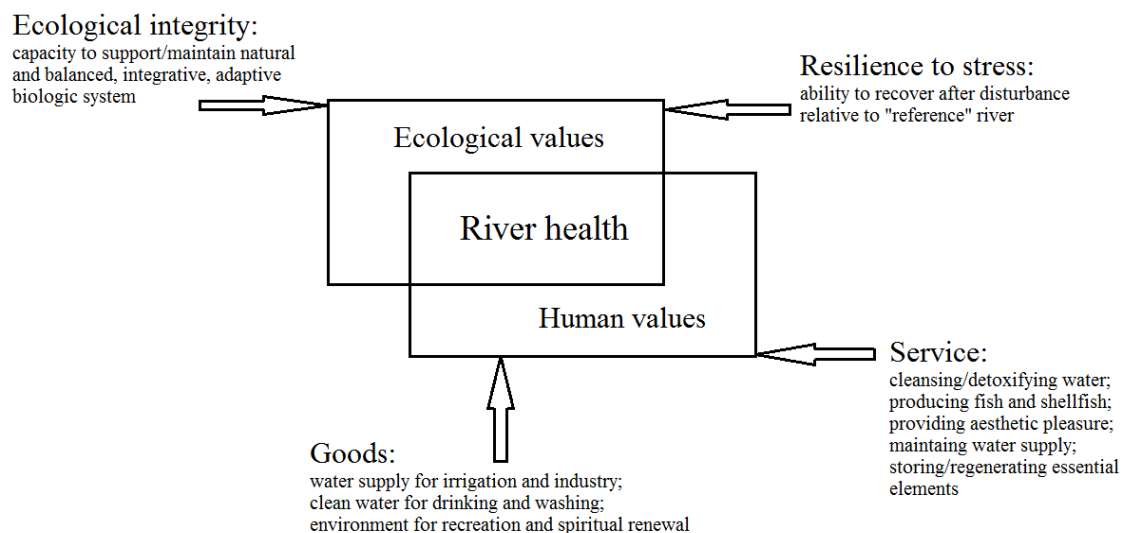
Diatom-based biomonitoring has progressed significantly in Australia recently so that these studies not only focus on palaeolimnological studies of lakes for understanding past climate changes (Gell et al. 2005) but also focus on water quality



monitoring with the development of comprehensive, diatom-based indices for streams and rivers (John 1983, 1993, Philibert et al. 2006, Chessman et al. 2007). Monitoring of diatom assemblages has the potential to provide evidence of stream condition important in programs designed to optimise water use. The taxonomic composition of benthic diatom communities has been widely used for monitoring water quality (Lowe and Pan 1996, Chessman et al. 2007). However, the majority of indices subsequently developed have used populations taken from substrates already growing at a site, with interpretations of the data based on the relative proportions of species present. Overall, a wide range of microalgae, including green algae, cyanobacteria and diatoms, are now being used to understand their ecological response to different flow regimes for the purpose of making decisions on the allocation of water to riverine ecosystems (Snow et al. 2000, Kotsedi et al. 2012).

## 2.7 River health

There are several specifications that can be used to define the health of a river. These include the physical structure of the channel, riparian condition, water chemistry, discharge and aquatic flora and fauna (Bunn et al. 1999, Karr 1999, Norris and Thoms 1999, Boulton et al. 2014). Therefore, the ecological perspective is different when compared to legal and management perspectives. In fact, the ecological concept of river health is related to the condition of the river and this relates to the ecological requirements for any organism and micro-organism living in the river and the ecosystem's ability to recover from the effect of all impacting stressors (Boulton 1999, Boulton and Brock 1999). Conversely, the perception of river health from a solicitor's and water manager's perspective mainly focusses on the use of the water for anthropogenic benefits including water supply for industry and agriculture, and urban usage (Figure 2.8).

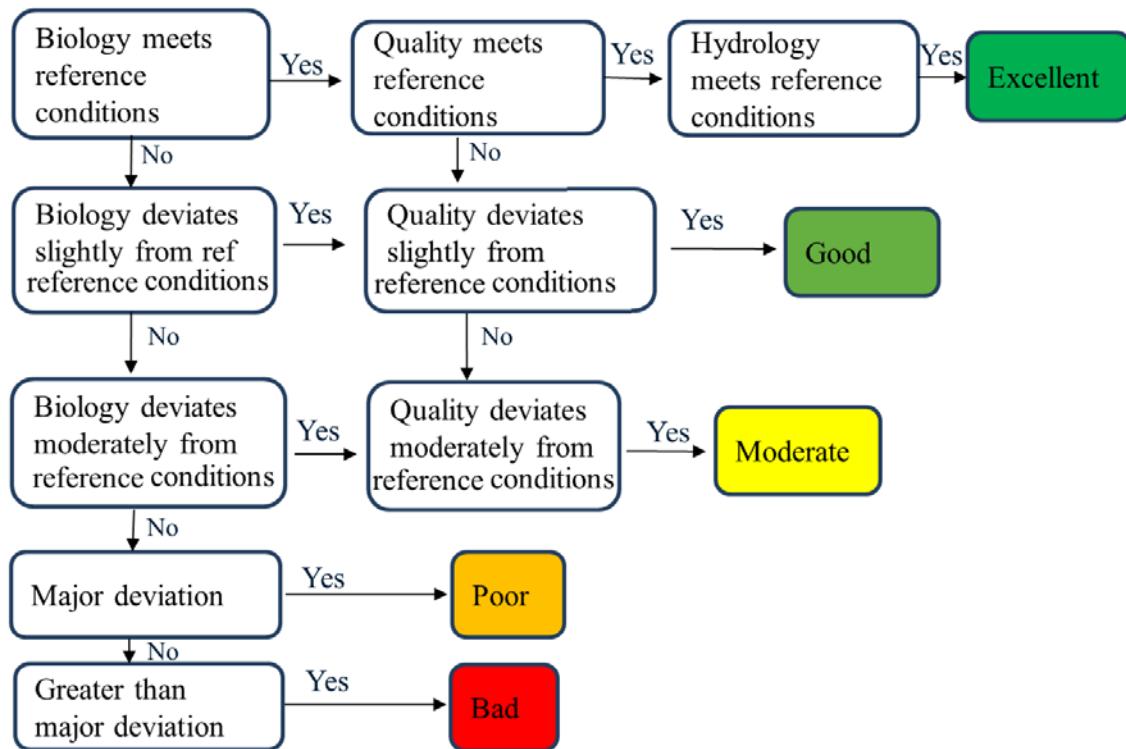


**Figure 2.7:** The concept of river health (redrawn from Boulton 1999)

Ecologists and water managers now agree that an environmental flow for a healthy working river must be considered from all aspects, including flow pattern, flow volume, flow connectivity, flow variability, water quality, water quantity and availability of water according to rainfall and consumption (Arthington 2012, Boulton et al. 2014). The rate of natural flow is central to sustaining a healthy working river so that if it is more than two-thirds of natural the probability of having a healthy working river is high and if the natural flow decreased to less than half natural, the probability of healthy working river is low (Jones et al. 2002).

## **2.8 Water body classification and eco-hydrology models**

Historically water quality monitoring programs have focused on water chemistry criteria (Karr 1991). Today, they are more likely to focus not only on water chemistry but also on biological and hydrological characteristics as well. This is because it is essential to demonstrate the effect of pollutants on biota as the effect on water quality is also influenced by the nature of the receiving waters. Water in rivers can be classified, based on the biology, hydrology and quality, into different ecological categories of conditions such as bad, poor, moderate, good or high (Figure 2.7) (Acreman and Ferguson 2010):



**Figure 2.8:** Water body classification in rivers (redrawn from Acreman and Ferguson 2010).

In the past, modelling of flow regimes in riverine ecosystems (e.g. rivers, streams, reservoirs, lakes and floodplains) was based mostly on engineering and mathematical models (Grigg 1996, Palmer et al. 2005). In fact, water engineers have applied various models including hydraulic and hydrologic models for describing flow regime responses, reactions and repercussions in different circumstances. The main aims in hydraulic modelling are the collection, regulation, measurement, control, storage, transfer and use of the water (Singh and Woolhiser 2002, Jelali and Kroll 2012, Roozbahani et al. 2015). The main purposes of hydrologic modelling are to describe the water's properties such as its physical and chemical characteristics. Hydraulic and hydrologic modelling employs conceptual models, logical models, physical models, statistical models, stochastic models, process-based models, math models and

quantitative models (e.g. HEC-RAS model, SWAT model, REALM model, Multi-objective optimisation models, Bayesian Network models, Artificial Neural Network, regression models) (Sorooshian and Dracup 1980, Gelhar 1986, Beck 1987, Beven 1989, Wurbs 1993, Refsgaard and Knudsen 1996, Laflen et al. 1997, Arnold et al. 1998, Varis and Kuikka 1999, Maidment and Djokic 2000, Tokar and Markus 2000, Downes et al. 2002, Hicks and Peacock 2005, Knebl et al. 2005, Kavetski et al. 2006, Weerts and El Serafy 2006, Yang et al. 2007, Perera 2008, Yilmaz et al. 2008, Webb et al. 2010b, Yang 2011). Today, water scientists and freshwater ecologists are using different types of the ecological response model.

**a) Simple linear models:** These models are being focused on relationship between flow regimes and biological properties to predict ecological outcomes from managed flow events (Driver et al. 2004).

**b) Generalised linear and nonlinear models:** These models are being used to incorporate the greater complexity within the general modelling framework. This allows analyses to incorporate features and avoids pseudo-replication in linear and nonlinear relationships, non-Gaussian residuals or non-normal response data (Mac Nally 2000, 2002, Arthington et al. 2007). The generalised linear and nonlinear models are applied to predict the relationship flow regimes and biological responses (Arthington 2012).

**c) Hierarchical models:** These models are being used to predict environmental outcome under incomplete data collection. In fact, the hierarchical aspect allows data from different sampling time to be combined to increase inferential strength. These models are popular due to high cost of frequently sampling and monitoring in the riverine ecosystem (Webb et al. 2015).

**d) Functional linear models:** These models are being used to predict relationship flow and ecological outcome spatially and temporally without need to quantify hydrologic

metrics. The results of the functional linear models are derived from mathematically functions (Stewart-Koster et al. 2010).

**e) Machine learning approaches:** machine learning is growing rapidly to generate accurate predictive models which help to solve problem in complex systems. Today, river scientist and water managers are using the machine learning approaches to identify and solve the complex issues in water management (Arthington et al. 2007).

Although the allocation of water for consumptive users (industry, agriculture, fisheries, urban, recreational and domestic usage) is based mainly on these engineering and mathematical models, the use of ecological models incorporating biological indices has great potential for improving the way water supply systems are operated and how transfers are made between storages. River scientists and managers are now beginning to appreciate that an over reliance on engineering models has not brought comprehensive solutions in maintaining and restoring riverine ecosystems (Palmer et al. 2005). Improving waterway condition based on ecological criteria is an ongoing challenge for ecologists and river scientists (Bunn et al. 2014).

The optimisation of consumptive flows to provide environmental benefits however, without compromising the consumptive user's values, is a new challenge for river scientists. One of the well-known eco-hydrology models is the Physical Habitat Simulation Model (PHABSIM) which simulates the relationship between stream flows and physical habitats, and so the rate of micro-habitat availability in flow modification (Gore et al. 1998, Booker and Dunbar 2004).

The difference between eco-hydrological models and hydrological models is related to the employment of different responses in the community, at family, genus and species levels. In fact, eco-hydrology is an integrative science which borrows from

ecology, biology, biogeochemical geomorphology, hydrology and hydraulics to describe ecosystem functions and hydrological process in different circumstances.

Under the working river approach, to ensure a river remains both healthy, and contributes to economic production, it is essential to reach a balance between environmental flows and consumptive flows. The environmental flow should be of adequate magnitude and variation to meet ecological requirements and management objectives for a river, accounting not only for the volume of flow through a river system but also a pattern of flow including quantity, timing of release and the quality of the released water (Arthington 2012) that serves the needs of the ecosystem. Whilst consumptive water is provided for urban, agricultural, industrial and recreational use, it is rarely accounted for in an assessment of environmental requirements (Powell et al. 2013) despite rivers being used to deliver/transfer water from upstream storages to water users or downstream storages. Simple modification to the timing and route of this water transfer can provide a wealth of environmental benefits with minimal impact on end users (VEWH 2012). Yet the optimisation of the water allocation amongst consumptive users remains a controversial topic as the driving factor that determines river ecosystem health and sustainability is the availability of water, and, as such, truly sustainable water management needs co-operation and balance between the social, economic and environmental values (Powell et al. 2013).

## **Chapter 3: Study area and study site characteristics**

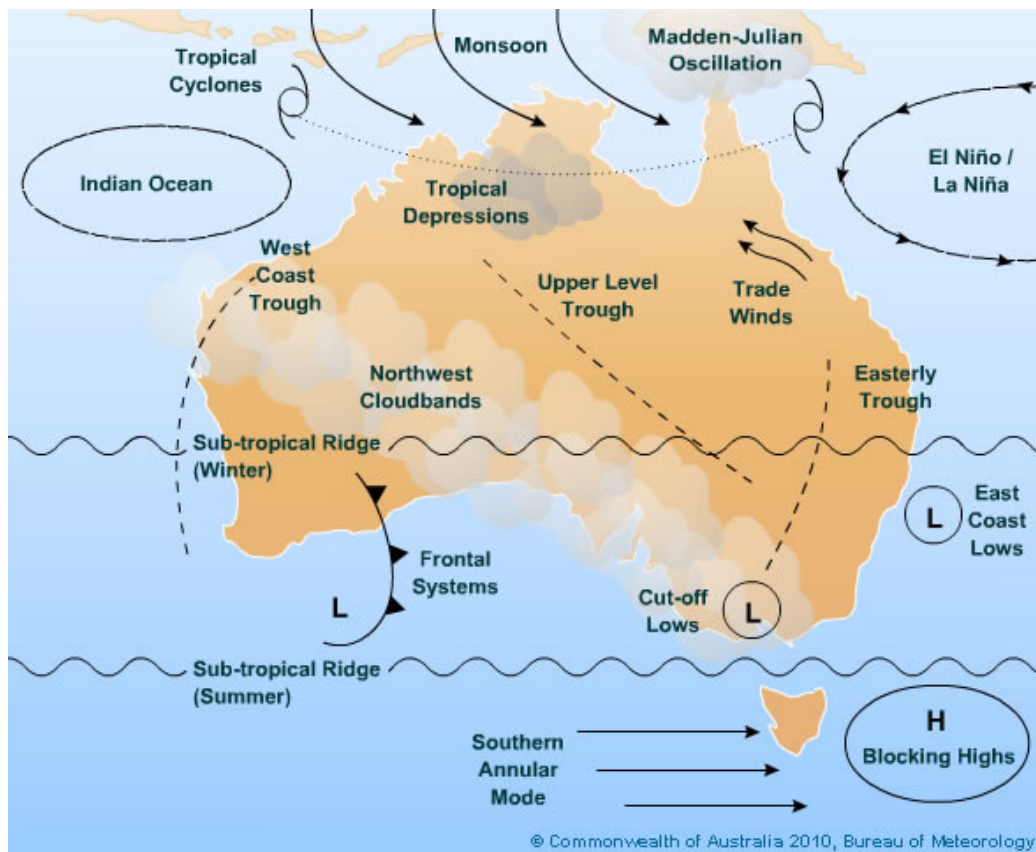
### **Chapter Outline**

In this chapter the environment, climate, vegetation, indigenous and European settlement history, stream flow patterns, water quality and water resources development in western Victoria, Australia is described. The last part of the chapter focuses on the MacKenzie River, a tributary of the Wimmera River located on the northern slopes of the Grampians Ranges in western Victoria.

### **3.1 Australian environment and climate**

The average annual rainfall across Australia is < 500 mm. However rainfall is highly variable across much of Australia with much inter-annual variability; as such periods of drought and flooding are very common (AusBOM 2014). The climate of southeast Australia (including western Victoria) is driven by three main climatic modes including: the El Niño-Southern Oscillation (ENSO), the Southern Annular Mode (SAM) and the Indian Ocean Dipole (IOD) (Nicholls 1988, Power et al. 1999, Kiem et al. 2003, Kiem and Franks 2004, Gillett et al. 2006, Meyers et al. 2007, Murphy and Timbal 2008, Barr 2010, AusBOM 2014) (Figure 3.1).





**Figure 3.1:** The drivers of the climate variability across Australia Source: AusBOM (2014)

ENSO plays a significant role in rainfall variability across eastern Australia (Nicholls 1988, Meyers et al. 2007). the variability of ENSO is depicted as the Southern Oscillation Index (SOI) which is calculated by the sea-level pressure difference between Tahiti and Darwin (Barr 2010, AusBOM 2014). In the neutral state the south east trade winds bringing warm and humid air towards Australia and the western Pacific and it keeps the central Pacific Ocean slightly cool. El Niño relates to the negative phase of ENSO and is associated with warm ocean water in the central and east-central regions of the equatorial Pacific Ocean. The consequence of prolonged El Niño phases for eastern Australia are extended periods of severe drought (Wang and Hendon 2007). La Niña is

the positive phase of ENSO and refers to the extensive cool ocean water off the central and eastern tropical Pacific Ocean. La Niña conditions strengthen the south-east trade winds which drive and enhance wet conditions across the eastern and northern regions of Australia. A larger than usual number of tropical cyclones from November to April (the cyclone season) are associated with La Niña activity (AusBOM 2014).

The Southern Annular Mode (SAM) is defined as a north-south movement of the westerly wind belt which is prevalent in the middle and high latitudes of the southern hemisphere (Marshall 2003). SAM (or the Antarctic Oscillation) is one of the main rainfall drivers in southern Australia. During periods of a positive SAM, the strong westerly wind belt contracts towards Antarctica. Therefore, the wind pressures across southern Australia are weaker than normal restricting the penetration of cold and wet weather across southern Australia (Karpechko et al. 2009). It has been reported that the positive SAM was the main contributor to the Millennium Drought which occurred in Australia between 1997 and 2010 (AusBOM 2014). During a negative SAM, the strong westerly wind belt expands towards the equator and leads to an increase in rainfall and storms across southern Australia (Hendon et al. 2007, AusBOM 2014). The strength of the SAM has been established by employing a number of statistical methods (Marshall 2003, Goodwin et al. 2004, Fogt and Bromwich 2006).

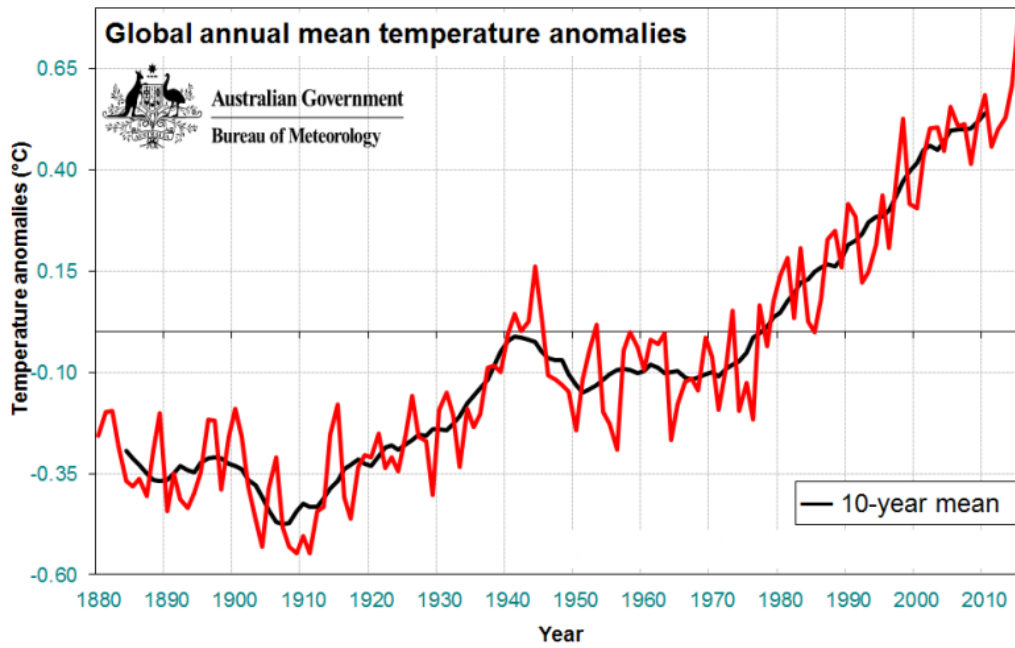
The Indian Ocean Dipole (IOD) manifests as the difference in sea surface water temperature between the eastern and western regions of the Indian Ocean (Feng and Meyers 2003, Cai et al. 2009). The activities of the IOD affect the countries around the Indian Ocean Basin such as Australia, Indonesia and Papua New Guinea. The mechanism of the IOD is similar to ENSO and it has a significant role influencing rainfall variability across Australia (Saji and Yamagata 2003). During a positive phase sea-surface temperatures increase in the western Indian Ocean and winds blow from the

east causing Australia to experience less rainfall as a result of the prevailing offshore winds. During a negative phase of the IOD the sea surface temperature increases in the eastern Indian Ocean and the prevailing winds are from the west toward Australia bringing onshore winds and more rain across Australia (Feng and Meyers 2003, Saji and Yamagata 2003, AusBOM 2014).

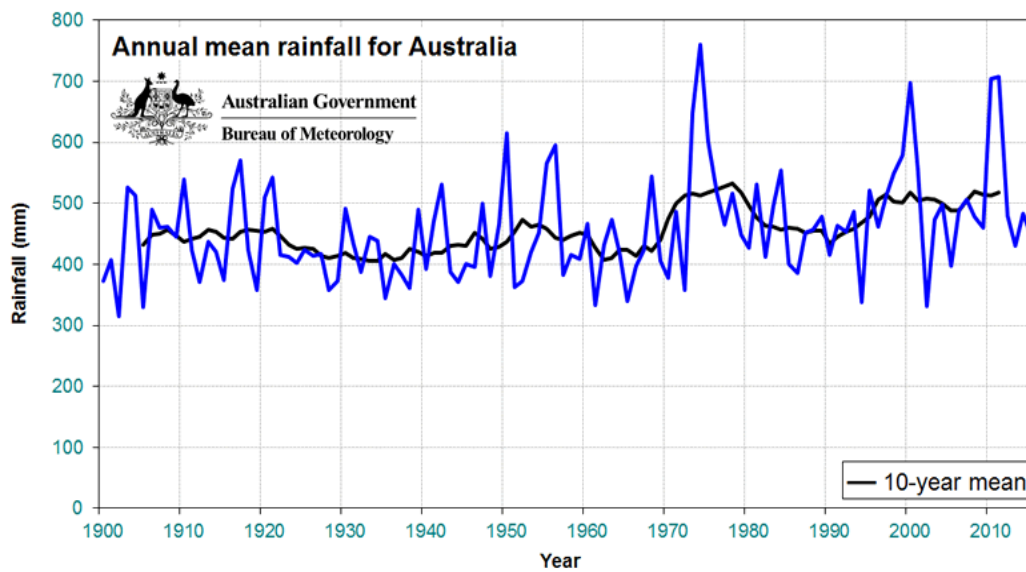
### **3.1.1 Climate change**

Australia is experiencing the impacts of climate change which is altering the intensity and frequency of ENSO (Hughes 2003, Downie 2006). It has been shown that the intensity of El Niño phases have increased whilst La Niña phases have subsequently decreased during the last century (Plummer et al. 1999, Hughes 2003). Since 1910, but particularly in the recent decades, the temperature has increased around 0.8°C globally (Figure 3.2) (Collins 2000, Hughes 2003, Suppiah et al. 2007, Coumou and Rahmstorf 2012, AusBOM 2015). The climatic warming occurs in winter and spring, and has led to a night-time temperature increase of approximately 0.96°C (Suppiah et al. 2007).

The average annual rainfall in Australia has fluctuated, with the highest falls occurring during 1973-75 (Figure 3.3) (Collins and Della-Marta 1999, Gergis et al. 2012, AusBOM 2015). Overall, climate change has influenced the annual temperature, the amount of precipitation, and the incidence of tropical cyclones across Australia (Hughes 2003, Knutson et al. 2010, AusBOM 2015).



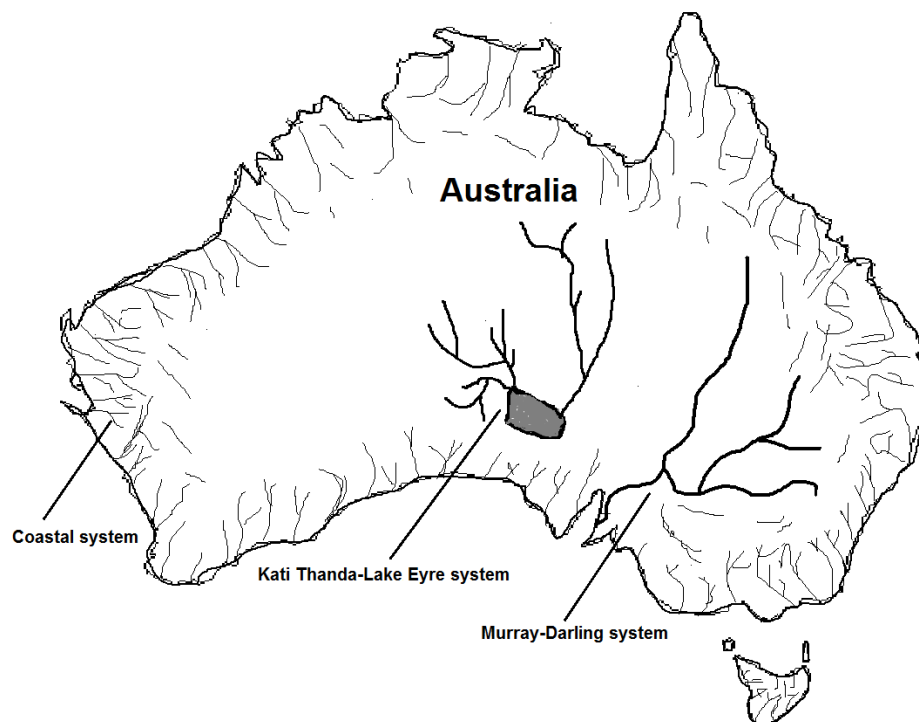
**Figure 3.2:** Global annual mean temperature anomalies. Source: AusBOM (2015)



**Figure 3.3:** Annual mean rainfall (mm) for Australia since 1900. The black line shows the 10-year moving average. Source: AusBOM (2015)

### 3.1.2 Australian freshwater ecosystems

Australian freshwater ecosystems have distinctive ecological features, aquatic biota, energy flow and physicochemical features when compared to other inland waters across the world (Lake et al. 1985, Lake et al. 2000, Nielsen et al. 2003, Bond et al. 2008, Lake 2011, Boulton et al. 2014). More than two thirds of Australia is arid or semi-arid and, and as a result, extensive areas can only support intermittent streams and shallow ephemeral lakes (Boulton and Brock 1999). The majority of rainfall (based on average annual totals) occurs in TAS, VIC, NSW, QLD, NT and northern WA. Therefore many Australian river systems have variable flows with low discharge rates (Lake et al. 1985, Boulton and Brock 1999). In this dry environment, perennial freshwater ecosystems are restricted to the humid eastern, southern and tropical zones which elevate their importance for use by humans, wildlife and stock. Overall, there are three main freshwater systems in Australia, namely: a) coastal systems; b) the Kati Thanda-Lake Eyre system; c) the Murray-Darling system (Figure 3.4).



**Figure 3.4:** Three types of Australian freshwater inland systems (Murray-Darling system, Kati Thanda-Lake Eyre system and coastal system)

The Murray-Darling system is the most iconic and important freshwater system in Australia owing to its high level of development for irrigated agriculture. The two main basins, the Darling in the north and the Murray in the south and east, are together known as Murray-Darling Basin, one of the largest river basins in the world. The Murray-Darling Basin spans approximately 1.056 million km<sup>2</sup> and contains 440,000 rivers and streams and 30,000 wetlands (MDBA 2014b). The Basin extends over NSW, ACT, VIC and the southeast parts of QLD and SA. The rivers of the Murray-Darling Basin experience variable flows both seasonally and inter-annually resulting in the formation of a variety of river types including; ephemeral, semi-arid and permanent rivers (Brandis et al. 2009). Most rivers in the southern basin are highly regulated due the construction of many weirs and dams over the last 90 years (MDBA 2014b).

Regulation and a high level of abstraction have combined with a highly variable climate and subdued topography to affect stream flows and the distribution of water across the basin. Therefore the hydrology, flow pattern, river forms, water quality characteristics, aquatic biota, physical processes, ecological attributes and ecosystem functions have changed across the basin (Gehrke et al. 1995, Maheshwari et al. 1995, Maier et al. 2001, Brandis et al. 2009, Kingsford 2011, Mosley et al. 2012).

### **3.2 Western Victoria**

The western Victorian region is situated between 36-38°S latitude and 141-144°E longitude. It is bounded by the Wimmera-Mallee in the north, Goldfields in the east, Southern Ocean and Bass Strait in the south and South Australia to the west (Figure 3.5). The region of western Victoria can be divided into two geomorphic zones (upland and lowland) based on environmental features and ecological characteristics; the uplands of the Great Diving Range and the Grampians and Otway Ranges (Gell 1997, Barr 2010), and the lower lying Volcanic Plains. The Otway Ranges lie to the south of

the Plains and are among the most humid parts of the state. The Great Dividing Range lies along the eastern margin of Australia but runs east-west in Victoria essentially dividing the state into the southern plains and, to the north, the plains of the southern Murray-Darling Basin.

The Grampians are an isolated range that is akin to a western extension of the Great Dividing Range. The highest peak within the Grampians Ranges, at 1167 m above the sea level, is Mount William (Clark 2010). The colonisation of western Victoria by Europeans (c. 1850 CE) dramatically increased the human population resulting in extensive anthropogenic modification to the regional environment (Clarke 2002).



**Figure 3.5:** Western Victoria is located between 36-38°S latitude and 141-144°E longitude.

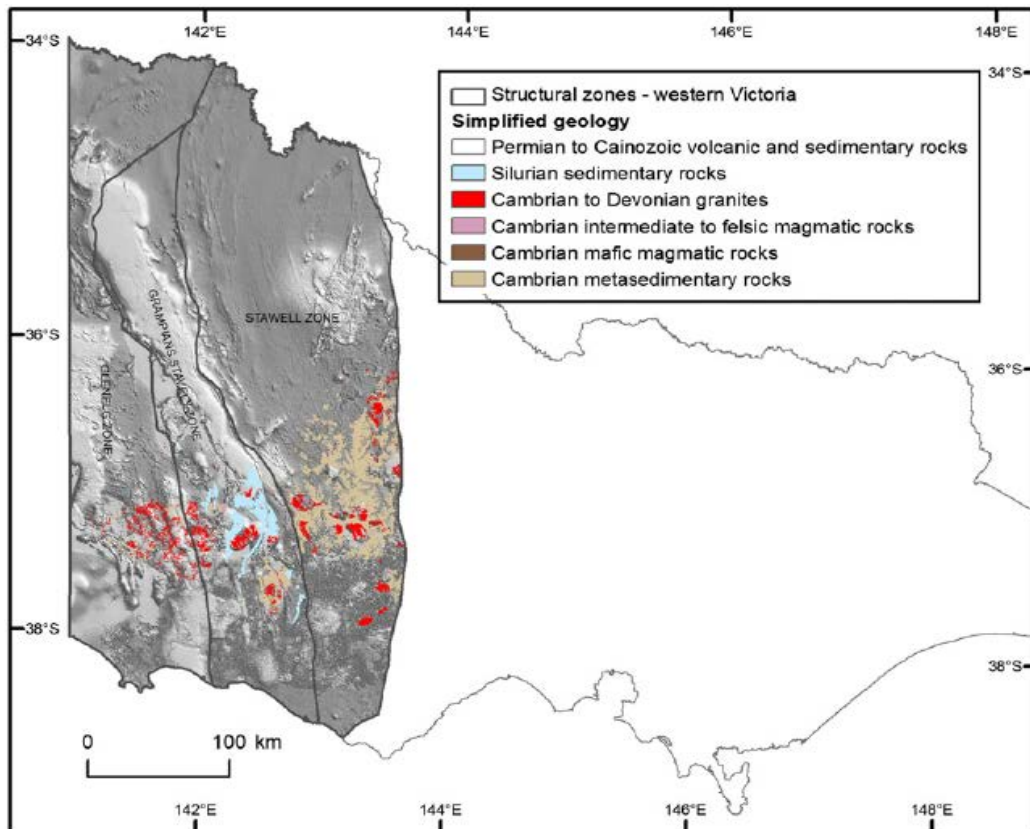
### 3.2.1 Geology, geomorphology and vegetation

The geology of western Victoria is diverse and mainly composed of Cainozoic and Palaeozoic sedimentary rocks of marine origin, with areas of intrusive igneous rocks

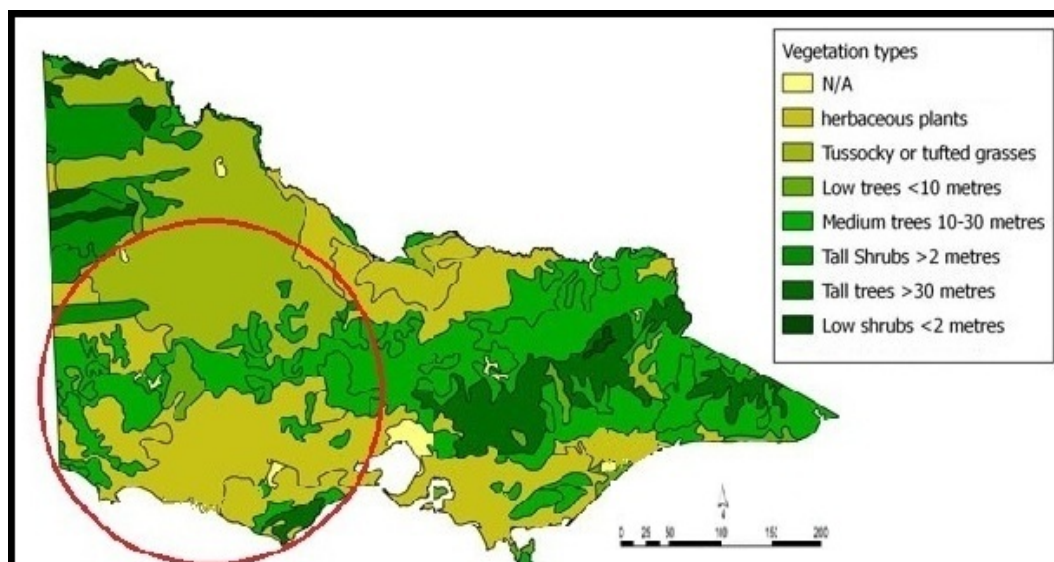
(Lawrence 1975, Cayley and Taylor 1997, Birch 2003). The geology of western Victoria was formed by Tasmanides which includes Cambrian Delamerian and Lachlan as a result of deformation of Ordovician to Silurian mudstone (Figure 3.6) (Birch 2003, Lisitsin et al. 2013). Therefore, western Victoria (including Grampians) lies on a diverse range of Cambrian bedrock (Cayley and Taylor 1997). The rock materials mainly contain sedimentary (mostly quartz, sandstone and red siltstone) and igneous (granites and Rocklands Volcanic) forms called the Grampians Group (Cayley and Taylor 1997, Birch 2003). The volcanoes in southwest Victoria can be divided into four types including lava volcanos, maars, scoria volcanos and volcanic complexes (Joyce 1988, Barr 2010). In the uplands of the Wimmera the geology ranges from Cambrian metasediments and Devonian granites while it changes to younger tertiary strata in the Wimmera plains.

Geomorphological studies have shown that western Victoria was created by tectonic forces and is, generally (including the uplands), much lower and is less rugged than eastern Victoria (Rosengren 1999). The uplands of western Victoria can be divided into three geomorphic units including: Dissected Uplands or Midlands, Prominent Ridges of the Grampians and Dissected tablelands or Dundas tablelands. The soil in western Victoria has different varieties, mostly sandy with moderate fertility which supports plant cover and vegetation (Willatt and Pullar 1984, Enright et al. 1997). Plant cover, vegetation and forest in eastern Victoria is greater than western Victoria (Figure 3.7).





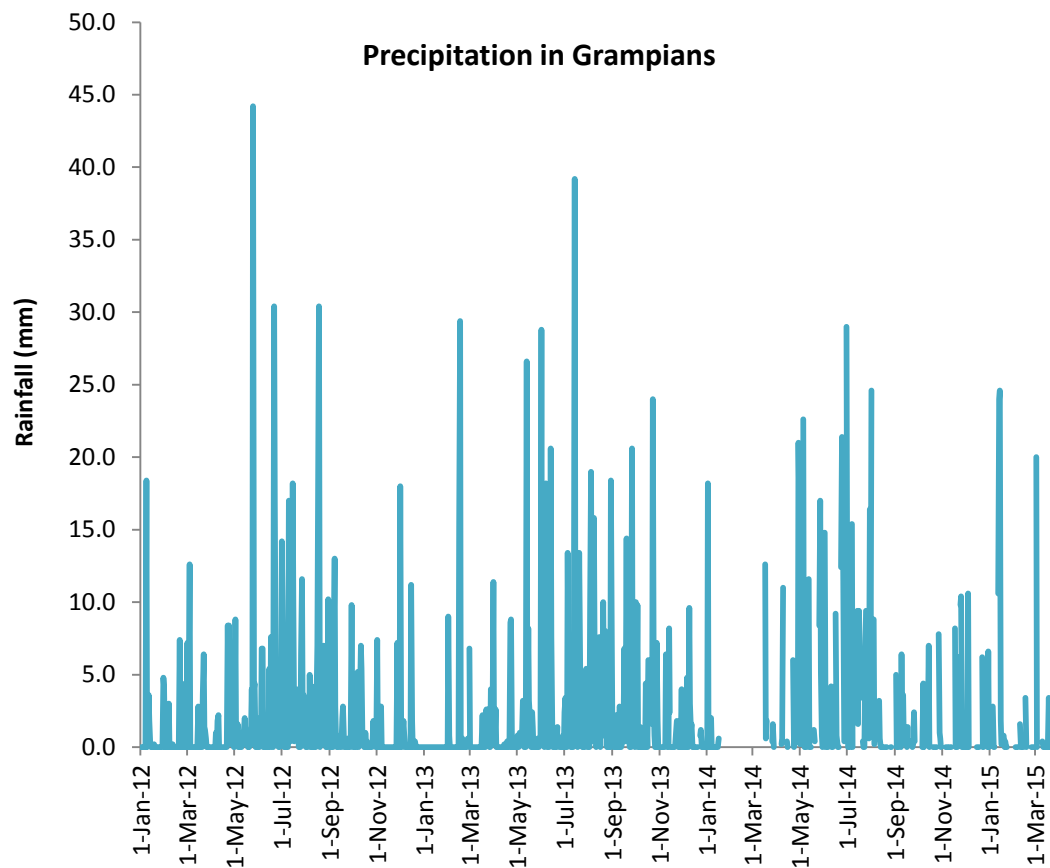
**Figure 3.6:** A simplified geology map of Western Victoria. Source: Lisitsin et al. (2013).



**Figure 3.7:** Vegetation and plant cover of Victoria including western Victoria. Source: Agriculture Victoria (2013).

### **3.2.2 Climate and weather**

Generally the climate of western Victoria is similar to the Mediterranean: cool and wet in winter, and warm to hot in summer. However the rainfall in western Victoria varies from more than 1600 mm in the southwest and the Grampians to less than 300 mm in the northern plains (WCMA 2004a), resulting in the northwest being semi-arid, and the southeast being very humid, particularly in the Otway Ranges (House et al. 2002). The majority of this precipitation occurs during late autumn (April-May), winter (June-August), and early spring (September). However rainfall also occurs in varying amounts during all other months of the year (Figure 3.8) (AusBOM 2014). The ENSO and IOD are prominent during summer and spring, and the SAM is very important during winter in Western Victoria. The ENSO brings warm and humid air to western Victoria under its natural phase. However, the negative phase of ENSO brings extended periods of severe drought particularly when it combines with a positive IOD. Conversely, the positive phase of the ENSO brings extensive cool ocean weather to western Victoria.

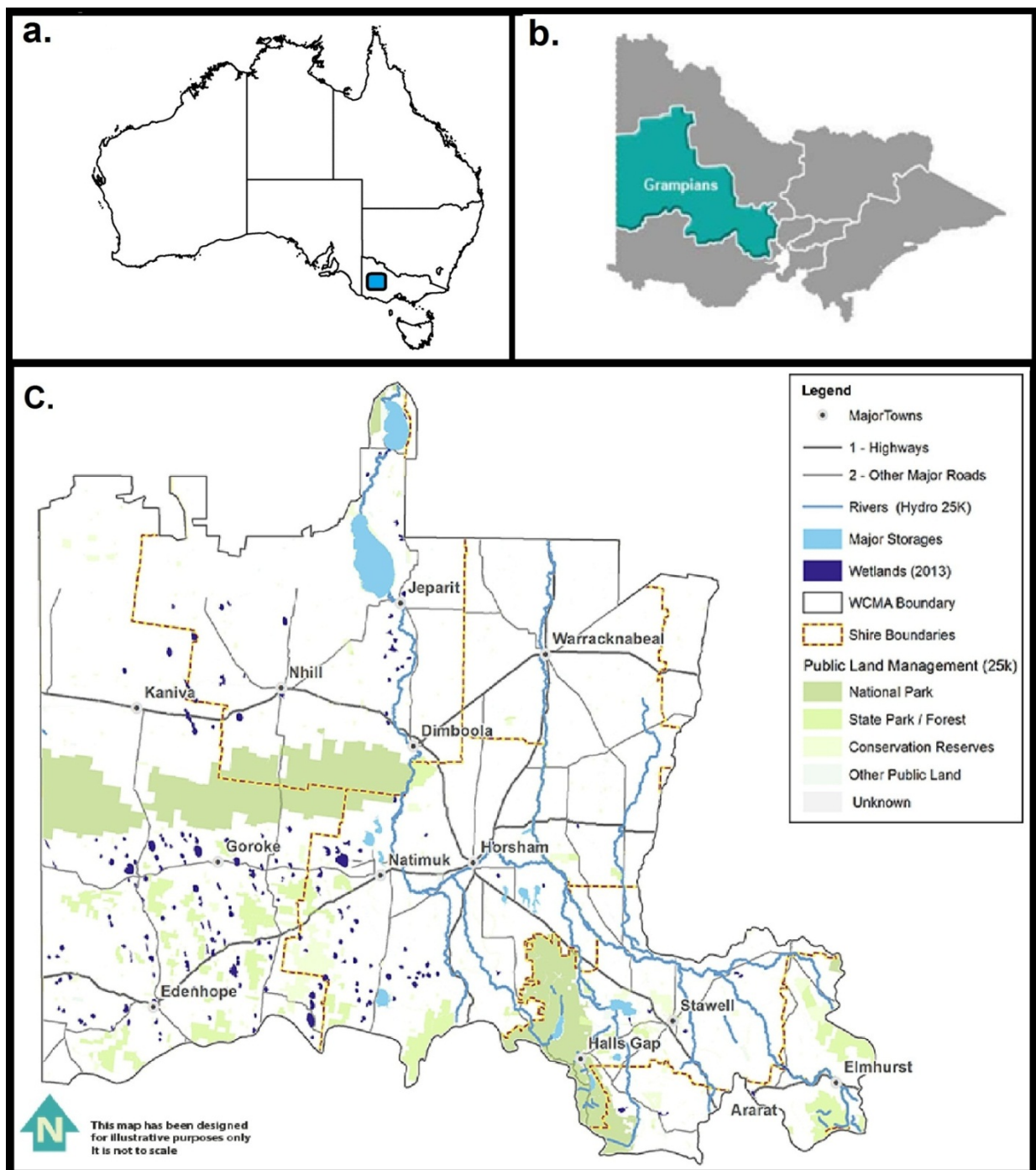


**Figure 3.8:** The rate of rainfall in the Grampians area from January 2012 to March 2015. Source: AusBOM (2015).

### **3.3 Wimmera catchment**

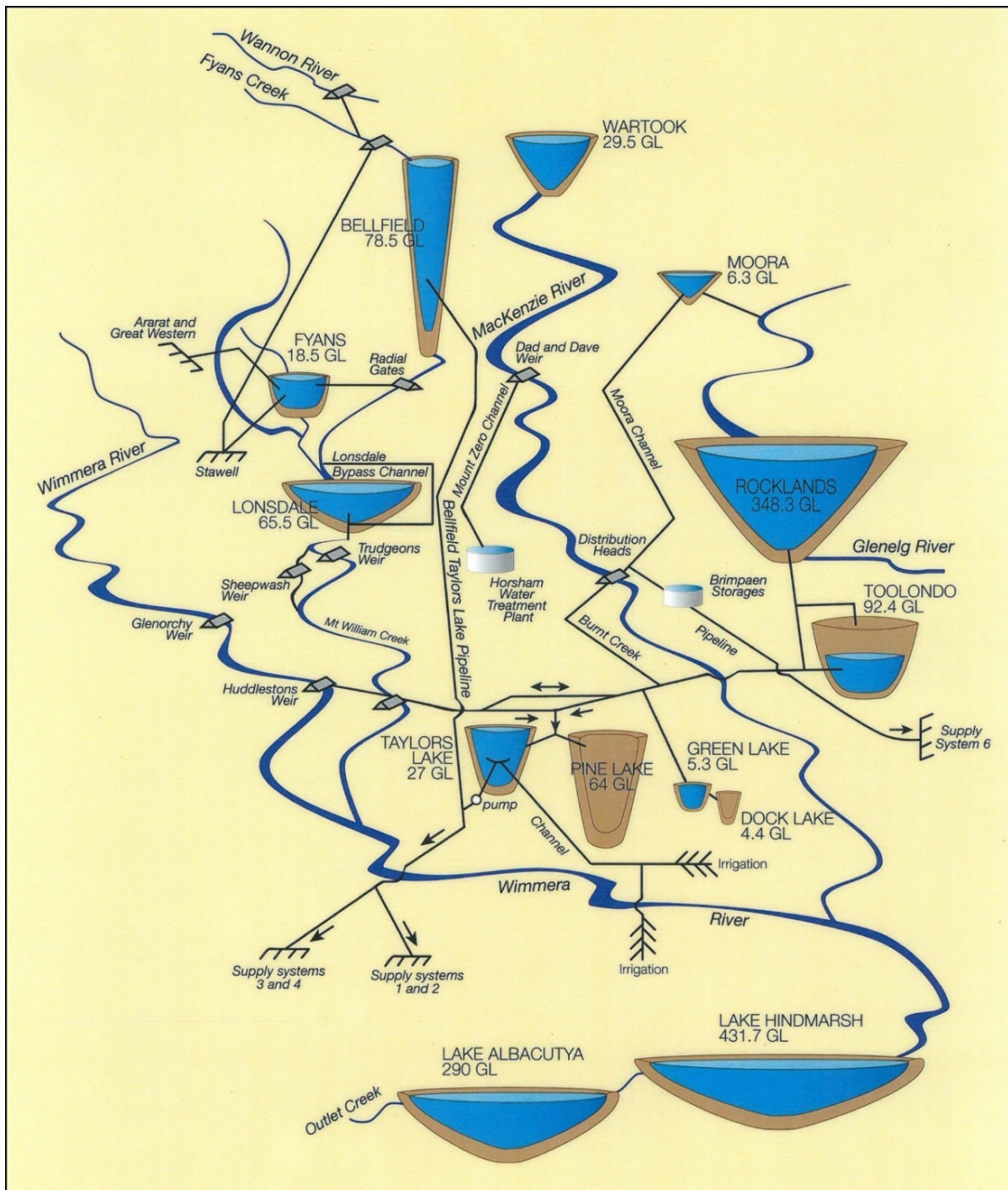
The Wimmera River is an inland flowing, intermittent river within a catchment area of 23,500 km<sup>2</sup> located in the Grampians region of western Victoria. Whilst it is situated in the Murray-Darling Basin its flow is limited by the dune fields of northwest Victoria and so its flow ends in terminal lakes (Alluvium 2013). The Wimmera River is one of the largest endoreic waterways in Victoria (SKM 2002b, WCMA 2004a, Alluvium 2013). The river rises in the Mt Buangor State Park and the Pyrenees Ranges and flows through Crowlands, Glenorchy, Horsham, Dimboola, Jeparit and finally terminates at Lake Hindmarsh, although water can penetrate into Lake Albacutya and the Outlet Creek system after extended wet periods (e.g. 1973-1975) (Figure 3.9). The Wimmera River has several tributaries including the Mt Cole Creek, Six Mile Creek, Seven Mile Creek, Sheepwash Creek, Mt William Creek, Glenpatrick Creek and Heifer Station Creek; but the main tributaries are the MacKenzie River, Burnt Creek and Norton Creek (Anderson and Morison 1989, WCMA 2004a, Alluvium 2013).

There are a number of channels, pipelines and waterways in the system which supply and deliver water to various consumptive users including: urban usage, irrigation, water storages, environmental and recreational needs. In the Wimmera-Glenelg system there are a number of water storages including: Lake Wartook, Lake Lonsdale, Lake Bellfield, Lake Taylor and Lake Fyans (Wimmera system), and Rocklands and Moora Moora Reservoirs (Glenelg System). The engineered Wimmera-Glenelg system is complex because of the water supply operation itself, the different sized water storages and the varying demands from customers and the environment for water delivery (VEWH 2015). The efficiency and flexibility of the system allows waterway managers to transfer water between reservoirs. Furthermore the facility of the system allows the off-stream storages to harvest water from channels and storages (Figure 3.10).



**Figure 3.9:** Wimmera River System (the map is adopted from WCMA website at: <http://www.wcma.vic.gov.au/about-us/Region>).





**Figure 3.10:** Schematic diagram of the complex water supply in the Wimmera-Glenelg system.

### **3.3.1 Environmental condition**

The Wimmera River catchment supports a diverse range of vegetation. The upper areas of the Grampians ranges are covered by dense woodlands dominated by *Eucalyptus* species. In the lower parts of the catchment there are areas of open grassland with woodland communities of Buloke, Native Cypress Pine and eucalypt (WRCLPB 1997, SKM 2002b). Since European settlement, the Wimmera River has been substantially modified, because the catchment land, particularly the fertile plains, have been extensively developed for agriculture. The Wimmera River catchment is used for crop and livestock production such as wheat, legumes and sheep (Fischer 1999, SKM 2002b). This intense agricultural activity including irrigation, land clearing for farming and cropping, and more recently industrial activities such as the construction of the Wimmera Mallee pipeline project development, define the catchment as highly modified (60%) between 1994-2004 (Nathan and Lowe 2012).

The upper parts of the Wimmera River have been regulated (SKM 2002b). Much of the length of the River has suffered from bank erosion caused by the clearing of plant cover and riparian vegetation (Anderson and Morison 1989). Furthermore, fluctuations in the natural flow regimes along the river and its tributaries encourage the transport of sediment from upstream to downstream, and the deposition of those sediments into the lower reaches (RWC 1991). The lower reaches of the Wimmera River flow through semi-arid regions resulting in water loss through evaporation, percolation and seepage (Anderson and Morison 1989, Alluvium 2013). The low-gradient of the catchment and water flow reduction restrict the River to a series of pools and small lakes in the mid and lower parts of the river, particularly in the drier months.

The Wimmera River and its tributaries (including the MacKenzie River, Burnt Creek and Norton Creek) provide habitat for different species including waterbirds, fish, platypus, amphibians, snails, mussels and macro-invertebrates; a number of which are

listed as vulnerable or considered threatened in Australia (SKM 2002b). Twelve fish species have been reported from the Wimmera River of which six are endemic to south-eastern Australia, including the River Blackfish (*Gadopsis marmoratus*) and Mountain Galaxias (*Galaxias olidus*) (DNRE 2000b, SKM 2002b). Four fish species, one frog species and many plant species in the Wimmera River system are considered threatened (DNRE 2000c, SKM 2002b, 2003). A number of other species in the catchment such as Platypus (*Ornithorhynchus anatinus*) and Water Rat (*Hydromys chrysogaster*) directly depend on the river environment for food and shelter. The aquatic ecosystem in the lower parts of the Wimmera River experiences stress due to poor water quality, largely as a result of increasing water salinity as the flow reaches the terminal lakes (SKM 2002b). The terminal lakes of the Wimmera River (Lake Hindmarsh and Albacutya), when full, play a significant role in providing habitat for approximately 50 waterbird species such as the Great Egret (*Ardea alba*) and Freckled Duck (*Stictonetta naevosa*) both of which are listed as endangered in Victoria (DNRE 2000a). Moreover Lakes Hindmarsh and Albacutya have unique and outstanding ecological characteristics such that Lake Albacutya is listed under the Ramsar Convention (SKM 2002b).

#### **3.3.1.1 Natural phenomena in the Grampians National Park**

One of the main natural phenomena in southeast Australia is bushfire. Bushfires (wildfires) often occur in dry conditions and dense vegetation when dry winds blow from central Australia to the southeast. Some trees, particularly *Eucalyptus*, are prone to fire because their leaves contain highly flammable oils (Gill and Moore 1996). The Grampians National Park has dense vegetation which is composed mostly of *Eucalyptus*. Two strong bushfires occurred in the early months of 2006 and 2014 which affected the most of the Grampians National Park. According to the Country Fire Authority (CFA), 55,000 hectares of the Grampians region was burnt in January 2014



(Figure 3.22). This greatly affected the vegetation of the MacKenzie River catchment such that the surrounds of Reach 1 and Reach 2 were burnt completely, with much of the canopy cover lost. The volume of large woody debris in the channel increased. It has been reported that bushfires play significant roles in structuring terrestrial plant communities and are a natural feature of Australian sclerophyll ecosystems (Whelan 1995). However, the impacts of the bushfire on freshwater ecosystems are not well documented (Cowell et al. 2006).



**Figure 3.11:** The extent of the bushfire in the Grampians National Park in early 2014 (left image adopted from NASA) and the impact on the ground and along the MacKenzie River.

### **3.3.2 Hydrological alterations**

The Wimmera catchment hydrology has been modified on account of the development of an irrigation system (e.g. Wimmera-Mallee pipeline), abstraction for the supply of water to urban, domestic and stock users, and the establishment of the Wimmera Mallee Domestic and Stock Supply System (WMDSS).

Grampians Wimmera Mallee Water (GWMWater) is the water agency responsible for the operation of the WMDSS which captures and distributes water in the Wimmera and Glenelg catchment regions. The WMDSS is a complex network of channels, pipes and storages which captures large portions of the water in the system. It has significant impacts on flow regime characteristics; decreasing the magnitude and frequency of the flow before reaching Lakes Hindmarsh and Albacutya (WCMA 2004a).

The Wimmera River has been regulated since the construction of Wartook Reservoir in 1887. The maximum annual flow of the river is 135,570 ML (Horsham station) (SKM 2002b). However the annual flow regime can change from no flow, as occurred during the El Niño of 1994; to 570,000 ML which occurred in the La Niña of 1956 (DWRV 1989, SKM 2002b). The natural flow of the river has been substantially modified due to the construction of reservoirs, weirs and locks along the catchment which together underpin the WMDSS, supplying water for agriculture, urban and domestic use (Anderson and Morison 1989, SKM 2002b, WCMA 2004a, Alluvium 2013).

The establishment of the WMDSS has resulted in further development of the Wimmera River system, which consequently has affected the flow regime in the Wimmera Catchment. The WMDSS diverts water from the Wimmera, Glenelg, Wannon, Murray and Goulburn Rivers and features 12 storages and 18,000 km channels (Western 1994, Overman 1996, Western et al. 1997, SKM 2002b). The transfer of water and the system operation of the Wimmera River are very complex and the majority of

the diversions have been organised from the Glenorchy and Huddleston weirs. The flow regime changes substantially below the Huddleston Weir and this affects the hydrology of the lower parts of the river (SKM 2002b).

The water quality of the Wimmera River varies downstream. Overall, the river water has high salinity and high nutrient concentrations, and low dissolved oxygen levels (Anderson and Morison 1989). The salinity of the water is high in both the upstream and downstream reaches but the salinity is less in the midstream due to the diluting effect of water entering from the WMDSS at the Glenorchy Weir (Anderson and Morison 1989, SKM 2002b).

Additionally, the intrusion of saline groundwater into the lowland reaches in conjunction with decreasing volume of the water in the dry season, and high temperatures increases the salinity of the water, and saline pools can reach >50,000 mg/L (Anderson and Morison 1989). The concentrations of phosphorus and nitrogen are moderate to high particularly in the downstream reaches (Alluvium 2013), and are correlated with fluctuations in rainfall and flow regime (SKM 2003). Increased algal growth due to eutrophication which creates restrictions within the channel lead to bank erosion further impacting on the aquatic biota and habitats (Anderson and Morison 1989, Craigie et al. 1999, SKM 2002b). The low concentration of dissolved oxygen is another major issue for the management of the Wimmera River. The depletion of dissolved oxygen in water to <3 mg/L is common along the river and deoxygenation occurs at depths greater than 2 m in the water column, especially below Huddleston Weir (Anderson and Morison 1989). It seems groundwater contributes to stratification which then contributes to the deoxygenation of the lower layer of the river.

### **3.4 The MacKenzie River**

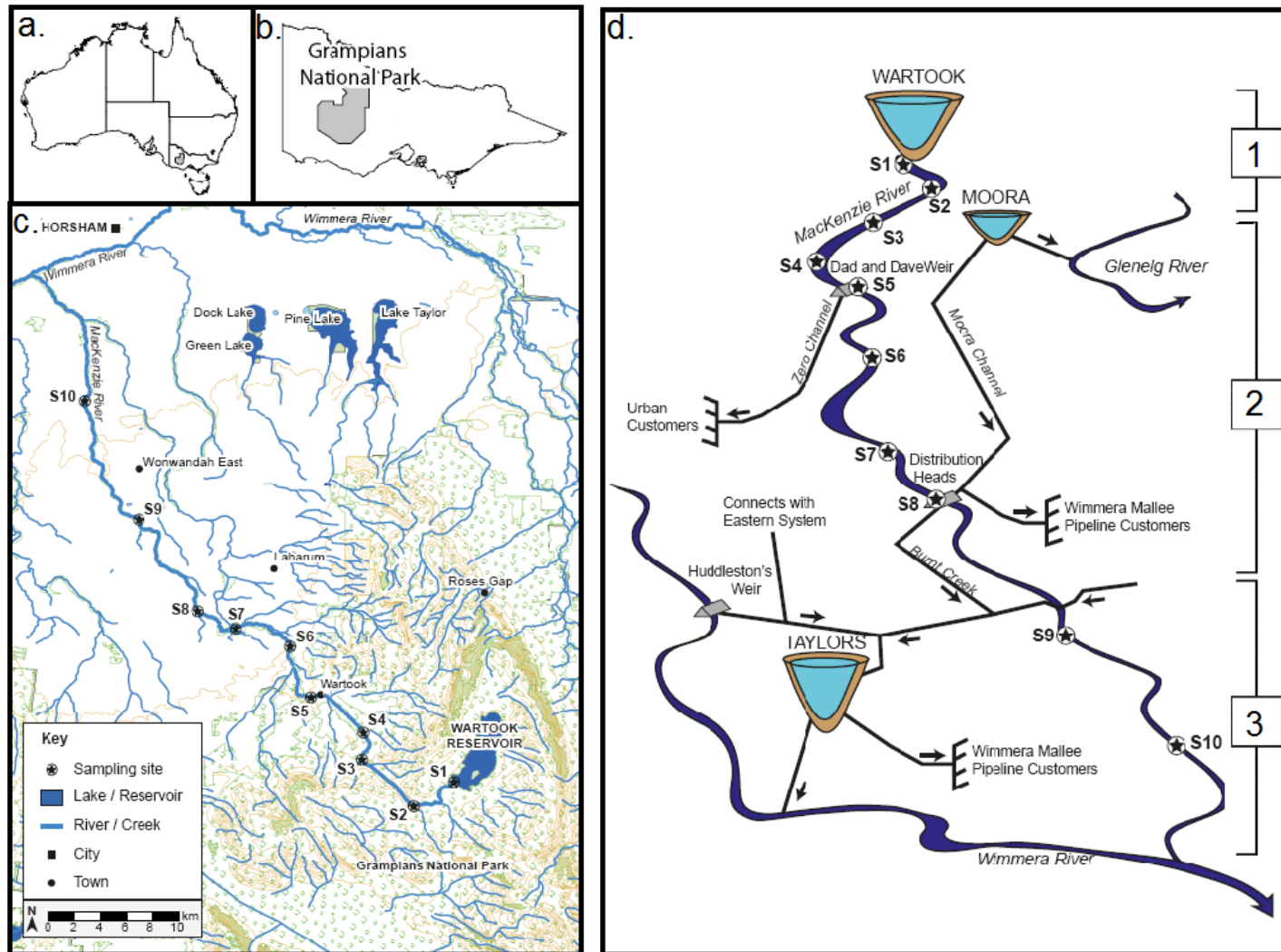
The MacKenzie River, which drains the northern slopes of the Grampians Ranges in western Victoria, is one of the main tributaries of the Wimmera River (Figure 3.11a-c). The headwaters feed into Lake Wartook in the Grampians National Park, which has a maximum capacity of 29,360 ML. The river flows approximately 50 km from Wartook Reservoir before its confluence with the Wimmera River. The catchment lies to the south of the city of Horsham and covers an area of approximately 597 km<sup>2</sup> (WCMA 2004b).

Water is released from Wartook Reservoir for multiple purposes; however, the water released is usually for consumptive use, and is directed through a network of distributary channels and associated structures at different locations along the system, including Mt Zero Channel and Distribution Heads. Flows vary from year to year depending on seasonal inflows, however typically between 7,000 ML and 10,000 ML is released each year from Lake Wartook into the upper MacKenzie River; of this volume one third of the released water was explicitly for environmental purposes. The remaining water was released to meet the consumptive demands and to transfer water to downstream reservoirs (GWMWater 2015). As a consequence of this anthropogenic modification, the MacKenzie River is classified as a highly modified river (GWMWC 2012, GWMWater 2015), where consumptive flows will dominate the flow regime in some years (Figure 3.11d)

The Wimmera Catchment Management Authority (WCMA) and GWMWater release water along the MacKenzie River to improve water quality, stream condition and river health especially in the downstream reaches (WCMA 2015). The upstream section (Reach 1) tends to receive water most days of the year due to releases to secure the requirements of water supply for the city of Horsham and its recreational and

conservation values, which is diverted into Mt Zero channel. Below this the middle and downstream sections (Reaches 2 and 3) receive a more intermittent supply.

Wartook Reservoir and the Grampians National Park are highly recognised for their natural and recreational values. The Park supports a wide range of flora and fauna providing suitable conditions for riparian vegetation growth, and the existence of woodland and aquatic biota. However the region is vulnerable to natural bushfire particularly in the dry season. Although there are large changes in the hydrology down the river, there is little variation in the geomorphological features. The main geomorphologic changes relate to vegetation encroachment on the channel. Dense riparian vegetation exists along the MacKenzie River from the headwaters in the Grampians National Park to the lower reaches nearer to Horsham. The vegetation is mostly composed of species of *Eucalyptus*, *Leptospermum* and *Acacia*. This area represents a suitable habitat for native biota such as: kangaroos and bird species like the Eastern Yellow Robin, Superb Fairy-wren, White Cockatoo and Rufous Night-heron. Furthermore the MacKenzie River supports a great diversity of aquatic species, especially native fish. It also supports exotic fish species including trout, redfin, carp and mosquito fish (Anderson and Morison 1989). Moreover there still remains an important population of platypus in this river (WCMA 2004b, Alluvium 2013). In addition the MacKenzie River has great potential for eco-tourism since, among a range of natural attractions, it has examples of Aboriginal and European heritage and is a good place for camping at Zumsteins and MacKenzie Falls, and recreational fishing along the river.



**Figure 3.12:** (a-c) Location of the ten sampling stations along the MacKenzie River system in the Wimmera catchment and (d) Schematic diagram showing the location of the three Reaches of the MacKenzie River within a complex water supply system



### 3.4.1 Reach 1: Lake Wartook to ‘Dad and Dave’ Weir

Reach 1 is located in the upstream section of the river below Lake Wartook (Figure 3.11d) and includes the sampling stations of Wartook Outlet (S1), Smiths Mill (S2), MacKenzie Falls (S3) and Zumsteins (S4). The Wartook Outlet (S1) is located at 37° 5'39.68"S, 142°26'1.21"E with an elevation around 442 m above sea level (asl) (Figure 3.12). The Wartook reservoir was built with concrete, boulders, rocks and stones. The Wartook Reservoir discharges into the MacKenzie River via the Lake Wartook outlet and the WCMA and GWM Water are responsible for controlling the operation of the flow regimes. The maximum capacity of the reservoir is 29,360 ML while it yields a long-term average of 25,565 ML (Barton et al. 2011). This area was affected by bushfires in 2006 and 2014 (see section 3.5 for more details).



**Figure 3.13:** The Wartook Reservoir outlet (S1) one of main modified sites in the MacKenzie River

Smiths Mill (S2) is located at 37° 6'29.77"S, 142°25'28.75"E with an elevation around 441 m asl (Figure 3.14). The river bank is in natural condition with abundant riparian and woodland vegetation.



**Figure 3.14:** The Smiths Mill (S2) site with abundant riparian and woodland vegetation.

The MacKenzie Falls (S3) site is located at 37° 6'38.59"S, 142°24'46.82"E with an elevation around 412 m asl (Figure 3.15). This site is in natural physical condition with abundant native riparian vegetation and woodland. The falls themselves have a natural landscape and draw many tourists for ecotourism and recreational activities.



**Figure 3.15:** Site MacKenzie Falls (S3) photos with abundant native riparian vegetation and woodland.



Zumsteins (S4) is located at 37° 5'30.62"S, 142°23'6.32"E with an elevation around 243 m asl (Figure 3.16). The site has healthy riparian vegetation which extends into the stream channel.



**Figure 3.16:** The Zumsteins site (S4) the river flows through a gorge.

### **3.4.2 Reach 2: Dad and Dave' Weir to Distribution Heads**

Reach 2 is located in the mid-stream section of the MacKenzie River. It includes the diversion point 'Dad 'n' Dave' (S5), Mt Zero Channel, Ewan Vale (S6), Tatlock Bridge (S7) and Distribution Heads (S8). In Reach 2 a significant portion of the MacKenzie River is diverted into Mt Zero channel, which then flows to a Water Treatment Plant to increase the water quality to a level suitable for consumption for the inhabitants of Horsham. Water which is not diverted via the 'Dad'n'Dave' Weir to the Mt Zero Channel continues to flow down the river via Ewan Vale (S6) and Tatlock Bridge (S7). It is then circulated to Distribution Heads (S8) where water can be diverted via a number of weirs to other waterways (e.g. Burnt Creek). Reach 2 has a diverse array of vegetation associations such as Shrubby Woodland, Plains Grassy Woodland, Riparian Scrub, Plains Sedgy Woodland and Shallow Freshwater Marsh (Anderson and Morison

1989, WCMA 2004b) . This reach provides habitats suitable for aquatic biota such as River Blackfish, Eastern Pigmy Perch and Platypus.

The Dad ‘n’ Dave site (S5) and Mt Zero Channel are located at 37° 3'54.63"S, 142°22'31.58"E with an elevation around 218 m asl (Figure 3.17). At Dad ‘n’ Dave (S5), the river bank supports a healthy riparian woodland which extends to the stream edge providing large, woody debris habitat. Mt Zero Channel (SMt) was constructed by GWMWater to supply water for Horsham city (Alluvium 2013).



**Figure 3.17:** Site Dad ‘n’ Dave (S5) and Mt Zero Channel (SMt) photos with healthy riparian woodland which extends to the stream edge providing large, woody debris habitat



The Ewan Vale site (S6) is located at 37°02'02" S, 142°20'25" E with an elevation around 207 m asl (Figure 3.18). Typically the river flows very slowly in this stretch of river due to a lower gradient and the greater depth and width. Riparian vegetation encroaches to the channel where there is much woody debris.



**Figure 3.18:** Site Ewan Vale (S6) photos with much woody debris

The Tatlock Bridge (S7) site is located at 36°59'50" S, 142°19'04" E with an elevation around 197 m asl (Figure 3.19). The channel has a high cover of woody debris with emergent and submerged plants present.



**Figure 3.19:** Site Tatlock Bridge (S7) photos with high cover of woody debris with emergent and submerged plants

Distribution Heads (S8) is located at 36°57'12.67"S, 142°16'31.71"E with an elevation of around 189 m asl (Figure 3.20). The channel is deep and wide and riparian vegetation extends into the channel and emergent and submerged plants are present.



**Figure 3.20:** Site Distribution Heads (S8) photos with riparian vegetation extends into the channel and emergent and submerged plants

### **3.4. 3 Reach 3: Distribution Heads to the Wimmera River**

Reach 3 is located in the downstream sections of MacKenzie River. It includes the sites of Graham's Bridge (S9) and Wonwondah East (S10). The hydrology of this reach has been substantially altered due to water diversions. The hydrologic alterations induced geomorphic changes including riparian vegetation encroachment which intercepts sediment being transported down the channel (SKM 2002b). As a result of sediment accumulation in the channel bed, weirs are in-filled reducing their capacity and leading them to dry out in summer. The vegetation associations in this reach include Shrubby Woodland, shallow Sands Woodland, Blackbox chenopod Woodland, Plains Grassy Woodland, Riparian Scrub, Plains Sedgy woodland and Shallow Freshwater Marsh (WCMA 2004b). This reach supports aquatic biota such as the River Blackfish and Eastern Pigmy Perch. In this reach most of the neighbouring land has been cleared and is used for agricultural activity including irrigation agriculture, pastoral farming and



cropping. In addition human activities, such as the harvesting of water under the Wimmera Mallee pipeline project, have been developed in this reach.

Graham's Bridge (S9) is located at 36°56'49.00"S, 142°14'7.00"E with an elevation around 177 m asl (Figure 3.21). Riparian vegetation extends into the channel which hosts much woody debris.



**Figure 3.21:** The Graham's Bridge site (S9) with extends into the channel which hosts much woody debris

The Wonwondah (S10) site is located at 36°52'41.00"S, 142°11'26.00"E with an elevation around 160 m asl (Figure 3.22). At this location riparian vegetation extends into the channel and much woody debris is present. The channel tends to dry completely during the dry season.



**Figure 3.22:** The Wonwondah site (S10) with riparian vegetation extends into the channel and much woody debris

### **3.5 Why the MacKenzie River?**

Over the last two decades ecological monitoring in the Wimmera catchment showed that flow regime and water quality are critical characteristics that affect the river's health, particularly in its lower reaches (Anderson and Morison 1989, Westbury et al. 2007).

One of the main priorities for the Wimmera Catchment Management Authority (WCMA) is to find a sustainable solution to mitigate the threats which are affecting the condition of the Wimmera River. For ecological risk assessment, predictive tools have been developed by the WCMA for managing environmental flow allocations in the Wimmera River (Chee et al. 2005, WCMA 2015). The allocations of water in the MacKenzie River system, (one of the main tributaries of the Wimmera River), fall within the Wimmera-Glenelg Bulk and Environmental Entitlements for which Grampians Wimmera Mallee Water (GWMWater) is the storage manager. Whilst coordinated use of entitlements is implied within their administrative arrangements, cooperation still proves difficult, particularly during times of water shortage when entitlement holders become focused on their individual requirements. Storage managers (GWMWater) have, however, a duty of care to the environment in the way they operate reservoir systems and manage water delivery to both consumptive and environmental entitlement holders. Biological indices can be useful tools for water resource managers in the assessment of river health and decision making with regards to water sharing amongst the consumptive users, in order to improve environment benefits and river health, whilst considering potential impacts on consumptive users.

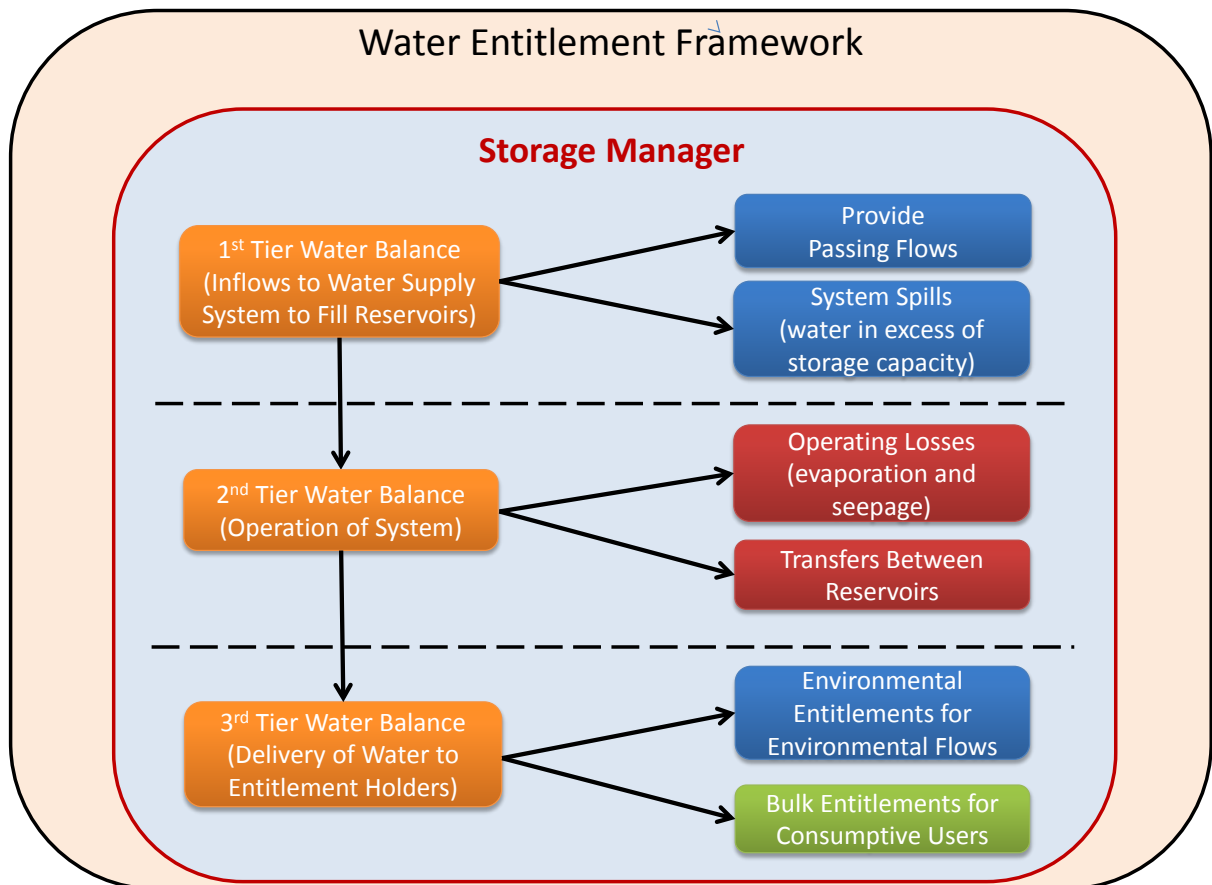
The water supply system in the Grampians was developed to deliver consumptive water for industry, agriculture and for domestic and stock consumption. Due to the recent drought-dominated regime in this area (south-east Australia), and the construction of the Wimmera-Mallee Pipeline, the share of water to users has changed significantly (Barton et al. 2011) and that available to the environment is limited. Therefore, in order

to gain best value from the volume available, the water supply system in the MacKenzie River needs to be optimised towards maximising environment benefits from a diminished volume of water.

A generic water supply system is depicted in Figure 3.23. In this figure, all water in this water supply system is a part of an entitlement framework. In the entitlement framework a storage manager is appointed to operate the supply system. Areas shaded blue are recognised as environmental water and are managed to obtain environmental objectives. This represents a situation in which environmental flows science is mostly developed and directed. Those parts in red can significantly affect the environment, but they are not managed to maximise ecosystem response.

This gap has been recognised by Department of Environment, Land, Water and Planning (formerly known as Department of Sustainability and Environment) (DELWP 2015), but it has not yet been completely addressed. Contemporary water supply system operations are guided by policy objectives and, for the Wimmera-Glenelg system, where the current project has been tested on the basis of the following policies:

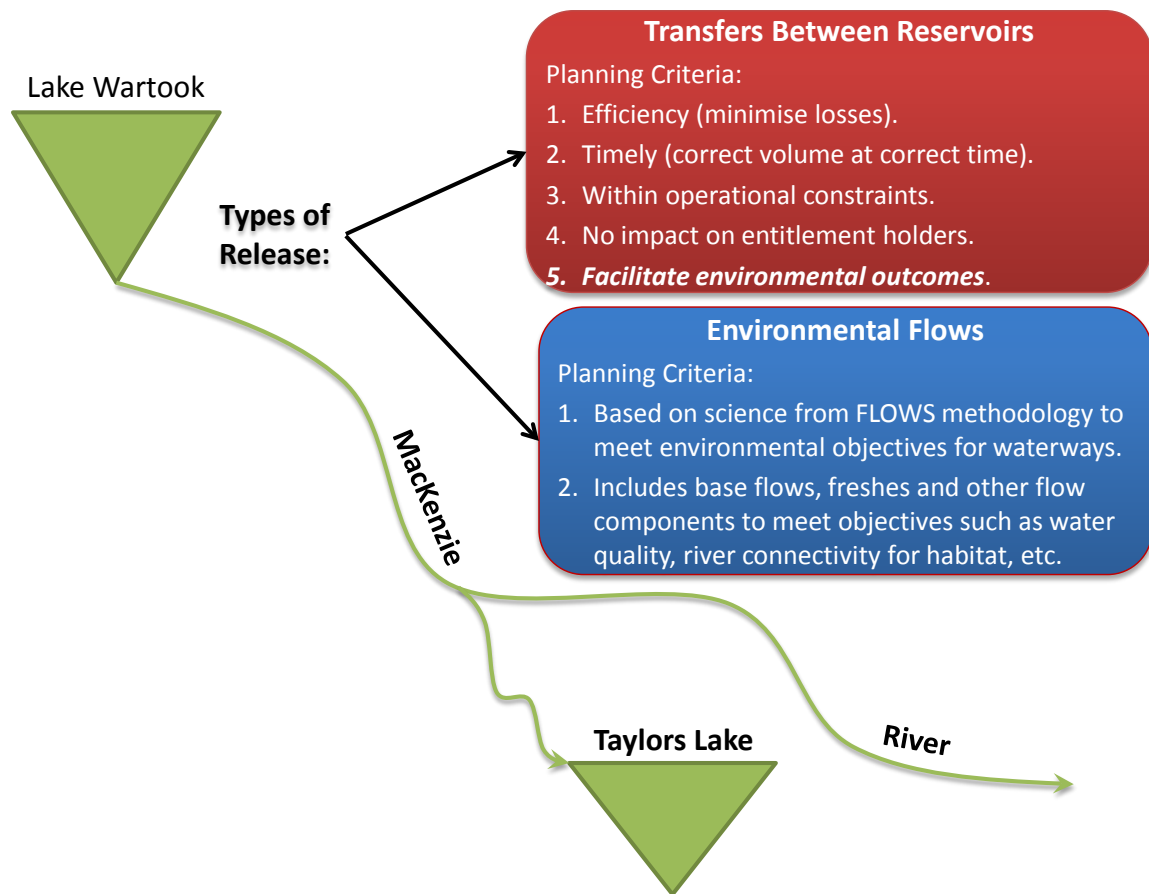
- Duty of care to the environment (Statement of Obligations) (DELWP 2007).
- To facilitate environmental outcomes (listed within the Storage Manager Instrument of Appointment) (DELWP 2015).
- To manage consumptive flows for environmental outcomes as well (listed as a policy within the recent Western Region Sustainable Water Strategy) (DELWP 2015).



**Figure 3.23:** Diagram showing a typical water supply system water balance.

The management of water supply systems can often be constrained by a range of competing objectives. Figure 3.24 shows how two typical types of releases can be different in environmental flows and transfers. Transfers between storages are constrained by management objectives of maintaining efficiency, for transfers to be delivered in a timely manner, within operational constraints (for example, at flow rates that do not exceed valve or weir capacities). These management objectives may not be always compatible with facilitating environmental outcomes.





**Figure 3.24:** Diagram demonstrating the different planning criteria for the two major types of release in the MacKenzie River system.

Annually, a total of 10,000 ML of water is released from Lake Wartook into the MacKenzie River. Of this volume, only about 4,000 ML (about one third) was released explicitly for environmental purposes. The remaining 6,000 ML (about two thirds) was released to meet consumptive demands and to transfer water to downstream reservoirs (personal communication; GWMWater). Routine water releases from Wartook Reservoir are up to 50 ML/day in summer and 15 ML/day in the winter. There can be occasional releases of up to 200 ML/day, or more, if the Reservoir is full and operators need to discharge excess water. Downstream, the Mt Zero channel has a capacity of about 30 ML/day. These are significant water volumes and they pose the question

“could this water be optimised to achieve positive environmental outcomes”? This comparison of volumes certainly demonstrates the potential for the MacKenzie River to be developed as a case study for a “healthy working river”. The timing and route of the transfer of the consumptive water could be explored to provide environmental benefit without impacting multiple water users (WCMA 2004b). This represents an opportunity to optimise these consumptive releases to achieve greater environmental benefits rather than relying on the smaller environmental releases alone. The use of consumptive water reduces the amount of extra environmental water needed to meet specific objectives. It seems that the 4,000ML (as environmental flow) may not be adequate to support all ecological requirements (Table 3.1 and Table 3.2). Therefore, the ecological requirements could be better served if the consumptive allocation was configured to also aid the environmental cause. In this project, the ecological requirements have been investigated to optimise flows, with the aim of addressing major environmental requirements.

**Table 3.1:** Environmental flow components for the MacKenzie River at Reach 1 and 2 (WCMA 2015)

Flow component	Timing	Magnitude	Climatic scenario	Frequency	Duration
Cease to flow	Dec-May	0 ML/day	Drought	As infrequently as possible	Less than 80 day in total
			Dry		Less than 30 days in total
			Average		
Base flow	Dec-May	2 ML/d Or natural	All	Continuous	Continuous
	Jun- Nov	7 ML/d	All	Continuous	Continuous
Freshes	Dec-May	5 ML/d	Drought	3 per period	4-7 days
			Dry	4 per period	4-7 days
	Dec-May	50 ML/d	Average	2 per period	2-7 days
			Wet	3 per period	2-7 days
High flow	Jun- Nov	55 ML/d	Drought	5 per period	2 days
			Dry	5 per period	4 days
			Wet	5 per period	5 days
			Average	5 per period	7 days
	Jun-Nov	130 ML/d	Drought	1 per period	1day
			Dry	3 per period	2days
			Wet	5 per period	3 days
			Average	5 per period	4 days
Bankfull	Any	500 ML/d	Average	1 per period	2 days
			Wet	1 per period	2 days
Overbank	Aug-Nov	900 ML/day	Wet	1 per period	1day

**Table 3.2:** Environmental flow components for the MacKenzie River at Reach 3  
(WCMA 2015)

Flow component	Timing	Magnitude	Climatic scenario	Frequency	Duration
Cease to flow	Dec-May	0 ML/day	Drought	As infrequently as possible	Less than 80 day in total
			Dry		Less than 30 days in total
			Average		
Base flow	Any	10 ML/d or natural	All	Continuous	Continuous
Freshes	Dec-May	35ML/d	Drought	3 per period	2-7 days
			Dry	3 per period	3-7 days
			Average	4 per period	3-7 days
			Wet	4 per period	3-7 days
	Jun-Nov	35 ML/d	Drought	5 per period	2 days
			Dry		4 days
			Average		5 days
			Wet		7 days
High flow	Jun-Nov	190 ML/d	Average	1 per period	1 days
			Wet		2 days
Bankfull	Any	500 ML/d	Wet	1 per period	1 day
Overbank	Aug-Nov	1000 ML/day	Wet	1 per period	1day

Overall, the MacKenzie River (Figure 3.25) was chosen as a case study for the following key reasons:

1. This River has been a substantially modified and regulated stream since the construction of the Wartook Reservoir by the Victorian Government in 1887;
2. The River is suitable for an assessment of spatial and temporal variations in water quality and the effect of humans on the condition of rivers and streams;
3. The MacKenzie River provides an appropriate field example for understanding of the amount of water that must be transferred to the environment to serve the ecological requirements to ensure a healthy working river;
4. The MacKenzie River provides a good case study to improve water allocation practices to multiple social, economic and environment benefits;
5. The operation of the field work and *in situ* experiments are well supported by the water manager, GWMWater (as a research and industry partner).



**Figure 3.25:** Schematic diagram showing the complex relationship of the MacKenzie River with the broader water supply system.

## **Chapter 4: Methods and Materials**

### **Chapter outline**

In this chapter the field techniques, laboratory methods, statistical analysis and model development approaches used to measure, evaluate and tailor the configuration of consumptive flows in the MacKenzie River are outlined. The first part of the chapter describes field techniques including the approach used to select study sites and sample collection methods (water, soft algae and diatoms) in different flow regimes along the river. The second part of the chapter describes the laboratory techniques used for water chemistry analysis, diatom and soft algae sample preparation, species identification and enumeration, and the measurement of biological properties such as biofilm dry mass, ash-free dry mass (AFDM), and chlorophyll-*a* concentration. The third part of the chapter describes the statistical methods which were used to analyse the water and biological data. The last part of the chapter explains the approach used to develop ecological models of the system.

### **4.1 Field techniques**

#### **4.1.1 Strategy for site selection**

The selection of sites along the river is critical as they needed to be representative of the physical condition (Barbour et al. 1999, Biggs and Kilroy 2000), chemical features (Barbour et al. 1999, Victoria EPA 2009) and biological properties (Lowe and Laliberte 1996, Barbour et al. 1999, Stevenson and Bahls 1999) of the reach. Ten sampling stations were established on the MacKenzie River to undertake response-to-flow analyses of water chemistry, biofilms, diatoms and ecosystem structure. The sampling

sites were also chosen to ensure the understanding of the impact of regulated flow regimes on the physical, chemical and biological environment downstream.

#### **4.1.2 Channel form and site records**

Site descriptions were carried out at each selected sampling site. This inventory comprised the physical character and algal periphyton following standard field inventory protocols (Barbour et al. 1999, Stevenson and Bahls 1999, Biggs and Kilroy 2000). At least one reference photograph of the each site was taken during each visit.

#### **4.1.3 Sampling strategy**

As outlined in Chapter 3, the MacKenzie River was divided into three Reaches and ten sampling locations were established. Reach 1 includes sampling stations S1, S2, S3 and S4 (Lake Wartook to ‘Dad and Dave’ Weir); Reach 2 includes sampling stations S5, S6, S7 and S8 (‘Dad and Dave’ Weir to Distribution Heads) and Reach 3 includes sampling stations S9 and S10 (Distribution Heads to the Wimmera River) (see Figure 11 in Chapter 3). The upstream section (Reach 1) tends to receive water most days of the year due to the requirements of water supply for the city of Horsham and is highly appraised for its recreational and conservation values. The middle and downstream sections (Reaches 2 and 3) receive a more intermittent supply. Therefore, the lower part of the river (Reach 3) has the potential to change greatly over time. Overall, the purpose of the water release from Wartook reservoir into the MacKenzie River is predominantly for consumptive users (Alluvium 2013, GWMWater 2015).

Samples were taken initially in different seasons (28 February 2012, 17 July 2012, 9 November 2012 and 25 May 2013) to obtain baseline information of the spatial and temporal variations of the algae communities, water quality, aquatic biota and stream condition before the first water release event. After the baseline sampling, samples were



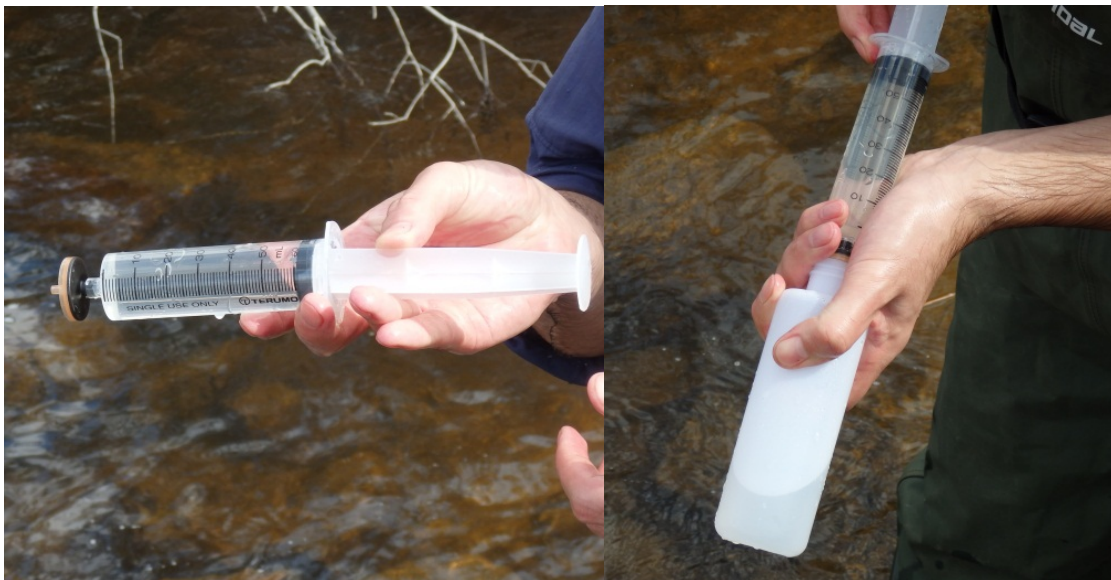
taken before, during and after three water release events (event 1: 18 October 2013, 21 October 2013, 25 October 2013; event 2: 16 December 2013, 19 December 2013, 23 December 2013, 3 January 2014, 16 April 2014; event 3: 29 October 2014, 1 November 2014, 8 November 2014 and 22 November 2014) (Table 4.1). Data of the flow regimes from 2011-2015 were provided by the WCMA and GWMWater.

**Table 4.1:** Sampling date under different flow regime and different seasons

Event	Sampling date	Flow rate
Baseline	28/02/2012	Base flow (10 ML/day)
		Cease to flow at site S10
	17/07/2012	Base flow (15 ML/day)
	9/11/2012	Base flow (15 ML/day)
	25/05/2013	Base flow (15 ML/day)
Event 1	18/10/2013	Before Freshes (15 ML/day)
	21/10/2013	During Freshes (35 ML/day)
	25/10/2013	After Freshes (15 ML/day)
Event 2	16/12/2013	Before Freshes (15 ML/day)
	19/12/2013	During Freshes (40 ML/day)
	23/12/2013	After Freshes (15 ML/day)
	3/01/2014	After Freshes (15ML/day)
	16/04/2014	After freshes (15 ML/day)
Event 3	29/10/2014	Before high flow (15 ML/day)
	1/11/2014	During High flow (55 ML/day)
	8/11/2014	After high flow (15 ML/day)
	22/11/2014	After high flow (15 ML/day)

#### 4.1.4 Water sampling

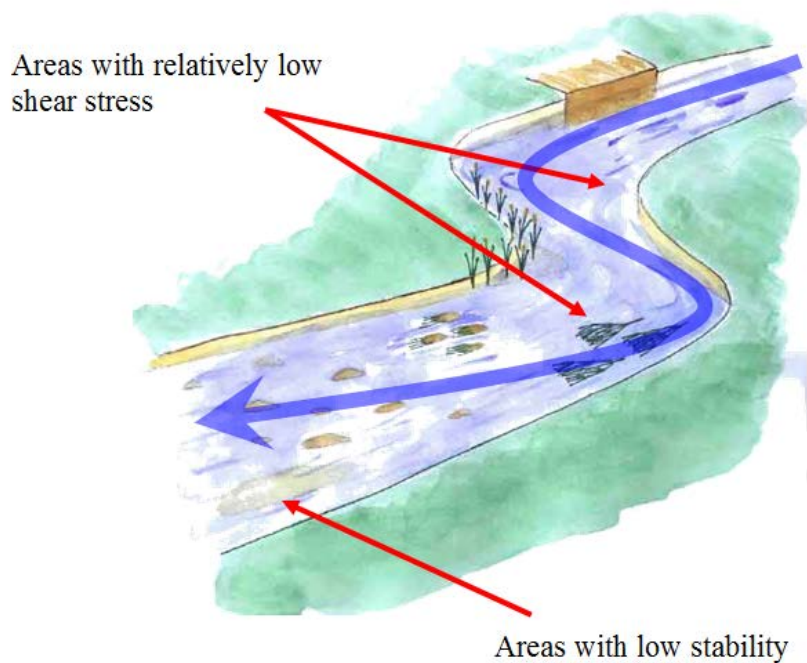
Water samples were first collected seasonally to obtain baseline data on water quality in the river. Then, water samples were collected under different flow regimes including low flows, freshes and high flows. Water samples were collected at points approximately 20 cm below the water surface using a 50 mL syringe. The samples were filtered (0.45  $\mu\text{m}$  filters) and placed in acid-washed bottles (Figure 4.1) using standard methods (APHA 2007, Victoria EPA 2009). The collected samples were stored in a cool box until returned to the Federation University laboratory for chemical analysis. *In situ* measurements of temperature (TEMP,  $^{\circ}\text{C}$ ), pH, electrical conductivity (EC,  $\mu\text{S cm}^{-1}$ ), turbidity (NTU), oxidation reduction potential (ORP, mV), total dissolved solid (TDS,  $\text{mg L}^{-1}$ ) and dissolved oxygen (DO,  $\text{mg L}^{-1}$ ) were obtained using an Horiba multimeter calibrated to 25 $^{\circ}\text{C}$  (Water checker U-52G).



**Figure 4.1:** Collecting water samples from river using syringe and filters.

#### 4.1.5 Algae sampling

At each sampling station multiple samples were collected for analysis of algal biomass standing crop and species composition. In all cases, samples were taken from areas with low hydraulic stress (Figure 4.2). Shading and depth of the water body were also considered in taking samples; samples were collected in shallow water where there was no shading (Figure 4.3).



**Figure 4.2:** Schematic diagram of a river showing areas of low stress and low stability

Source: DARES (2003)



**Figure 4.3:** The areas of river suitable, and not suitable, for collecting periphyton samples

Algal periphyton communities can have great diversity, yet their structure and composition may vary depending on the nature of the substrate. Diatoms and soft algae colonise natural substrates such as cobbles, stones, mud, rock, woody debris, emergent and submerged plants, (Kelly et al. 1998, Stevenson and Bahls 1999, Taylor et al. 2007a). Small boulders and pebbles were used for taking samples if cobbles were not available (Kelly et al. 1998).

In the present study, algal periphyton samples were collected (three replicates) from emergent and submerged surfaces including cobbles, pebbles or rocks, woody debris, aquatic plants and mud at points within each of the established sampling sites where the water velocity was relatively low (0.1- 0.9 m/s) (Figure 4.4). The algal periphyton was scraped from an area of 20-30 cm<sup>2</sup> of a substrate using a soft toothbrush. The sample was washed into a plastic tray with river water and the resulting algal suspension rinsed into a 250 mL collection bottle (Figure 4.5). Separate samples were collected for estimations of biomass and for the identification and counting of diatoms and soft algae. Six replicates were collected for estimation of dry mass, ash-free dry mass and chlorophyll-*a* (Nusch 1980, Lowe and LaLiberte 2006). The algal samples were preserved by adding two drops of Lugol's iodine (2 g potassium Iodide, 1 g resublimed Iodine , 10 mL glacial acetic acid and 300 mL distilled water) as a fixative (Wehr et al. 2015).





**Figure 4.4:** Stretches of MacKenzie River showing substrates suitable for collecting samples (cobbles, emergent and submerged plants).



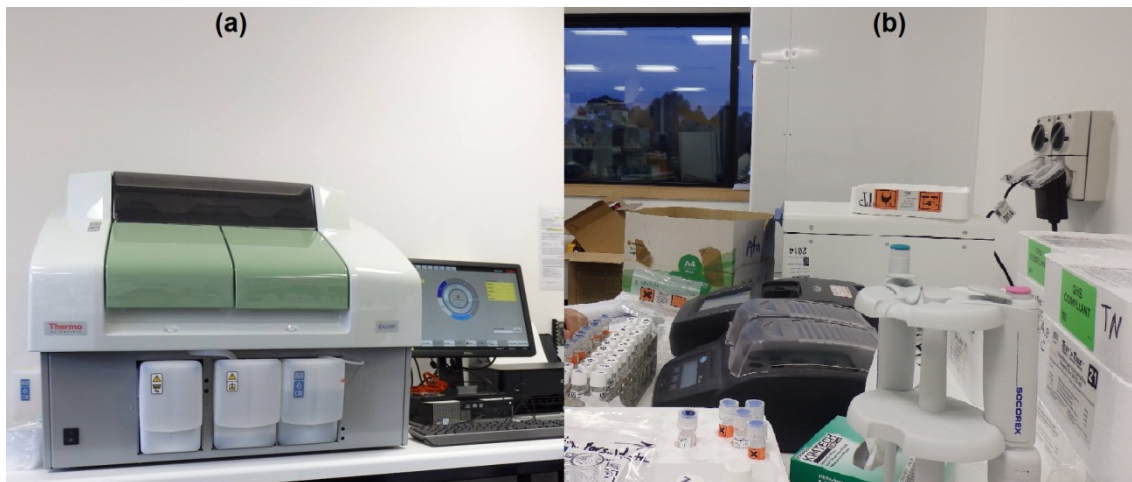
**Figure 4.5:** The author collecting algal periphyton samples from MacKenzie River

## **4.2 Laboratory techniques**

The techniques employed in the laboratory included water quality measurement, biomass analyses, diatom and soft algae slide preparation, the identification and enumeration of diatom and soft algae, and the calculation of ecological and biological indices.

### **4.2.1 Water quality analysis**

Water quality measurements obtained in the laboratory, included total suspended solids (TSS,  $\text{mg L}^{-1}$ ), total oxidized nitrogen (TON,  $\text{mg L}^{-1}$ ) and total phosphorus (TP,  $\text{mg L}^{-1}$ ), total nitrogen (TN,  $\text{mg L}^{-1}$ ) ammonia ( $\text{NH}_3$ ,  $\text{mg L}^{-1}$ ), silica ( $\text{SiO}_2$ ,  $\text{mg L}^{-1}$ ) cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{mg L}^{-1}$ ) and anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{mg L}^{-1}$ ). Measurements were undertaken using Gallery Automated Photometric Analyser and Hach DR 2800 spectrophotometer (Figure 4.6) following standard methods (Victoria EPA 2003b, APHA 2007, Victoria EPA 2009). The method of expressing water quality that offers a simple, stable and reproducible unit of measure which responds to changes in the principal characteristics of water quality. The relationship among different environmental variables was examined. Then the results compared with The Australian and New Zealand Environment Conservation Council (ANZECC and ARMCANZ 2000). Then, the information is then used to classify to excellent, good, medium, poor and bad (Chapman 1996, Barbour et al. 1999, Biggs and Kilroy 2000, APHA 2007, Victoria EPA 2009).



**Figure 4.6:** (a) Gallery Automated Photometric Analyser; (b) Hach DR 2800 spectrophotometer

#### 4.2.2 Soft algae and diatom analysis

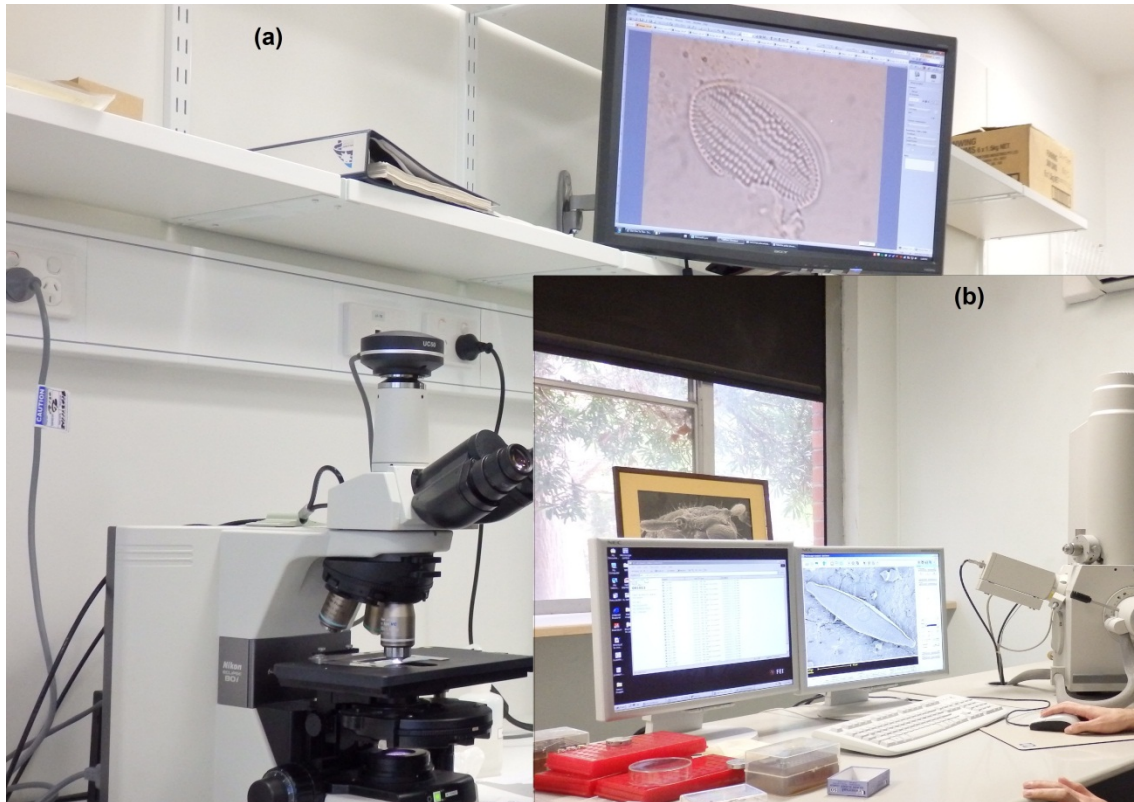
Temporary slides were prepared for soft algae to identify the species composition and to enumerate algal groups. The relative abundance of the different algal groups (green algae, cyanobacteria, diatoms and other algae) was calculated by placing 1 mL of each sample into a Sedgwick-Rafter counting chamber. Cells were counted using a Nikon Eclipse 80i microscope at 100-400× magnification.

For diatom species identification and enumeration, the samples were prepared following the method of Battarbee (1986). Samples were digested with 10% hydrogen peroxide in a beaker at 90°C on a hotplate for 2 hours, after which two drops of 10% hydrochloric acid were added. The beakers were filled with distilled water and left to settle overnight after which the supernatant was discarded. This process was repeated four times. Subsamples of 400 µl were air-dried on coverslips and mounted using Naphrax (Battarbee 1986). At least 300 diatom valves were identified and counted per slide using a Nikon Eclipse 80i microscope with differential interference contrast at 1000× magnification (Figure 4.7a).



For scanning electron microscopy (SEM), the rinsed samples were resuspended in a solution of deionised water and household bleach (50:1) for 30 minutes and rinsed three times in distilled water. Diatom suspensions were dried directly on 22 mm aluminium stubs and gold coated with a Dynavac Xenosput sputter coater. Images of frustules were taken in a Philips XL30 field-emission scanning electron microscope (Figure 4.7b).

Both soft algae and diatoms were identified in the laboratory using global and regional algal identification keys (Foged et al. 1976, Foged 1978, 1979, John 1983, Krammer and Lange-Bertalot 1986, 1988, Krammer and Lange-Bertalot 1991a, Krammer and Lange-Bertalot 1991b, Vyverman 1991, Lange-Bertalot and Moser 1994, Hodgson et al. 1997a, Biggs and Kilroy 2000, Camburn and Charles 2000, Krammer 2000, Ling and Tyler 2000, Sonneman et al. 2000, John et al. 2002). Cells were identified to species level, or where this was not possible, to the lowest taxonomic level possible.



**Figure 4.7:** (a) Nikon Eclipse 80i light microscope connected to camera and monitor;  
(b) Philips XL30 field-emission scanning electron microscope

#### 4.2.3 Analysis of biomass

Samples for the determination of dry mass (DM) were oven-dried for 24 hours at 60°C and weighed. Samples were then combusted at 525°C in a muffle furnace for four hours, and reweighed. Ash-free dry mass (AFDM) was estimated as the difference in the mass before and after combustion and expressed as mg.cm<sup>-2</sup> of the original substratum (Steinman et al. 1996, Biggs and Kilroy 2000, Lavoie et al. 2004):

$$DM = \frac{(W1 - W2)}{A}$$

Where:

DM= dry mass

W1= dried algae on filter (mg)

W2= filter weight (mg)

A= area of substrate (cm<sup>2</sup>)

$$AFDM = \frac{(W1 - W2) - W3}{A}$$

Where:

AFDM= ash-free dry mass

W1= dried algae on filter (mg)

W2= filter weight (mg)

W3= material on filter (mg) after combustion

A= area of substrate (cm<sup>2</sup>).

#### 4.2.4 Analysis of chlorophyll-*a*

For chlorophyll-*a* analysis, the samples were transferred into tubes containing 10 ml of 95% ethanol (Nusch 1980, Snow et al. 2000). The samples were stored overnight in a freezer and then allowed to return to room temperature. The absorbance of the supernatant at 665 nm was determined before and after adding two drops of 0.1N HCl using a Shimadzu UV 1800 spectrophotometer. The chlorophyll-*a* concentration was determined using the Hilmer's equation (Hilmer 1990) that had been derived from the Nusch's equation (Nusch 1980).

$$\text{Chlorophyll } a \text{ (mg.m}^{-2}\text{)} = (E_{b665} - E_{a665}) \times 29.6 \times \left(\frac{V}{A}\right) \times 1000$$

Where:

$E_{b665}$  = absorbance at 665 nm before acidification

$E_{a665}$  = absorbance at 665 nm after acidification

$A$  = area of the sample ( $\text{mm}^2$ )

$V$  = volume of solvent used for the extraction (mL)

29.6 = constant calculated from the maximum acid ratio (1.7) and the specific

absorption coefficient of chlorophyll *a* in ethanol ( $82 \text{ g.L}^{-1}.\text{cm}^{-1}$ )

1000 = correction factor ( $\mu\text{g}.\text{mm}^{-2}$  to  $\text{mg}.\text{m}^{-2}$ )

#### **4.2.5 Diatom Species Index for Australian Rivers (DSIAR)**

The ecological condition of MacKenzie River was evaluated using the DSIAR as a local diatom index (Chessman et al. 2007). DSIAR was developed using data from south east mainland Australia including ACT, VIC, NSW, SA and QLD for stream condition survey. Sensitivity Values (SV) in this index evaluate the tolerance of each species to anthropogenic stress (e.g. industry, agriculture, urban and any other manipulation in the catchment). The SV of all species are used to calculate numerical scores for each sample in the dataset, weighted by the proportional abundance of the each species.

$$DSIAR = \sum_i^n SV_s \times RA$$

Where:

DSIAR= Diatom Species Index for Australian Rivers

SVs= Sensitivity values

RA= Relative abundance

High DSIAR scores signify a flora which is 'less impacted' by anthropogenic modification of the aquatic ecosystem. In contrast, low scores are interpreted as indicating a greater anthropogenic stress (Chessman et al. 2007). The sensitivity values of species to anthropogenic stressors in the MacKenzie River were used to calculate algae-based index scores for each sample in the datasets.

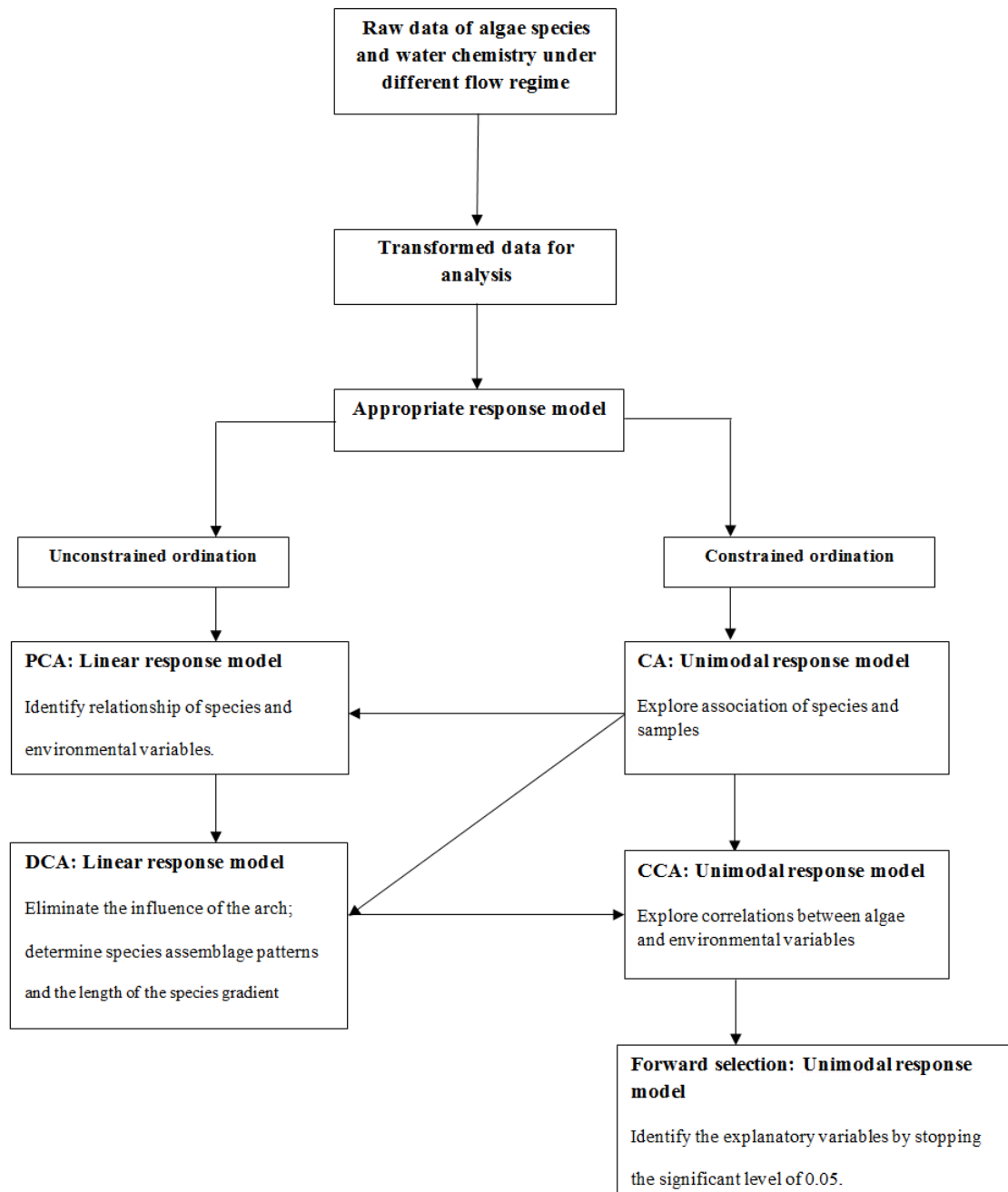
### **4.3 Statistical techniques**

Approaches directed at analysing ecological data have been discussed extensively over the last two decades (Gerritsen 1995, Norris 1995, Van den Brink and Ter Braak 1998, De'ath and Fabricius 2000, Lepš and Šmilauer 2003, Clark 2007, Goslee and Urban 2007). One of the main aims of data analysis in freshwater ecology is the development of tools to inform decisions for water resource management and operations (Barbour et al. 1999). Data can also be used to develop indices suited to biological monitoring (Karr 1987, Barbour et al. 1999). For example, multivariate and multi-metric approaches (multivariate statistical approaches) have been developed by river scientists and waterway agencies to help in river assessment (Norris and Georges 1993, Wright et al. 1993, Barbour et al. 1999).

In the present study, the statistical techniques employed for data analysis consisted of data screening to determine the need to transform data, and the application of techniques comprising Pearson's correlation coefficient, principal component analysis (PCA), correspondence analysis (CA), detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA). Ultimately, forward selections were used to find the most significant variables of water quality and algal response under different flow regimes for input into an ecological response model.

The statistical analyses were undertaken in SPSS Statistics 20 (SPSS 2011), CANOCO software for Windows 4.5 (ter Braak and Šmilauer 2002, Lepš and Šmilauer 2003), C2 software (Juggins 2003), SigmaPlot 12.0, and Microsoft Excel 2010. SPSS was used to calculate the Pearson's correlation matrix and standard deviations in the dataset. CANOCO was used to assess algae assemblage patterns under different flow regimes using PCA, CA, DCA, CCA and forward selection (Figure 4.8).

In this study, three different ecological models were developed including statistical scatter models, quantitative regression models and conceptual models, based on algal responses to different flow regimes.



**Figure 4.8:** Main steps for data analysis, ordinations and modelling

#### **4.3.1 Data screening and data transformations**

Datasets of water chemistry, biological properties and species composition were assembled from analyses spanning three years of field survey and laboratory analysis. Therefore, the dataset comprised values from biological analysis (DM, AFDM and Chl-*a*), diatoms and soft algae enumeration, the DSIAR calculation, water quality measurements, rainfall, and daily flow regime information from the local agency.

During data analysis screening was undertaken to understand the data distribution (i.e. normal vs non-normal) to ensure that the species composition data met the assumptions of parametric (e.g. linear regression) statistical tests. In addition to this, the water quality data were in different units (e.g. mg L<sup>-1</sup>, µS cm<sup>-1</sup>). This required the majority of the data to be transformed in order to ‘normalise’ the data to (1) ensure the data met the assumptions of the statistical techniques (e.g. diatom data) or (2) ensure the data were directly comparable by removing the artefact of unit of measurement (e.g. water quality data). The data were either log transformed or square-root transformed to create a normal distribution.

#### **4.3.2 Pearson’s correlation**

The Pearson correlation matrix is a statistical procedure which investigates the relationship between two variables (Taylor 1990, Zar 2012). In the present study, the Pearson’s correlation was examined to determine the level of significance ( $p < 0.05$ ) in the relationships between two variables. Pearson *r* correlation is a bi-variable measure of the association between two variables. A positive co-efficient between two variables indicates that there is a direct relationship and a negative co-efficient indicates an indirect relationship (Wright 1934). The following factors were analysed to generate a Pearson’s correlation matrix under different flow regimes: hydrological, including flow



regime; water quality and biological characteristics; pH; conductivity (Cond.); turbidity (Turb.); total phosphorus (TP); total nitrogen (TN); silica (Si); cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ); anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ); Chlorophyll-*a* (Chl-*a*); dry mass (DM); ash-free dry mass (AFDM) and DSIAR.

#### **4.3.3 Principal component analysis**

Principal component analysis (PCA) is a statistical method which illustrates the covariance of the variables. It allows for the identification of the principal directions (gradients) of the variations in the data in order to find the patterns in ecological datasets (Jackson 1993, Jolliffe 2002). Furthermore, PCA (indirect analyses) is an unconstrained linear response model used to explore relationships between species and environmental variables. After transformation, the data was used to generate a PCA ordination for the diatom and soft algae community patterns under low flow conditions, freshes and high flows using CANOCO (ter Braak and Šmilauer 2002, Lepš and Šmilauer 2003). If the gradient of axis 1 (the strongest axis) was recorded as  $<2$  (this number was obtained by a DCA, see section 4.3.5), then a linear ordination was deemed an appropriate analysis. However, in some cases a correspondence analysis (CA) was applied to explore the weighted average of species (Table 4.2).

**Table 4.2:** Ordination techniques for statistical analysis

	<b>Linear response model</b>	<b>Unimodal response model</b>
<b>Unconstrained</b>	Principal components analysis (PCA)	Correspondence analysis (CA),
<b>Constrained</b>	Detrended correspondence analysis (DCA),	Canonical correspondence analysis (CCA), forward selection

#### **4.3.4 Correspondence analysis**

Correspondence analysis (CA) is a statistical method which is used to find the association of species and samples. CA is an indirect gradient analysis utilising the unconstrained weighted averaging method (Lepš and Šmilauer 2003) and is applied to data that have a unimodal distribution. The CA test was applied using transformed data to find the association of the algal community structure under different flow regimes. Often, CA may cause an arch in the data. This is caused by nonlinearity of distributions along gradients, and obscures the interpretation of the data, whereupon a detrended correspondence analysis is often applied.

#### **4.3.5 Detrended correspondence analysis**

Detrended correspondence analysis (DCA) was applied to species (unimodal) data that exhibited an arch when analysed using the computationally simple CA (Hill and Gauch 1980). This often occurred where the axis 1 gradient was  $>2$ . In addition, the interpretation of the DCA is easier than other techniques because the main axes are

aligned to the biological gradients (Lepš and Šmilauer 2003, Palmer 2016). In the present study, the DCA was performed on the algal dataset using CANOCO (ter Braak and Šmilauer 2002, Lepš and Šmilauer 2003) to determine species assemblage patterns and the length of the species gradient.

#### **4.3.6 Canonical correspondence analysis**

Canonical correspondence analysis (CCA) was used to understand direct correlations between algal assemblages and productivity, and the environmental variables under different flow regimes. This technique was also used to investigate the relationship between the community and the environmental variables (ter Braak and Verdonschot 1995, ter Braak and Šmilauer 2002, Anderson and Willis 2003, Zare-Chahouki 2012, Paliy and Shankar 2016, Palmer 2016).

Forward selection was then used in the CCA to find the most significant environmental variables that influenced flow, water quality and algal community structure.

#### **4.3.7 Forward selection**

Forward selection is a constrained and unimodal response model that is used to estimate the amount of the proportion of variance that can be explained in the response data (e.g. diatoms) by each predictor variable (e.g. water chemistry parameter) (Douglas and Smol 1995, ter Braak and Verdonschot 1995, Ryves et al. 2002). Forward selection is used to select significant environmental variables, and so find a minimal subset of environmental variables to model a multivariate community structure. In the present study, after examination of the CCA for hydrological, water chemistry and biological data, forward selection was used to develop the models among flow regime, water

quality and algal community structure to find the strongest and most significant environmental variables in the system. Patterns of algal communities and environmental variables (water chemistry) under different flow regimes were investigated by using unconstrained and constrained ordination analyses in CANOCO 4.5 (ter Braak and Šmilauer 2002). The CCA was performed on the full dataset using a Monte Carlo permutation test ( $n= 999$  unrestricted permutations). Forward selection, using a Bonferroni adjustment, identified a subset of significant variables ( $p < 0.05$ ).

## **4.4 Modelling**

### **4.4.1 Regression models**

Generally, regression models are used to predict a response from a subset of significant variables (or drivers) and are a powerful tool in the hind casting of past, and forecasting of future scenarios. Regression models are used to reduce and simplify complicated datasets to make them more understandable and manageable (Mac Nally 2000). In addition, such models describe the relationship between environmental variables and species in ecology and environmental science (De'Ath 2002), allowing an unknown response (dependent variable) to be determined from a set of predictor (independent) variables.

Regression models were developed based on the algal responses under different flow regimes. The models were used to examine algal relative abundance, biomass, species composition, and DSIAR within a wide hydrological gradient along the river. The mean values and standard error of data for each parameter were calculated and used to produce a regression model using SigmaPlot 12.0 software. Furthermore, regression models were used to predict algal biomass and water quality under different flow regimes.

#### **4.4.2 Conceptual model**

The ecological response models were configured to assess the influence of flow regimes on water quality, algal biomass, chlorophyll-*a* concentration and species composition.

This information has been used to evaluate river health and thereby assist the storage manager in configuring consumptive flows down the MacKenzie River to provide greater ecological benefit. The models were based on the relationship between algae responses to differences in flow regime. The conceptual models were created using water quality data, algal biomass, chlorophyll-*a* concentration, species composition, flow regimes and the ecosystem function in the MacKenzie River. Moreover, different types of flow regime including cease to flow, low flows, freshes, high flows, bankfull flows and overbank flows were used to classify the water body along the river by evaluating water quality and biological properties. The flow components can improve the water quality and biological properties of the river and, combined with a biological index, assist the development of ecological response models.

In summary, statistical scatter models, quantitative regression models and conceptual models were applied to derive consumptive flows and sustainable water allocations for environmental benefits. Therefore, operational protocols, recommendations and strategic rules were suggested in three different reaches along the MacKenzie River. The conceptual models were delineated based on the relationship between ecological models, flow regimes and river health. The conceptual ecosystem response model based on the functional relationships between stream hydrology, water quality, biology and ecosystem function, has been developed as a tool to assist in the future configuration of flows in the MacKenzie River.

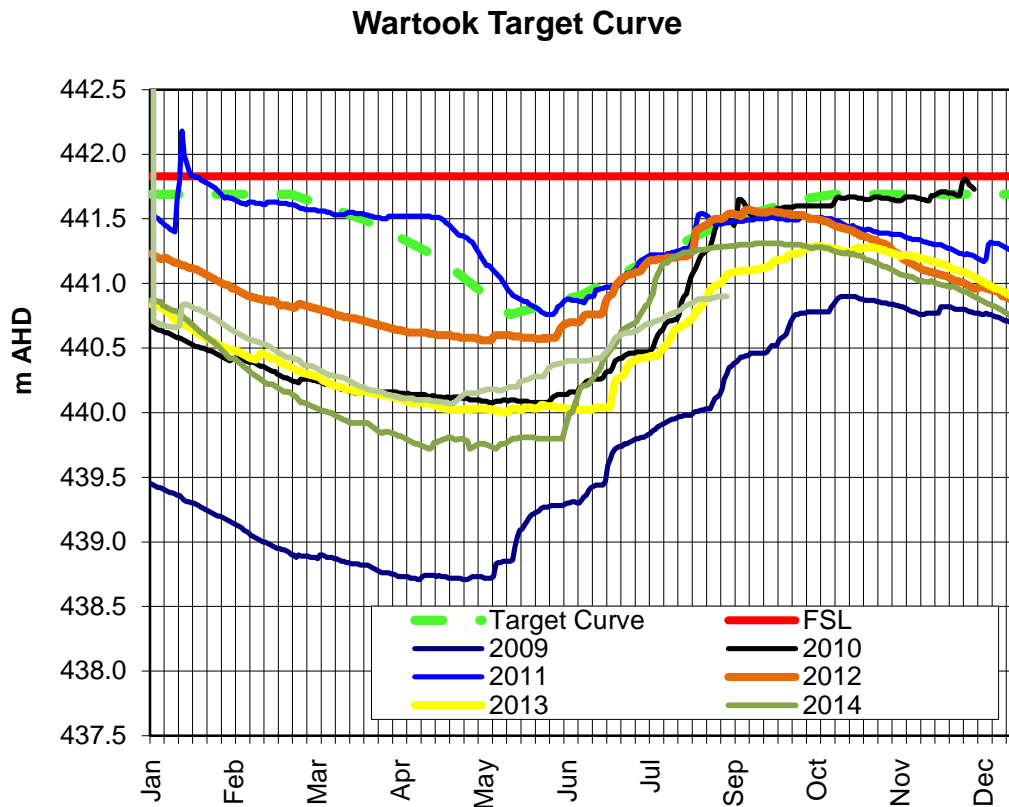
## **Chapter 5: Results**

### **Chapter outline**

The measured responses of water quality and algae under different seasons and different flow regimes are presented here. Firstly, variations in flow and water quality in the MacKenzie River are presented. Secondly, the response of algae in different seasons (under existing baseline flows) is presented and described in terms of biomass and species composition, community structure, and ecosystem function. Then, analyses of the response of the algae to manipulated flow regimes (before, during and after water release events) are described.

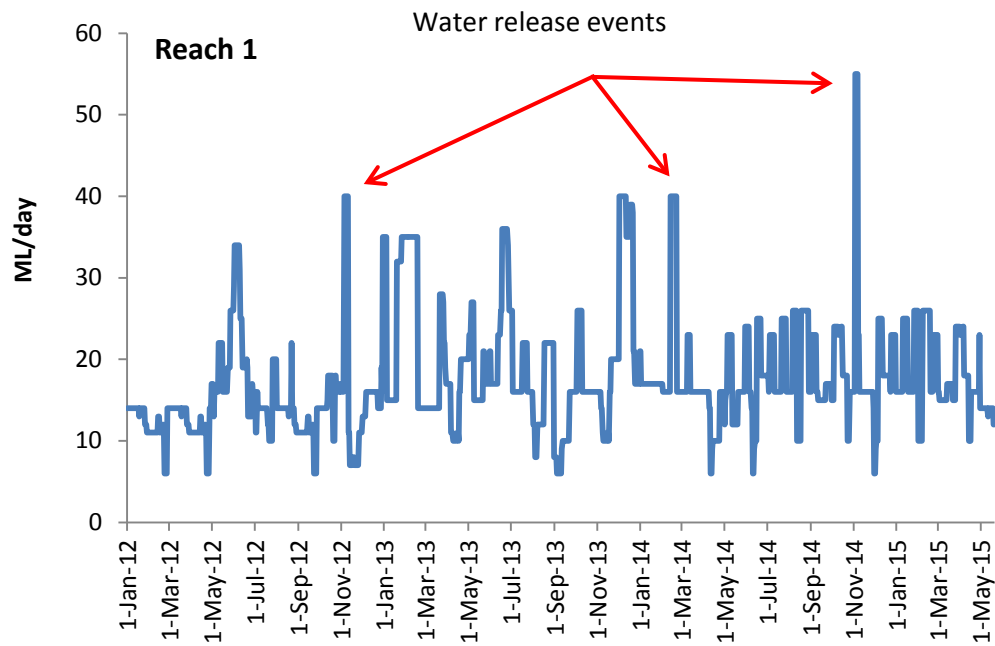
### **5.1 Flow regime variations in the MacKenzie River**

The MacKenzie River is subject to a seasonal climate and so the lower sections have limited flow in the dry season rendering it discontinuous. River flows vary downstream temporally and are greatly influenced by the use of the river channel for the transfer of water for consumptive users, including the supply of water to Horsham city, and the Wimmera Mallee domestic and stock supply system. Water discharge data at Wartook Reservoir and the head gauge of the MacKenzie River show that water levels vary seasonally, particularly over the last few years. The lowest and highest volume of the reservoir/discharge from Wartook was reported in 2009 and 2011 respectively (Figure 5.1).



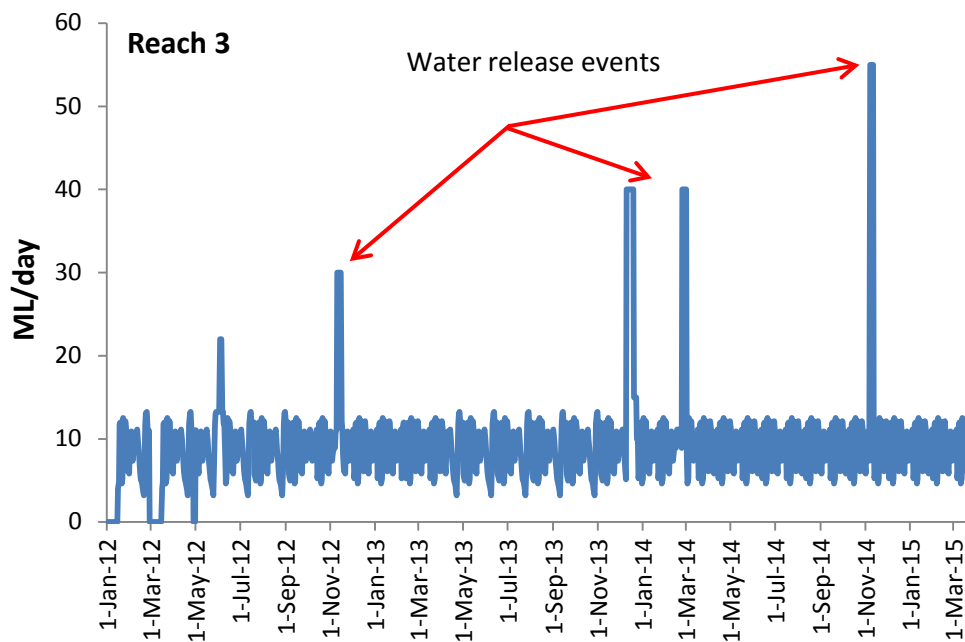
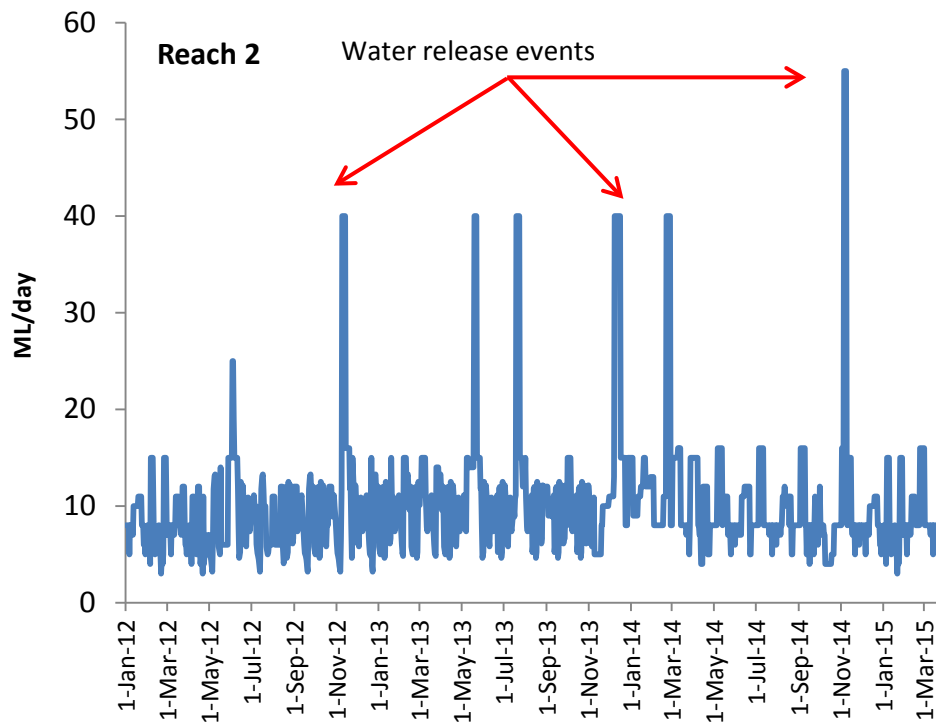
**Figure 5.1:** Water heights of Wartook Reservoir measured at the head gauge since 2009

Often, the flow of the MacKenzie River is controlled by water authorities. The base flow regime for the MacKenzie River is 10-15 ML/day which includes consumptive and environmental flows. Occasionally, every two or three months for three days, the flow is increased to 35-40 ML/day to meet environmental flow requirements. The upstream section (Reach 1) of the River tends to receive water most days of the year (Figure 5.2) due to the requirement of providing a regular water supply to the city of Horsham. Flow regimes in Reach 2 are affected by diversions of the MacKenzie River to Mt Zero Channel at the ‘Dad and Dave’ off take point and so Reach 2 receives a more intermittent supply (Figure 5.3). Due to infiltration and evaporation, flows tend to be lower downstream (Figure 5.4) increasing the risk to poor water quality.



**Figure 5.2:** Flow releases from Lake Wartook outlet (Reach 1) into the MacKenzie River. Measured at the Wartook outlet (S1) from January 2012 to March 2015.





## 5.2 Seasonal changes of water quality

The water quality results show the pH gradually increases down-stream in different seasons. It was observed that the up-stream sites (S1-S4) have lower conductivity and lower nutrient concentrations (TN, TP, TON, ORP) compared to downstream (Table 5.1; Table 5.2; Table 5.3; Table 5.4). Furthermore, the concentrations of cations ( $\text{Mg}^{+2}$ ,  $\text{Ca}^{+2}$ ) and anions ( $\text{SO}^{-4}$ ,  $\text{Cl}^{-1}$ ) increased down-stream. The changes in water quality observed during water release events were such that the water quality downstream was almost comparable to that upstream. Nevertheless, the results show some similarities and dissimilarities occurred in water quality characteristics along the river. For examples pH was more acidic in November 2012 (Table 5.3). In addition, temperature, Oxidation Reduction potential, Total Dissolved Solid, Total Oxidative Nitrogen, total phosphorus and total nitrogen were different in different seasons. However, some of the water quality characteristics such as cations and anions were relatively similar in different seasons. Whilst the water quality does vary during different seasons, the main changes in water quality are observed during the water release events. Therefore, flow regimes are known to have a significant influence on water quality (oxygen level, temperature, suspended solid, organic matters and other nutrients), biotic structure and function and the metabolism of rivers and streams.

**Table 5.1:** Physical and chemical water quality characteristics at the sampling stations  
on the MacKenzie River in February 2012

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>pH</b>	-	6.5	6.3	6.6	6.8	7.2	7.3	7.2	7.5	7.2	dry
<b>Temp.</b>	°C	18.9	18.7	17.8	17.6	19.8	18.2	19.0	21.2	22.2	dry
<b>Cond.</b>	µS cm <sup>-1</sup>	75	78	79	76	80	82	82	85	88	dry
<b>Turb.</b>	NTU	8.2	9.3	8	9	8.5	12	15	16	15	dry
<b>Depth</b>	m	0.5	0.6	0.5	0.6	0.8	1.5	1.2	1.1	1.2	dry
<b>ORP</b>	mV	222	250	256	261	281	258	279	284	261	dry
<b>DO</b>	mg L <sup>-1</sup>	8.3	98.8	9.8	8.4	9.2	8.8	7.8	8.5	7.3	dry
<b>TDS</b>	mg L <sup>-1</sup>	45	48	43	55	65	55	71	71	71	dry
<b>TSS</b>	mg L <sup>-1</sup>	5	6.3	7.4	8	7	8.2	7.3	7.8	8.2	dry
<b>TN</b>	mg L <sup>-1</sup>	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.5	0.7	dry
<b>TP</b>	mg L <sup>-1</sup>	0.05	0.05	0.06	0.05	0.07	0.05	0.08	0.08	0.08	dry
<b>Mg<sup>2+</sup></b>	mg L <sup>-1</sup>	1.3	1.7	2	2.2	2.8	2.5	3.2	3.3	3.3	dry
<b>NH<sub>3</sub></b>	mg L <sup>-1</sup>	0.1	0.1	0.08	0.09	0.07	0.09	0.07	0.08	0.07	dry
<b>SO<sub>4</sub><sup>2-</sup></b>	mg L <sup>-1</sup>	0.22	0.36	0.46	0.48	0.55	0.54	0.63	0.55	0.45	dry
<b>Ca<sup>2+</sup></b>	mg L <sup>-1</sup>	1.6	1.8	2.2	3.3	3.2	3.3	3.1	3.2	3.4	dry
<b>SiO<sub>2</sub></b>	mg L <sup>-1</sup>	0.24	0.35	0.23	0.25	0.39	0.45	0.55	0.66	0.68	dry
<b>TON</b>	mg L <sup>-1</sup>	0.5	0.08	0.07	0.07	0.05	0.07	0.08	0.08	0.08	dry
<b>Cl<sup>-</sup></b>	mg L <sup>-1</sup>	27	25	25	28	33	32	38	38	34	dry

(Temp = temperature, ORP= Oxidation Reduction potential, TDS= Total Dissolved Solid, TON= Total Oxidative Nitrogen).

**Table 5.2:** Physical and chemical water quality characteristics at the sampling stations  
on the MacKenzie River in July 2012

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>pH</b>	-	6.5	6.8	6.7	6.6	6.9	7.2	7.4	7.3	7.4	7.5
<b>Temp.</b>	°C	14	13	12	15	16	15	15	15	13	14
<b>Cond.</b>	µS cm <sup>-1</sup>	70	75	78	71	75	140	150	120	110	150
<b>Turb.</b>	NTU	10	92	12	11	14	12	15	16	15	15
<b>Depth</b>	m	0.5	0.6	0.5	0.6	0.8	1.4	1.5	1.8	1.8	1.5
<b>ORP</b>	mV	222	250	256	261	281	258	279	284	261	260
<b>DO</b>	mg L <sup>-1</sup>	8.3	98.8	9.8	8.4	9.2	8.8	7.8	8.5	7.3	7.5
<b>TDS</b>	mg L <sup>-1</sup>	45	48	48	55	65	55	71	71	71	75
<b>TSS</b>	mg L <sup>-1</sup>	5.6	6.3	7.4	8	7	8.2	7.3	7.8	8.2	8.3
<b>TN</b>	mg L <sup>-1</sup>	0.2	0.2	0.2	0.3	0.3	0.55	0.4	0.5	0.7	0.8
<b>TP</b>	mg L <sup>-1</sup>	0.05	0.05	0.06	0.05	0.07	0.05	0.08	0.08	0.08	0.09
<b>Mg<sup>2+</sup></b>	mg L <sup>-1</sup>	1.3	1.7	2	2.2	2.8	2.5	3.2	3.3	3.3	3.8
<b>NH<sub>3</sub></b>	mg L <sup>-1</sup>	0.2	0.1	0.08	0.07	0.06	0.07	0.07	0.08	0.07	0.08
<b>SO<sub>4</sub><sup>-2</sup></b>	mg L <sup>-1</sup>	0.22	0.36	0.46	0.48	0.55	0.5	0.63	0.62	0.45	0.45
<b>Ca<sup>+2</sup></b>	mg L <sup>-1</sup>	1.6	1.8	2.2	3.3	3.1	3.3	3.1	3.2	3.4	0.36
<b>SiO<sub>2</sub></b>	mg L <sup>-1</sup>	0.22	0.22	0.46	0.25	0.39	0.45	0.55	0.66	0.68	0.65
<b>TON</b>	mg L <sup>-1</sup>	0.04	0.08	0.07	0.07	0.05	0.07	0.08	0.08	0.08	0.08
<b>Cl<sup>-1</sup></b>	mg L <sup>-1</sup>	21	18	29	23	41	36	38	38	34	36

(Temp = temperature, ORP= Oxidation Reduction potential, TDS= Total Dissolved Solid, TON= Total Oxidative Nitrogen).

**Table 5.3:** Physical and chemical water quality characteristics at the sampling stations  
on the MacKenzie River in November 2012

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>pH</b>	-	6.2	6.3	6.4	6.5	6.6	7.1	6.9	7.2	7.6	7.4
<b>Temp.</b>	°C	18	18	19	20	22	21	18	21	21	23
<b>Cond.</b>	µS cm <sup>-1</sup>	80	85	85	86	97	114	115	150	145	163
<b>Turb.</b>	NTU	8	11	15	12	14	17	15	15	19	18
<b>Depth</b>	m	0.8	0.6	0.8	1.1	1.2	1.5	1.1	1.0	1.2	0.8
<b>ORP</b>	mV	202	203	214	225	235	280	269	299	280	287
<b>DO</b>	mg L <sup>-1</sup>	8	8.2	9.2	10.2	9.8	8.2	7.7	8.2	7.8	8.1
<b>TDS</b>	mg L <sup>-1</sup>	42	55	42	66	59	68	81	82	85	87
<b>TSS</b>	mg L <sup>-1</sup>	5.2	5.6	5.4	7.1	8.2	7.2	8.2	8.8	8.9	7.9
<b>TN</b>	mg L <sup>-1</sup>	0.1	0.3	0.2	0.3	0.5	0.6	0.4	0.60	0.8	0.9
<b>TP</b>	mg L <sup>-1</sup>	0.04	0.05	0.06	0.05	0.06	0.06	0.06	0.08	0.07	0.1
<b>Mg<sup>2+</sup></b>	mg L <sup>-1</sup>	1.8	2.2	2.3	2.6	4.4	3.4	5.5	5.1	5.3	5.4
<b>NH<sub>3</sub></b>	mg L <sup>-1</sup>	0.1	0.1	0.1	0.08	0.08	0.07	0.08	0.09	0.09	0.08
<b>SO<sub>4</sub><sup>-2</sup></b>	mg L <sup>-1</sup>	0.23	0.25	0.33	0.3	0.45	0.66	0.8	0.7	0.6	0.8
<b>Ca<sup>+2</sup></b>	mg L <sup>-1</sup>	1.2	1.2	1.3	2.2	1.3	3.3	5.2	3.3	3.3	4.8
<b>SiO<sub>2</sub></b>	mg L <sup>-1</sup>	0.1	0.2	0.3	0.4	0.5	0.4	0.6	0.8	0.7	0.9
<b>TON</b>	mg L <sup>-1</sup>	0.06	0.06	0.04	0.05	0.05	0.4	0.08	0.09	0.1	0.1
<b>Cl<sup>-1</sup></b>	mg L <sup>-1</sup>	22	23	28	35	37	42	45	46	42	44

(Temp = temperature, ORP= Oxidation Reduction potential, TDS= Total Dissolved Solid, TON= Total Oxidative Nitrogen).

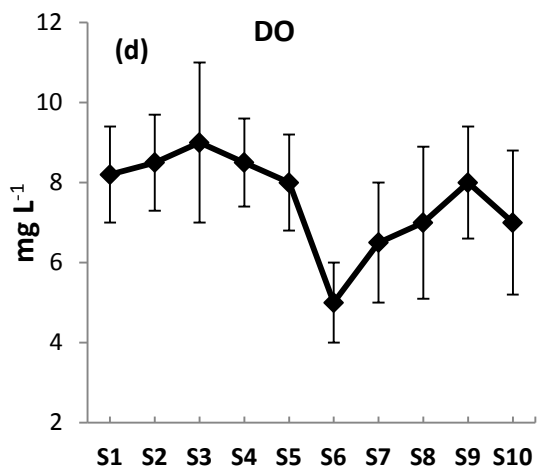
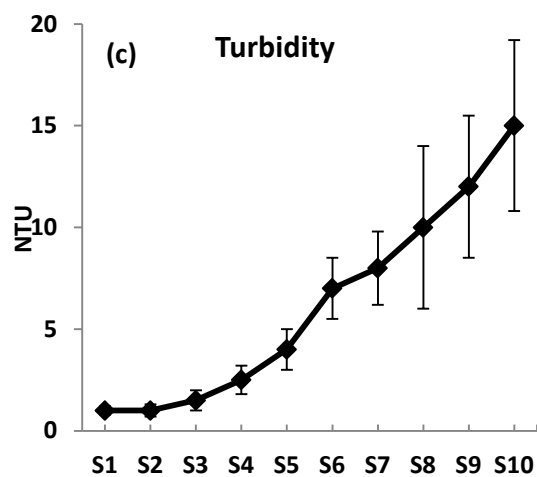
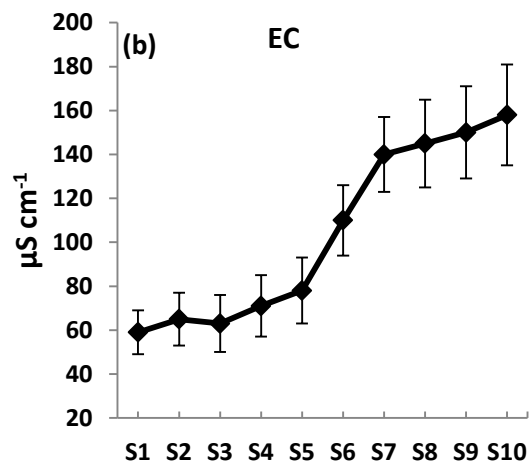
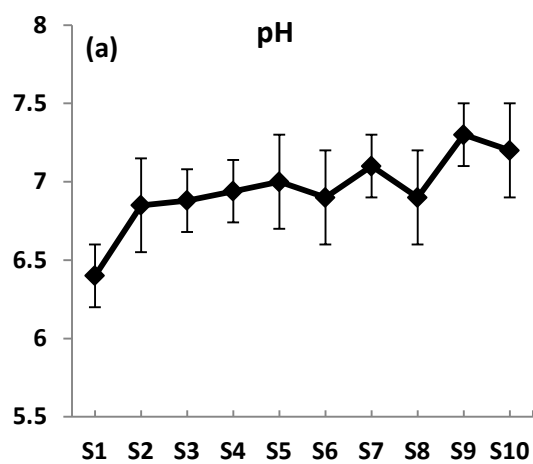
**Table 5.4:** Physical and chemical water quality characteristics at the sampling stations on the MacKenzie River in June 2013

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>pH</b>	-	6.8	6.7	6.6	6.9	7.2	7.3	7.2	7.3	7.2	7.7
<b>Temp.</b>	°C	13	12	15	16	15	18.2	19.8	18.2	19.0	22
<b>Cond.</b>	µS cm <sup>-1</sup>	75	78	71	75	140	82	80	82	82	85
<b>Turb.</b>	NTU	92	12	11	14	12	12	8.5	12	15	21
<b>Depth</b>	m	0.6	0.5	0.6	0.8	1.4	1.5	0.8	1.5	1.2	1.9
<b>ORP</b>	mV	250	256	261	281	258	258	281	258	279	285
<b>DO</b>	mg L <sup>-1</sup>	98.8	9.8	8.4	9.2	8.8	8.8	9.2	8.8	7.8	6.9
<b>TDS</b>	mg L <sup>-1</sup>	48	48	55	65	55	55	65	55	71	72
<b>TSS</b>	mg L <sup>-1</sup>	6.3	7.4	8	7	8.2	8.2	7	8.2	7.3	7.6
<b>TN</b>	mg L <sup>-1</sup>	0.2	0.2	0.3	0.3	0.55	0.3	0.3	0.3	0.4	.45
<b>TP</b>	mg L <sup>-1</sup>	0.05	0.06	0.05	0.07	0.05	0.05	0.07	0.05	0.08	0.09
<b>Mg<sup>2+</sup></b>	mg L <sup>-1</sup>	1.7	2	2.2	2.8	2.5	2.5	2.8	2.5	3.2	4.8
<b>NH<sub>3</sub></b>	mg L <sup>-1</sup>	0.1	0.08	0.07	0.06	0.07	0.09	0.07	0.09	0.07	0.09
<b>SO<sub>4</sub><sup>-2</sup></b>	mg L <sup>-1</sup>	0.36	0.46	0.48	0.55	0.5	0.54	0.55	0.54	0.63	0.84
<b>Ca<sup>+2</sup></b>	mg L <sup>-1</sup>	1.8	2.2	3.3	3.1	3.3	3.3	3.2	3.3	3.1	3.9
<b>SiO<sub>2</sub></b>	mg L <sup>-1</sup>	0.22	0.46	0.25	0.39	0.45	0.45	0.39	0.45	0.55	0.65
<b>TON</b>	mg L <sup>-1</sup>	0.08	0.07	0.07	0.05	0.07	0.07	0.05	0.07	0.08	0.1
<b>Cl<sup>-1</sup></b>	mg L <sup>-1</sup>	18	29	23	41	36	32	33	32	38	49

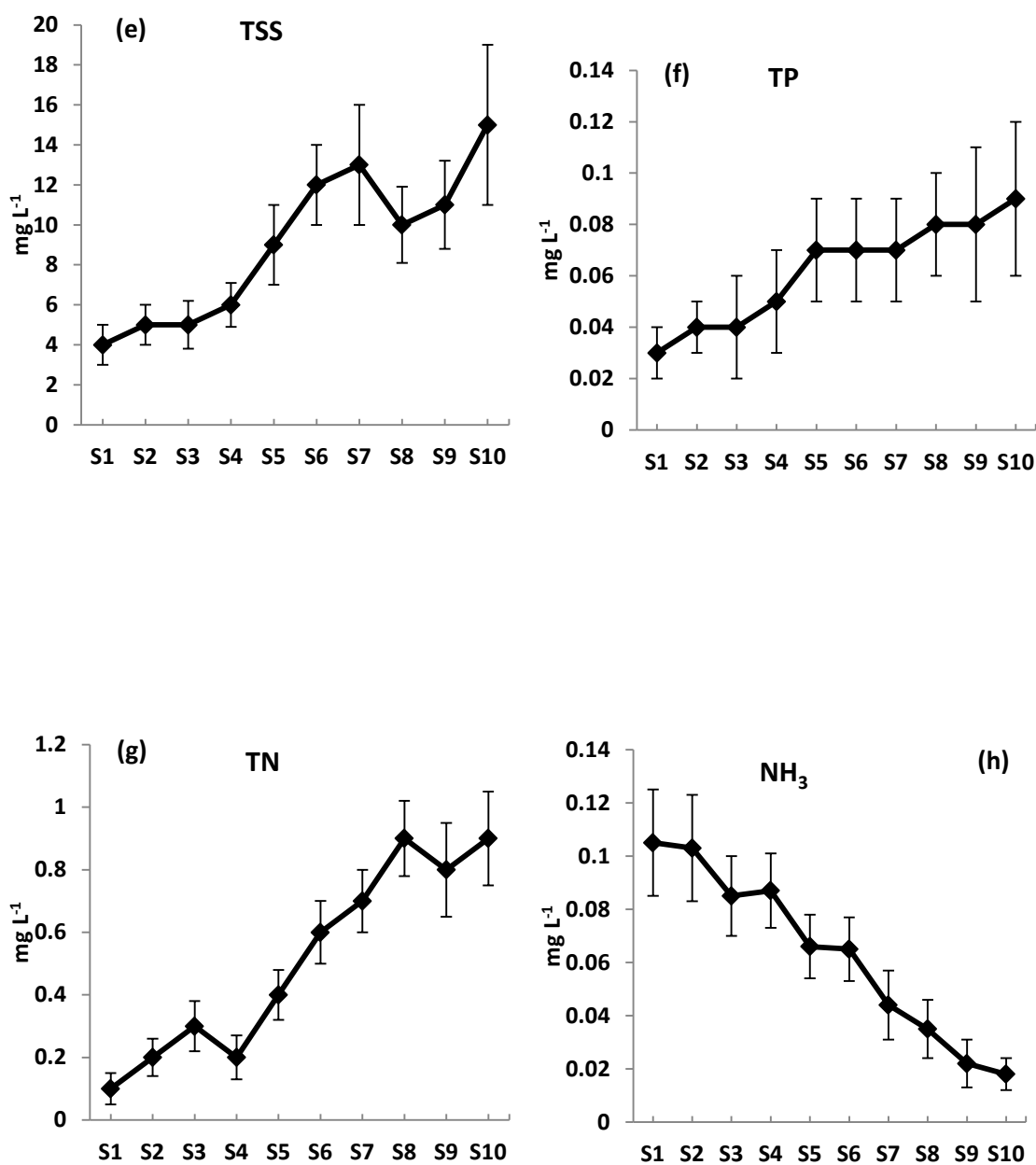
(Temp = temperature, ORP= Oxidation Reduction potential, TDS= Total Dissolved Solid, TON= Total Oxidative Nitrogen).

### **5.2.1 Average annual water chemistry in the MacKenzie River**

There were downstream trends in most of the physical and chemical water parameters measured during the different flow regimes. Typically, the pH gradually increased (became more alkaline) downstream (Figure 5.5a). The upstream sites (S1-S4) also had lower conductivity compared to those further downstream (Figure 5.5b). Turbidity increased greatly downstream (Figure 5.5c) and the concentration of dissolved oxygen (DO) changed along the river where low concentrations were observed mid-stream due to standing water in the middle of the river (Figure 5.5d). Total suspended solid (TSS) values also increased in the lower parts of the river particularly during water release events (Figure 5.5e). The concentrations of nutrients (TN and TP) also increased downstream (Figure 5.5f-g). The concentrations of cations ( $\text{Mg}^{+2}$ ,  $\text{Ca}^{+2}$ ) and anions ( $\text{SO}_4^{-2}$ ,  $\text{Cl}^{-1}$ ) also increased downstream (Table 5.1) consistent with an increase in total salinity and the concentration of Oxidation Reduction Potential (ORP), Total Dissolved Solid (TDS) and Total Oxidative Nitrogen (TON). In contrast, the concentration of ammonia decreased from upstream to downstream (Figure 5.5h).







**Figure 5.5:** Physical and chemical characteristics of water at the sampling stations (S1-S10) along the MacKenzie River: **(a)** pH; **(b)** Electrical Conductivity; **(c)** Turbidity; **(d)** Dissolved Oxygen; **(e)** Total Suspended Solid; **(f)** Total Phosphorus; **(g)** Total Nitrogen; **(h)** Ammonia. Data indicate means  $\pm$  SD

**Table 5.5:** Average annual physical and chemical water quality characteristics at the sampling stations on the MacKenzie River from February 2012 to Nov 2014 (Temp = temperature, ORP= Oxidation Reduction potential, TDS= Total Dissolved Solid, TON= Total Oxidative Nitrogen). Data indicate means  $\pm$  SD.

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>Temp</b>	°C	14 $\pm$ 7	16 $\pm$ 6	16 $\pm$ 7	16 $\pm$ 8	17 $\pm$ 6	18 $\pm$ 4	19 $\pm$ 8	19 $\pm$ 6	19 $\pm$ 7	19 $\pm$ 6
<b>Depth</b>	m	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.3	0.7 $\pm$ 0.4	0.6 $\pm$ 0.3	1 $\pm$ 0.5	0.8 $\pm$ 0.4	1 $\pm$ 0.3	0.5 $\pm$ 0.2	0.8 $\pm$ 0.5
<b>ORP</b>	mV	212 $\pm$ 22	222 $\pm$ 32	223 $\pm$ 35	258 $\pm$ 41	245 $\pm$ 42	255 $\pm$ 64	255 $\pm$ 58	244 $\pm$ 51	299 $\pm$ 75	298 $\pm$ 74
<b>TDS</b>	mg L <sup>-1</sup>	22 $\pm$ 11	35 $\pm$ 15	41 $\pm$ 22	68 $\pm$ 31	71 $\pm$ 23	86 $\pm$ 32	91 $\pm$ 33	98 $\pm$ 38	106 $\pm$ 41	111 $\pm$ 35
<b>Mg<sup>2+</sup></b>	mg L <sup>-1</sup>	1.1 $\pm$ 0.5	1.8 $\pm$ 0.8	2.2 $\pm$ 0.9	2 $\pm$ 0.8	3 $\pm$ 0.5	3 $\pm$ 0.5	3 $\pm$ 1	4 $\pm$ 1.2	4 $\pm$ 1.2	4.1 $\pm$ 1.3
<b>SO<sub>4</sub><sup>2-</sup></b>	mg L <sup>-1</sup>	0.55 $\pm$ 0.08	0.51 $\pm$ 0.1	0.52 $\pm$ 0.1	0.55 $\pm$ 0.1	0.61 $\pm$ 0.1	0.44 $\pm$ 0.09	0.64 $\pm$ 0.1	0.63 $\pm$ 0.08	0.55 $\pm$ 0.09	0.45 $\pm$ 0.1
<b>Ca<sup>+2</sup></b>	mg L <sup>-1</sup>	1.5 $\pm$ 0.5	2.1 $\pm$ 0.6	2.5 $\pm$ 0.5	3 $\pm$ 0.6	3.2 $\pm$ 0.5	3.2 $\pm$ 0.6	3.6 $\pm$ 0.2	3.4 $\pm$ 0.5	3.5 $\pm$ 0.5	3.9 $\pm$ 0.6
<b>SiO<sub>2</sub></b>	mg L <sup>-1</sup>	0.22 $\pm$ 0.05	0.28 $\pm$ 0.06	0.36 $\pm$ 0.09	0.38 $\pm$ 0.06	0.49 $\pm$ 0.08	0.65 $\pm$ 0.05	0.75 $\pm$ 0.04	0.79 $\pm$ 0.09	0.78 $\pm$ 0.08	0.88 $\pm$ 0.09
<b>TON</b>	mg L <sup>-1</sup>	0.07 $\pm$ 0.01	0.08 $\pm$ 0.02	0.07 $\pm$ 0.01	0.06 $\pm$ 0.02	0.08 $\pm$ 0.02	0.07 $\pm$ 0.03	0.07 $\pm$ 0.01	0.08 $\pm$ 0.04	0.1 $\pm$ 0.05	0.1 $\pm$ 0.05
<b>Cl<sup>-1</sup></b>	mg L <sup>-1</sup>	19 $\pm$ 8	22 $\pm$ 8	23 $\pm$ 4	28 $\pm$ 8	29 $\pm$ 7	30 $\pm$ 10	30 $\pm$ 6	29 $\pm$ 8	33 $\pm$ 8	40 $\pm$ 10

The changes in water quality observed during water release events were such that the water quality downstream was relatively similar to that upstream. Nevertheless, the results show this phenomenon (similarity of the water quality between upstream and downstream reaches) is only temporary in nature and antecedent conditions return within a few days of water release. Whilst the water quality does vary during different seasons, the main changes in water quality are observed during the water release events (Table 5.2).

**Table 5.6:** Physical and chemical water quality characteristics during water release events at the sampling stations on the MacKenzie River (Temp. = temperature, Cond. = conductivity, Turb.= Turbidity, ORP= Oxidation Reduction potential, DO = Dissolved Oxygen, TDS= Total Dissolved Solid, TSS: Total Suspended Solid, TN= Total Nitrogen, TON= Total Oxidative Nitrogen).

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
<b>pH</b>	-	6.7	6.7	6.7	6.8	6.8	6.8	6.7	6.5	6.6	6.7
<b>Temp.</b>	°C	16.2	16.5	16.3	16.2	17.4	17.2	17.5	17.2	18.1	17.9
<b>Cond.</b>	µS cm <sup>-1</sup>	75	76	79	76	79	82	82	85	88	86
<b>Turb.</b>	NTU	8.5	9.3	8.2	10	8.5	12	15.2	16	14.2	14.5
<b>Depth</b>	m	0.8	0.8	0.7	1.2	1.3	1.5	1.6	1.7	1.2	1.3
<b>ORP</b>	mV	250	251	256	264	261	258	269	274	261	271
<b>DO</b>	mg L <sup>-1</sup>	8.5	9.2	9.8	8.4	9.2	7.2	7.8	8.5	7.8	8.2
<b>TDS</b>	mg L <sup>-1</sup>	45	46	42	55	65	64	71	75	71	85
<b>TSS</b>	mg L <sup>-1</sup>	6	6.2	7.4	6.6	7	8.2	7.3	6.8	8.2	7.1
<b>TN</b>	mg L <sup>-1</sup>	0.2	0.2	0.3	0.2	0.3	0.3	0.4	0.5	0.7	0.8
<b>TP</b>	mg L <sup>-1</sup>	0.05	0.05	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.08
<b>Mg<sup>2+</sup></b>	mg L <sup>-1</sup>	1.2	1.7	2	2.2	2.1	2.5	2.6	3.3	3.3	3.3
<b>NH<sub>3</sub></b>	mg L <sup>-1</sup>	0.1	0.1	0.09	0.09	0.08	0.09	0.07	0.08	0.07	0.09
<b>SO<sub>4</sub><sup>2-</sup></b>	mg L <sup>-1</sup>	0.45	0.52	0.46	0.48	0.55	0.54	0.62	0.55	0.42	0.56
<b>Ca<sup>2+</sup></b>	mg L <sup>-1</sup>	1.6	1.8	2.2	3.3	3.2	3.3	3.1	3.2	3.4	3.6
<b>SiO<sub>2</sub></b>	mg L <sup>-1</sup>	0.024	0.25	0.23	0.25	0.39	0.51	0.55	0.66	0.62	0.77
<b>TON</b>	mg L <sup>-1</sup>	0.06	0.08	0.07	0.08	0.05	0.07	0.06	0.08	0.08	0.08
<b>Cl<sup>-1</sup></b>	mg L <sup>-1</sup>	22	25	23	25	31	32	33	28	29	28

### 5.3 Algal response under base flow (10-15 ML/day)

This investigation showed that during base flow the algal flora of the MacKenzie River was composed of typical acidic taxa, especially in the upper reaches, while more alkaline taxa were recorded in lower reaches. In this study, 126 diatom species (43 genera), 44 green algae species (23 genera), 24 cyanobacteria (10 genera), and 9 other algae (6 genera) were recorded from samples collected during base flow conditions. The diatom community (species composition) was the most abundant and dominated the river samples, displaying high diversity in the upstream sites while the relative proportions of green algae and cyanobacteria increased in the mid and downstream reaches.

The most common algal species in the upstream reaches (S1-S5) were diatoms (based on relative abundance ) including: diatoms - *Brachysira brebissonii*, *Eunotia bilunaris*, *Frustulia rhomboides*, *Gomphonema affine*, *Melosira arentii*, *Navicula heimansioides*, *Tabellaria flocculosa*; green algae - *Stigeoclonium flagelliferum*, *Ulothrix flacca*; and cyanobacteria - *Tolypothrix tenuis*. In the downstream reaches (S6-S10), the most common algal species were: diatoms - *Eunotia serpentina*, *Nitzschia capitellata*, *Planothidium frequentissimum*, *Surirella angusta*; green algae - *Oedogonium undulatum*; and Charophytes - *Chara* sp., *Nitella cristata* and *Schizothrix arenaria* (Table 5.3).

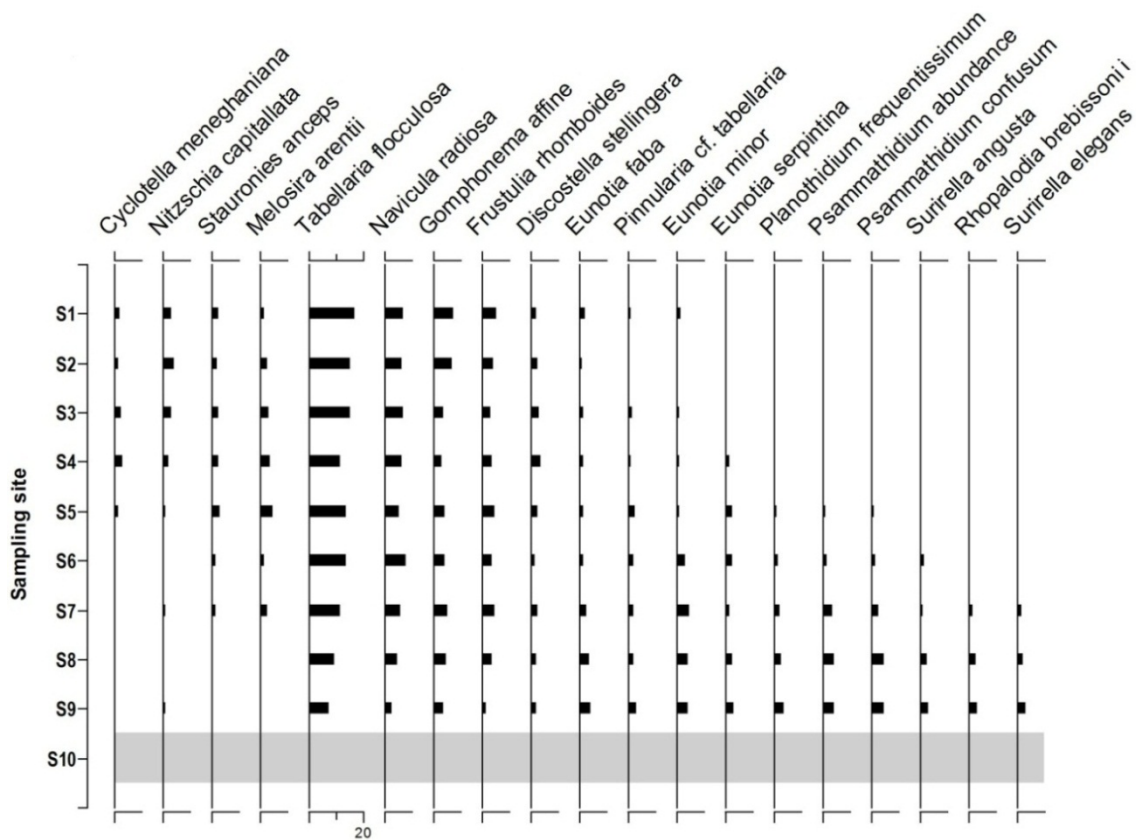
**Table 5.7:** Taxonomic composition of algae by genus and number of taxa present in each genus, in the MacKenzie River

<b>Bacillariophyta</b>	<b>Taxa No.</b>	<b>Chlorophyta</b>	<b>Taxa No.</b>	<b>Cyanophyta</b>	<b>Taxa No.</b>	<b>Other groups</b>	<b>Taxa No.</b>
<i>Achnanthes</i>	2	<i>Ankistrodesmus</i>	1	<i>Anabaena</i>	2	<i>Cryptomonas</i>	1
<i>Achnanthidium</i>	1	<i>Bambusina</i>	1	<i>Chroococcus</i>	1	<i>Ceratium</i>	2
<i>Asterionella</i>	1	<i>Bulbochaete</i>	1	<i>Lyngbya</i>	3	<i>Dinobryon</i>	2
<i>Aulacosira</i>	2	<i>Chara</i>	2	<i>Merismopedia</i>	1	<i>Euglena</i>	1
<i>Brachysira</i>	6	<i>Chlorella</i>	1	<i>Nodularia</i>	1	<i>Gymnodinium</i>	1
<i>Brevisira</i>	1	<i>Cladophora</i>	1	<i>Nostoc</i>	1	<i>Peridinium</i>	2
<i>Caloneis</i>	2	<i>Closterium</i>	3	<i>Oscillatoria</i>	5		
<i>Cocconeis</i>	1	<i>Cosmarium</i>	4	<i>Phormidium</i>	2		
<i>Craticula</i>	1	<i>Euastrum</i>	3	<i>Schizothrix</i>	1		
<i>Cyclostephanos</i>	1	<i>Gonium</i>	1	<i>Tolypothrix</i>	1		
<i>Cyclotella</i>	2	<i>Micrasterias</i>	1	<i>Nostoc</i>	1		
<i>Cymbella</i>	3	<i>Monoraphidium</i>	1	<i>Oscillatoria</i>	5		
<i>Cymboplectra</i>	4	<i>Nitella</i>	2				
<i>Diatoma</i>	3	<i>Oedogonium</i>	2				
<i>Diplonies</i>	1	<i>Oocystis</i>	2				
<i>Discostella</i>	1	<i>Pediastrum</i>	2				
<i>Encyonema</i>	2	<i>Rhizoclonium</i>	1				
<i>Epithemia</i>	1	<i>Scenedesmus</i>	6				
<i>Eunotia</i>	16	<i>Spirogyra</i>	1				
<i>Envekadea</i>	1	<i>Staurodesmus</i>	1				
<i>Fragilaria</i>	4	<i>Staurostrum</i>	5				
<i>Frustulia</i>	5	<i>Stigeoclonium</i>	1				
<i>Gomphonema</i>	7	<i>Ulothrix</i>	1				
<i>Gyrosigma</i>	2						
<i>Hantzschia</i>	1						
<i>Luticola</i>	1						
<i>Melosira</i>	1						
<i>Navicula</i>	8						
<i>Neidium</i>	4						
<i>Nitzschia</i>	5						
<i>Pinnularia</i>	9						
<i>Planothidium</i>	1						
<i>Psammothidium</i>	2						
<i>Pseudostaurosira</i>	1						
<i>Rhopalodia</i>	1						
<i>Sellaphora</i>	1						
<i>Stauroforma</i>	1						
<i>Stauroneis</i>	7						
<i>Staurosira</i>	1						
<i>Stenopterobia</i>	3						
<i>Surirella</i>	3						
<i>Synedra</i>	3						
<i>Tabellaria</i>	3						
<b>Total</b>	<b>126</b>		<b>44</b>		<b>24</b>		<b>9</b>

### 5.3.1 Diatom variations under base flow (10-15ML/day)

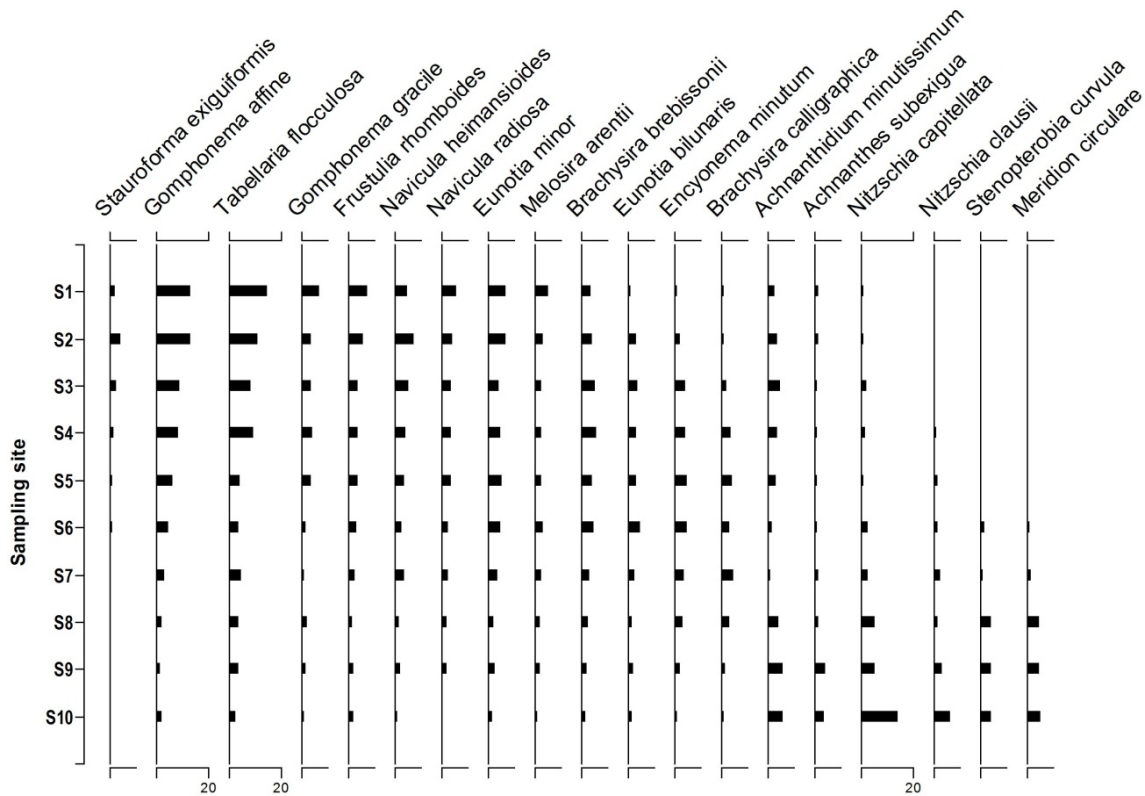
Diatom species composition varied from upstream to downstream and between seasons.

The summer (February 2012) samples presented in Figure 5.6 showed *F. rhomboidia*, *G. affine*, *N. radiosa*, *T. flocculosa* to be common in the upstream reaches, however, the relative abundance of those diatoms decreased downstream. In contrast, those most strongly associated with downstream reaches were *Eunotia serpentina*, *Planothidium abundans*, *Rhopalodia brebissonii* and *Surirella elegans*.



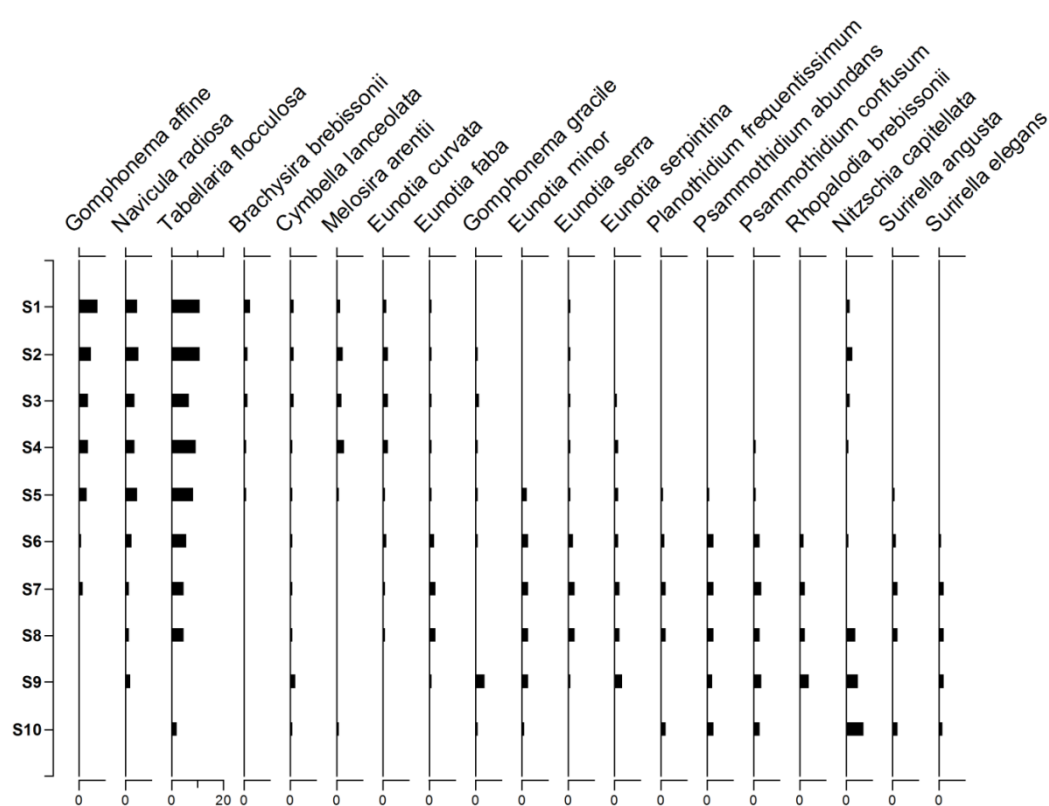
**Figure 5.6:** Results of diatom analysis along the MacKenzie River sampling stations in February 2012 (>4% in any one sample, sorted by weighted-averaging [ascending]). Station 10 was dry at the time of sampling.

In contrast, the winter (July 2012) samples showed the upstream sites supported *F. rhomboides*, *G. affine*, *G. gracile*, *N. heimansioides*, *N. radiosa* and *T. flocculosa* while the downstream sites supported assemblages including *N. capitellata*, *N. clausii*, *M. circulare* and *. curvula* (Figure 5.7).



**Figure 5.7:** Results of diatom analysis along the MacKenzie River sampling stations in July 2012 (>4% in any one sample, sorted by weighted-averaging [ascending]).

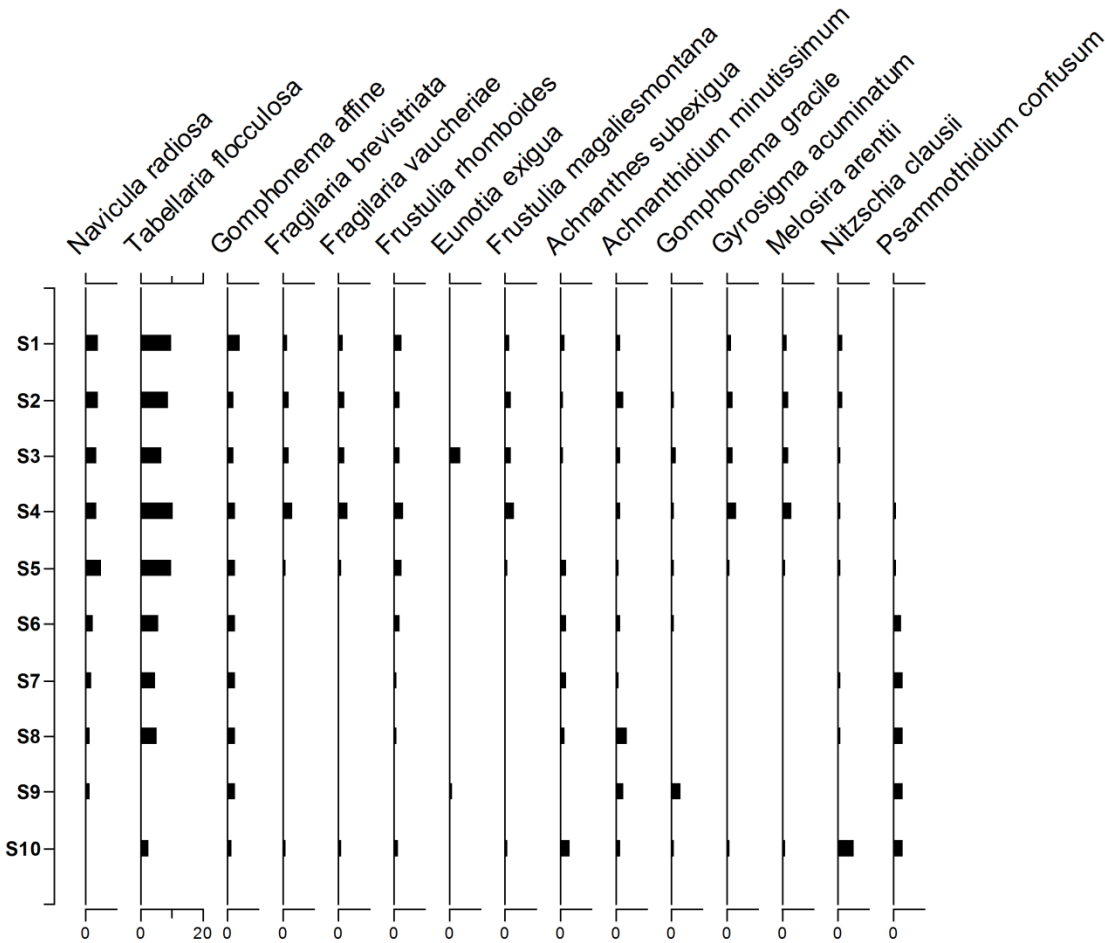
The results of the spring survey (November 2012) showed the algal community to be similar to those in summer. The most common diatom taxa collected in the upstream (S1-S5) samples were *T. flocculosa*, *G. affine*, *N. radiosa* and *Melosira arentii* while in the downstream reaches the most common species were *Eunotia serpentina*, *Psammothidium abundans*, *Psammothidium confusum*, *N. capitellata* and *Surirella angusta* (Figure 5.8).



**Figure 5.8:** Results of diatom analysis along the MacKenzie River sampling stations in November 2012 (>4% in any one sample, sorted by weighted-averaging [ascending]).



The results of the diatom community structure in the following winter, in June 2013, showed the most common diatom species in upstream sites (S1-SX) are *T. flocculosa*, *G. affine*, *N. radiosa*, while in the downstream section (SX-S10) the most common species were *Achnantheidium minutissimum*, *N. clausii* and *Psammothidium confusum* (Figure 5.9)



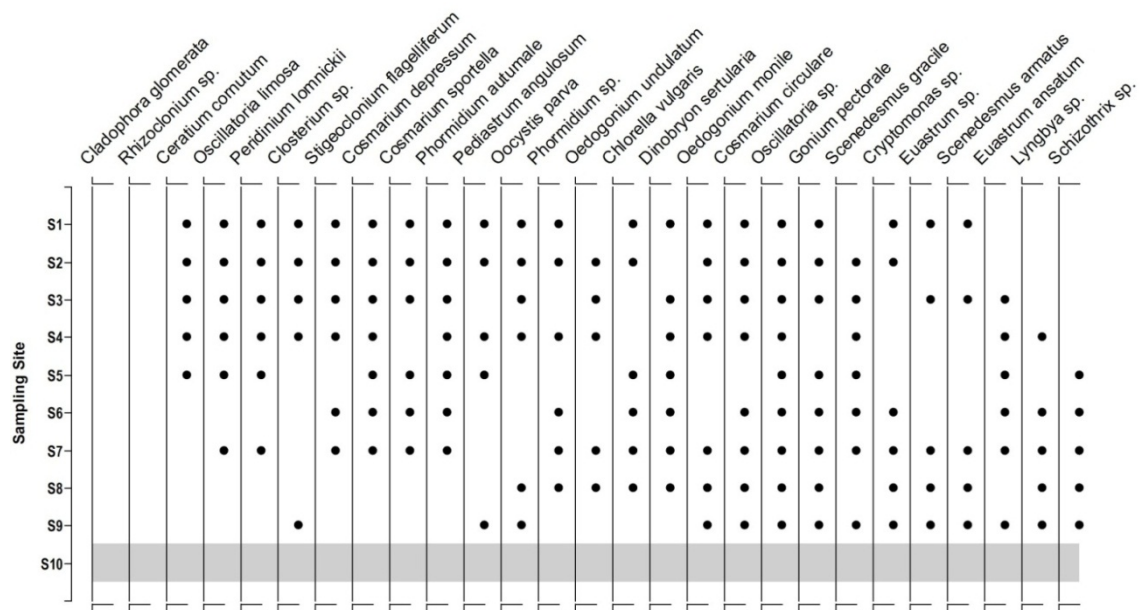
**Figure 5.9:** Results of diatom analysis along the MacKenzie River sampling stations in June 2013 (>4% in any one sample, sorted by weighted-averaging [ascending]).

### 5.3.2 Soft algae variations under base flow (10-15 ML/day)

The soft algal species composition differed along the MacKenzie River and between seasons. The soft algae were classified into three different groups comprising Chlorophyta (green algae), Cynobacteria (blue-green algae) and other algae (Chrysophyta, Charophyta and Euglenophyta) (Table 5.3). The results showed that the algal assemblages differed between sites, and from upstream to downstream. The unicellular species were mostly found in the upstream sites while the downstream sites (S6-S10) supported both unicellular and filamentous algae.

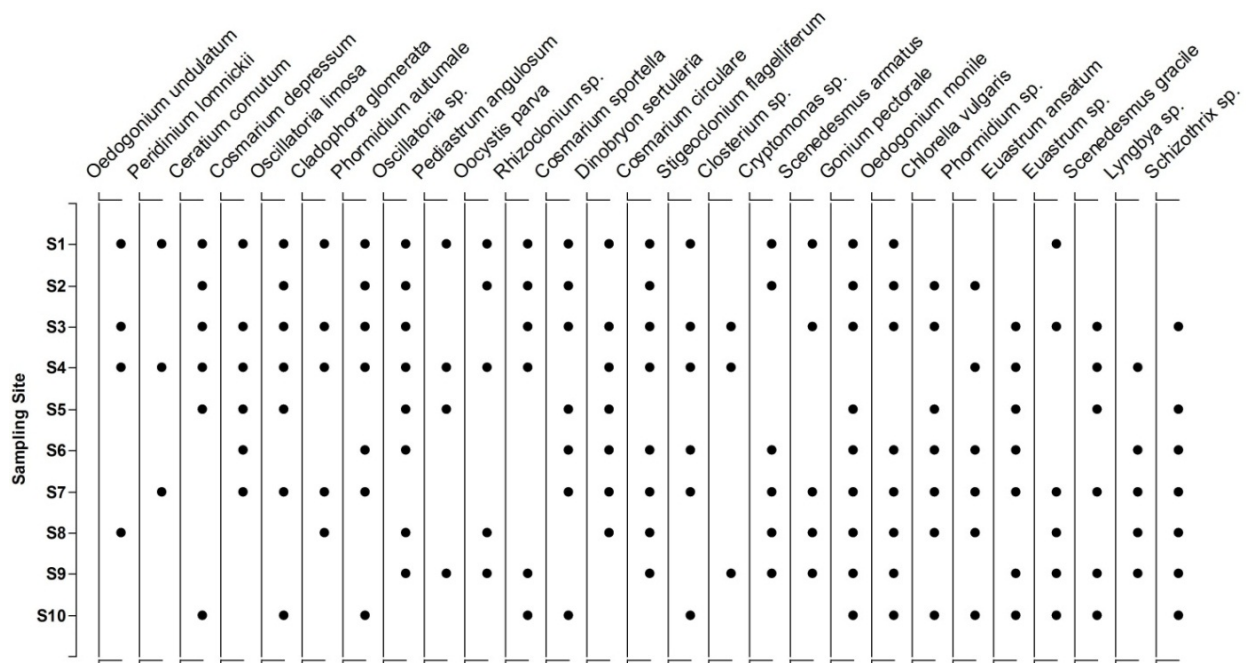
Soft algae species composition was different from upstream to downstream and between the seasons. The summer results (February 2012) showed *Chlorella vulgaris*, *Closterium* sp., *Dinobryon sertularia*, *Oocytis parva*, *Pediastrum angulosum* and *Peridinium lomnickii*, were the most common of the soft algae in the upstream sites whilst *C. vulgaris*, *Cosmarium sportella*, *O. parva*, *P. angulosum* and *Stigeoclonium flagelliferum* were common in the midstream sections. In contrast, filamentous algae like *Lyngbya* sp. and *Schizothrix* sp. were more often present downstream (Figure 5.10).

The winter results (July 2012) showed upstream sites of the river had more unicellular soft algae including *Ceratium cornutum*, *C. vulgaris*, *Closterium* sp., *D. sertularia*, *Peridinium lomnickii*, *P. angulosum* and *O. parva* while filamentous soft algae species that were found to be more abundant in the downstream sites included *C. glomerata*, *Lyngbya* sp., *Oedogonium undulatum*, *Oedogonium monile* and *Schizothrix* sp. (Figure 5.11).



**Figure 5.10:** Taxonomic composition of soft algae in MacKenzie River

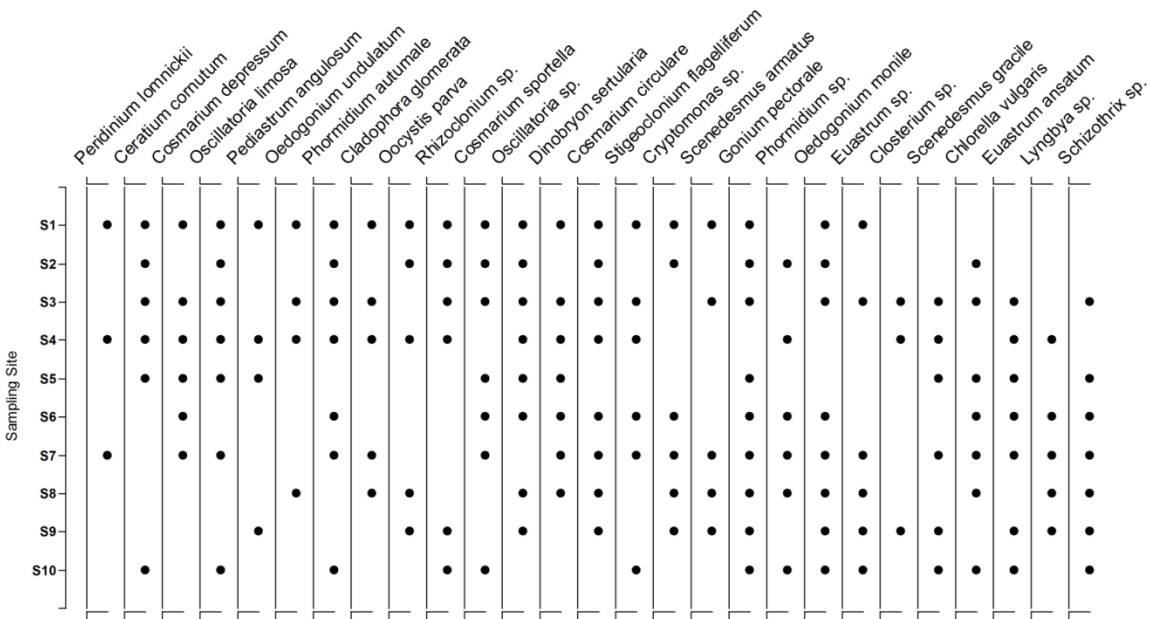
(presence/absence data) stations in February 2012 (Station 10 was dry at the time of sampling).



**Figure 5.11:** Taxonomic composition of soft algae along MacKenzie River

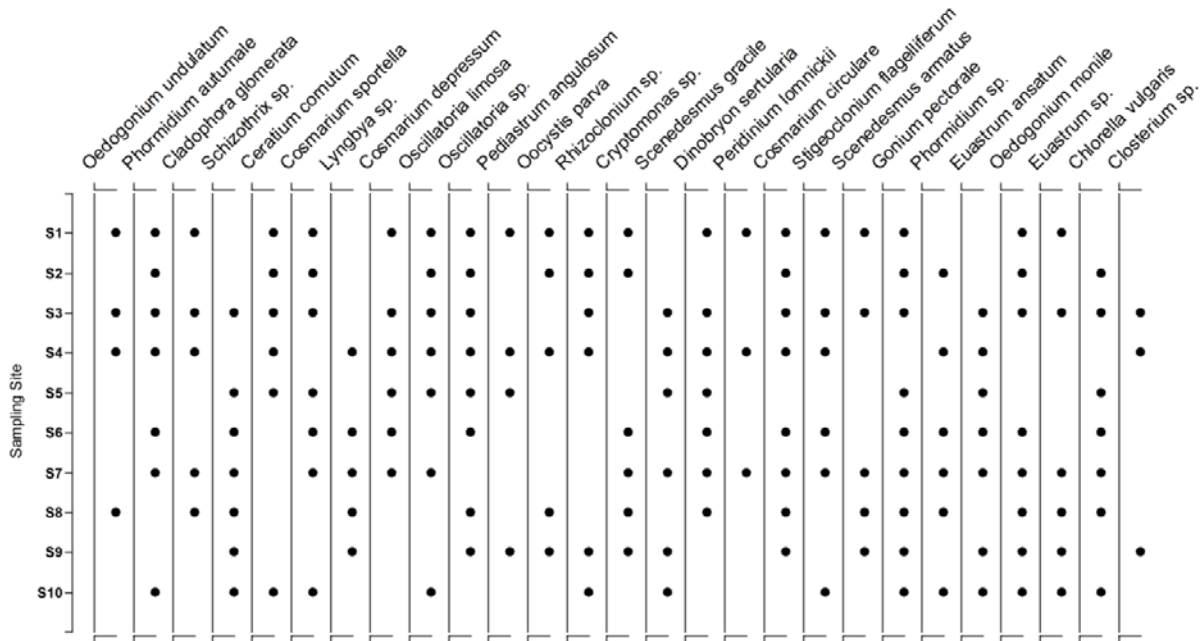
(presence/absence data) in July 2012.

The results of November 2012 showed *C. cornutum*, *C. depressum*, *O. parva* and *Rhizoclonium* sp. were more commonly found in upstream sites while *Lyngbya* sp. and *Schizothrix* sp. were present in downstream (S8-10) (Figure 5.12).



**Figure 5.12:** Taxonomic composition of soft algae along the MacKenzie River (presence / absence data) in November 2012.

The results of June 2013 showed that unicellular soft algae dominated upstream sampling sites while filamentous algae dominated the downstream sites (S8-10) (Figure 5.13).



**Figure 5.13:** Taxonomic composition of soft algae along the MacKenzie River (presence / absence data) in June 2013.

### 5.3.3 Relative abundance of the algal groups under base flow (10-15 ML/day)

Base flow sampling results showed that relative abundances of the algal groups varied by stream reach and also by season. Relative abundance of diatoms was highest in the upstream sites while filamentous green algae were more abundant downstream. The taxonomic composition of the algae varied among reaches along the river. In February 2012, diatoms represented the highest proportion of algal cells (~48%) upstream (S1 and S2) but decreased gradually from the midstream (~30%) to the lower reaches

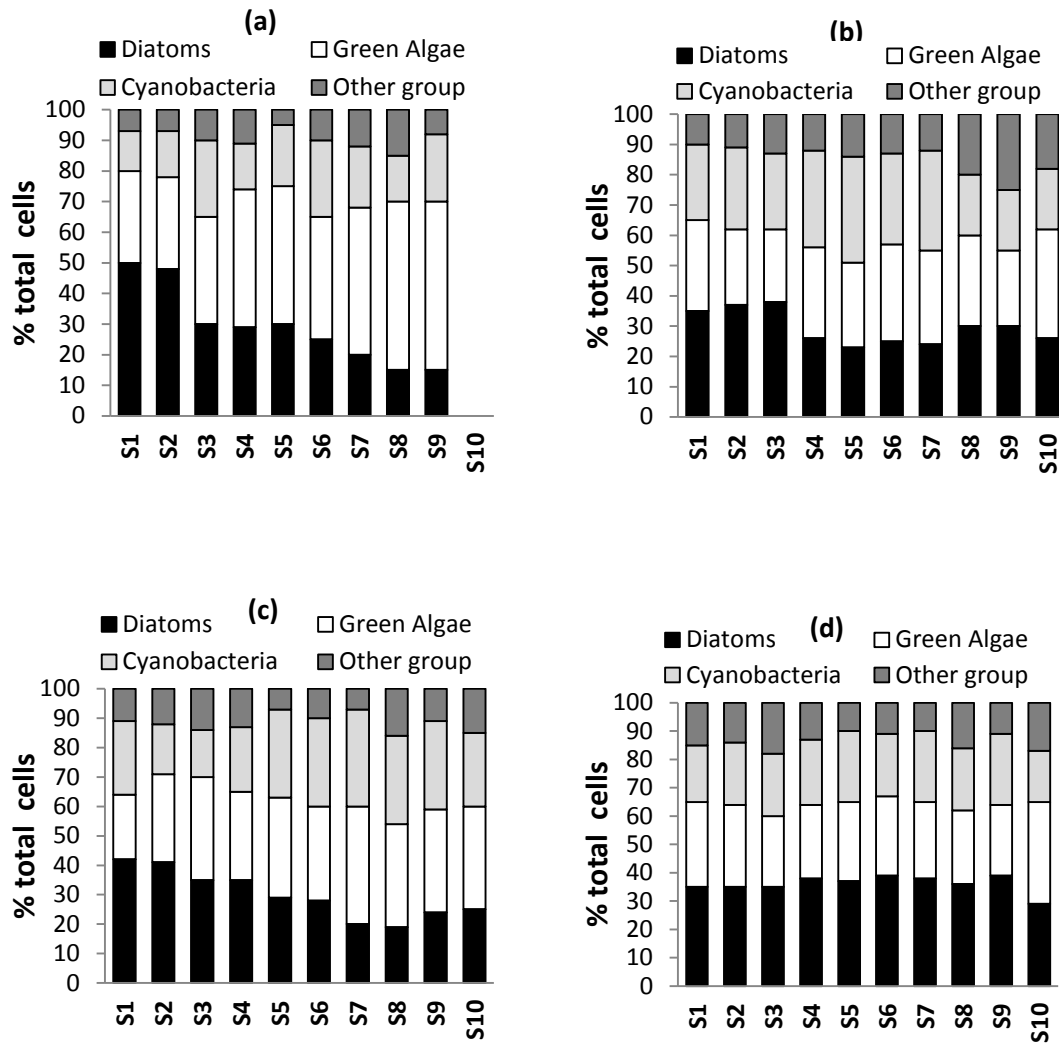
(~20%). In contrast, the relative abundance of green algae increased from approximately 20% in upstream sites to 40% in downstream sites, while the relative abundance of cyanobacteria increased slightly from 15% upstream to 20% downstream. The other algae (Chrysophyta, Charophyta and Euglenophyta) varied slightly between sites (Figure 5.14a).

The algal group assemblages in the July 2012 samples (Figure 5.14b) showed that the relative abundance of diatoms, green algae, and cyanobacteria were approximately 35%, 30% and 25% respectively in the upstream sites (S1-S3). The relative abundances of diatoms and green algae were slightly lower in the midstream sites with both approximating 25%, whereas cyanobacteria and 'other algae such as Chrysophyta' increased slightly to 35% and 15% respectively. In the downstream sites in July the relative abundance of the diatoms slightly increased (~30%) while that of the cyanobacteria decreased (~20%). The other algae groups also increased slightly in relative abundances downstream, but they were not dominant in the MacKenzie system.

The relative abundances of the algal groups again changed downstream seasonally, and in November 2012 (spring) (Figure 5.14c) diatoms were more abundant in the upstream sites while green algae and cyanobacteria were more abundant in downstream sites. The relative abundance of the cells of diatom, green algae, and cyanobacteria and other algae were 42%, 23%, 22% and 13% respectively in the upstream sites, changing to 25%, 30%, 25% and 20% respectively downstream. Together, the soft algal groups (especially filamentous and colonial algae) increased markedly in mid and downstream reaches in spring and summer whilst diatoms had a higher relative abundance in upstream sites in the same seasons.

The results of the June 2013 sampling (Figure 5.14d) showed diatoms and cyanobacteria to be the most abundant algal groups in the upstream reaches of the

MacKenzie River, with diatom abundance decreasing downstream. The relative abundance of diatoms, green algae, cyanobacteria and other algae groups was approximately 35%, 24%, 26% and 15% respectively in the upstream sites whilst downstream their relative abundances were 30%, 35%, 18% and 17% respectively.



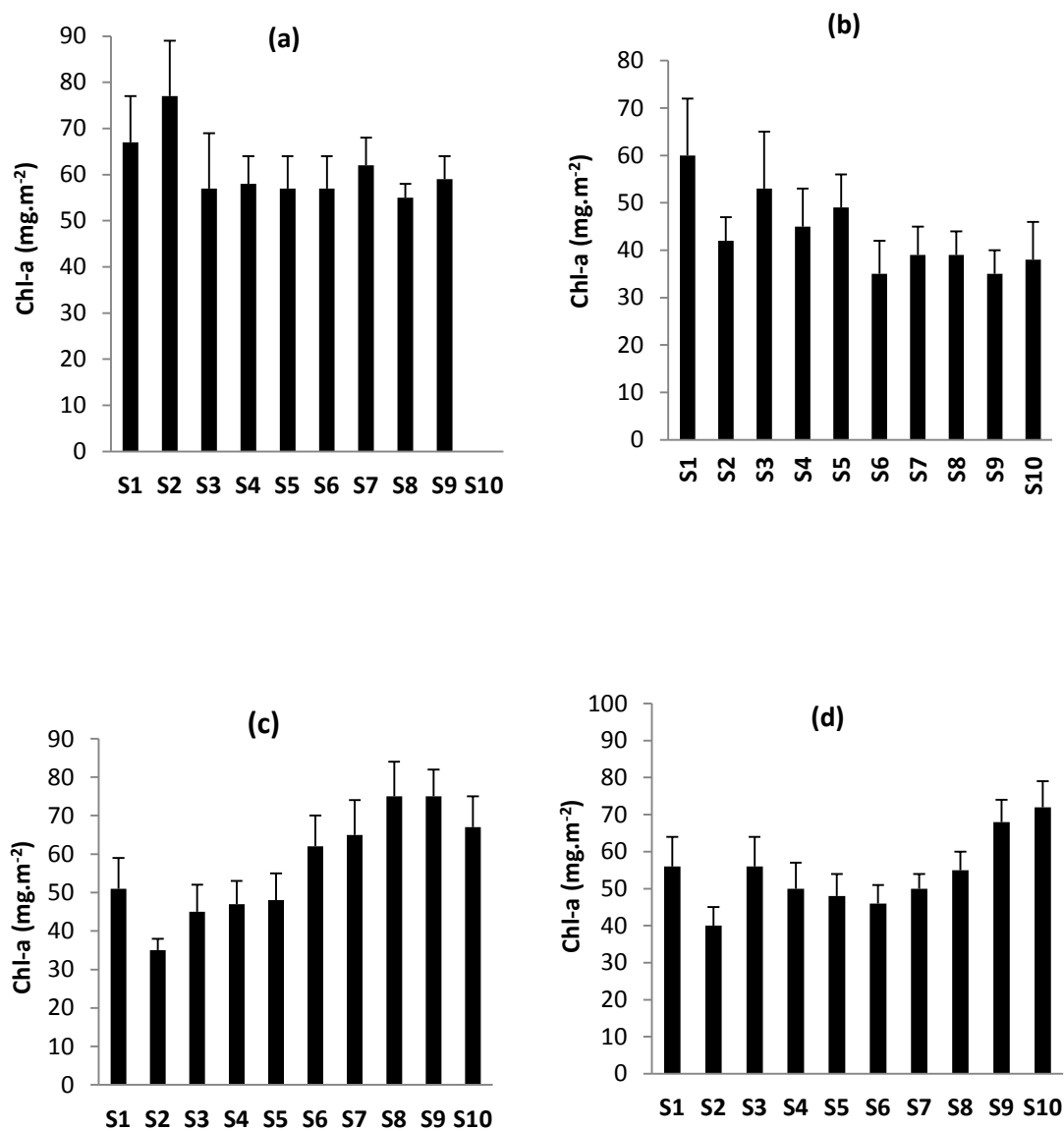
**Figure 5.14:** Percent of total cells of the algal communities in the MacKenzie River: (a) February 2012; (b) July 2012; (c) November 2012; (d) June 2013. (Station 10 was dry in February 2012).

#### **5.3.4 Biological properties of algae under base flow (15 ML/day)**

The results of the analyses of biomass vary significantly between sampling sites and events. The dry mass and ash-free dry mass (AFDM) showed values varied downstream in February 2012. The value of dry mass in the upstream, midstream and downstream reaches in the February 2012 samples was  $7\text{mg.cm}^{-2}$ ,  $5\text{mg.cm}^{-2}$ , and  $4\text{mg.cm}^{-2}$  respectively. The river was dry in February 2012 at the most downstream site (S10) and so dry mass samples could not be taken for S10 in February 2012 (Figure 5.15a)

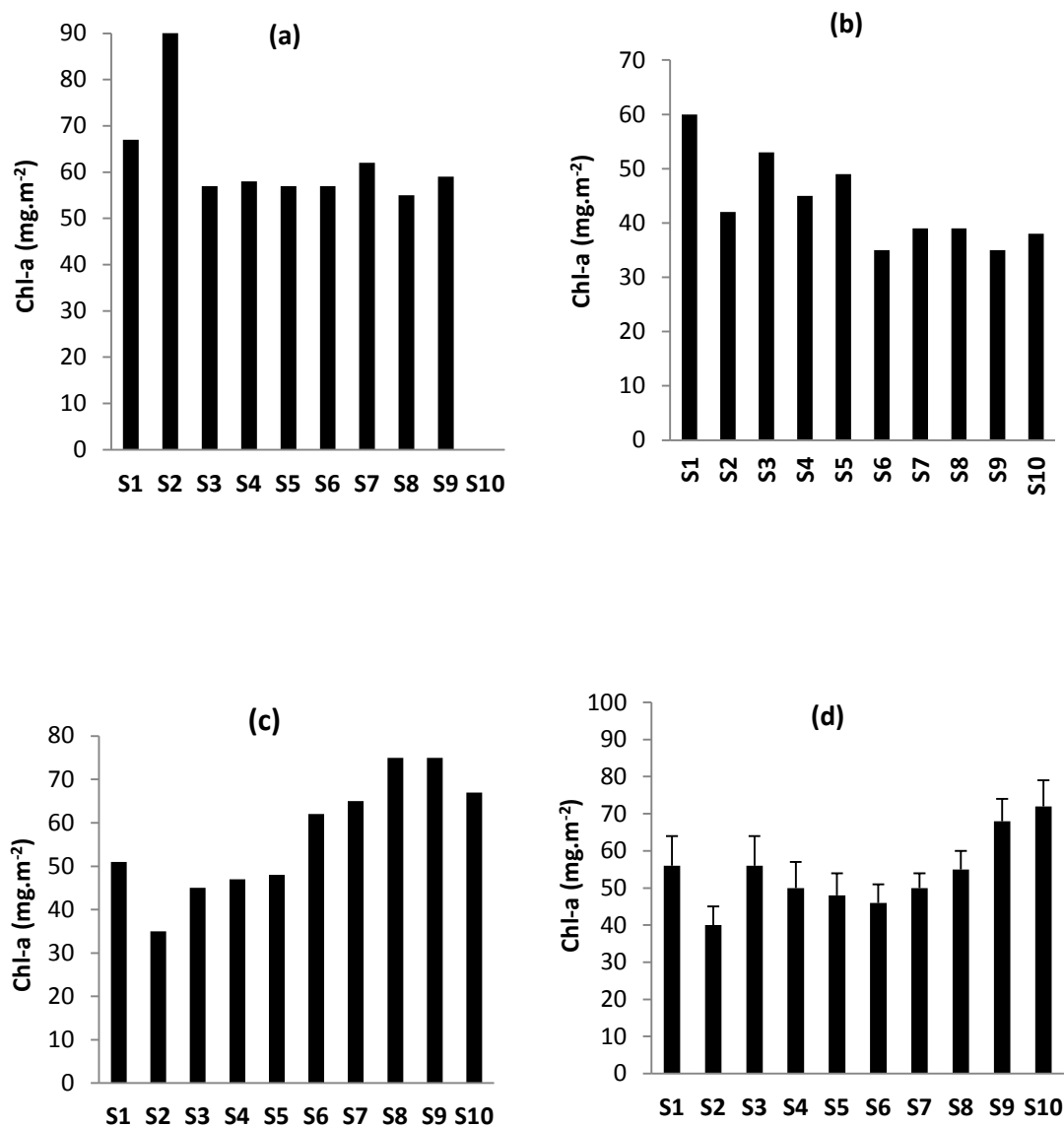
In July 2012, the accumulation of dry mass and AFDM also were the highest in the uppermost site (S1) but had sharply decreased by S2. At S3 the dry mass and ADFM results were higher than S2, but gradually decreased further towards the mid and lower parts of the river (Figure 5.15b). In contrast, there was a trend of dry mass and ADFM results decreasing with distance downstream in November 2012 (Figure 5.15c). There was no clear trend for the dry mass and AFDM results in June 2013 (Figure 5.15d).





**Figure 5.15:** The concentration of chlorophyll-*a* at each of the sampling stations along the MacKenzie River; **(a)** February 2012; **(b)** July 2012; **(c)** November 2012; **(d)** June 2013. Data indicate means  $\pm$  SD. (Station 10 was dry in February 2012).

The upstream and downstream values for chlorophyll-*a* concentrations in February 2012 ranged from approximately 90 mg.m<sup>-2</sup> (highest value in S2) and 60 mg.m<sup>-2</sup> (mid and lower of the river) with no real pattern evident (Figure 5.16a). The results from July 2012 showed that chlorophyll-*a* concentration gradually decreased downstream. The highest chlorophyll-*a* concentration was greatest upstream with approximately 60 mg.m<sup>-2</sup> recorded at S1, with the concentration gradually decreasing through mid and downstream reaches correlated negatively with turbidity (Figure 5.16b). In contrast, the concentration of chlorophyll-*a* increased markedly downstream in November 2012 (Figure 5.16c), ranging from approximately 35 mg.m<sup>-2</sup> in the upstream sites increasing to 80 mg.m<sup>-2</sup>. Overall, it seems the algal productivity is greater in spring, especially in the lower parts of the river. The chlorophyll-*a* concentrations for June 2013 (Figure 5.16d) showed an unusual pattern of gradual increases (S2 to S5; S6 to S10), with substantial drops at downstream sites (S1 to S2; S5 to S6).



**Figure 5.16:** The concentration of chlorophyll-*a* at each of the sampling stations along the MacKenzie River; **(a)** February 2012; **(b)** July 2012; **(c)** November 2012; **(d)** June 2013. Data indicate means ± SD. (Station 10 was dry in February 2012).

## **5.4 Algal response under manipulated flow regimes (Fishes and high flow)**

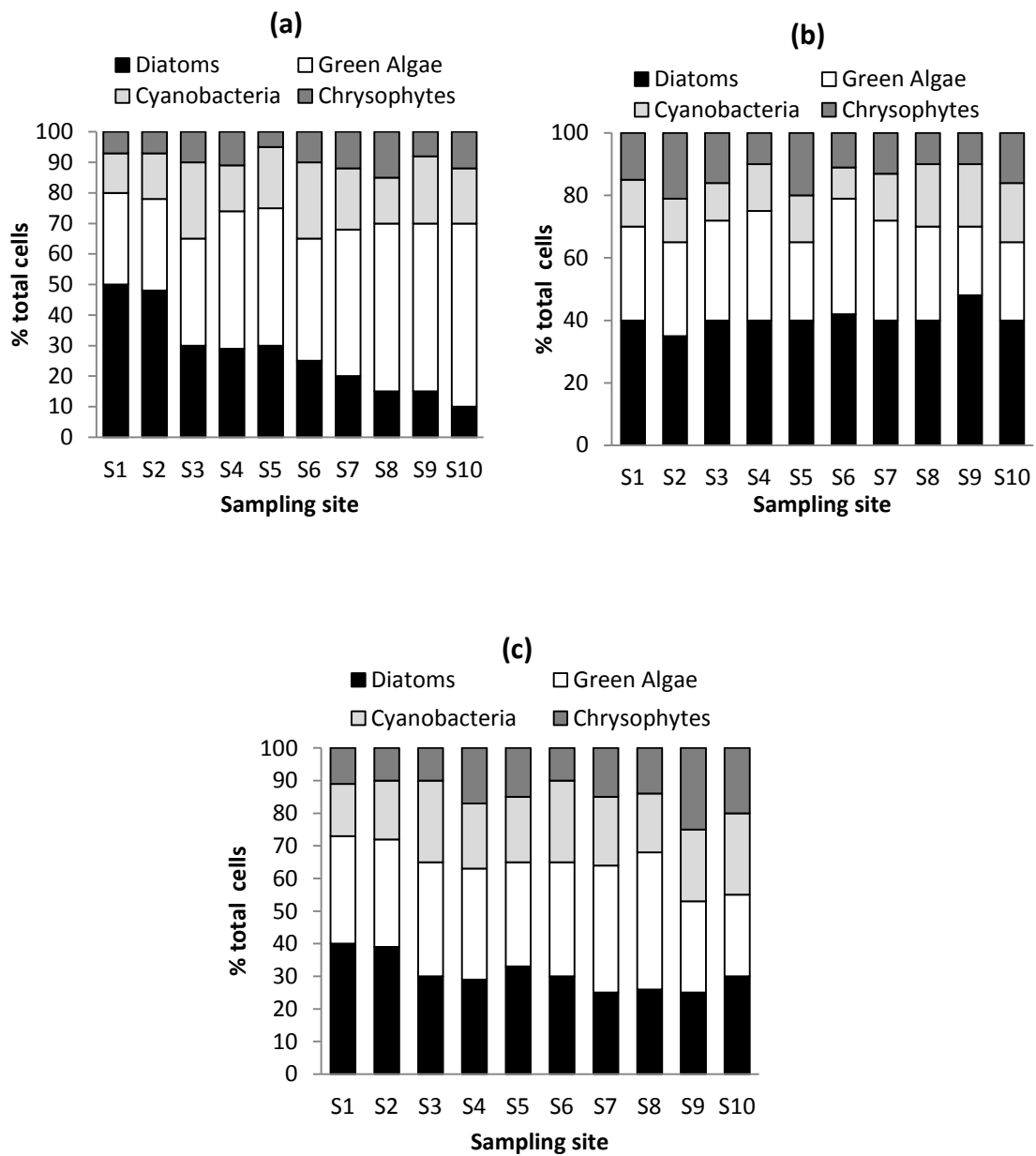
Algae and water quality were monitored under different water release regimes to determine their response to river flow and so its effect on river health.

Algal community structure was documented in terms of the major algal groups and then biological properties of the algal periphyton communities were measured before, during and after water release events (1 week before water release, 3 days during water release and three weeks after water release (3 times)).

### **5.4.1 Algal community structure before, during and after Fishes (35-40 ML/day)**

Algal species composition varied between sites under fishes (35-40 ML/day). The algal composition shifted downstream after water release events. Diatoms were the most abundant group (50% of cells) upstream (Site 1 and 2 in Reach 1) before the water release whilst green algae were most abundant downstream (55% of cells at Site 10 in Reach 3). The proportion of green algae and cyanobacteria tended to be greater downstream before the water release, whereas diatoms had lower relative abundance downstream (to 10% at site S10). However, cyanobacteria and other algae were relatively more abundant (25% and 15% respectively) in some mid-stream sites (Reach 2) (Figure 5.17a).

There were substantial changes in algal communities during and after water release events. Diatom cell relative (40%) abundance increased during and after fishes, whilst green algae decreased (23%) downstream. In other words, the proportion of green algae during and after fishes were less abundant towards the downstream sites, therefore the algal taxonomic composition became more uniform across the reaches following a release event. For example, blooms of green algae, typical of base flow conditions in downstream reaches, were reduced by the fishes (Figure 5.17b-c).



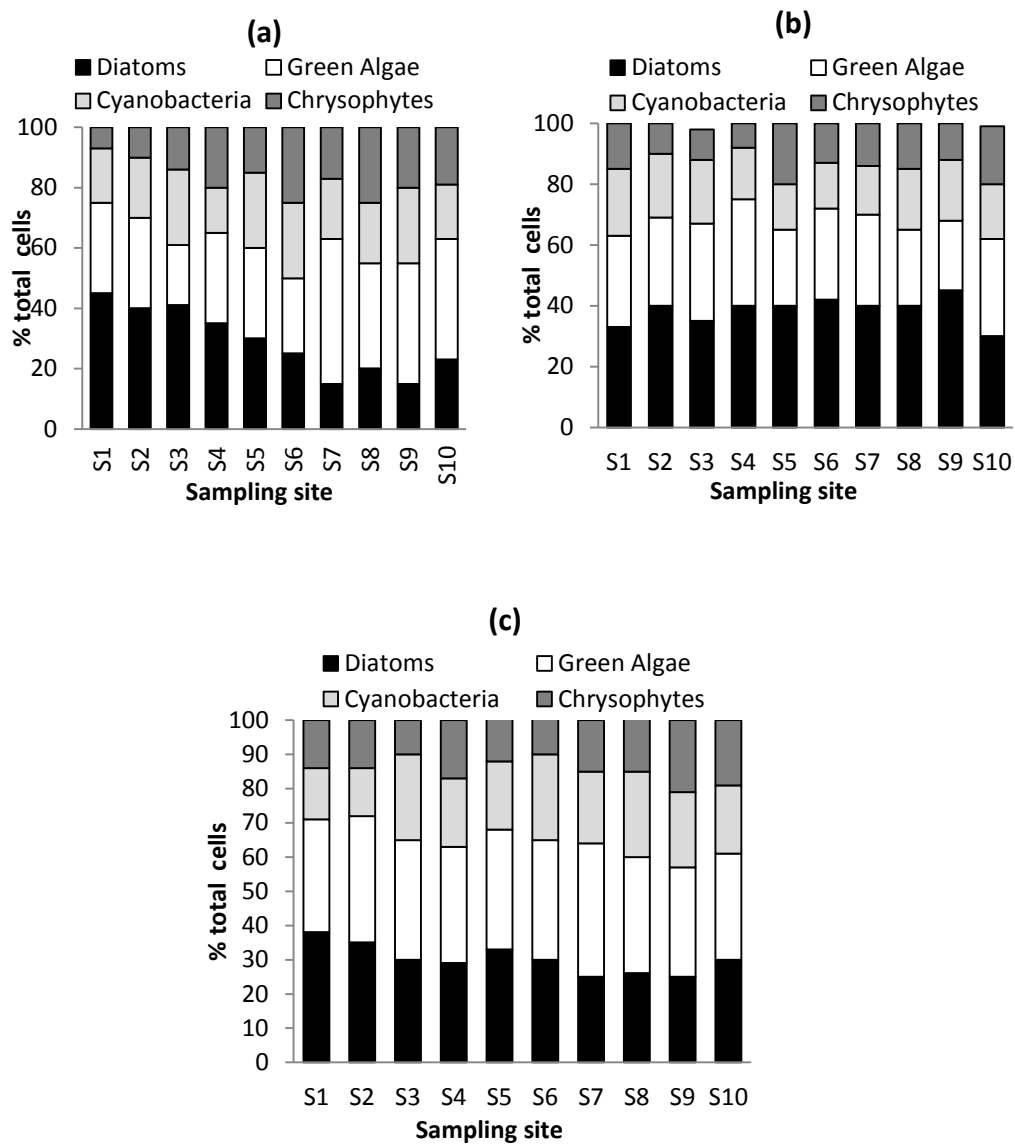
**Figure 5.17:** Relative abundance of total cells of algae in sampling sites along the MacKenzie River; **(a)** before Freshes (15 ML/day); **(b)** during Freshes (35-40 ML/day); **(c)** after Freshes (15ML/day).

#### **5.4.2 Algal community structure before, during and after high flow (55 ML/day)**

Diatoms were common in upstream sites (45%), while green algae (33%), cyanobacteria (12%) and chrysophyta (10%) were less abundant before high flow (Figure 5.18a).

However this pattern in the algae community structure changed gradually downstream. In the midstream (Reach 2), the percentage of diatoms, green algae and cyanobacteria were similar. In contrast to the upstream sites green algae were more abundant from sites 7 to 10.

Diatoms had higher relative abundances downstream (sites 5 to 10) during the high flow (approximately 40%) and after the high flow (approximately 35%). In contrast, green algae and cyanobacteria relative abundances decreased downstream (approximately 30% and 20% respectively). The results showed that high flows had a major influence on algae communities. The abundance of cyanobacteria and Chrysophyta increased from upstream to downstream during base flow and before high flow, whilst their composition was relatively uniform spatially during and after high flow. Overall the algal taxonomic composition became more uniform along the MacKenzie River during and after the high flow (Figure 5.18b-c).



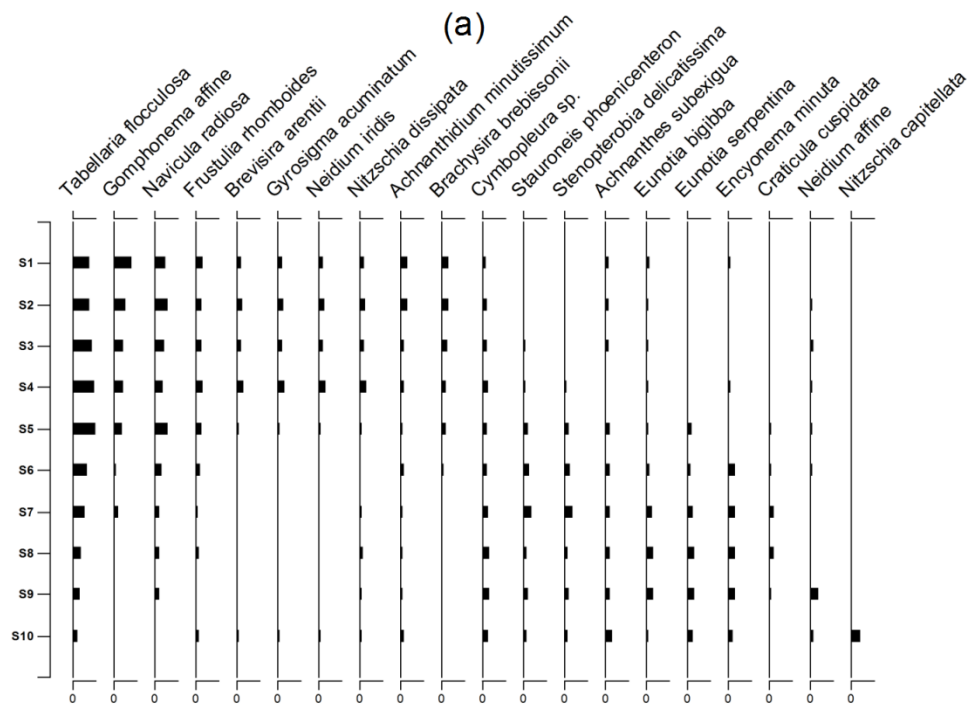
**Figure 5.18:** Relative abundance of cells of algae from sampling sites along the MacKenzie River; **(a)** before high flow (15 ML/day); **(b)** during high flow (55 ML/day); **(c)** after high flow (15ML/day).

## 5.5 Diatom variations under manipulated flow regimes

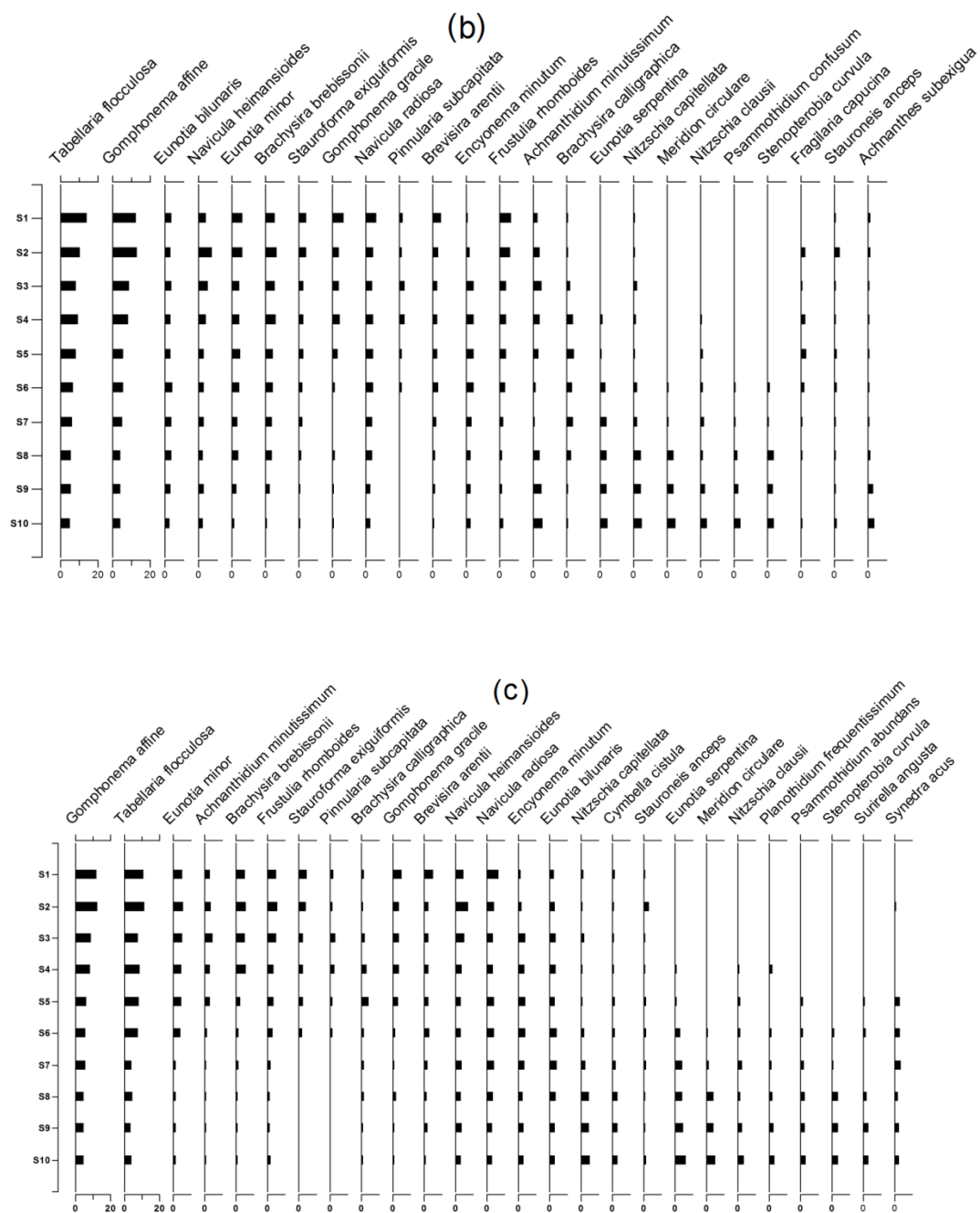
### 5.5.1 Diatom variations under Freshes (35-40 ML/day)

The diatom species composition after freshes at upstream sites was different to those at downstream sites. The most common diatom taxa found at the upstream sites before freshes were *Brevisira arentii*, *F.rhomboides*, *G. affine*, *N. radiosa*, *N. iridis*, *T. flocculosa* whilst at downstream sites the common diatoms found were *Encyonema minuta*, *Eunotia bigibba*, *E. serpentina*, *N. capitellata* and *S. delicatissima* (Figure 5.19a).

These community structures changed during and after Freshes. The most abundant diatoms in the upstream reach during and after the water release were *Brachysira brebissonii*, *B. arentii*, *E. minor*, *G. affine*, *N. heimansioides*, *S. .exiguiformis* and *T. flocculosa* whilst those sampled from the downstream reach were *E. serpentina*, *N. capitellata*, *N. clausii*, and *S. curvula* (Figure 5.19b-c).





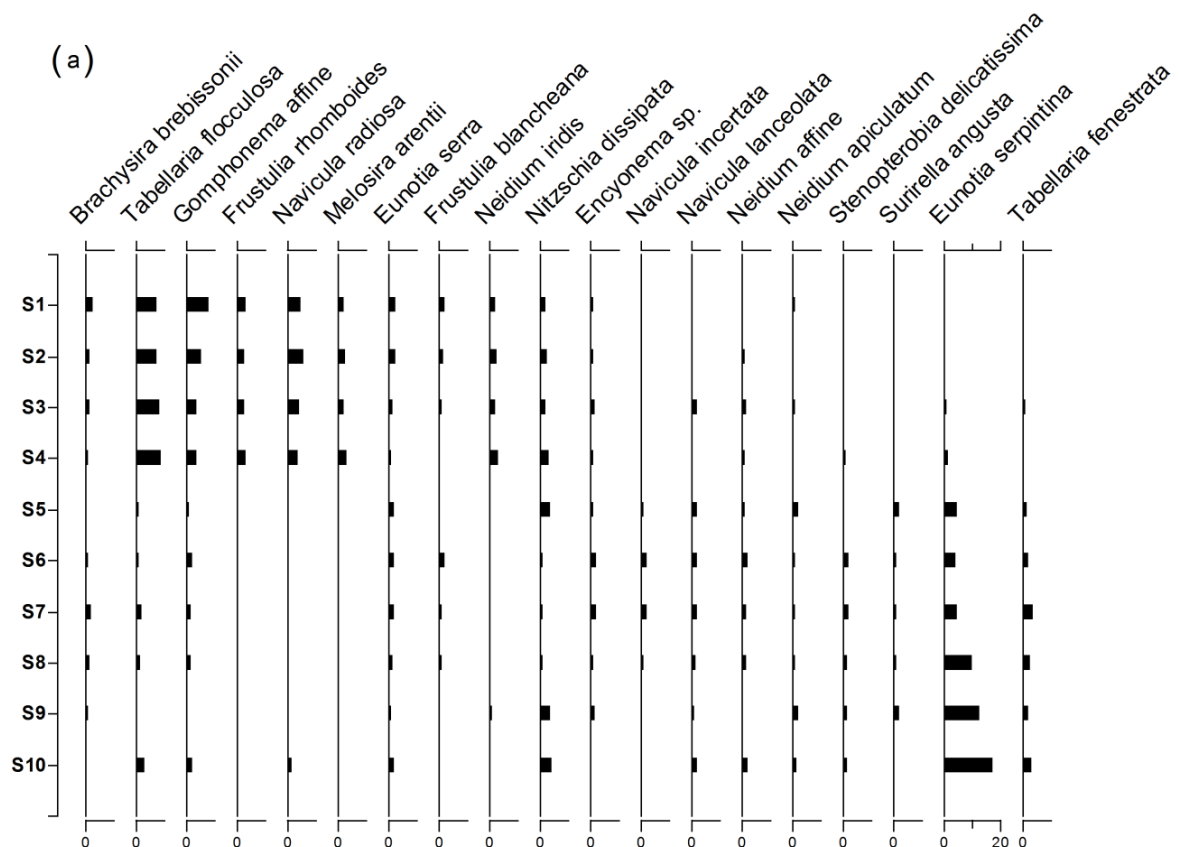


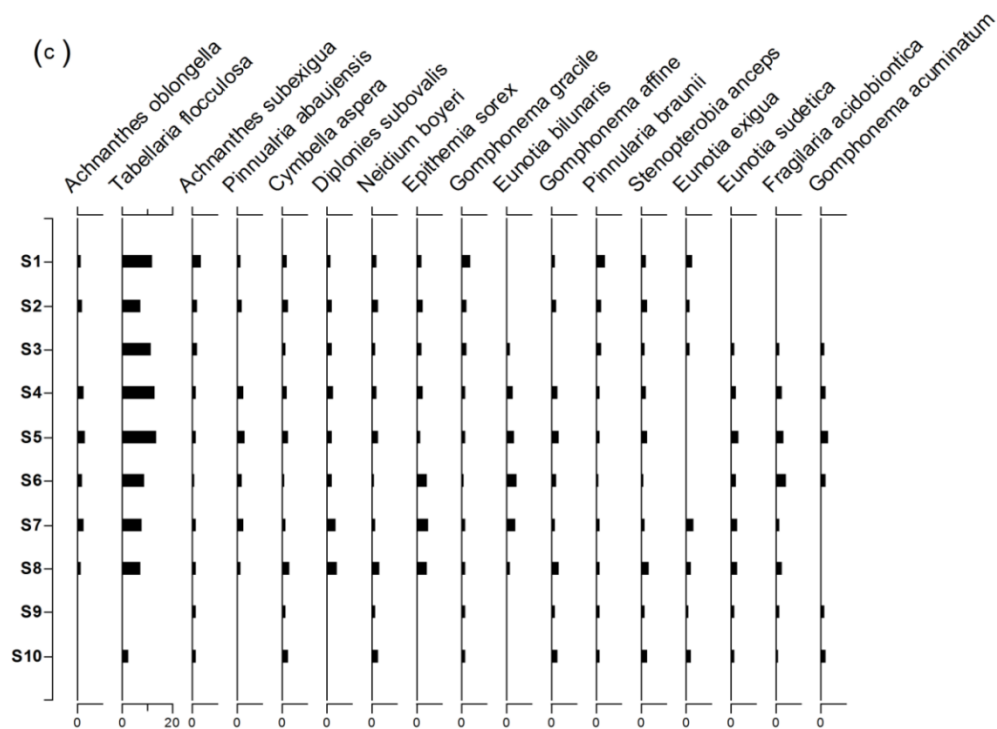
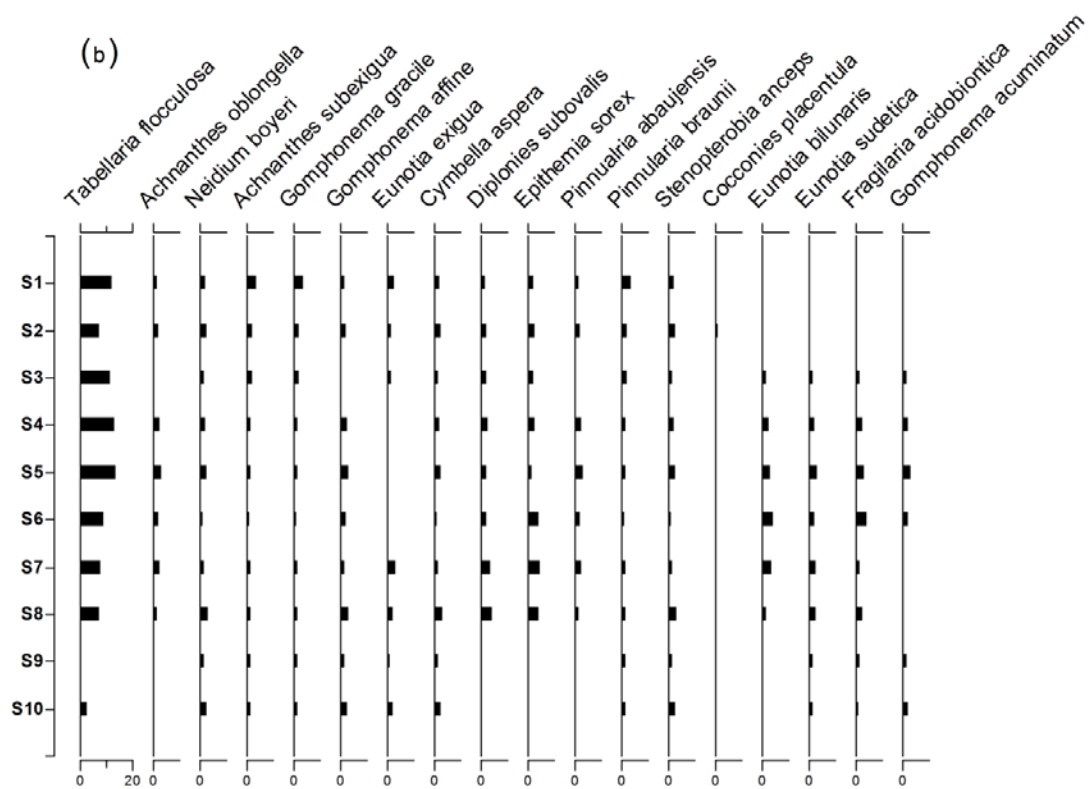
**Figure 5.19:** Relative abundance of diatom taxa along the MacKenzie River in December 2013; (a) before Freshes (15 ML/day); (b) during Freshes (35-40ML/day); (c) after Freshes (15ML/day).

### 5.5.2 Diatom variations under high flows (55 ML/day)

The diatom species composition showed change along the river prior to the release of high flows (Figure 5.20a), with *T. flocculosa*, *G. affine* and *N. radiosa* being the most abundant species. *F. rhomboides*, *M. arentii*, *N. iridis* and *N. dissipata* were also relatively abundant in the upstream sites. Most of these species displayed a strong affinity for the upper half of the sites, with little or no representation in sites 6 to 10. The most common diatom species downstream were *E. serpentina*, *T. fenestrata* and, to a lesser extent, *S. delicatissima* (Figure 5.20a).

Although *T. flocculosa* was again the most abundant species during and after the high flows (Figure 5.20b-c), the general distribution showed a far more evenly spread pattern. In contrast, the diatom species composition during high flow and after were *A. subexigua*, *E. sores*, *Eunotia bilunaris*, *G. affine* and *G. gracile* (Figure 5.20b-c).





**Figure 5.20:** Relative abundance of selected diatom taxa along the MacKenzie River in November 2014 (a) before high flows (15 ML/day); (b) during high flows (55 ML/day); (c) after high flows (15ML/day).

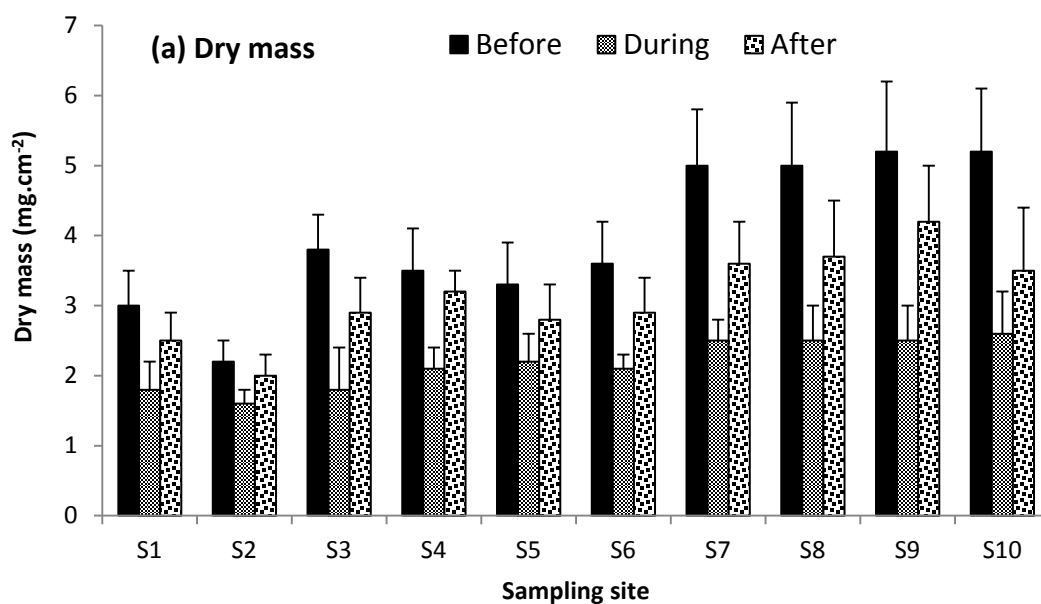


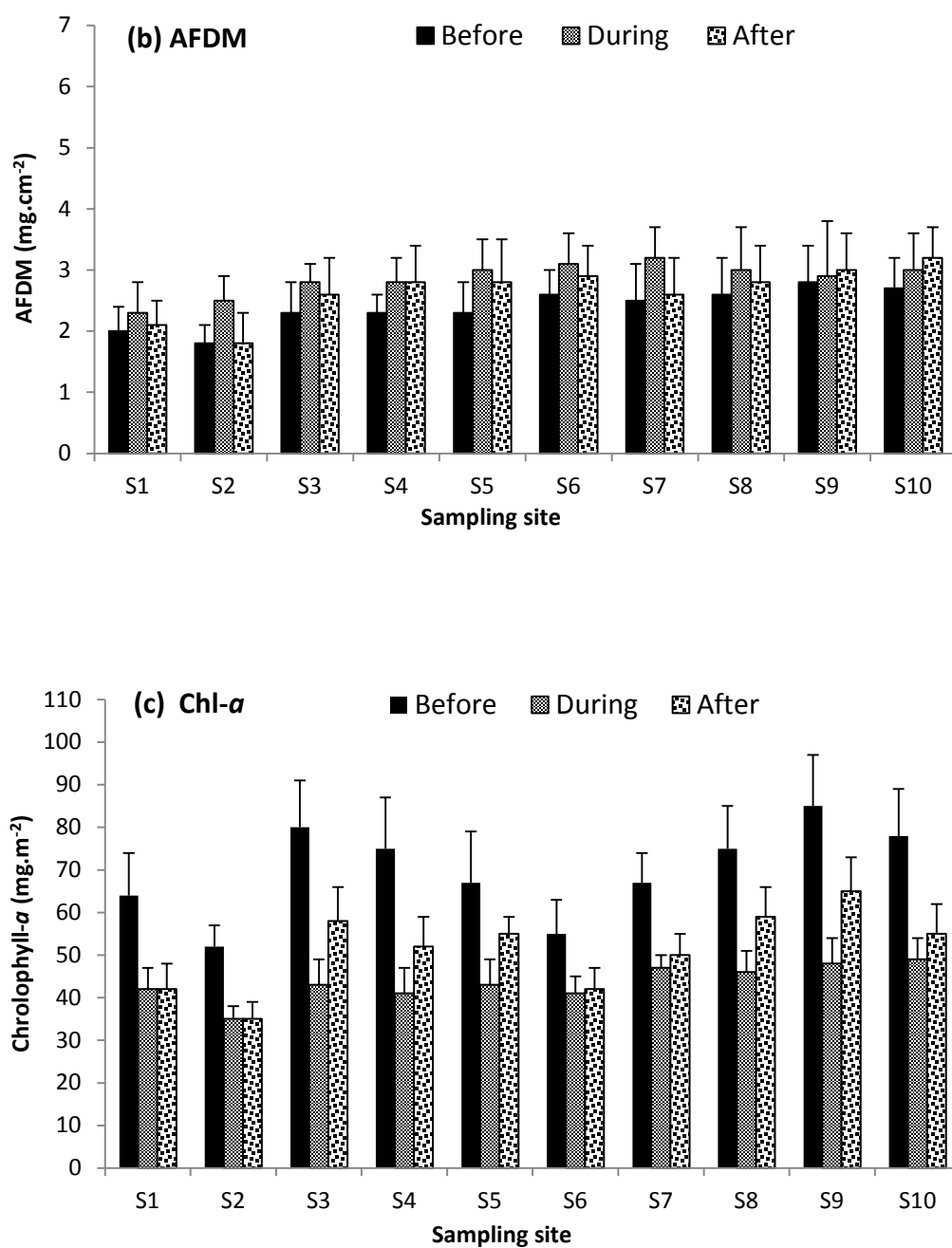
## 5.6 Algal biomass under manipulated flow regimes

### 5.6.1 Algal biomass under Freshes (35-40 ML/day)

The accumulation of dry mass, AFDM and chlorophyll-*a* concentration were measured before, during and after freshes. The accumulation of dry mass was typically greater in the downstream sites for each flow scenario and was always lower during and after freshes (35-40ML/day) (Figure 5.21a). These values were at their lowest midstream after water release events. The accumulation of AFDM also typically increased from upstream to downstream before, during and after freshes (Figure 5.21b). However, AFDM increased at all sites during and after freshes, a notable difference observed when compared with the dry mass results.

Changes in chlorophyll-*a* concentration under freshes demonstrated a general increase from upstream to downstream. In other words, the concentration of chlorophyll-*a* increased from upstream to downstream before freshes whilst it decreased during freshes along the river. The concentration of chlorophyll-*a* increased slightly after freshes along the river (Figure 5.21c).





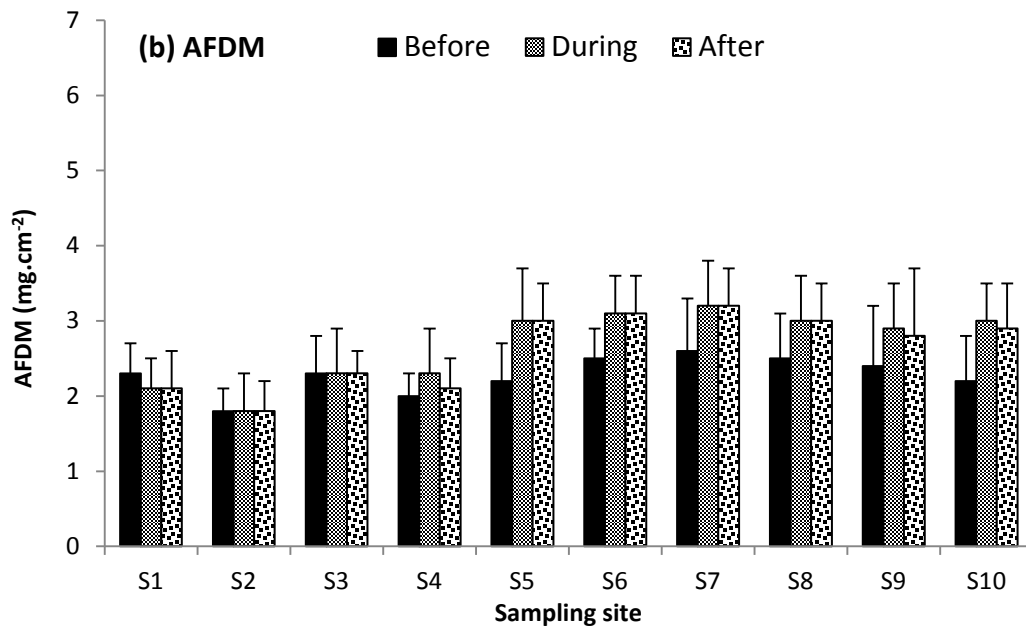
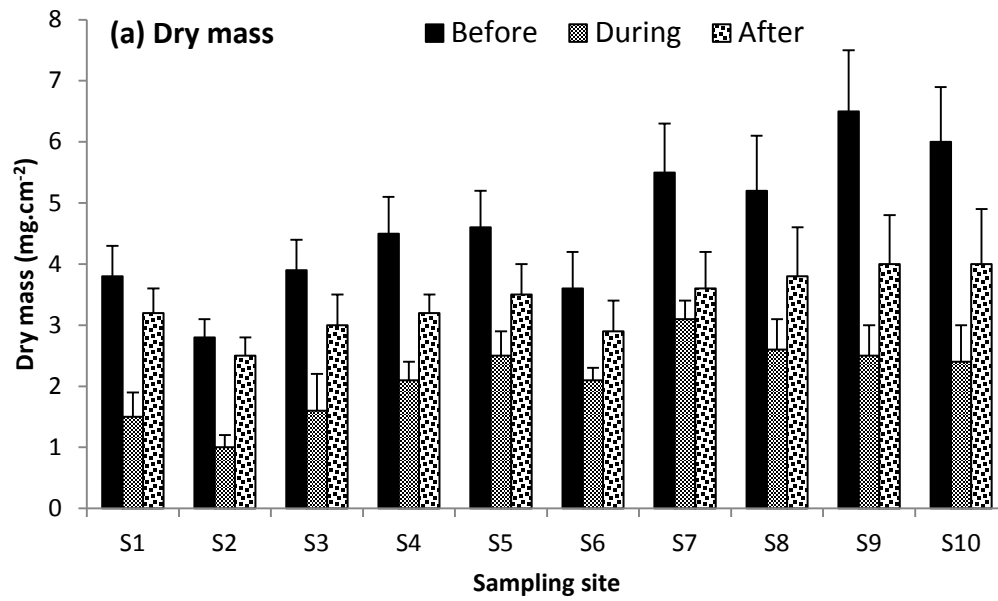
**Figure 5.21:** Accumulation of algal biomass before Freshes (15 ML/day), during Freshes (35-40ML/day) and after Freshes (15ML/day) at each of the sampling station along the MacKenzie River; **(a)** Dry mass; **(b)** AFDM; **(c)** Chlorophyll-*a*. Data indicate means  $\pm$  SD.

### 5.6.2 Algal biomass under high flows (55 ML/day)

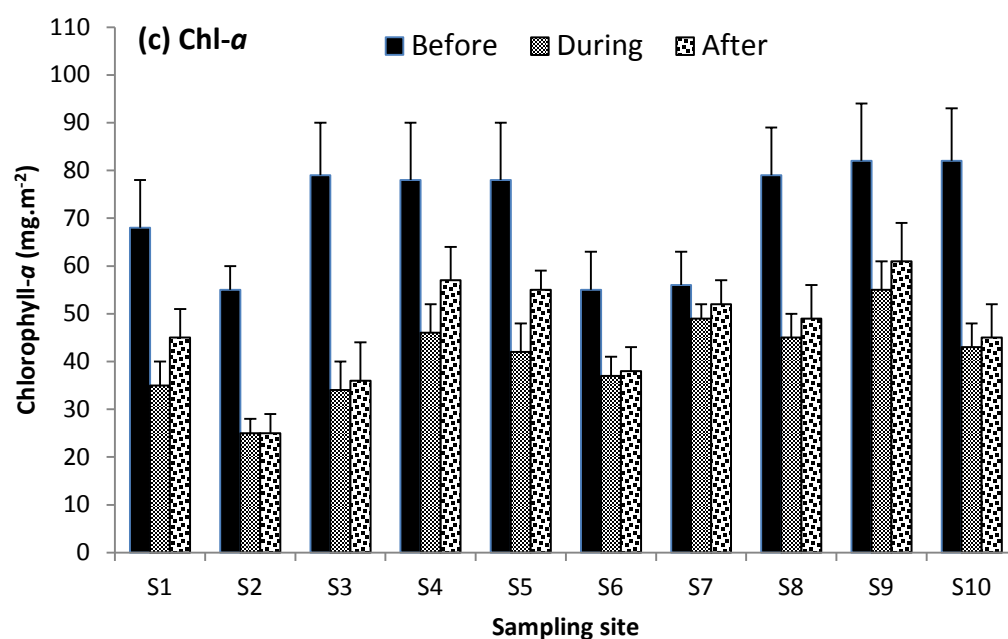
Before the high flows (55ML/day) were released, dry mass generally increased from the upstream to the downstream sites with the highest value of dry mass ( $6.5 \text{ mg.cm}^{-2}$ ) found at site 9; under the same conditions however, there was more fluctuation evident at midstream and downstream sites in the values of the dry mass. Dry mass decreased dramatically during high flows and partially recovered after the high flows (Figure 5.22a). Overall the accumulation of dry mass decreased during high flow conditions within the system.

The accumulation of AFDM increased in upstream and midstream and subsequent decreased in downstream sites before high flows while during high flows the AFDM decreased at site 1, remained unchanged at sites 2 and 3 and increased at the other sites. After the high flows, AFDM values were almost the same as during high flows (Figure 5.22b).

The chlorophyll-*a* concentration fluctuated downstream (before, during and after high flows); chlorophyll-*a* concentration increased before water release (high flows) but decreased substantially during high flows and then partially increased again afterwards but still did not reach the same concentrations as before the releases (Figure 5.22c).







**Figure 5.22:** Accumulation of algal biomass before high flows (15 ML/day), during high flows (55ML/day) and after high flows (15ML/day) at each of the sampling station along the MacKenzie River; (a) Dry mass; (b) AFDM; (c) Chlorophyll-*a*. Data indicate means  $\pm$  SD.

## **5.7 DSIAR**

The Diatom Species Index for Australian Rivers (DSIAR) was calculated to classify the condition of the waterway. The results showed sites S1 to S4 to be in good condition with a DSIAR score above 60 under all flow regimes. During baseflow conditions, sites S5 to S8 were in moderate condition with DSIAR scores between 41-60 and downstream sites ranged from moderate to poor condition (Table 5.4). The DSIAR scores varied more in Reaches 2 and 3 compared to those upstream (Reach 1). Overall the upstream sites had the highest scores (least impacted), with DSIAR scores decreasing downstream. The lowest site (S10) typically returned the lowest, or near lowest, DSIAR score. However, during and after water release events (freshes and high flows) the DSIAR score increased downstream to scores reflective of good and moderate conditions, albeit temporarily (Table 5.4).

**Table 5.8:** The Results of modified Diatom Species Index for Australian Rivers

(DSIAR) at sampling sites along the MacKenzie River in different flow regimes. The scores were used to classify the waterway as bad (0-20), poor (21-40), moderate (41-60), good (61-80) and high (81-100).

	Reach	1				2				3	
	Site										
	Date	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Base flow (10 ML/day)	28/02/12	78	75	71	68	56	49	49	48	35	-
Base flow (15 ML/day)	17/07/12	77	72	72	66	58	48	42	48	42	38
Base flow (15 ML/day)	9/11/12	78	65	76	68	59	48	43	47	49	41
Base flow (15 ML/day)	25/05/13	75	72	75	67	55	49	45	49	47	48
During freshes (35 ML/d)	21/10/13	82	78	78	66	65	65	61	62	65	62
After freshes (15 ML/d)	25/10/13	81	75	79	69	66	65	61	63	66	65
Before freshes (15 ML/d)	16/12/13	76	74	72	71	55	42	46	48	37	38
During freshes (40 ML/d)	19/12/13	86	75	78	75	64	66	69	63	66	62
After freshes (15 ML/d)	23/12/13	78	75	72	72	57	56	55	55	59	52
After freshes (15 ML/d)	3/01/14	79	75	78	71	59	57	55	45	41	39
After freshes (15 ML/d)	16/04/14	74	71	68	62	61	49	45	41	41	39
Before high flow (15 ML/d)	29/10/14	71	72	73	74	55	42	46	48	37	38
During high flow (55 ML/d)	1/11/14	82	75	78	75	71	68	69	65	66	67
After high flow (15 ML/d)	08/11/14	78	75	72	72	67	66	65	61	59	52
After high flow (15 ML/d)	22/11/14	79	75	68	68	59	57	55	65	45	37

## **5.8 Data analysis and interpretation**

The species and water quality data were transformed to reduce skewness and to, as far as possible, ensure normal distribution of the data sets. The untransformed species composition data do not meet an assumption of parametric (e.g. linear regression) statistical tests because they were distributed in an abnormal shape. Furthermore, the water quality data units were completely different (e.g.  $\text{mg L}^{-1}$ ,  $\mu\text{S cm}^{-1}$ ). Therefore, it was essential to transform data into a format of log or square-root to normalise them and make them comparable. Therefore, both water chemistry data and species data were log and/or square-root transformed before analysis.

Pearson's correlation matrix and constrained and unconstrained ordinations (PCA, CA, DCA, CCA and forward selection) were performed on data relating to water quality measurements, biological properties and species composition to evaluate the influence of the different flow regimes on algal communities.

### **5.8.1 Pearson's correlation**

The Pearson's correlation matrix of the environmental data showed significant relationships ( $p < 0.05$ ) between the water quality measures of pH, conductivity, total nitrogen, turbidity, phosphorus, and other characteristics under different flow regimes. The water quality measurements showed Wartook Reservoir, as an anthropogenic modification, may affect most physical and chemical characteristics of the river including water pH, electrical conductivity, turbidity and total suspended solids values along the river. This can be concluded from a comparison of base flow and water release events. The water quality characteristics were analysed to derive a Pearson's correlation matrix under different flow regimes (Table 5.5).

Generally, on the basis of high correlations between key variables, the lowland sites are deeper, more alkaline, and have higher concentrations of nutrients and salts.

Specifically, the statistical results showed that the flow regime has significant relationships with most of the water quality and biological characteristics including pH ( $r = 0.487$ ), temperature ( $r = 0.455$ ), conductivity ( $r = 0.318$ ), turbidity ( $r = 0.586$ ), ORP ( $r = 0.487$ ), DO ( $r = 0.465$ ), TDS ( $r = 0.702$ ), TSS ( $r = 0.586$ ), Mg ( $r = 0.528$ ), silica ( $r = 0.416$ ), TN ( $r = 0.488$ ), TON ( $r = 0.425$ ), TP ( $r = 0.489$ ), dry mass ( $r = 0.427$ ) and chlorophyll-*a* ( $r = 0.356$ ). Furthermore, the results showed that physical, chemical and biological characteristics correlated significantly with each other. For example, temperature and pH are highly correlated ( $r = 0.994$ ) highlighting that warmer, lowland sites tend to be more alkaline (Table 5.5).

**Table 5.9:** Pearson’s correlation matrix of the water chemistry and biological properties in the MacKenzie River (\*Significant correlation at the 0.05 level).

	Flow	pH	Temp	Cond.	Turb.	Depth	ORP	DO	TDS	TSS	Cl	Mg	Ca	SO <sub>4</sub>	SiO <sub>2</sub>	TN	NH3	TON	TP	DM	AFDM	Chl- <i>a</i>
Flow	1																					
pH	.487*	1																				
Temp	.455*	.994*	1																			
Cond.	.318*	.773*	.733*	1																		
Turb	.586*	.460	.410	.829*	1																	
Depth	.375	.793*	.821*	.370	-.096	1																
ORP	.659*	.950*	.936*	.883*	.625*	.683*	1															
DO	.465*	.907*	.940*	.487	.165	.872*	.822*	1														
TDS	.702*	.954*	.933	.777*	.552	.685*	.961*	.853*	1													
TSS	.569*	.393	.417	.628*	.464	.199	.499*	.279	.306	1												
Cl	.322	.943*	.927*	.702*	.436	.717*	.851*	.824*	.880*	.372	1											
Mg	.528*	.979*	.981*	.786*	.536	.747*	.970*	.908*	.961*	.466	.913*	1										
Ca	.318	.960*	.947*	.867*	.603*	.704*	.945*	.796*	.897*	.525	.924*	.957*	1									
SO <sub>4</sub>	.315	.756*	.781*	.565	.125	.747*	.683*	.740*	.584	.378	.657	.687*	.715*	1								
SiO <sub>2</sub>	.416*	.596*	.602	.716*	.810*	.188	.734*	.510	.659*	.615	.574	.727*	.686*	.284	1							
TN	.488*	.713*	.668*	.823*	.760*	.191	.764*	.483	.762*	.448	.737*	.716*	.718*	.442	.701*	1						
NH3	-.358	.452	.495	.001	-.481	.754*	.306	.633*	.325	.015	.344	.365	.303	.639*	-.197	-.075	1					
TON	.425*	.357	.408	-.044	-.336	.708*	.283	.584	.256	-.128	.286	.341	.281	.586	.044	-.187	.673*	1				
TP	.489*	.680*	.680*	.748*	.511	.369	.642*	.483	.531	.795	.716*	.661*	.764*	.631	.537	.696*	.148	-.105	1			
DM	-.427*	.667*	.644*	.432	.136	.554	.599	.622	.639*	.066	.699*	.599	.602	.600	.278	.531	.600	.495	.343	1		
AFDM	.233	.808*	.820*	.498	.108	.772*	.739*	.837*	.734*	.267	.752*	.766	.733*	.783	.377	.474	.771	.630	.470	.908*	1	
Chl- <i>a</i>	-.356*	.802*	.779*	.509	.096	.772*	.704*	.741*	.756*	.045	.777*	.702*	.671*	.748*	.156	.567	.533	.425	.415	.676*	.702*	1

(Temp. = temperature, Cond. = conductivity, Turb. = Turbidity, ORP= Oxidation Reduction potential, DO = Dissolved Oxygen, TDS= Total Dissolved Solid, TSS=Total Suspended Solid, TN= Total Nitrogen, Total phosphorus, TON= Total Oxidative Nitrogen, DM= Dry mass, AFDM= Ash-free dry mass, Chl-*a* = Chlorophyll-*a*)

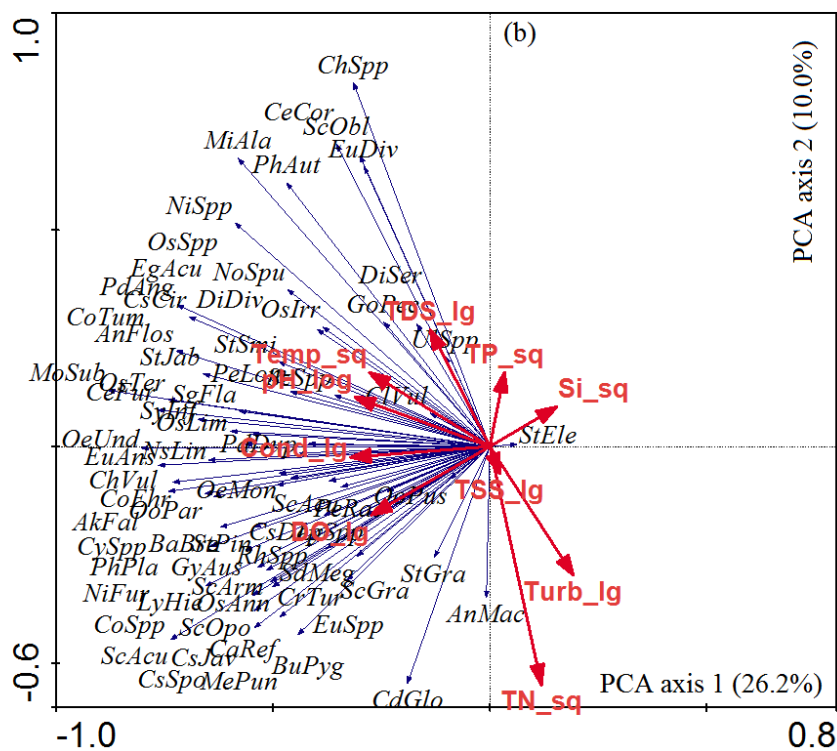
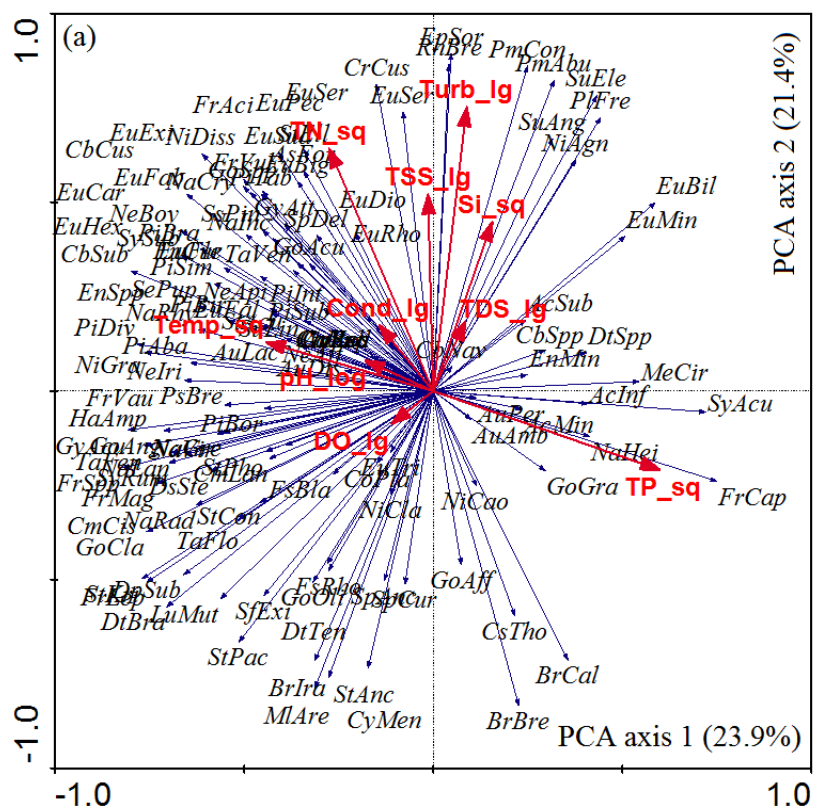
### 5.8.2 Principal Component Analysis (PCA)

Principal components analysis (PCA) is an indirect and unconstrained ordination technique which identifies the relationship between predictor and response variables. It allows for the identification of the principal directions (gradients) of the variations in the data in order to explore patterns in ecological datasets. Furthermore, PCA identifies relationships between environmental variables and species in a dataset. The PCA carried out for diatoms under base flow (10-15 ML/day) conditions showed that axis 1 accounted for 23.9% of the variation in the dataset and axis 2 for 21.4% of the variation in the dataset (Table 5.6). From the data plot, perhaps as a result of antecedent rainfall, the diatom community changed in July 2012 while the February 2012, November 2012 and June 2013 the species compositions were more similar. The projection of environmental variables showed that the most important variables that correlated with the species data were TP, turbidity, TSS, Si, TN and temperature. The PCA results showed that upstream species, such as *F. rhomboidia*, *G. affine*, *N. radiosa* and *T. flocculosa* were mostly associated with low temperature, low TP, high DO, and low pH while the downstream species, such as *E. serpentina*, *P. abundans*, *R. brebissoni* and *S. elegans* were mostly associated with high TN, turbidity, silica and TSS (Figure 5.23a). Furthermore, the results revealed TP and temperature correlated negatively with the first axis while turbidity, TSS and TN had a positive correlation with the second axis.

The PCA test performed on soft algae revealed that axis 1 accounted for 26.2% of the variation and axis 2 accounted for 10.0% of the variation in the data set. The eigenvalues of the PCA were 0.251 and 0.120 for axes 1 and 2 respectively (Table 5.6). The results revealed that the most important variables influencing soft algae were turbidity, conductivity, TN, TDS, pH, DO and temperature. Moreover, data plots showed the ecological pattern of soft algae under base flow in different seasons and

showed that the abundance upstream species *Closterium* sp., *D. sertularia*, *O. parva*, *P. angulosum* and *P. lomnickii* were positively correlated with DO and conductivity. Conversely, downstream species *C. sportella*, *O. parva*, *P. angulosum*, *S. flagelliferum* *Lyngbya* sp. and *Schizothrix* sp. were positively correlated with TDS, temperature and pH (Figure 5.23b). The results for species composition of soft algae in July 2012 under base flow (10- 15 ML/day) showed different patterns in comparison with other seasons.





**Figure 5.23:** Principal Correspondence Analysis (PCA) under 15 ML/day along the MacKenzie River: **(a)** Diatoms response; **(b)** Soft algae response.

The PCA analysis carried out before freshes, during freshes (35-40 ML/day) and after freshes revealed diatom species composition changes during freshes along the river. The eigenvalues of the PCA were 0.220 and 0.215, for axes 1 and 2 respectively (Table 5.6). The PCA indicated that during and after freshes the upstream species, such as *B. brebissonii*, *B. arentii*, *E. minor*, *G. affine*, *N. heimansioides*, *S. exiguiformis* and *T. flocculosa*, were associated with high DO (Figure 5.24a). In contrast, downstream species, such as *E. serpentina*, *N. capitellata*, *N. clausii*, and *S. curvula*, were associated with high turbidity, TSS, conductivity, TN, Si, pH, and TDS (Figure 5.24a). The analysis revealed the variation was 22.0% for axis 1 and 21.5% for axis 2. The results showed that the most important variables were TN, TDS, temperature, TSS, turbidity, pH and conductivity. Furthermore, the results revealed TP and temperature and DO had a negative correlation with turbidity, conductivity and TSS on the second axis.

The eigenvalues of the soft algae were 0.188 and 0.142 for axes 1 and 2 respectively (Table 5.6). The analysis indicated the variation was 22.3% for axis 1 and 15.6% for axis 2. PCA explored the ecological relations between the soft algae and environmental variables under water release events (35-40 ML/day) and it revealed that species composition patterns change during freshes. The results showed that the most important variables influencing soft algae were TP, conductivity, pH, TSS and turbidity. The PCA results showed that upstream species such as *S. opoliensis* var. *caudatus* and *S. elegans* were associated with high DO, low TP and low TDS whilst downstream species (e.g. *P. autumnale*, *C. tumidulum* and *O. irrigua*) had positive relationships with TSS, turbidity, pH, conductivity (Figure 5.24b).

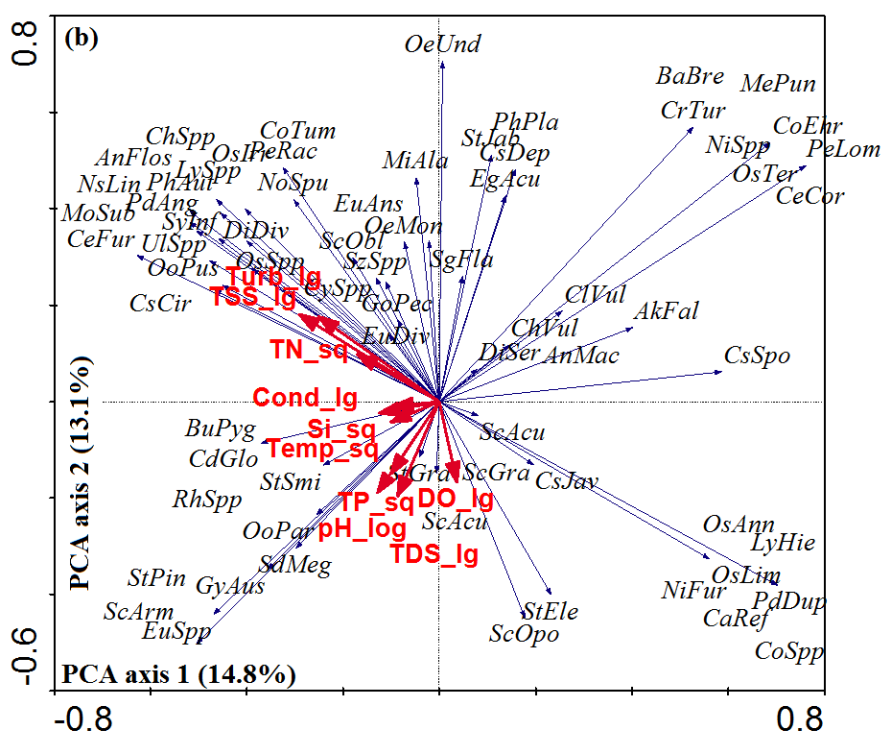
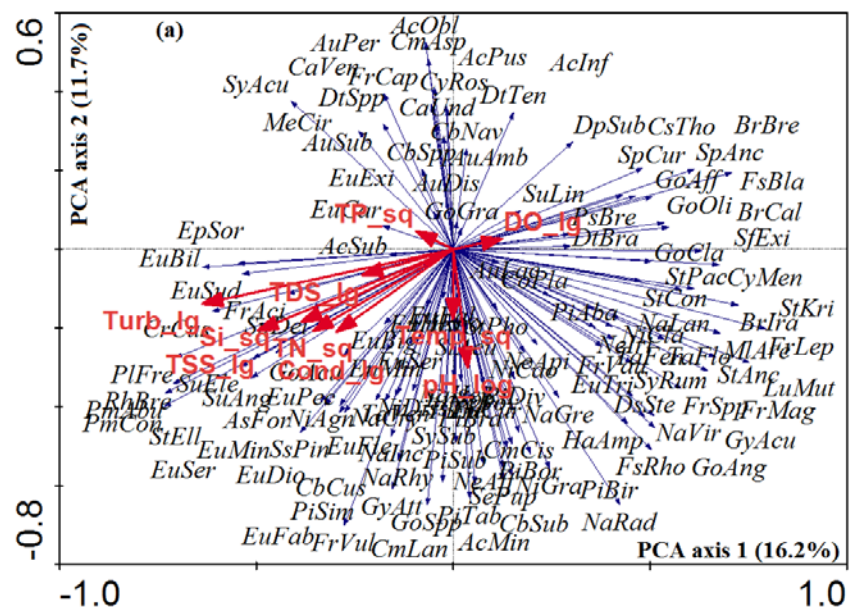


PCA was performed on data collected before high flows, during high flows (55 ML/day) and after high flows to illustrate the relationship between species and environmental variables. The results showed diatom species composition patterns were different before, during and after high flows. The analysis revealed that axis 1 explained 21.5% of the variation in the diatom data and axis 2 17.6%. The data visualisation showed that the most important variables were TP, pH, Si, TDS, TN and temperature. The PCA showed *G. affine*, *F. rhomboides*, *N. heimansioides*, *N. radiosa* and *T. flocculosa* were associated with high DO, low pH, and low TP while downstream species such as *E. serpentina* and *S. delicatissima* were associated with higher Si, TDS, turbidity and temperature (Figure 5.25a). The eigenvalues of the PCA were 0.215 and 0.176, for axes 1 and 2 respectively (Table 5.6). In addition, the results revealed DO had a negative correlation with temperature, TDS and turbidity. The analysis showed that soft algae also change under high flows where eigenvalues were 0.180 and 0.165, for axes 1 and 2 respectively (Table 5.6). PCA results showed species composition and biomass of soft algae were mostly associated with TSS, turbidity, pH, and conductivity under high flows (Figure 5.25b).



The PCA analysis carried out on the diatom data set under all flow regimes (different flow regimes in different seasons) revealed diatom species composition changes along the river mostly in association with turbidity, TSS, pH, Si and conductivity. The eigenvalues of the PCA were 0.162 and 0.117, for axes 1 and 2 respectively (Table 5.6). The PCA revealed species such as *B. brebissonii*, *E. minor*, *G. affine*, *N. heimansioides*, *S. exiguiformis* and *T. flocculosa* were associated with low pH, temperature, and conductivity but high DO, whilst downstream species such as *E. serpentina*, *N. capitellata*, *N. clausii*, and *S. curvula* were associated with high turbidity, TSS, TN, and TDS (Figure 5.26a). The analysis revealed 16.2% of variation for axis 1 and 11.7% of variation for axis 2 (Table 5.6).

The PCA performed on the data set of soft algae under all flow regimes (different flow regimes in different seasons) revealed soft algae changes along the river were mostly associated with turbidity, TSS, TN and TP. The analysis indicated 14.8.3% of variation for axis 1 and 13.1% of variation for axis 2. The PCA results showed that upstream species such as *S. acuminatus*, *Euastrum* sp. and *S. armatus* were associated with high DO, and low TP and TDS, whilst downstream species *A. flos-aquae*, *M. subclavatum*, *O. pusila* were associated with high TSS, turbidity, pH, and conductivity (Figure 5.26b). The eigenvalues of the soft algae were 0.148 and 0.131 for axes 1 and 2 respectively (Table 5.6).



**Figure 5.26:** Principal Correspondence Analysis (PCA) altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River: **(a)** Diatoms response; **(b)** Soft algae response.

**Table 5.10:** Comparison of PCA eigenvalues and cumulative percentage variance under different flow regimes

		Diatoms		Soft algae	
		Axis 1	Axis 2	Axis1	Axis 2
10-15 ML/d	Eigenvalues	0.239	0.214	0.251	0.120
	Cumulative % variance	23.9	21.4	26.2	10.0
35-40 ML/d	Eigenvalues	0.220	0.215	0.188	0.142
	Cumulative % variance	22.0	21.5	22.3	15.6
55 ML/d	Eigenvalues	0.215	0.176	0.180	0.165
	Cumulative % variance	22.7	20.0	25.5	9.2
altogether	Eigenvalues	0.162	0.117	0.148	0.131
	Cumulative % variance	16.2	11.7	14.8	13.1

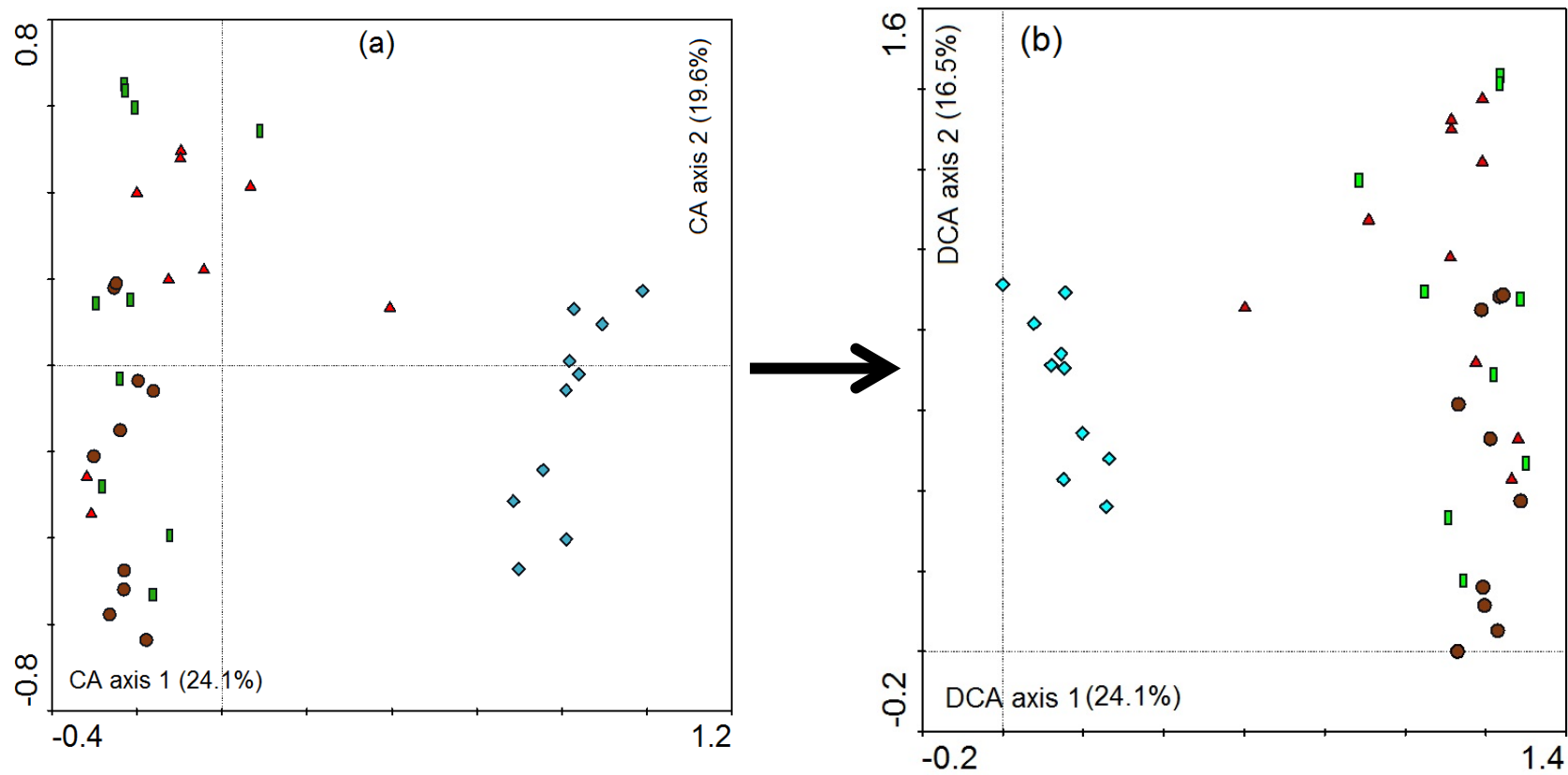


### **5.8.3 Correspondence Analysis (CA) and Detrended Correspondence Analysis (DCA)**

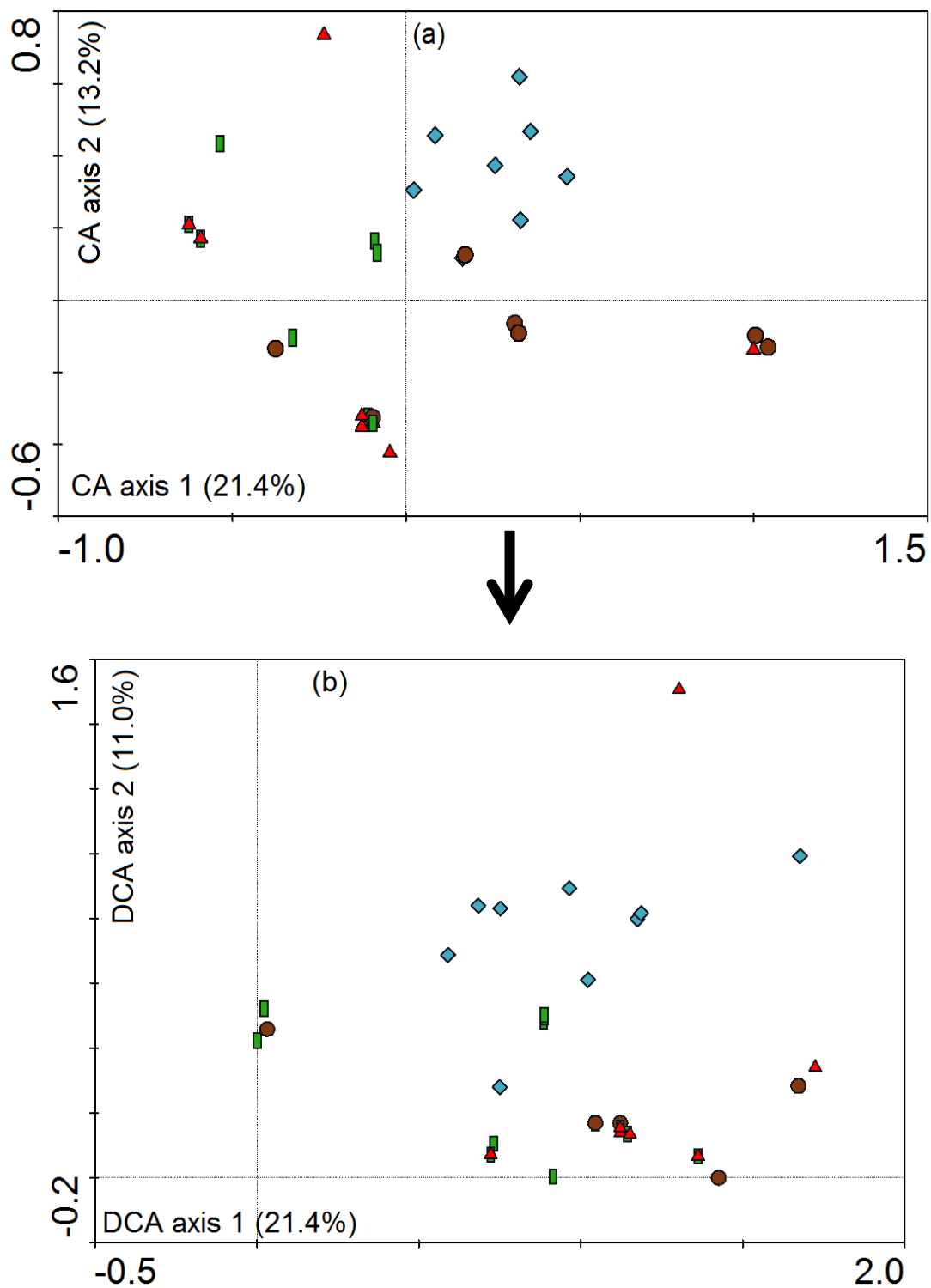
In order to reveal associations between species and samples, correspondence analysis (CA) was carried out on data set. The CA was applied using transformed data to find the association of the algal community structure under different flow regimes. The CA test revealed the non-linearity of species within the unconstrained condition. Exploration of the diatom and soft algae assemblage data used the computationally simple, unconstrained ordination technique of CA. Nonetheless, the CA sometimes suffers from two problems including the arch effect and edge effect (compression of the gradient ends) due to unimodal species response curves. The DCA was also used to determine the gradient length and identify patterns of species and samples. Furthermore, the DCA reveals the nature of the diatom and soft algae species composition variation between flows and also between seasons.

The CA test carried out for diatom species collected under 10-15 ML/day found that there were two strong gradients in the datasets, with both axes 1 and 2 explaining a large portion of the variance in the diatom and algal community data. In the diatom data there was a clear split between the assemblages observed during water releases and those observed at 10-15 ML/day. The CA of diatom species accounted for 24.1% of the species variation on axis 1 while 19.6% of the variation was accounted for on axis 2 (Figure 5.27a). The eigenvalues of the CA were 0.163 and 0.132, for axes 1 and 2 respectively (Table 5.7). The DCA was displayed under base flow (10-15 ML/day) and showed the gradient length to exceed 2 and that eigenvalues were 0.163 and 0.111 for axes 1 and 2 respectively (Table 5.8). The results revealed 24.1% of the variance was explained by axis 1 and 16.5 % by axis 2 (Figure 5.27b). The data analyses revealed the results of indirect gradient analysis (CA) and direct gradient analysis (DCA) were almost the same. The results indicated the diatom species assemblages in July 2012 were in contrast to those sampled in other seasons.

At the same time, the CA test was carried out on soft algae data and this revealed the split in the diatom assemblage data is not reflected in the soft algae data, where far more scatter in the data is evident (this may be due to lower counts in this dataset). The results revealed 21.4% of the variance was explained by axis 1 and 13.2% by axis 2 (Figure 5.28a). The eigenvalues of the CA were 0.103 and 0.079, for axes 1 and 2 respectively (Table 5.7). The data plot of this CA revealed an arch effect for June 2013 (Figure 5.28a) and so detrended correspondence analysis (DCA) was applied. The DCA results for soft algae showed that soft algae assemblages varied between seasons in the MacKenzie River. The results also showed that the species assemblage in July 2012 was different to that from other seasons (Figure 5.28b). The results revealed 21.4% of the variance was explained by axis 1 and 11.0% by axis 2 (Figure 5.28b). The eigenvalues of the DCA were 0.103 and 0.075, for axes 1 and 2 respectively (Table 5.8).



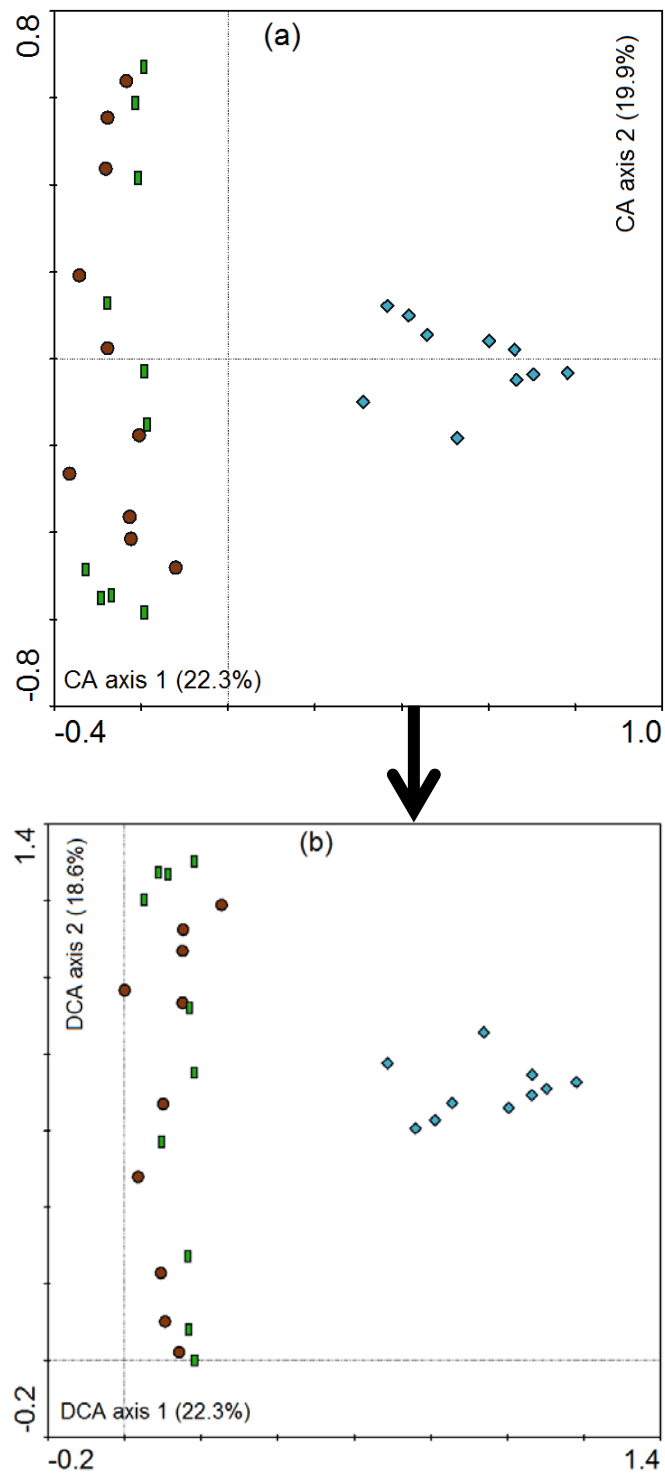
**Figure 5.27:** (a) Correspondence Analysis (CA) of diatoms under 10-15 ML/day along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of diatoms under 10-15 ML/day along the MacKenzie River: February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red triangle)



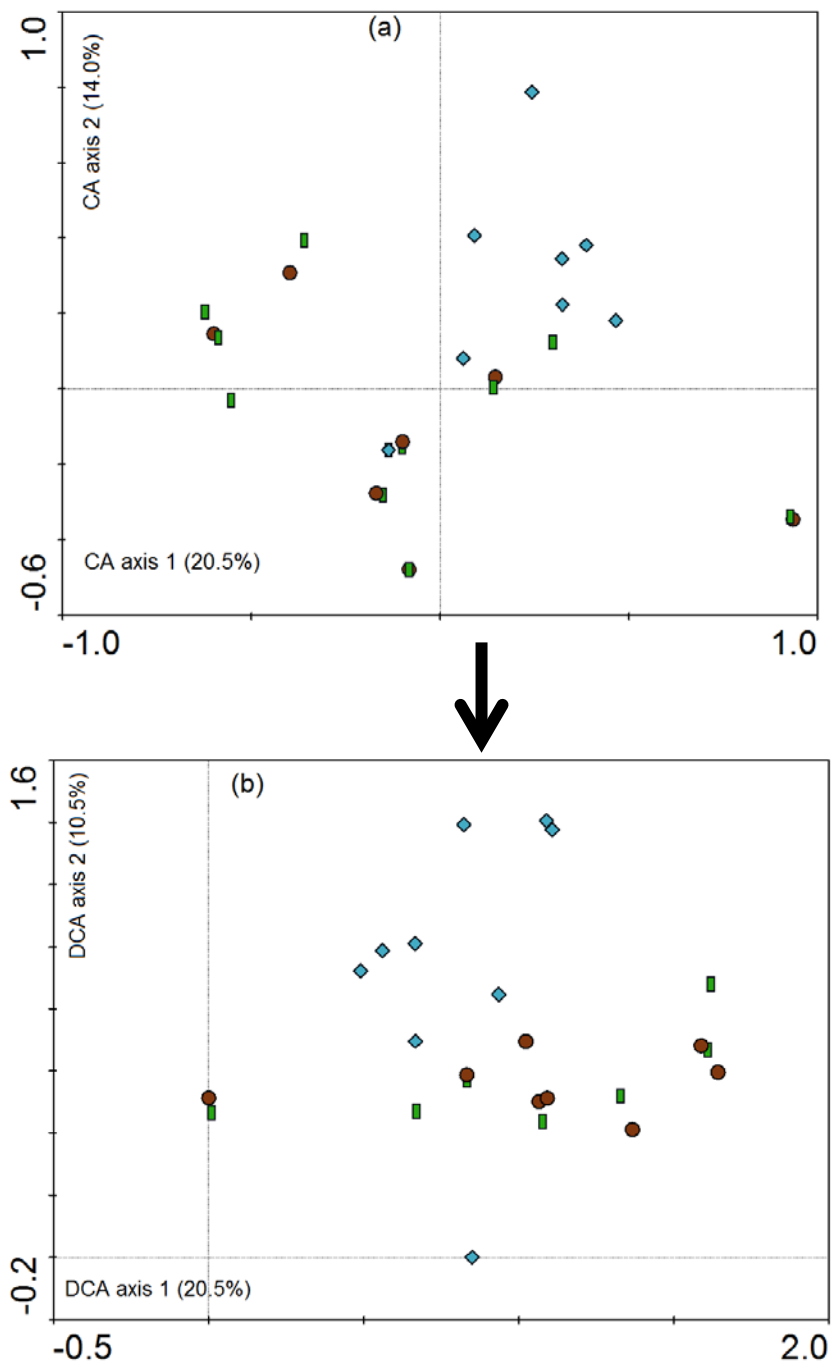
**Figure 5.28:** (a) Correspondence Analysis (CA) of soft algae under 10-15 ML/day along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of soft algae under 10-15 ML/day along the MacKenzie River: February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red triangle)

The CA test was also performed on diatom samples before, during (35-40 ML/day) and after fresh releases. The diatom results showed 22.3% of variation was explained by axis 1 and 19.9% by axis 2. The eigenvalues of the CA were 0.143 and 0.128, for axes 1 and 2 respectively (Figure 5.29a; Table 5.7). At the same time, the DCA results for freshes (35-40 ML/day) showed the diatom species assemblages are different during water release events from Wartook Reservoir into the MacKenzie River. The eigenvalues were 0.143 and 0.120 for axes 1 and 2 respectively (Table 5.8). The DCA of diatom species showed that axis 1 accounted for 22.3% of the variation and 18.6% for axis 2 respectively (Figure 5.29b). The results of indirect gradient analysis (CA) and direct gradient analysis (DCA) were similar.

The CA test was also carried out on soft algae samples before, during (35-40 ML/day) and after the release of freshes. The soft algae result showed 20.5% of variation was explained by axis 1 and 14.0% by axis 2 (Figure 5.30a). The eigenvalues of CA for axes 1 and 2 were 0.140 and 0.095 respectively (Table 5.7). The DCA of soft algae species showed that axis 1 accounted for 20.5% of the variation and 10.5% for axis 2 respectively (Figure 5.30b; Table 5.8). The arch artefact was observed in the CA of soft algae which was eliminated by analysis using DCA (Figure 5.30a-b).



**Figure 5.29:** (a) Correspondence Analysis (CA) of diatoms under 35-40 ML/day along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of diatoms under 35-40 ML/day along the MacKenzie River: Before Freshes (brown circle), during Freshes (blue diamond) and after Freshes (green box)

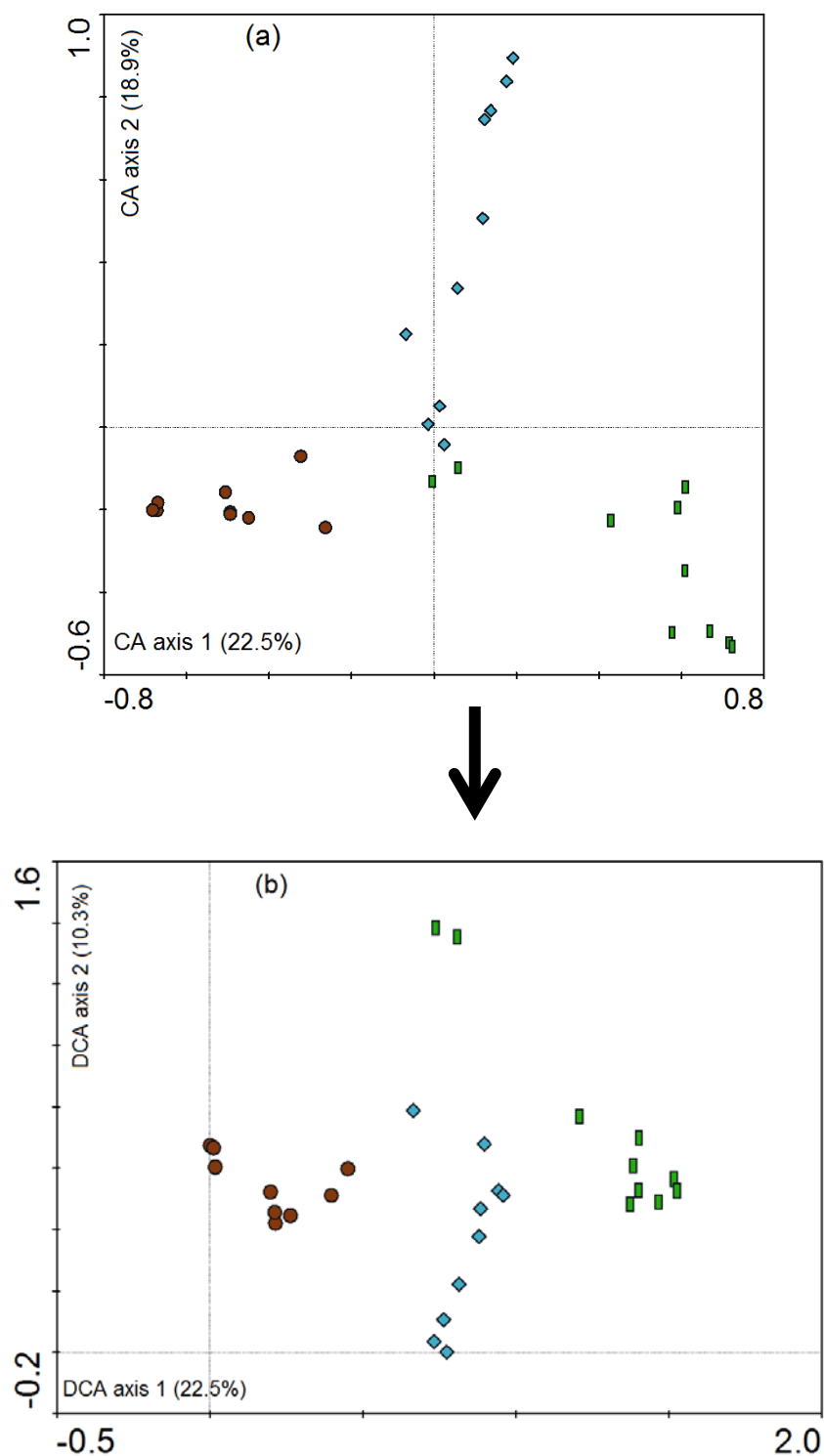


**Figure 5.30:** (a) Correspondence Analysis (CA) of soft algae under 35-40 ML/day along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of soft algae under 35-40 ML/day along the MacKenzie River: Before Freshes (brown circle), during Freshes (blue diamond) and after Freshes (green box)

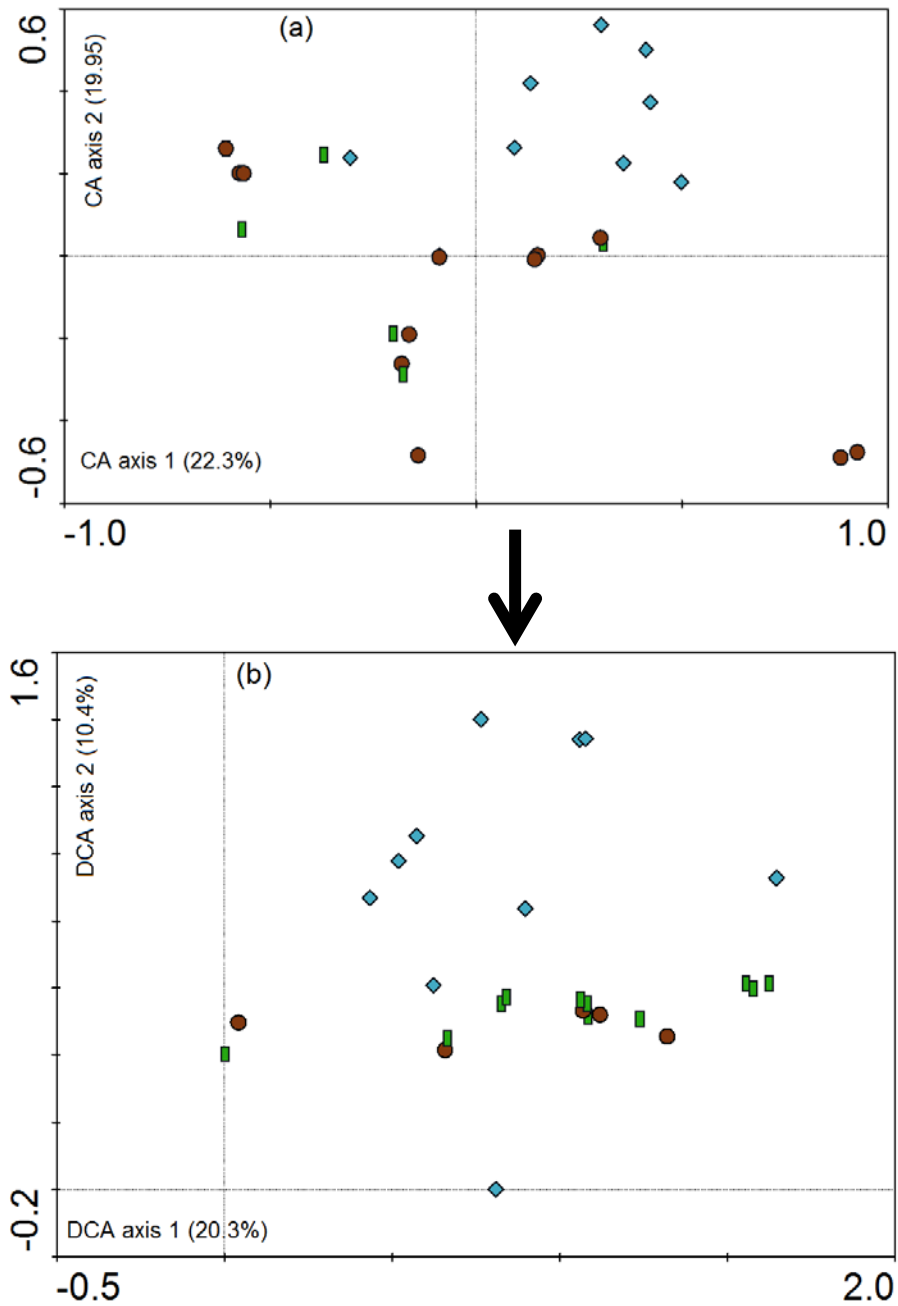
The CA of the diatom samples collected before, during (55 ML/day) and after high flows showed axis 1 explaining 22.5% of the variation, whilst axis 2 explained 18.9% (Figure 5.31a). The eigenvalues of the CA for axes 1 and 2 were 0.180 and 0.152 respectively (Table 5.7). Simultaneously, the DCA carried out on diatom species data from before, during (55 ML/day) and after high flows showed that axis 1 accounted for 22.5% of the variation and axis 2 10.3% (Figure 5.31b, Table 5.8).

The CA of the soft algae samples indicated that axis 1 explained 22.3% of the variation whilst axis 2 explained 19.9% (Figure 5.32a). The DCA result from the 55 ML/day flow showed again that the diatom species assemblage and soft algae community differed between before, during and after high flows. The soft algae result showed that axis 1 explained 20.3% of the variation and axis 2 10.4%. The eigenvalues of DCA for axes 1 and 2 were 0.143 and 0.073 respectively (Figure 5.32b). The arch effect was also observed in the CA of the soft algae and this was eliminated by detrending (in the DCA) (Figure 5.32a-b, Table 5.8).





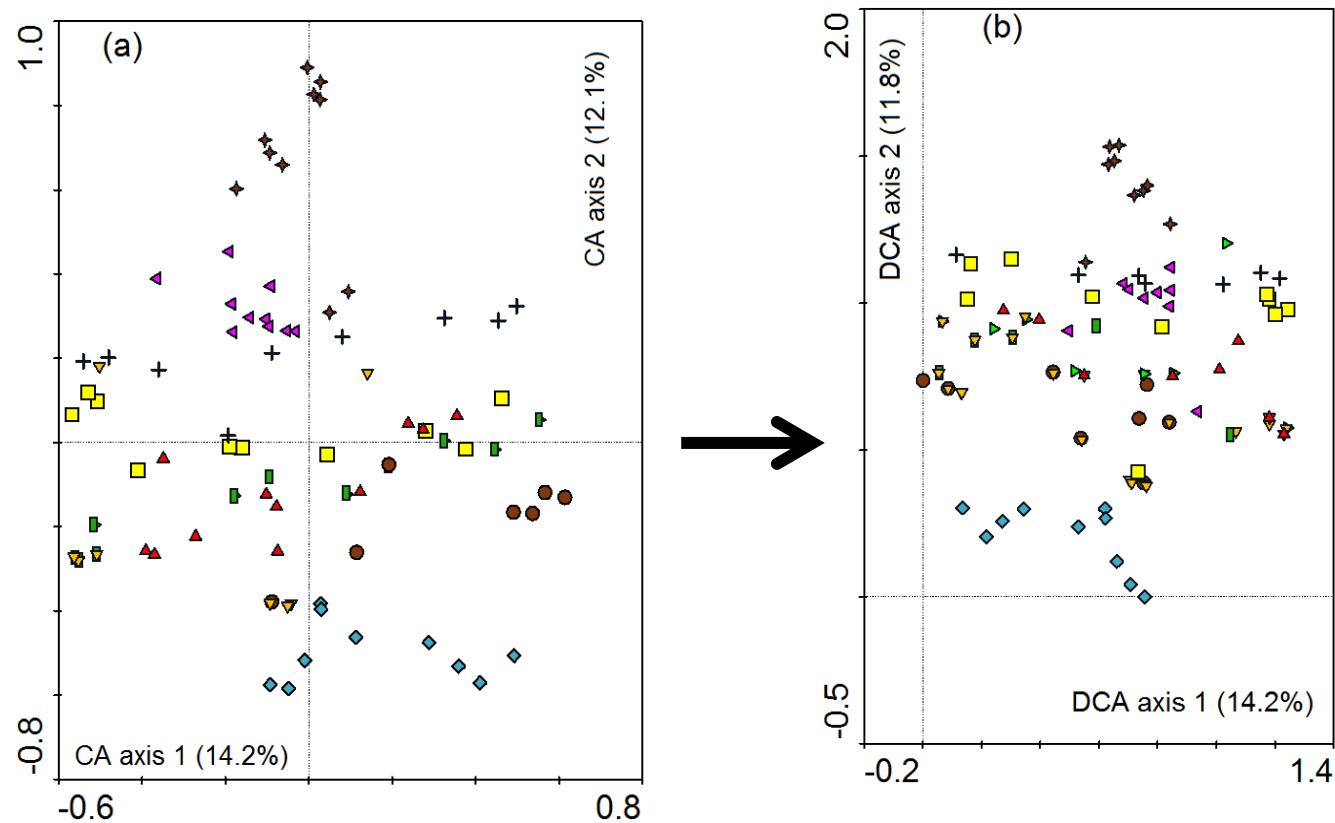
**Figure 5.31:** (a) Correspondence Analysis (CA) of diatoms under 55 ML/day along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of diatoms under 55 ML/day along the MacKenzie River: Before high flows (brown circle), during high flows (blue diamond) and after high flows (green box)



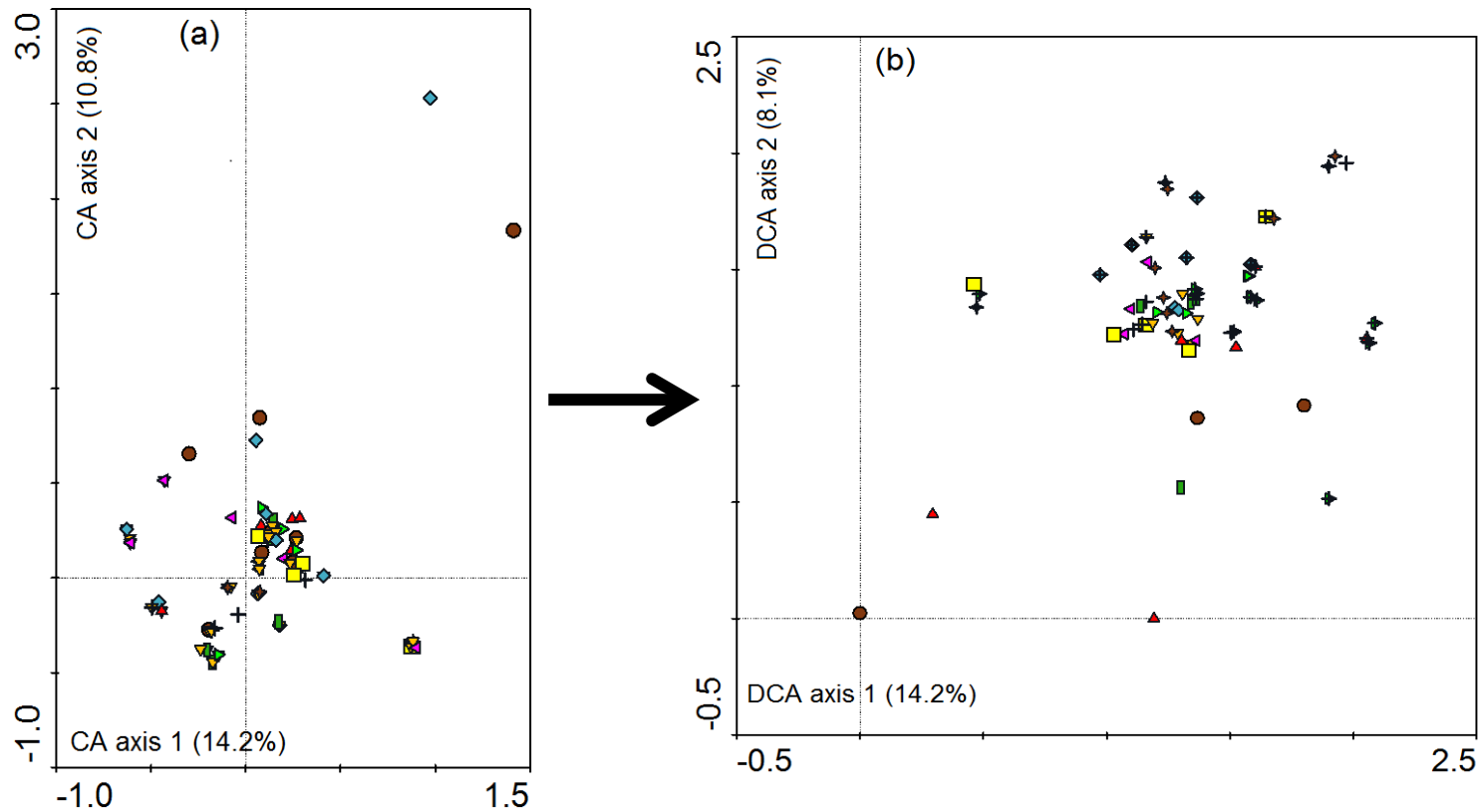
**Figure 5.32:** (a) Correspondence Analysis (CA) of soft algae under 55 ML/day along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of soft algae under 55 ML/day along the MacKenzie River: Before high flows (brown circle), during high flows (blue diamond) and after high flows (green box)

The CA test also was carried out on diatom species under all flows across different seasons. The results of all flows indicated strong gradients in the datasets, with both axes 1 and 2 explaining a large portion of the variance in the diatom communities. The analysis revealed that 14.2% of variation for axis 1 and 12.1% of variation for axis 2 (Figure 5.33a). The eigenvalues for axes 1 and 2 were 0.109 and 0.093 respectively (Table 5.7). Meantime, the DCA result of all flows revealed 14.2% of variation was explained by axis 1 and 11.8% by axis 2 (Figure 5.33b; Table 5.8).

The CA test was also applied to data of soft algae species under all flows across different seasons with axis 1 explaining 14.2% of the variation and axis 2 10.8% (Figure 5.34a; Table 5.7). The arch effect was observed and so a DCA was applied. The results of the DCA showed that 14.2% of the variation was explained by axis 1 and 8.1% by axis 2 (Figure 5.34b). The eigenvalues for axes 1 and 2 were 0.106 and 0.060 respectively (Table 5.8). The DCA results indicate that there is a strong unimodal response of species to flow regime. In other words, the DCA test suggests that Canonical correspondence analysis (CCA) would be an appropriate means for exploring the relations between the species and environmental data sets.



**Figure 5.33:** (a) Correspondence Analysis (CA) of diatoms altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of diatoms altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red up-triangle), before Freshes (yellow square), during Freshes (pink left-triangle), after Freshes (green right-triangle), before high flow (yellow down-triangle), during high flow (cross) and after high flow (brown star)



**Figure 5.34:** (a) Correspondence Analysis (CA) of soft algae altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River; (b) Detrended Correspondence Analysis (DCA) of soft algae altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red up-triangle), before Freshes (yellow square), during Freshes (pink left-triangle), after Freshes (green right-triangle), before high flow (yellow down-triangle), during high flow (cross) and after high flow (brown star)

**Table 5.11:** Comparison of CA eigenvalues and cumulative percentage variance under different flow regimes

		Diatoms		Soft algae	
		Axis 1	Axis 2	Axis1	Axis 2
10-15 ML/d	Eigenvalues	0.163	0.132	0.103	0.079
	Cumulative % variance	24.1	19.6	21.4	13.2
35-40 ML/d	Eigenvalues	0.143	0.128	0.140	0.095
	Cumulative % variance	22.3	19.9	20.5	14.0
55 ML/d	Eigenvalues	0.180	0.152	0.143	0.100
	Cumulative % variance	22.5	18.9	22.3	19.9
altogether	Eigenvalues	0.109	0.093	0.106	0.081
	Cumulative % variance	14.2	12.1	14.2	10.8



**Table 5.12:** Comparison of DCA eigenvalues and cumulative percentage variance under different flow regimes

		Diatoms		Soft algae	
		Axis 1	Axis 2	Axis1	Axis 2
10-15 ML/d	Eigenvalues	0.163	0.111	0.103	0.075
	Cumulative % variance	24.1	16.5	24.1	11.0
35-40 ML/d	Eigenvalues	0.143	0.120	0.140	0.071
	Cumulative % variance	22.3	18.6	20.5	10.5
55 ML/d	Eigenvalues	0.180	0.083	0.143	0.073
	Cumulative % variance	22.5	10.3	20.3	10.4
altogether	Eigenvalues	0.109	0.091	0.106	0.060
	Cumulative % variance	14.2	11.8	14.2	8.1

#### 5.8.4 Cononical Correspondence Analysis (CCA)

Canonical Correspondence Analysis (CCA) was used to determine the direct relationship between diatom and soft algae communities and the environmental variables. CCA is a constrained ordination which uses *a priori* hypotheses (in contrast with unconstrained tests which do not use *a priori* hypotheses). The CCA analyses were applied to determine the most important variables influencing the diatom and soft algae communities under different seasons and under different flows along the MacKenzie River. Furthermore, the CCA analyses were performed to examine how algal species respond to a range of variables under different flows. The environmental variables applied to the CCA analyses included pH, conductivity, turbidity, temperature, dissolved oxygen, total suspended solid, dissolved solid, total nitrogen, and total phosphorus under different flow regimes.

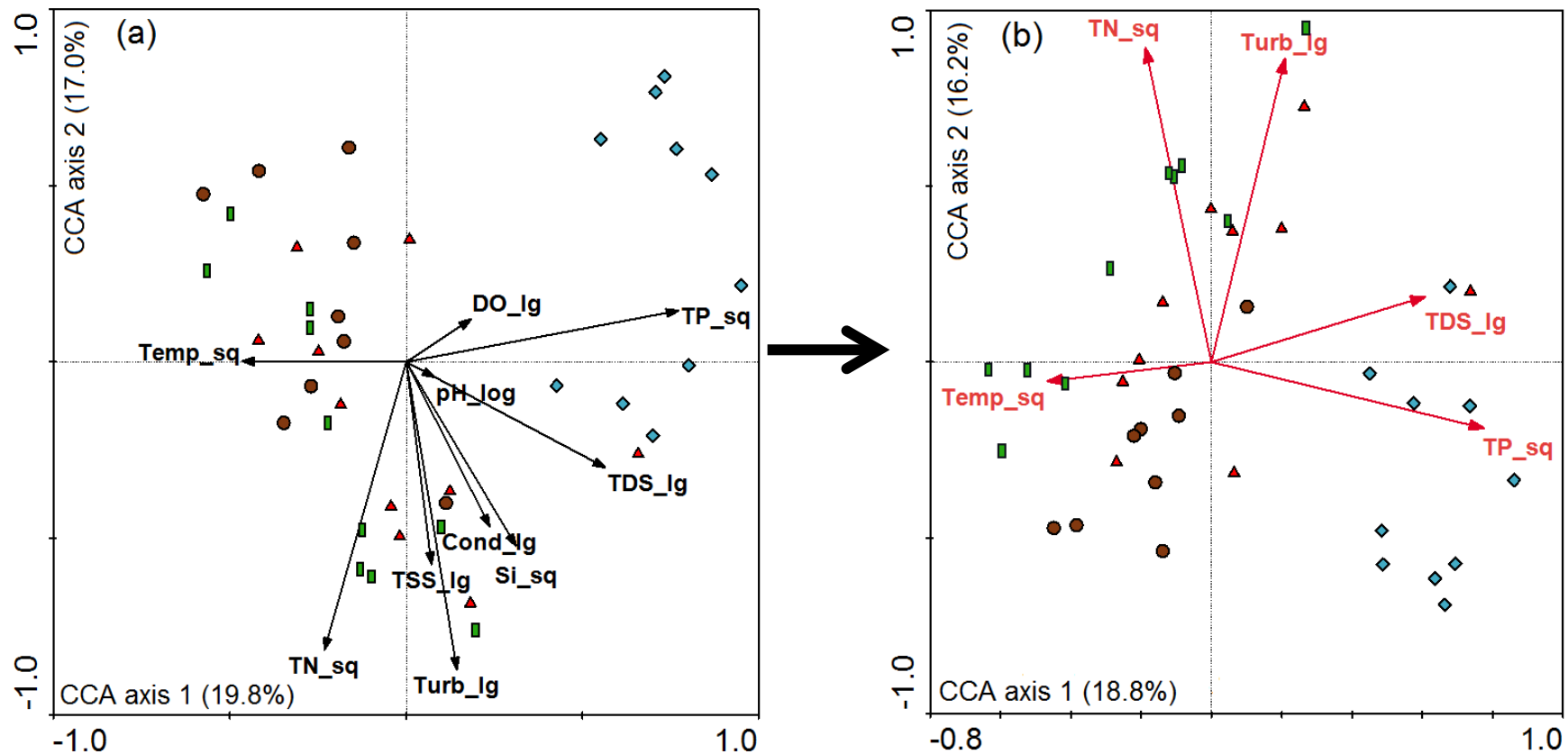
Due to potential co-variation between variables, the number of explanatory variables was restricted using a forward-selection process and Bonferroni-adjustment test. The forward selection option is used to identify the significant environmental variables. The iteration of forward selection chooses the variables that explain the greatest variation. To restrict the number of explanatory variables a Bonferroni-adjustment was applied to the forward-selection process, where the *p*-value is divided by the number of variables included (e.g. the first significant variable must have a *p*-value of  $<0.05$  ( $0.05/1$ ); the second significant variable must have a *p*-value of  $<0.025$  ( $0.05/2$ ) for it to be significant).

The CCA analyses of diatom and soft algae data under base flow (10-15 ML/day) showed that species assemblages were different under different flow regimes. In July 2012 the most influential environmental factors were TP, TDS and DO while the most influential factors in February 2012, November 2012 and June 2013 were conductivity, TSS, turbidity, silica, total nitrogen and temperature (Figure 5.35a). The eigenvalues for axes 1 and 2 were 0.134 and 0.115 respectively (Table 5.9). The significant environmental variables, including

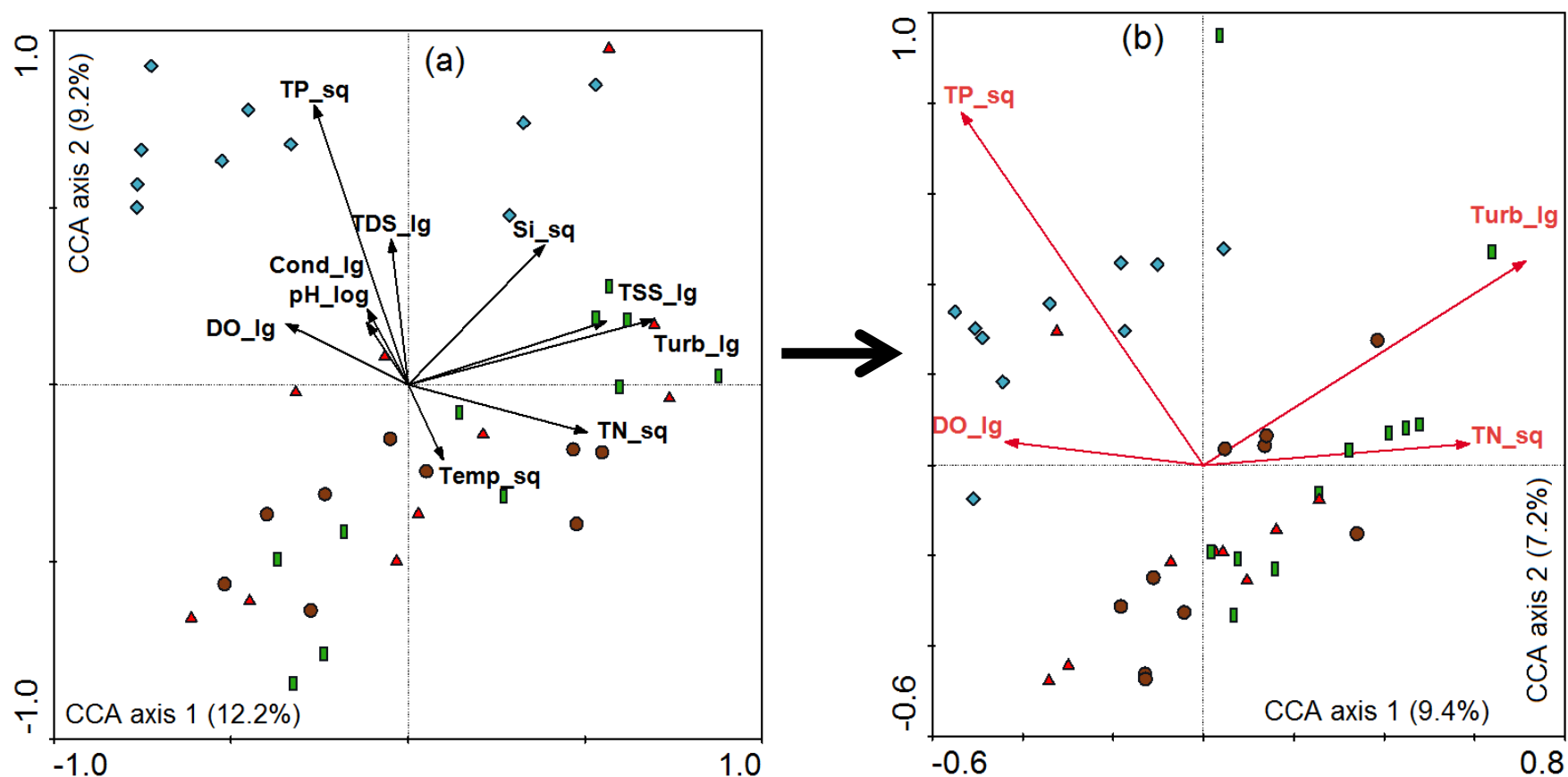
TP, TN, temperature and TDS, were recognised using manual forward selection. After forward selection, the eigenvalues derived from the CCA were 0.113 and 0.104 for axes1 and 2 respectively (Figure 5.35b; Table 5.10).

The CCA carried out on soft algae revealed the percentage variance of the species-environment relationship explained was 9.4% and 7.2% for axes1 and 2 respectively (Figure 5.36a; Table 5.9). As mentioned earlier several environmental variables were used to understand the relationship between species composition and environmental variables. Under 15 ML/day the significant environmental variables for soft algal distributions and abundances were turbidity, DO, TP and TN (Figure 5.36b; Table 5.10).



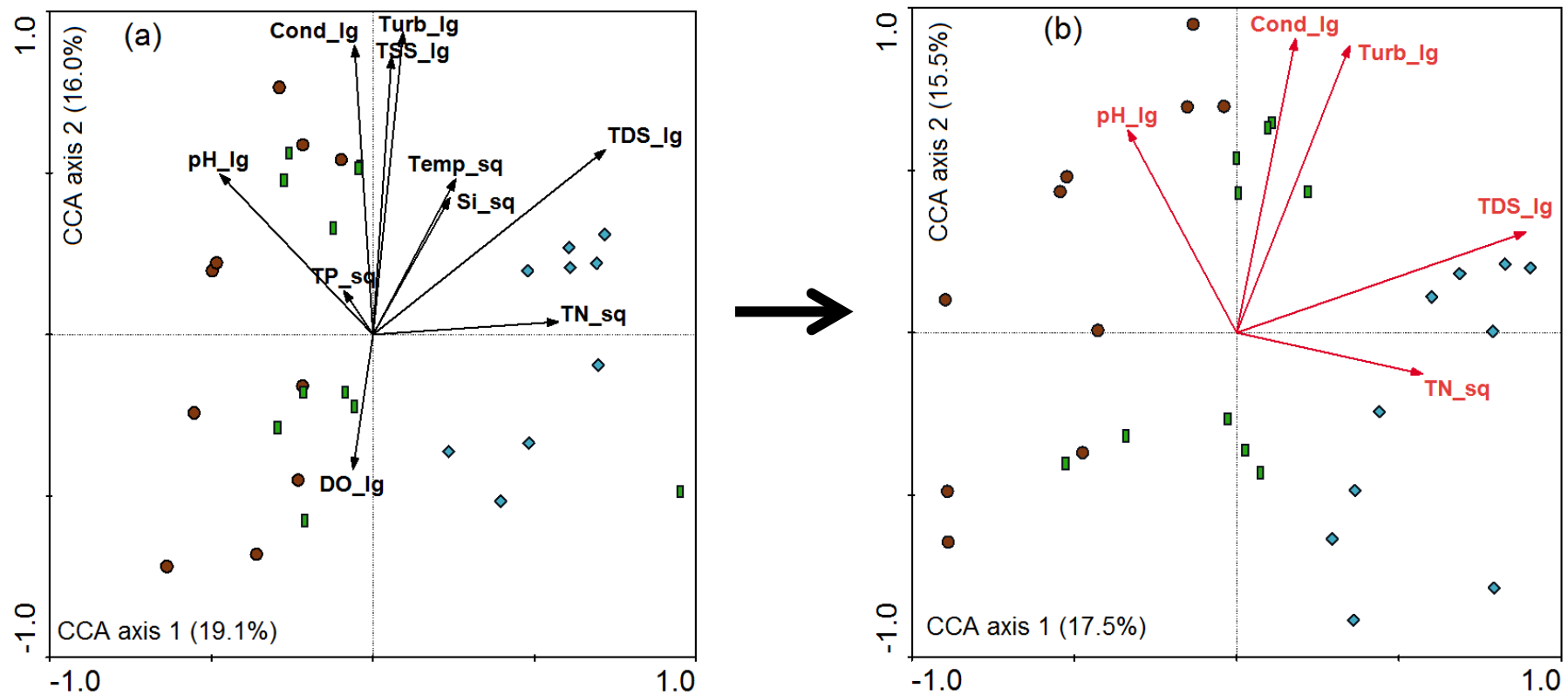


**Figure 5.35:** Cononical Correspondence Analysis (CCA) of diatoms under 15 ML/day along the MacKenzie River: (a) with all environment variables; (b) significant ( $p < 0.05$ ) variables after forward selection. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red triangle)

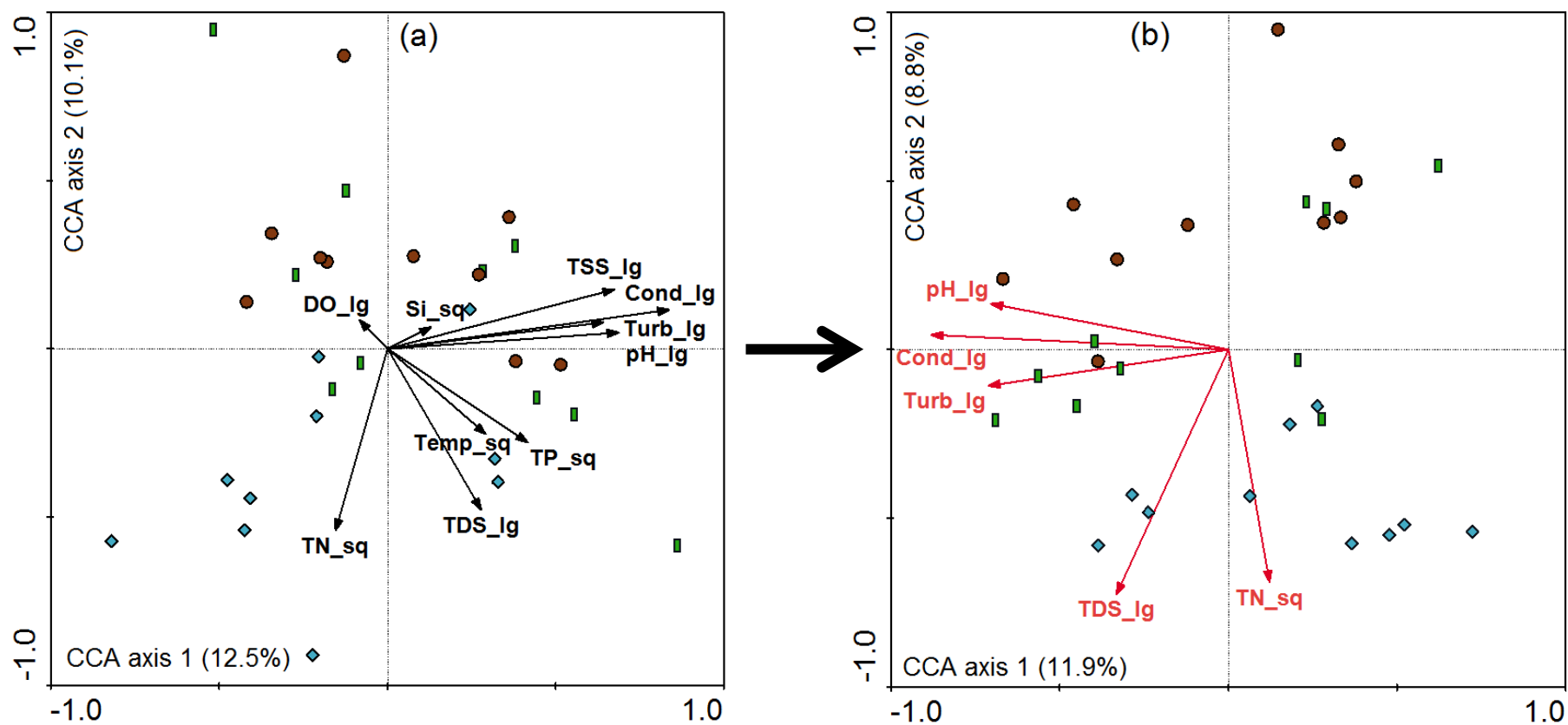


**Figure 5.36:** Cononical Correspondence Analysis (CCA) of soft algae under 15 ML/day along the MacKenzie River: **(a)** with all environment variables; **(b)** significant ( $p < 0.05$ ) variables after forward selection. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red triangle)

CCA was performed on diatom species and environmental variable data associated with the 35-40 ML/day flow and the eigenvalues derived from the CCA were 0.121 and 0.105% for axes 1 and 2 respectively (Table 5.9). The CCA results showed that pH, TP, TDS, turbidity, temperature and DO were the most significant environmental variables influencing diatom assemblages across seasons and sites. The percentage variance of the species-environment relationship explained were 17.5% in axis 1 and 15.5% by axis 2 (Figure 5.37). Conductivity, pH, TDS and TN were significant in the analysis. CCA carried out on soft algae revealed pH, conductivity, turbidity, TDS and TN were significant environmental variables in Freshes (35 ML/day) (Figure 5.38; Table 5.10).



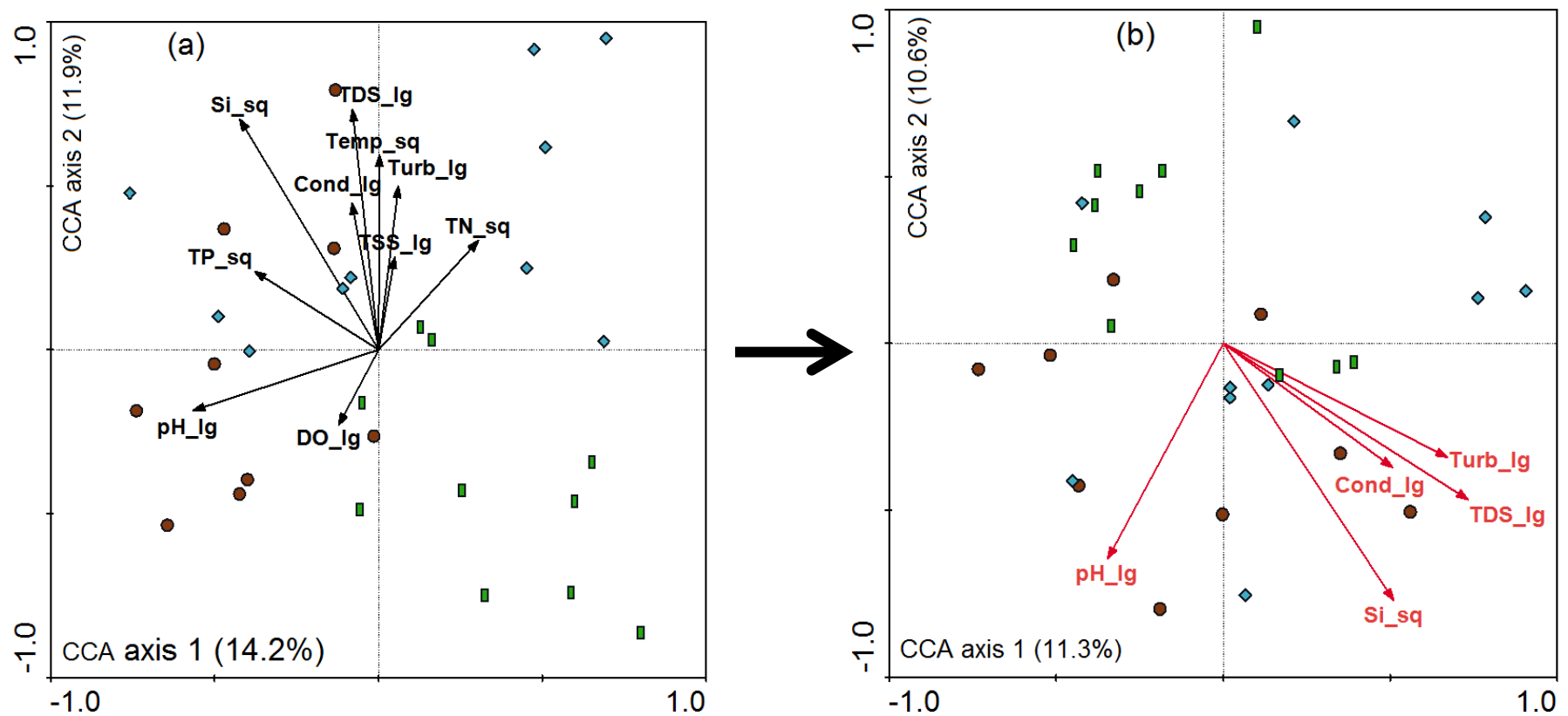
**Figure 5.37:** Cononical Correspondence Analysis (CCA) of diatoms under 35 ML/day along the MacKenzie River: **(a)** with all environment variables; **(b)** significant ( $p < 0.05$ ) variables after forward selection. Before Freshes (brown circle), during Freshes (blue diamond) and after Freshes (green box)



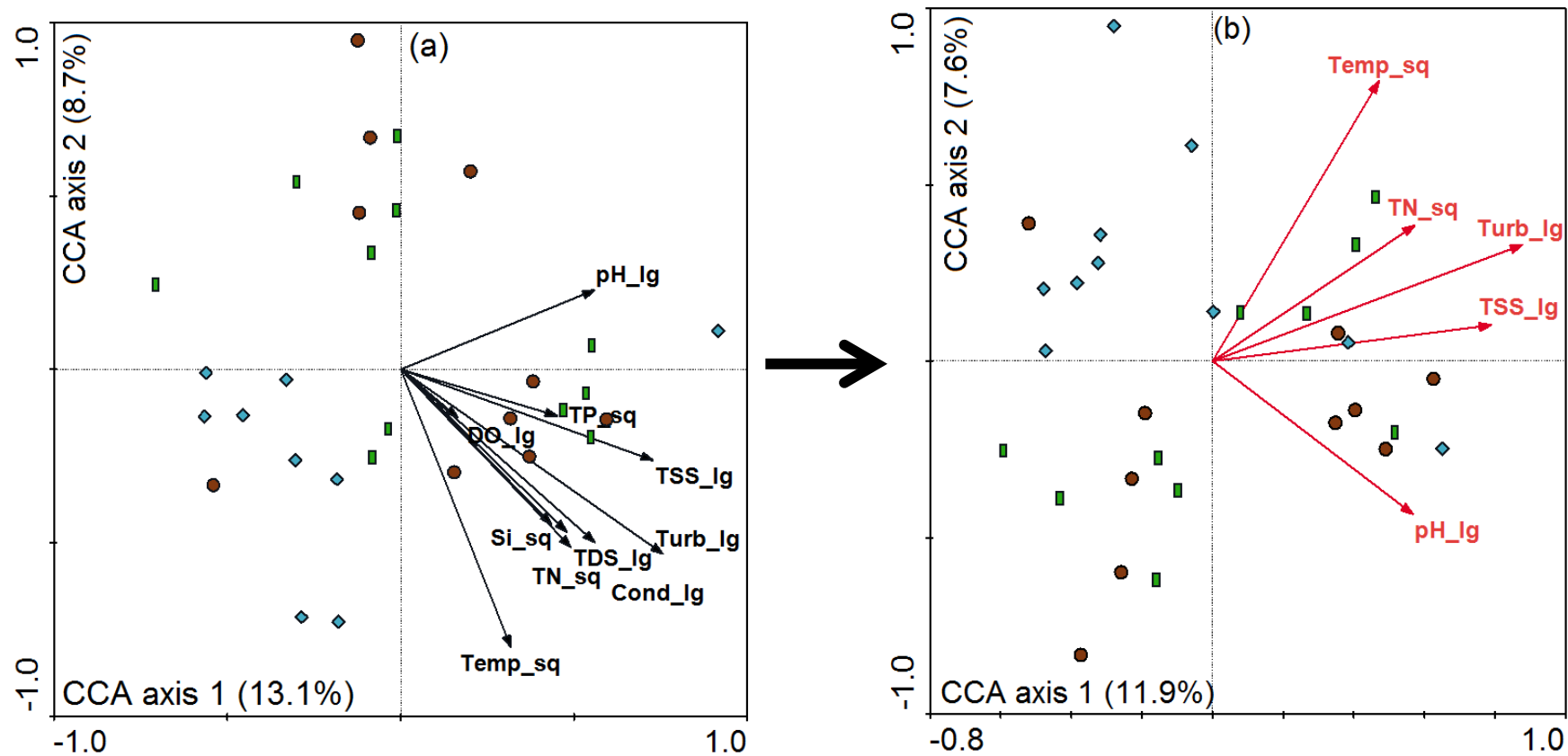
**Figure 5.38:** Cononical Correspondence Analysis (CCA) of soft algae under 35 ML/day along the MacKenzie River: (a) with all environment variables; (b) significant ( $p < 0.05$ ) variables after forward selection. Before Freshes (brown circle), during Freshes (blue diamond) and after Freshes (green box)

CCA analyses were applied to diatom and environmental data under high flows (55 ML/day). Five environmental variables (silica, conductivity, pH and TDS and turbidity) were found to correlate significantly (after forward selection) with the diatom data; 11.3% of the species-environment interactions was accounted for by axis 1 and 10.6% by axis 2 (Figure 5.39). Three significant variables (TP, pH and conductivity) were identified in the soft algae CCA, with axis 1 accounting for 11.9% of the variance and axis 2 7.6% (Figure Figure 5.40).

The CCA was applied using forward-selection to determine the most influential variables, environmental drivers of flow water chemistry and biological properties for all flows. Water chemistry had a significant influence on the diatom communities along the MacKenzie River under these circumstances. The CCA results showed that the diatom assemblage pattern was different during high flow compared with all the other flow scenarios. Diatom assemblages were associated with TDS and turbidity, parallel with axis 1 (9.9%), and pH and TP, temperature and DO parallel with axis 2 (6.8%) (Figure 5.41). The soft algae also were evaluated under all flows conditions and their responses to environmental variables analysed (Figure 5.42). This accounted for 8.5 % of the species-environment interactions on axis 1 and 3.0% on axis 2 (Table 5.10). The results highlight that nutrient availability is the major driver in soft algal assemblages in the MacKenzie River.

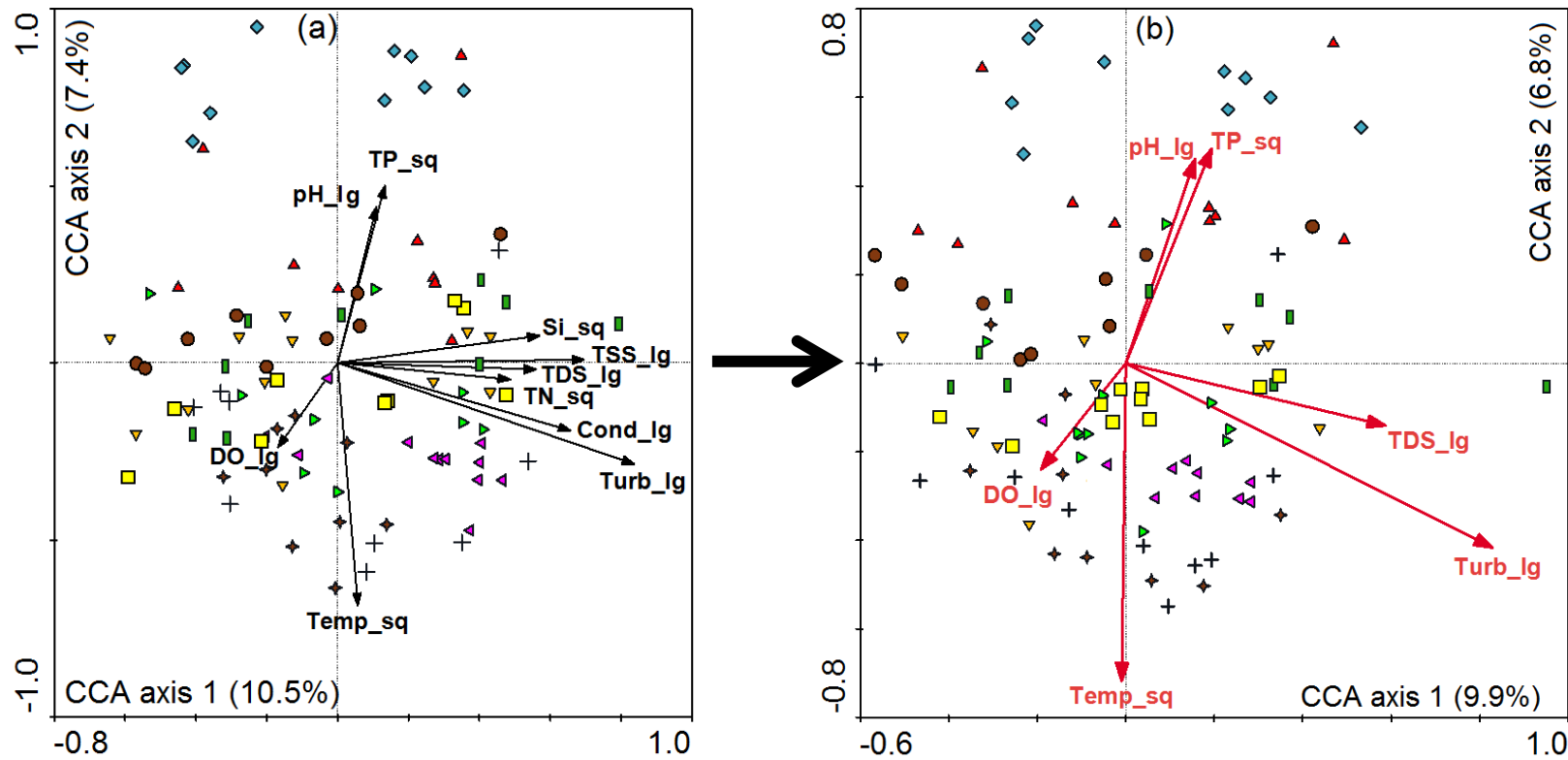


**Figure 5.39:** Cononical Correspondence Analysis (CCA) of diatoms under 55 ML/day along the MacKenzie River: **(a)** with all environment variables; **(b)** significant ( $p < 0.05$ ) variables after forward selection. Before high flows (brown circle), during high flows (blue diamond) and after high flows (green box)

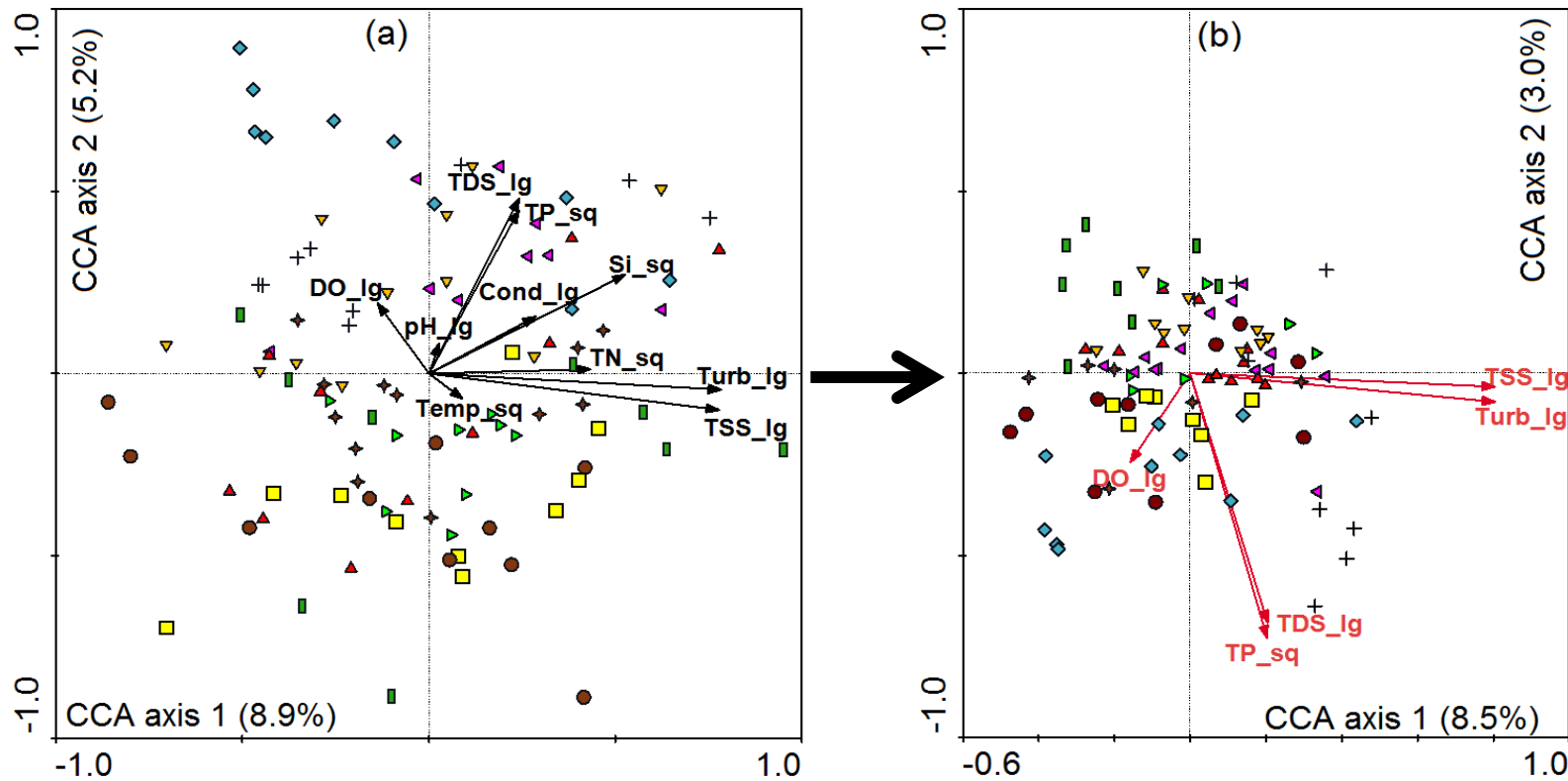


**Figure 5.40:** Cononical Correspondence Analysis (CCA) of soft algae under 55 ML/day along the MacKenzie River: **(a)** with all environment variables; **(b)** significant ( $p < 0.05$ ) variables after forward selection. Before high flows (brown circle), during high flows (blue diamond) and after high flows (green box)





**Figure 5.41:** Cononical Correspondence Analysis (CCA) of diatoms altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River: **(a)** with all environment variables; **(b)** significant ( $p < 0.05$ ) variables after forward selection.. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red up-triangle), before freshes (yellow square), during freshes (pink left-triangle), after freshes (right-triangle), before high flow (yellow down-triangle), during high flow (cross) and after high flow (star).



**Figure 5.42:** Cononical Correspondence Analysis (CCA) of soft algae altogether in different seasons under baseline flows and under different treatment flow regimes along the MacKenzie River: (a) with all environment variables; (b) significant ( $p < 0.05$ ) variables after forward selection. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red up-triangle), before freshes (yellow square), during freshes (pink left-triangle), after freshes (right-triangle), before high flow (yellow down-triangle), during high flow (cross) and after high flow (star)

**Table 5.13:** Comparison of CCA eigenvalues and cumulative percentage variance under different flow regimes

		Diatoms		Soft algae	
		Axis 1	Axis 2	Axis1	Axis 2
10-15 ML/d	Eigenvalues	0.134	0.115	0.068	0.052
	Cumulative % variance	19.8	17.0	12.2	9.2
35-40 ML/d	Eigenvalues	0.121	0.105	0.082	0.060
	Cumulative % variance	19.1	16.0	12.5	10.1
55 ML/d	Eigenvalues	0.114	0.095	0.075	0.061
	Cumulative % variance	14.2	11.9	13.1	8.7
altogether	Eigenvalues	0.109	0.092	0.075	0.063
	Cumulative % variance	10.5	7.4	8.9	5.2



**Table 5.14:** Comparison of CCA (after forward selection) in eigenvalues and cumulative percentage variance under different flow regimes

		Diatoms		Soft algae	
		Axis 1	Axis 2	Axis1	Axis 2
10-15 ML/d	Eigenvalues	0.113	0.104	0.062	0.049
	Cumulative % variance	18.8	16.2	9.4	7.2
35-40 ML/d	Eigenvalues	0.113	0.097	0.080	0.043
	Cumulative % variance	17.5	15.5	11.9	8.8
55 ML/d	Eigenvalues	0.084	0.082	0.062	0.049
	Cumulative % variance	11.3	10.6	11.9	7.6
altogether	Eigenvalues	0.076	0.053	0.046	0.029
	Cumulative % variance	9.9	6.8	8.5	3.0

## **Chapter 6: Discussion and synthesis of results**

### **Chapter outline**

In this chapter the interactions between the flow in the MacKenzie River and its water quality are discussed. These are used to describe and account for the response of the stream ecosystem focussing on algae as a principal indicator of stream condition. Observations of the relationships between the physical, chemical and biological characteristics of the MacKenzie River are then compared with observations in comparable systems to determine the degree to which the MacKenzie River responds in a similar fashion to other regulated streams. This evidence is then used to produce generalised observations of stream response to flow releases in order to generate guidelines for future water release operations in the MacKenzie River. The contributions of this study to a broader understanding of the nature and benefits of consumptive flow releases for environmental benefit are then discussed.

### **6.1 How does the MacKenzie River interaction under conditions of base flow (10-15 ML/day) in different seasons?**

Owing the seasonal nature of rainfall in the Grampians region the MacKenzie River is subject to extended periods of low flow. While this has always been so, the highly regulated nature of the system has likely extended these phases, and has impacted on the stream condition through these base flow conditions. In this section, the relationships

between flow, water quality and algal community structure and general ecosystem function are described and discussed under typical base flows (e.g. 10-15 ML/day).

#### **6.1.1 Physicochemical factors and nutrient flux under base flow**

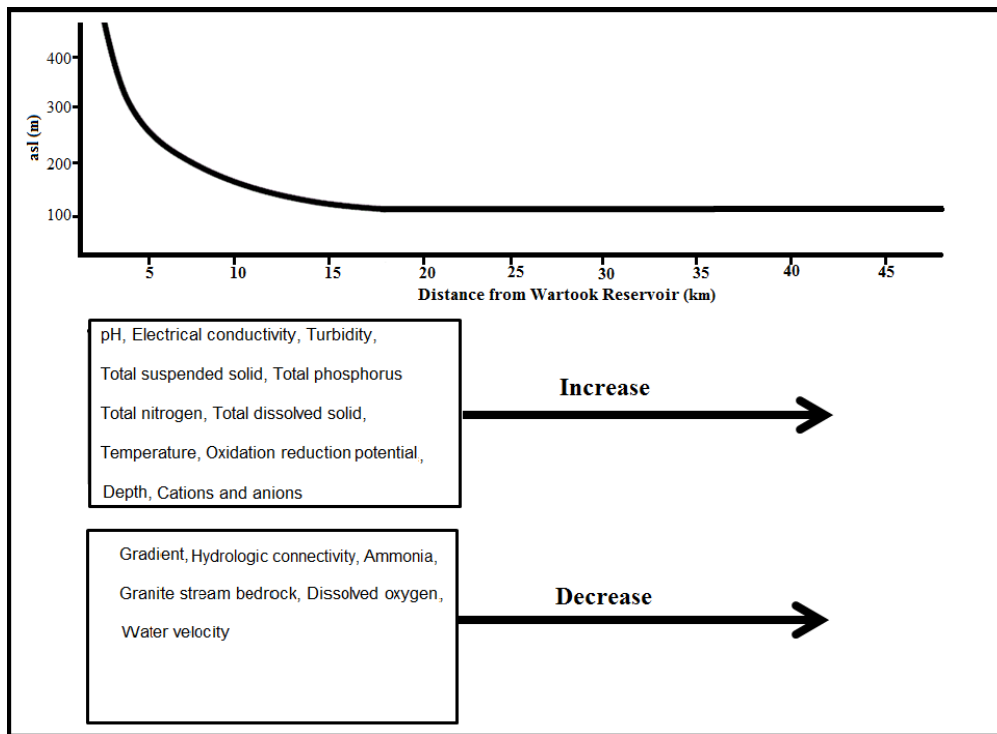
The pH of the MacKenzie River increases with distance downstream transitioning from slightly acid conditions upstream to alkaline in the lower reaches. The low pH conditions in the upper MacKenzie River coincide with lower turbidity and so greater light transparency which contributes to photosynthetic production by benthic algae. The pH can also impact on nutrient bioavailability and dynamics (uptake /release / transformations). For example, Wu et al. (2014) found the release of phosphorus decreased due to low pH in Lake Xuanwu (China). Therefore, it can be anticipated the release of phosphorus from sediment in the upstream of the MacKenzie River is likely lower due to low pH.

The temperature and dissolved oxygen of water in the MacKenzie River change spatially and temporally. The results of the present study also show that when temperature increases, the concentration of dissolved oxygen (DO) decreases which is consistent with the decreasing capacity of warmer water to hold oxygen (Hunt and Christiansen 2000, Allan and Castillo 2007). At the same time, electrical conductivity (EC) increases due to the greater dissolved mineral content entering the system. The temperature interacts with other water chemistry factors to affect the rate of photosynthesis and respiration down the MacKenzie River.

Turbidity, total suspended solids (TSS), cations and anions also increased with distance downstream along the MacKenzie River, particularly downstream of points of sediment input and water abstraction in reach 2 and 3, a result presented by the Victoria EPA (2003b) and corroborated here. The range of turbidity, TSS, cations and anions

were less in the MacKenzie River in comparison with the Wimmera and Glenelg Rivers (Anderson and Morison 1989, Chee et al. 2009, Alluvium 2013, VEWH 2015, WCMA 2015) as the MacKenzie River sits higher in the catchment.

The concentrations of nutrients increase with distance downstream due to agricultural activities and land use which contribute to increases in the concentration of nitrogen and phosphorus in the MacKenzie River. Land use from Zumsteins, and wastes from stock, which range across much of the catchment, are the likely source of nutrients in the middle and lower reaches of the river. Nutrients (nitrogen and phosphorus) and light are the main resources to promote algal productivity in the river. These nutrient changes have an influence on the condition of the MacKenzie River. The physical and chemical parameters (e.g. turbidity, temperature, TSS, dissolved organic matter) increase with distance from the Wartook Reservoir. Conversely, some other factors (e.g. gradient, mean particle size and dissolved oxygen) decline downstream (Figure 6.1)



**Figure 6.2:** The changes of the physical and chemical characteristics of the MacKenzie River.

The physical and chemical characteristics of the river are controllers for algal community structure and function. In fact biological structure, ecological processes and ecosystem function and metabolism can change due to flow patterns, water quality and climatic variability in Australian rivers and globally. The results presented here for the MacKenzie River results show some similarities and dissimilarities to the conclusions of Boulton et al. (2014) and the River Continuum Concept (Vannote et al. 1980). For example, the DO dramatically decreases in the middle of the river and increases slightly downstream (see Chapter 5; Figure 5.5d), the water velocity is stagnant in the middle of the river whilst the River Continuum Concept suggests that average velocity increases in downstream of rivers. These changes can appear naturally or as a consequence of anthropogenic modification of lotic systems due to water abstractions, diversions and

evaporations. As aforementioned the DO dramatically decreased in the middle reaches due to bacterial activity, high turbidity and standing water. However, the DO slightly increased downstream because of increasing flow velocity and photosynthesis of aquatic plants and algae. It is, therefore, reasonable to conclude that the community metabolism of the river declines, presumably due to imbalance between photosynthesis and respiration, in the middle and lower reaches (Table 6.1).

The results of this study were compared with the study of Anderson and Morison (1989) on the Wimmera River which revealed that most physicochemical parameters had increased in the catchment over the last two decades. Nevertheless, the concentration of measured dissolved ions did not exceed the limits defined in ANZECC and ARMCANZ (2000) for any reach. This study conforms with other published reports (e.g. Alluvium (2013)) that reveal the water quality and stream condition of the MacKenzie River are influenced by flow modifications (e.g. construction of Wartook Reservoir in 1887, human settlement in Zumsteins and lower reaches of the river, water diversion to the Mt Zero Channel, water storages and water plant treatment). In general, water quality varies in riverine ecosystems (spatially and temporally) and these changes can provide good evidence to assist in understanding the effects of human impacts and modifications (Heathwaite 2010). To summarise, the physical and chemical components of the environment interact with each other in the river. Flow regimes and physicochemical features are primary drivers which govern and maintain the biological patterns and structure of the river, and this is discussed further below.



**Table 6.1:** Water quality changes along the MacKenzie River under base flow based on water quality classification of ANZECC and ARMCANZ (2000)

Site	Condition
S1	Good
S2	Good
S3	Good
S4	Good
S5	Moderate
S6	Moderate
S7	Moderate
S8	Moderate
S9	Poor
S10	Poor

### 6.1.2 Algal response under base flow

Algae are abundant and widespread in their distribution and have a broad range of structural and functional attributes (Burns and Ryder 2001, Victoria EPA 2003a).

Furthermore, algal colonisation and structure are known to be highly responsive to shifts in water quality and flow variation (Ryder et al. 2006, Robson et al. 2008, Chester and Robson 2014). Here, biofilm assemblages and algal indicators such as chlorophyll-*a* have been used to understand the in-stream ecological and water quality response to flows in the MacKenzie River.

#### **6.1.2.1 Algal species composition and DSIAR under base flow**

The responses of algae to water chemistry have been long established (Stevenson 2014). For instance, Hustedt (1937) examined diatoms of Bali, Java and Sumatra in Indonesia and classified them into five groups based on their response to pH. Likewise, ter Braak and van Dame (1989) inferred the range of pH from diatoms in some European rivers. Winterbourn et al. (1992) reported acidic species (e.g. *Eunotia* sp.) as common and dominant taxa in some British streams. This has been observed widely in Australia as well (Philibert et al. 2006). The pH range provides thresholds for various diatom species and so shifts from a *T. flocculosa* dominated assemblage to samples dominated by circumneutral to alkaliphilous taxa (e.g. *C. cistula*) is expected. The results from the present study also showed similar evidence in term of acidophilous species in the lower pH waters in the upstream sections of the MacKenzie River under base flows (see Chapter 5).

Increased levels of turbidity and TSS will have adverse effects on primary productivity of benthic algae and metabolism of the fluvial community and ecosystem function due to their contribution as an inhibitor for light capturing by benthic algae. According to Watts et al. (2009b), a high diversity of algal periphyton species (biofilms) is an indicator of good river health. In other words, river health improves with increasing algal periphyton diversity in rivers, particularly epiphytic species (Guo et al. 2016a, Poikane et al. 2016). For the MacKenzie River, ecosystem health was examined by relating algal species composition to flow regime. The base flow results revealed middle and downstream reaches have low algal diversity despite a high incidence of algal blooms. Watts et al. (2009b) found similar evidence in the Mitta Mitta River for increasing incidence of algal blooms downstream under constant base flows. Flows

monitored as part of this study seemed to be insufficient to prevent or reduce algal blooming (mostly of green algae and cyanobacteria) in the lower parts of the river.

The PCA results (Chapter 5; Figure 5.29; Table 5.9) showed the upstream species are more associated with the concentration of dissolved oxygen in comparison with downstream species under base flow. Furthermore, the CCA results showed that temperature has an influence on the diatom community whilst the DO is less important for soft algae.

According to Bond et al. (2012), one of the main requirements for river health is the stream's ability to retain its biodiversity and ecological integrity. The current results reveal the upstream reaches of the MacKenzie River to have a higher diversity of diatoms and fewer blooms of filamentous green algae and cyanobacteria, and so can be considered to be in good condition.

The DSIAR results show the upstream reaches have the highest scores (least impacted), with DSIAR scores decreasing downstream. The calculated DSIAR shows more variation in the downstream reaches (Reaches 2 and 3) compared to upstream (Reach 1). Therefore, it is reasonable to conclude that stream condition declines along the river due to the ephemeral nature of the river and human modification. Indeed, during dry seasons, the health and condition of the river shifts from moderate to poor in the lowermost parts of the river. Overall, the middle and downstream reaches are under stress because of the low discharge and poorer quality of the water under base flow. The river does not retain its biodiversity under low flow whilst it does recover it under freshes.

#### **6.1.2.2 Biological properties and river productivity under base flow**

The CCA results show that certain environmental variables (e.g. turbidity, low concentration of nutrients) have impacts on algal biomass and primary production in the lower parts of the MacKenzie River. Therefore, these phenomena have impacts on food webs and higher trophic levels. The indicators of river health -primary productivity (algal biomass) and species composition (key species assemblages) can be controlled by environmental variables. The PCA results show the upstream species are correlated with low pH, low temperature and high DO, while the downstream species are correlated with high turbidity, TSS, EC, TN and TDS. Furthermore the results show that TP, temperature and DO correlate negatively with turbidity, TSS and conductivity. Overall the results show flow regimes, water quality and other environmental factors can affect algal biomass (measured as dry mass, AFDM, chl-*a*) and species composition along the river under different seasonal conditions (spatial and temporal).

#### **6.1.2.3 Stream ecosystem metabolism under base flow**

The pH has not only a substantial correlation with algal community structure but also has a broader influence on metabolism and productivity of the riverine community. Kuwabara (1992), for example, found that the metabolism of the fluvial community can change as pH varies across the catchment. Generally stream ecosystem metabolism (processes of synthesis and decomposition of any organic materials and production (autochthonous and allochthonous)) can be measured by the concentration of oxygen in a river (Odum 1956, Grimm and Fisher 1984, Uehlinger and Naegeli 1998, Allan and Castillo 2007, Grace et al. 2015). It has been reported that increasing turbidity of water inhibits the growth of benthic algae as the energy source for photosynthesis declines for benthic primary producers (e.g. benthic algae). Comparing the results of the present

study with others, it is reasonable to conclude that pH affects algal community structure which changes naturally and as a consequence of anthropogenic inputs or activities along the MacKenzie River. While the use of metabolism chambers is a more direct method for measurement of stream community metabolism, the measurement of benthic community metabolism is an important approach for the monitoring of river health (Bunn et al. 1999, Bunn and Davies 2000, Fellows et al. 2006) and indirect inference using DO appears sufficient here to demonstrate these effects.

#### **6.1.2.4 Food webs under base flow**

The current results indicate the diatom relative abundance is high in upstream reaches while green algae and cyanobacteria are more abundant downstream (see Chapter 5). It has been previously reported that epiphytic diatoms and cryptophytes are favoured food sources for stream invertebrates because freshwater algae have a substantial concentration of polyunsaturated fatty acids (Torres-Ruiz et al. 2007, Guo et al. 2016a, Guo et al. 2016b). The role of benthic algae is pivotal in both lotic and lentic systems because they are a major component of stream food webs (Stevenson et al. 1996, Burns and Ryder 2001). In the MacKenzie River, algae synthesize organic matter (carbon) so that the latter enters the food web, eventually to reach high trophic consumers (e.g. fish and platypus). Thus, it is reasonable to conclude that the algal community present upstream is healthier for consumers than the cyanobacterial community downstream. In fact, most of the carbon synthesized by cyanobacteria is not transferable to higher trophic levels as they are not a food source of sufficient quality for second order (e.g. macroinvertebrate) and ultimately higher trophic levels (e.g. fish, platypus and waterbirds) of stream food webs.

### 6.1.3 Key findings on MacKenzie River under base flow

The base flow regime for the MacKenzie River is 10-15 ML/day; occasionally the flow is increased to 35-40ML/day every two or three months for three days to meet environmental and consumptive flow requirements. The results show there were trends along the ten sampling sites during the different flow regimes. The physico- chemical analyses revealed longitudinal differences downstream which reflect a decline in stream condition, with lower oxygen levels and elevated nutrients, salinity and pH. While this can be expected under the River Continuum Concept it is clear that, under low flow, the water quality of the lower MacKenzie River is relatively poor. This condition improves after water releases whereby the full length of the River improves, however this result is temporary with the lower reaches returning to before-release conditions within a few days.

- Water quality declines from upstream to downstream in the MacKenzie River, particularly in summer and autumn;
- Algal species composition and relative abundance differs along the river such that diatoms are abundant upstream while green algae and cyanobacteria are abundant in the middle and lower reaches;
- DSIAR scores are high upstream which indicates good stream condition, but are low downstream, indicating poor condition;
- Algal blooms (*Cladophora* and *Chara*) occur in surface waters downstream which prevents penetration of light to the benthos;
- Although the rate of primary productivity is high downstream because of algal blooms, most synthesised carbon is unsuited to transfer to higher trophic consumers. Therefore bacterial activity increases to decompose the algal blooms which require

more oxygen from water column. Presumably the rate of respiration is high in the lower reaches of the river;

- Benthic metabolism changes along the river course such that downstream food webs are less effective and/or efficient than those upstream under conditions of base flow.

## **6.2 How does the MacKenzie River function under manipulated flow regimes?**

In this section hydrologic connectivity, physicochemical factors, nutrients dynamics, algal biomass, algal community assemblage, food webs and ecosystem metabolism within the MacKenzie River are discussed in the context of flow freshes and pulses and similar flow manipulations up to 55-60 ML/d.

### **6.2.1 Physicochemical factors and nutrients flux under freshes and high flows**

The results revealed that the water quality of the MacKenzie River has varied due to the regulation of flows for consumptive users at different points along the River. Results from the Pearson's correlation matrix (Chapter 5; Table 5.8) revealed that most water quality characteristics responded significantly to freshes and high flows. Furthermore, it seems that Wartook Reservoir has a substantial impact on most physical and chemical characteristics of the River. This can be concluded by comparing the results from base flow monitoring with those from higher flow water release events.

The results of this study under water release events (freshes and high flows) showed that the pH of the upstream and downstream reaches became similar. In other words, water release events likely bring acidic water from Wartook Reservoir to the lower parts of the MacKenzie River, reducing the alkalinity of that reach. The pH can determine solubility and availability of nutrients (e.g. N and P) and other chemical elements (e.g. heavy metals) in rivers and streams. The concentration of dissolved oxygen (DO) changes substantially along the river, particularly in the midstream where the DO increased greatly in response to water release events. However, the phenomenon returns to antecedent conditions after the passing of these events. It seems Wartook Reservoir upstream of the MacKenzie River has substantially, adversely impacted on the concentration of dissolved oxygen and temperature of the river. For instance the



water temperature decreased approximately 2°C (See Chapter 5; Table 5.2) in the lower part of the river due to the water release from Wartook Reservoir into the river. Watts et al. (2009b), found similar evidence for declining oxygen concentration at Dartmouth dam and the lower reaches of the Mitta Mitta River. It has been reported the respiration will increase with increasing temperature (Stevenson et al. 1996). The concentration of the dissolved oxygen in the MacKenzie River indicates the primary productivity of upstream is higher than in the midstream and downstream reaches. This phenomenon occurs because of natural features and human modifications along the river. In other words, the exchange of the oxygen in the atmosphere is a result of the mixing of the water which increases the concentration of oxygen due to the higher slope in the upstream reaches (see Chapter 3; Section 3.4.1). Conversely water flows more slowly due to the lower slope downstream (see Chapter 3; Section 3.4.3). The metabolism of the river is influenced by the cold water and oxygen release from Wartook Reservoir.

The transport of sediment from upstream and midstream to downstream, and the deposition of those sediments into downstream reaches, resulted in elevated turbidity and TSS in the downstream sections of the MacKenzie River. Increasing turbidity has been reported in most Australian rivers particularly in dry land rivers (Hamilton et al. 2005, Bunn et al. 2006a, Bunn et al. 2006b, Ward et al. 2013). By comparing the turbidity and pH it is reasonable to conclude that the low pH (acidic) correlates with the level of turbidity in rivers. This phenomenon was observed in the upstream of the MacKenzie River where it has low pH and low turbidity.

Nutrients (P, N, Si) are a main source of chemical energy for both autotrophic (e.g. cyanobacteria) and heterotrophic (bacteria) microbes in rivers and streams (Allan and Castillo 2007). Flow alteration and human activities (e.g. agriculture) profoundly influenced nutrient dynamics along the MacKenzie River. The nutrients enter the

MacKenzie River as dissolved materials from the atmosphere, lithosphere and hydrosphere. The nutrients also enter the MacKenzie River in organic form via biological assimilation (nitrogen assimilation by cyanobacteria). The contribution of the dissolved inorganic and organic nutrients can be more than that of organic materials under water release events, due to greater sediment input into the water. The statistical analyses showed that total nitrogen (TN) and total phosphorus (TP) were high under water release events.

To conclude, water quality is one of the main indicators of good ecological status in rivers. The ecological monitoring in the Wimmera River showed that the flow regime and water quality are critical characteristics that affect river health, particularly in the lower reaches (Anderson and Morison 1989, Westbury et al. 2007, Alluvium 2013). Therefore improving water quality in the MacKenzie River not only brings benefits for the MacKenzie River, but also brings benefits to the Wimmera River simultaneously (Table 6.2)

**Table 6.2:** Water quality changes along the MacKenzie River under freshes and high flows based on water quality classification of ANZECC and ARMCANZ (2000).

Site	Condition
S1	Good
S2	Good
S3	Good
S4	Good
S5	Good
S6	Good
S7	Good
S8	Good
S9	Moderate
S10	Moderate

### **6.2.2 Algal response under manipulated flow regimes**

In the following sections, the algal response in terms of algal species composition, algal productivity, their contribution to food webs and river metabolism under different flow regimes are discussed.

#### **6.2.2.1 Algal species composition and DSIAR under manipulated flows**

The results from algal monitoring surveys revealed the key indicator taxa (indicators of river health) increased under freshes and high flows. Indeed, the relative abundance of diatoms increased during and after freshes and high flows, especially epiphytic diatoms such as *T. flocculosa*, *G. affine*, *N. radiosa*, and *E. minutum* (epiphytic diatoms) (see Chapter 5). Biggs and Hickey (1994) and Ryder et al. (2006) found diatoms to increase while soft algae decreased under water release events. In contrast, Davie and Mitrovic (2014) found diatom abundance to decline while filamentous green algae and cyanobacteria increased downstream with high water releases in the Severn River (NSW). The CA and DCA results showed that algal community assemblages were different during high flow events compared to low flow events (see Chapter 5). The results showed that downstream species were more closely associated with turbidity, TSS and conductivity. The CCA (Chapter 5: Figures 5.41-43) results indicated that turbidity is a significant factor affecting both soft algae and diatoms. Biggs and Hickey (1994) found physiognomy of algal periphyton changed under different hydraulic gradients in the Ohau River, South Island, New Zealand. These authors also found the diatoms most abundant in the river were *Cymbella kappii*, *Synedra ulna* and *Gomphoneis herculeana*.

The results also showed cyanobacteria have more tolerance and resilience than green algae under flow discharges along the MacKenzie River. Blenkinsopp and Lock (1994) also found similar evidence in the Glywedog River (North Wales, UK). In other words, the cyanobacteria are more resilient under water releases, similar with findings of this study (see Chapter 5).

It has been documented that flow regulation has a detrimental impact on riverine biotic communities and structure (Bunn and Arthington 2002, Allan and Castillo 2007, Boulton et al. 2014). Of particular relevance to the current findings, Robinson et al. (2003) found macro-invertebrate community changes with distance downstream on the Spol River (Switzerland). These authors reported that benthic macro-invertebrate assemblages changed from Grammaridae and Turbellaria to the more tolerant Simuliidae and Chironomidea. Therefore, it can be concluded that biological structure can change under long term flow regime modification (e.g. as a consequence of dams or reservoirs).

In the present study the algal species composition, and DSIAR, showed the lower reaches of the river to be in poor condition under low flows, but this condition improved under flows of 35 ML/day, as indicated by the reduction in green algae and cyanobacteria and an improvement in DSIAR scores.

#### **6.2.2.2 Algal biomass and productivity under manipulated flows**

It was found that the pulsed flows decreased the occurrence of algal blooming and the algal biomass in the river as higher flows were able to scour and flush the river of green algae and cyanobacteria. Bourassa and Cattaneo (1998) and Watts et al. (2009b) reported similar results in terms of decreasing algal biomass during water release events.

In the present study, algal biomass is used as a surrogate of gross primary productivity, and it was found that levels increased as a consequence of flow alteration and higher concentration of nutrients. However, the accumulation of biomass decreased during and after high flows. Observations showed that dry mass was greatest at downstream monitoring sites. The accumulation of AFDM (ash-free dry mass) also increased from upstream to downstream under manipulated flows. The concentration of chlorophyll-*a* decreased under high flows along the river. This study were consistent with others which also reported that algal biomass decreased during and immediately subsequent to high flows (Horner et al. 1990, Biggs and Gerbeaux 1993, Jowett and Biggs 1997, Ryder et al. 2006, Watts et al. 2006, Flinders and Hart 2009, Davie and Mitrovic 2014). For example, it has been reported that low flow discharges in the Mitta Mitta River were insufficient to be effective in algal scouring along the river (Watts et al. 2006).

Some scientists have reported that sub-scouring flows can enhance the algal biomass in riverine ecosystems (Grimm and Fisher 1989, Stevenson et al. 1996) and so relationships between flow rates, velocity and thresholds required for scouring are important to understand algae responses under different flow regimes. The similarities and dissimilarities of the algal response in the present study with other published materials resummarised in (Table 6.5).

**Table 6.3:** The response of algal biological properties under pulsed flow regimes

Author (year)	Biological properties	Change	Algal group	Locality
Horner et al. (1990)	Chlorophyll-a	Decrease	Diatoms and Cyanobacteria	USA
Biggs and Gerbeaux (1993)	Chlorophyll-a	Decrease	Diatoms and Green algae	New Zealand
Biggs and Stokseth (1996)	AFDM	Increase	Diatom and filamentous green algae	New Zealand
Jowett and Biggs (1997)	Biomass	Decrease	Diatoms	New Zealand
Biggs et al. (1998)	AFDM,	Increase	Diatom and filamentous green algae	New Zealand
	Chlorophyll-a	Decrease		
Bourassa and Cattaneo (1998)	Chlorophyll-a	Decrease	Algal periphyton	Quebec, Canada
Townsend and Padovan (2005)	Biomass	Increase	Cyanobacteria	NT, Australia
Ryder et al. (2006)	Dry mass,	Decrease	Diatom and filamentous green algae	NSW, Australia
	AFDM,	Decrease		
	Chlorophyll-a,	Decrease		
	Species composition	Increase		
Watts et al. (2006)	Biomass	Decrease	Cyanobacteria and Green algae	NSW, Australia
Watts et al. (2009b)	Biomass	Decrease	Biofilms	NSW, Australia
Davie and Mitrovic (2014)	Chlorophyll-a and taxonomic composition	Decrease	Diatoms, Cyanobacteria and Green algae	NSW, Australia
This study	Dry mass,	Decrease	Diatoms, Cyanobacteria, Green algae and Chrysophytes	Vic, Australia
	AFDM,	Increase		
	Chlorophyll-a	Decrease		
	Species composition	Increase		

### **6.2.2.3 Stream ecosystem food webs and metabolism under manipulated flows**

Algae are an important component of the aquatic food web (Bunn et al. 1999, Bunn et al. 2006a, Davies et al. 2008) with microalgae being the main food source for many aquatic fauna (e.g. mayfly and snail) in freshwater ecosystems. Indeed, algae play an important role in producing and synthesising organic matter (carbon) so that carbon can enter the food web and be available for higher order trophic consumers (e.g. fish and waterbirds) (Bunn et al. 2006a, Guo et al. 2016a). This makes algae an essential part of the food web and biogeochemical cycling in freshwater ecosystems (Stevenson 2014).

As mentioned in Section 6.1.2.4, under the base flow synthesised carbon likely was not effectively transferred to higher trophic levels due to the low quality of food. In contrast, however, results from the pulsed flow experiments showed that the algal taxonomic composition of the population shifted from green algae dominated community to Bacillariophyceae in the downstream reaches of the MacKenzie River. This likely increased the quality of food for consumers.

The ordination results in this study showed that some environmental variables (e.g. turbidity, concentration of nutrients) had impact on algal biomass and primary production in the lower parts of the MacKenzie River. It can be concluded that high turbidity has adverse impacts on food webs and high trophic levels due to its impact on light availability.

### **6.2.3 Key findings under manipulated flow regimes**

Algal species composition changed along the river under different flow regimes and different seasons. Under base flow, diatoms were more abundant upstream and filamentous green algae were more abundant downstream. The results showed that the algal composition shifted downstream after water release events. Green algae,

cyanobacteria and Chrysophyta gradually increased from upstream to downstream under base flow conditions, and before water releases, whereas diatoms were greater upstream and increased downstream after water releases. However, cyanobacteria and Chrysophyta have the highest percentages in some sites in the mid-stream (Reach 2). Furthermore, the results showed that cyanobacteria were more resistant and tolerant under water release events. Conversely, the tolerance and resilience of green algae were low under water release events. The observation showed that blooms of filamentous green algae and cyanobacteria were reduced by the water release in downstream reaches. High diversity within the algal community along the river indicates a healthy river with good ecological status. These patterns are also reflected by algal diversity, which declined downstream, also indicative of declining stream health. After water release events numbers of cyanobacteria and filamentous green algae decline and these were replaced by mostly diatoms. Again, the pre-flow assemblages gradually returned when base flows returned. Similarly, DSIAR scores revealed a pre-release pattern of a gradient from good quality water to moderate and poor conditions in the lower reaches. These conditions too changed after flow releases with scores reflecting consistently good status throughout. DSIAR scores remained high after releases events ceased but they gradually declined after several days in the lower sections of MacKenzie River. The biological properties of the algal periphyton communities varied between sites under different flow regimes. The accumulation of dry mass (not ash-free) decreased downstream during freshes. However, the accumulation of AFDM (ash-free dry mass) gradually increased from upstream to downstream. The results showed that the concentration of chlorophyll-*a* decreased from upstream to downstream under water release events.



The Pearson's correlation matrix of the hydrological characteristics and biological properties was developed under the four different flow scenarios. The results revealed significant relationships ( $p < 0.05$ ) between flow regime and the water quality measures of pH, conductivity, total nitrogen, turbidity, phosphorus, total nitrogen, and the biological measures of chlorophyll-*a*, dry mass, ash-free dry mass and DSIAR. The relationships between the environmental variables were also examined revealing that pH and turbidity had significant ( $p < 0.05$ ) relationships with most other water quality and biological measures.

The PCA plot revealed patterns in ecological datasets and showed relationships between the environmental variables and algal species in the dataset. The PCA indicated the upstream species were associated with low pH and temperature and higher DO. In contrast downstream species were associated with higher turbidity, TSS, conductivity, TN, and TDS. Furthermore, the results revealed TP and temperature and DO correlated negatively with turbidity, TSS and conductivity.

Exploration of the diatom and soft algae assemblage data using the computationally simple, unconstrained ordination technique of CA indicated that there were two strong gradients in the data sets. In the diatom data there was a clear split between the assemblages observed during water release events and those observed at other flows. This split in the diatom assemblage data is not reflected in the soft algae data and there is far more scatter in the data. The DCA was used when gradients length  $> 2$  and to eliminate the arch effects for appropriate interpretation. The results of the CA and DCA of the diatom data were also similar whilst the results of the CA and DCA were not similar in some data visualisation plots due to arch effect observed in CA plots where DCA applied to remove the arch effect.

Finally, constrained ordination (CCA) was used to determine the direct relationships between diatom and soft algal communities and the water chemistry (environmental variable) data under different flows. A subset of significant environmental variables for both the diatom and soft algal data was determined using manual forward selection. To identify the most important of the closely related variables the number of explanatory variables was reduced using a Bonferroni-adjustment applied to the forward-selection process, where the  $p$ -value is divided by the number of variables included. For the diatom and soft algae data five environmental variables were found to have significant influence (e.g. pH, TSS, Turbidity, TN and TP).

- Water quality (e.g. dissolved oxygen) improved along the MacKenzie River under freshes and high flows;
- Algal species composition shifted from green algae-dominated, to a mix of diatoms, green algae and cyanobacteria in downstream reaches of the MacKenzie River;
- The stream condition improved under flows of 35 ML/day (optimal flow for the MacKenzie River) and high flows (55 ML/day), as indicated by the reduction in green algae and cyanobacteria and an improvement in DSIAR scores;
- Pulsed flows created suitable conditions for algal assemblage changes, then likely higher quality and more suitable synthesised carbons were transferred to higher trophic consumers;
- Depending on water availability, environmental watering plans often seek to release water along the MacKenzie River in order to improve water quality, stream condition and river health, especially for the downstream reaches. Therefore, this work demonstrates that elevated flows improve the condition of the river, and so there is good prospect for improving the condition by manipulating the nature of releases.

### **6.3 Modelling**

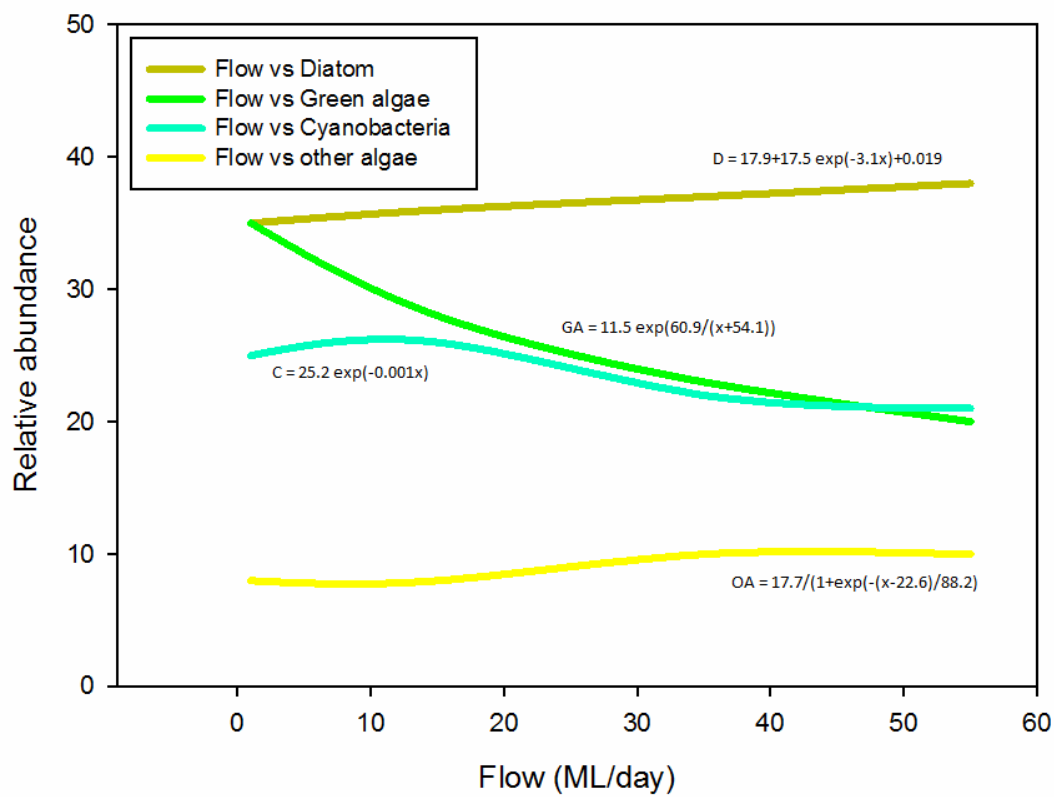
Regression models were developed to describe the relationships between the relative abundance of cyanobacteria, green algae, and diatoms and different flow regimes along the MacKenzie River. The mean values and standard deviation for each parameter were calculated and used to produce a regression model. Regression models are used to simplify complicated datasets to make them more understandable and manageable (Mac Nally 2000, 2002). In addition, Klaar et al. (2014) found that regression models have a set of capabilities suited to the application of ecological monitoring to the development of hydroecological models for environmental flow standards. In the present study, the models were also used to examine the response of algae within a wide hydrological gradient at three reaches along the MacKenzie River.

#### **6.3.1 Algal response models in Reach-1**

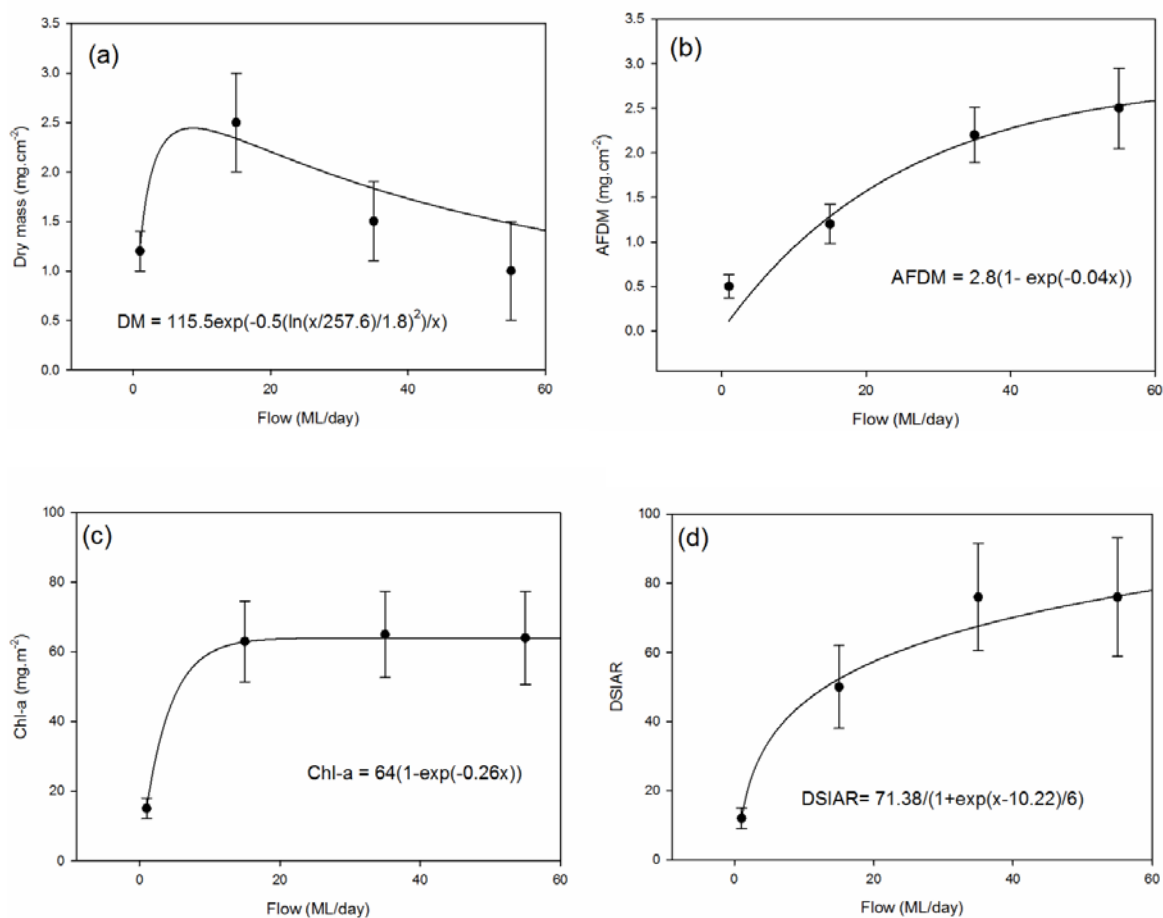
The algae-based models have been developed as a tool to assist in the future configuration of flows in the river. Algal response models, in different hydraulic gradients, can be useful in water management efforts to find sustainable solutions for a healthy working river. Figure 6.2 shows that the relative abundance of diatoms increased gradually and consistently with increasing flow in Reach 1, while green algae and cyanobacteria decreased under freshes and high flows. The models also highlighted that dry mass decreased in this reach after water release events (Figure 6.3a).

Furthermore, the models predict the relative abundance of algal communities (diatom, green algae and cyanobacteria) under cease to flow, low flows, freshes, high flows as well as between two different flows (e.g. between low flows and freshes). The relationships between flow and biological properties (dry mass, AFDM and chl-*a*) were measured and then empirical data applied to develop the regression models (Figure

6.3b-c). The models infer the fluctuations of the biological properties. Moreover, there was a strong positive relationship between DSIAR and flow (Figure 6.3d), particularly in response to pulsed or high flow water release events, confirming that stream conditions changed during and after high flow water release events.



**Figure 6.3:** Algal response (measured as relative abundance) to flow regimes in Reach 1 at the MacKenzie River: D= diatom, GA= green algae, C= cyanobacteria, OA= other algae.

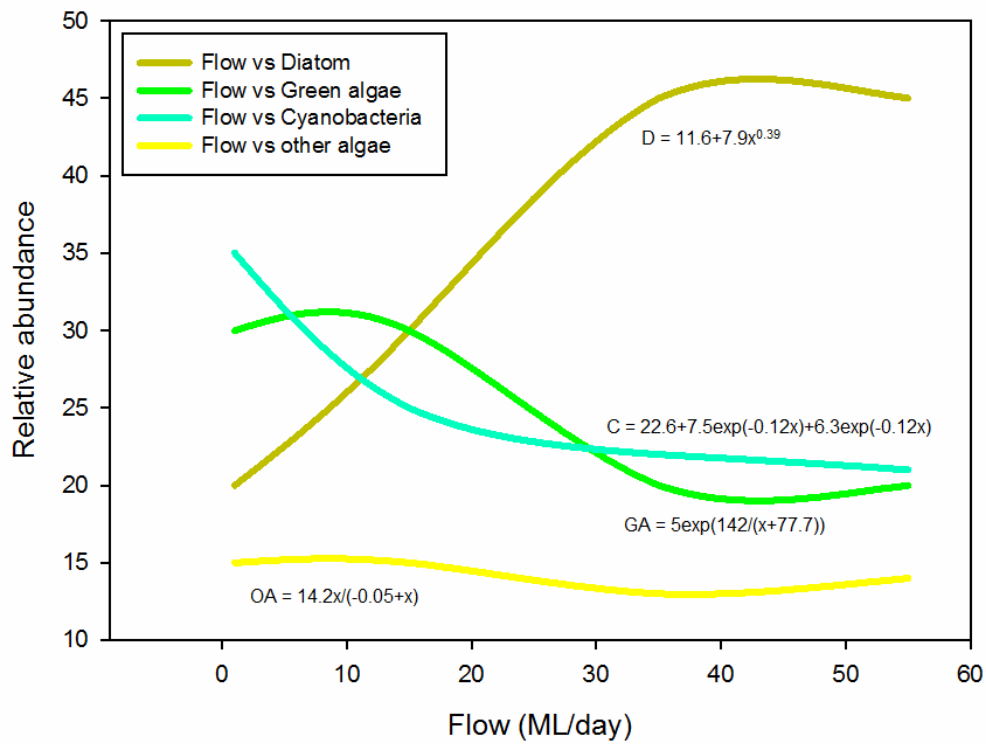


**Figure 6.4:** Response of algae indicators to flow in Reach-1 of the MacKenzie River: **(a)** relationship between dry mass and flow; **(b)** relationship between AFDM and flow; **(c)** relationship between chlorophyll-*a* and flow; **(d)** relationship between DSIAR and flow. Data indicate means  $\pm$  SD

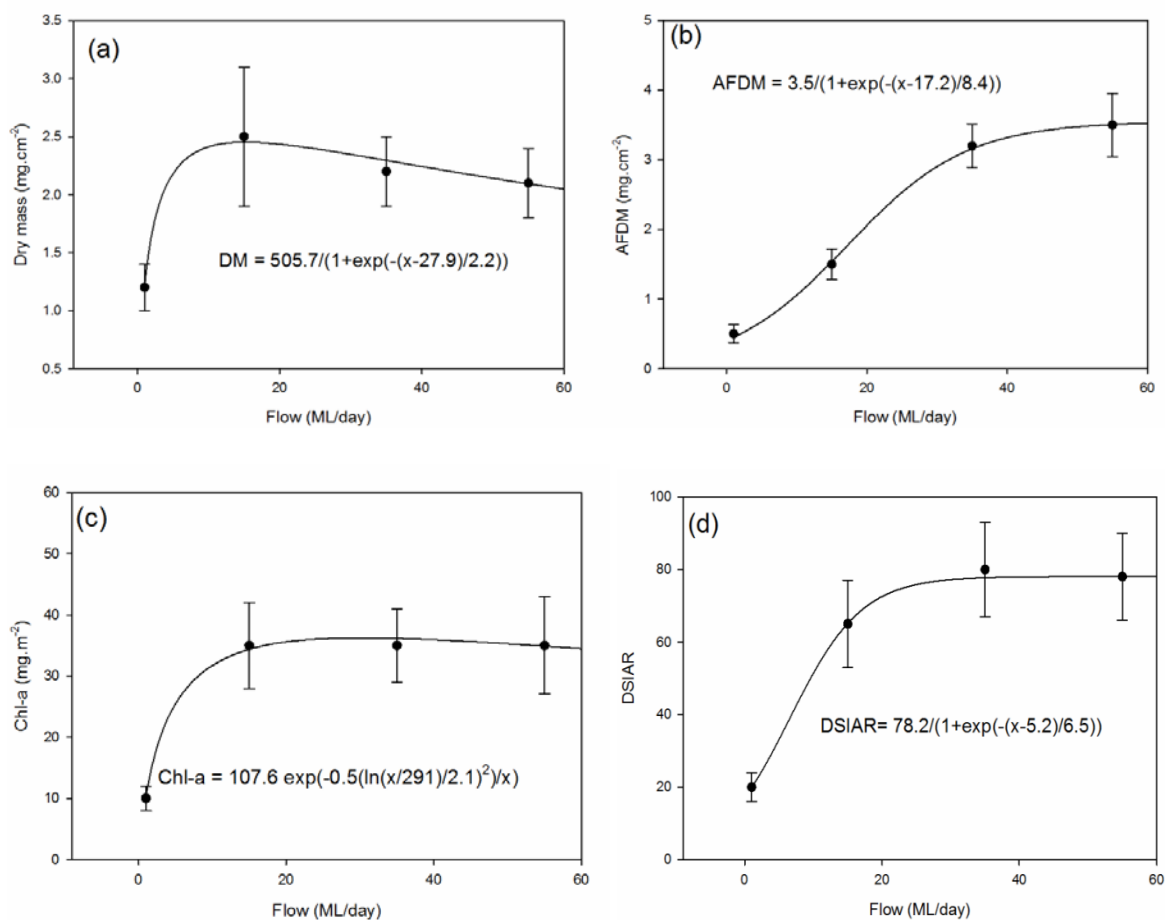
### 6.3.2 Algal response models in Reach-2

Analysis shows that flow regime has a substantial influence on algal community structure and biomass in the mid-stream (Reach 2) sections of the MacKenzie River where soft algae decreased while diatoms increased in relative abundance after releases (Figure 6.4). In addition, the results indicated that stream condition (as measured by DSIAR) can be improved by freshes and high flows. Cuffney et al. (2011) developed multi regression models based on algae and invertebrate response to human modification (e.g. agricultural developments) in rivers of the USA. The authors showed algae and invertebrate communities change due to the degradation of riverine ecosystems.

The data showed that dry mass decreases while AFDM increases after water release events (Figure 6.5a-b). The concentration of chlorophyll-*a* slightly decreases while DSIAR scores increase strongly, then reach a plateau at 35 ML/day (Figure 6.5 c-d). The models indicate that Reach 2 is more readily influenced by flow regime. Therefore it is in a less stable ecological state and is more easily perturbed with varying flow regimes due to the higher sensitivity of this reach to flow. The models predict the response of algae under different flow regimes can infer responses even to bankfull and overbank flows. Mac Nally (2000) illustrated that regression modelling is a useful and reliable approach in ecology and conservation biology. The models showed that 35 ML/d is a threshold flow level in the response of algal biodiversity, DSIAR score and the quality of food source for second order consumers in the food web. Therefore, this study showed 35 ML/d is an optimal flow above which only marginal benefits accrue for the MacKenzie River.



**Figure 6.5:** Algal response to flow in Reach-2 of the MacKenzie River: D= diatom, GA= green algae, C= cyanobacteria, OA= other algae.



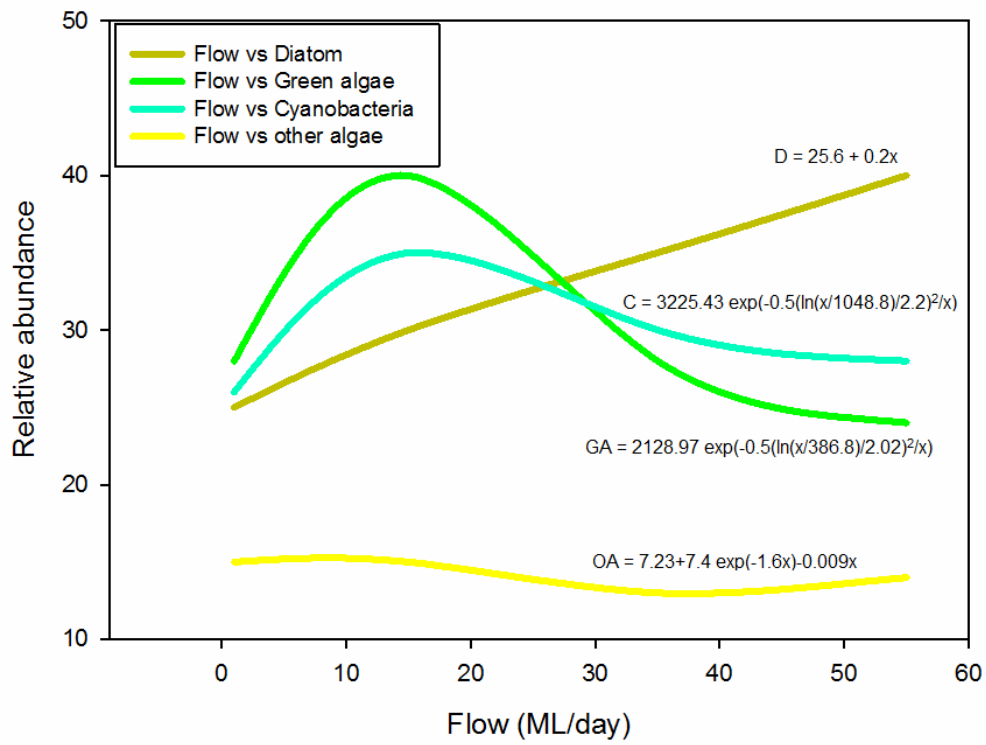
**Figure 6.6:** Response of algae indicators to flow for Reach-2 of the MacKenzie River:

(a) relationship between dry mass and flow regimes; (b) relationship between AFDM and flow regimes; (c) relationship between chlorophyll-*a* and flow regimes; (d) relationship between DSIAR and flow regimes. Data indicate means  $\pm$  SD.

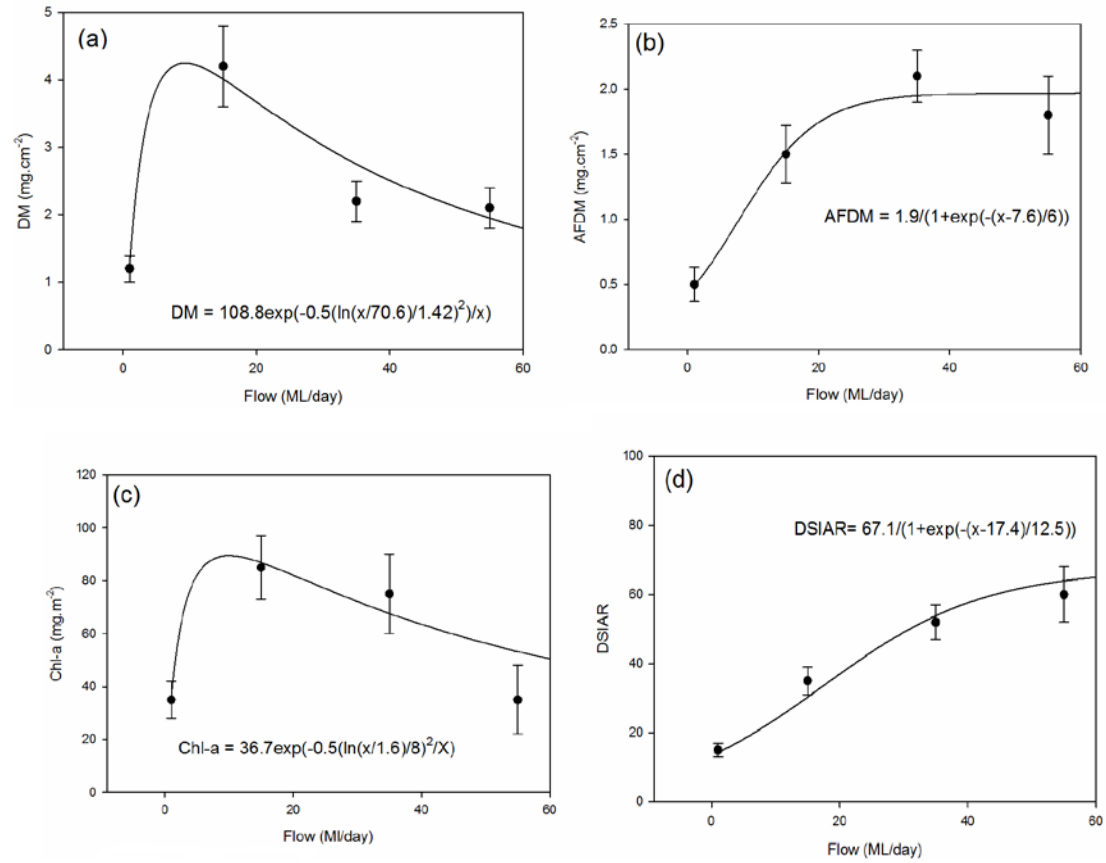


### 6.3.3 Algal response models in Reach-3

The lower part of the river (Reach 3) has shown the potential to change greatly over time, as evidenced during the persistent drought conditions between 1998 and 2009 (GWMWater pers. comm. 2012). Observations showed that high flows and freshes had a significant impact on the lower sections of the MacKenzie River in Reach 3 where green algae and cyanobacteria decreased while diatoms increase in relative abundance (Figure 6.6). In addition, the models showed that stream condition and water quality can be improved by freshes and high flows. In Reach 3 the accumulation of dry mass, AFDM and Chlorophyll-*a* concentration declined under higher flows of 15 ML/day or more (Figure 6.7a-c). DSIAR consistently improved with higher flows (Figure 6.7d) reflecting the increased abundance of sensitive diatom flora. This in turn indicated relatively good condition of the river throughout its length. There is generally a stronger positive response to flows indicating that Reach 3 is influenced by flow regime. Therefore it is in a less stable ecological state and, while more vulnerable to low flows, can readily reap benefits from enhanced flow regimes. The models showed that 35 ML/d is threshold to improve algal biodiversity, DSIAR score and food sources. Hence, 35 ML/d is optimal flow brings for the lower reach of the MacKenzie River above which there is only marginal benefit.



**Figure 6.7:** Algal response to flow in Reach-3 at MacKenzie River: D= diatom, GA= green algae, C= cyanobacteria, OA= other algae.



**Figure 6.8:** Response of algae indicators to flow in Reach-3 at MacKenzie River: **(a)** relationship between dry mass and flow regimes; **(b)** relationship between AFDM and flow regimes; **(c)** relationship between chlorophyll-*a* and flow regimes; **(d)** relationship between DSIAR and flow regimes. Data indicate means  $\pm$  SD.

## 6.4 Application of the models

The utilisation of ecological modelling has provided a powerful tool for the evaluation of the impact that catchment disturbance and hydrological changes have had on riverine ecosystems. According to Stevenson (2014), there are four phases for an evaluation of the interaction of natural resources and human activities including designing the ecological assessment, characterising the condition of the stream, diagnosing causes and threats to its condition, and comparing management options to select logical and rational options. The ecological models have great potential to monitor these impacts in riverine ecosystems (see chapter 2; section 2.9). In the present study, the ecological models (based on the functional relationships between stream hydrology, water quality, species composition, algal biomass, chlorophyll-*a* concentration and ecosystem function) were developed as tools to assist in the future configuration of flows for the MacKenzie River. The models showed the stream condition improved as indicated by the reduction in green algae and cyanobacteria, an improvement in water quality, increase in algal biodiversity and in the performance of food webs and metabolism.

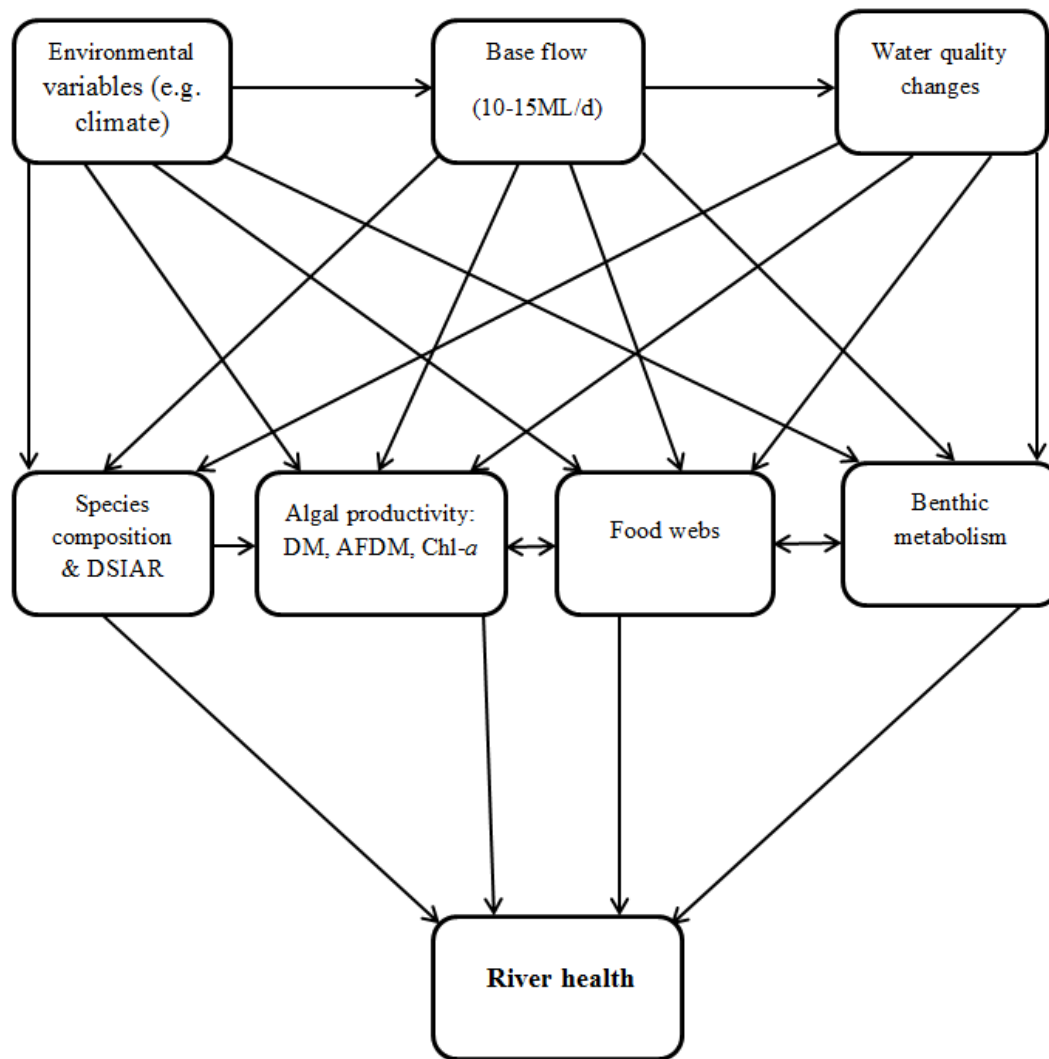
The use of flow releases within the MacKenzie River provides an opportunity to measure and assess responses to inform manipulations of the nature of releases, allowing for fine-tuning, over short periods, under an adaptive approach. The results revealed the upstream reaches of the MacKenzie River to have higher diversity of diatoms, higher DSIAR scores, less filamentous algae, and so can be considered to be in relatively good condition. However, the midstream and downstream sections were under stress because of the low flows and poorer quality of the water (Table 6.6). Indeed, the health and condition of the river shifts from moderate to poor condition classes towards the lowermost parts of the river in dry seasons.

**Table 6.4:** Ecological condition under base flows and recommendations for the adaptive management in the MacKenzie River.

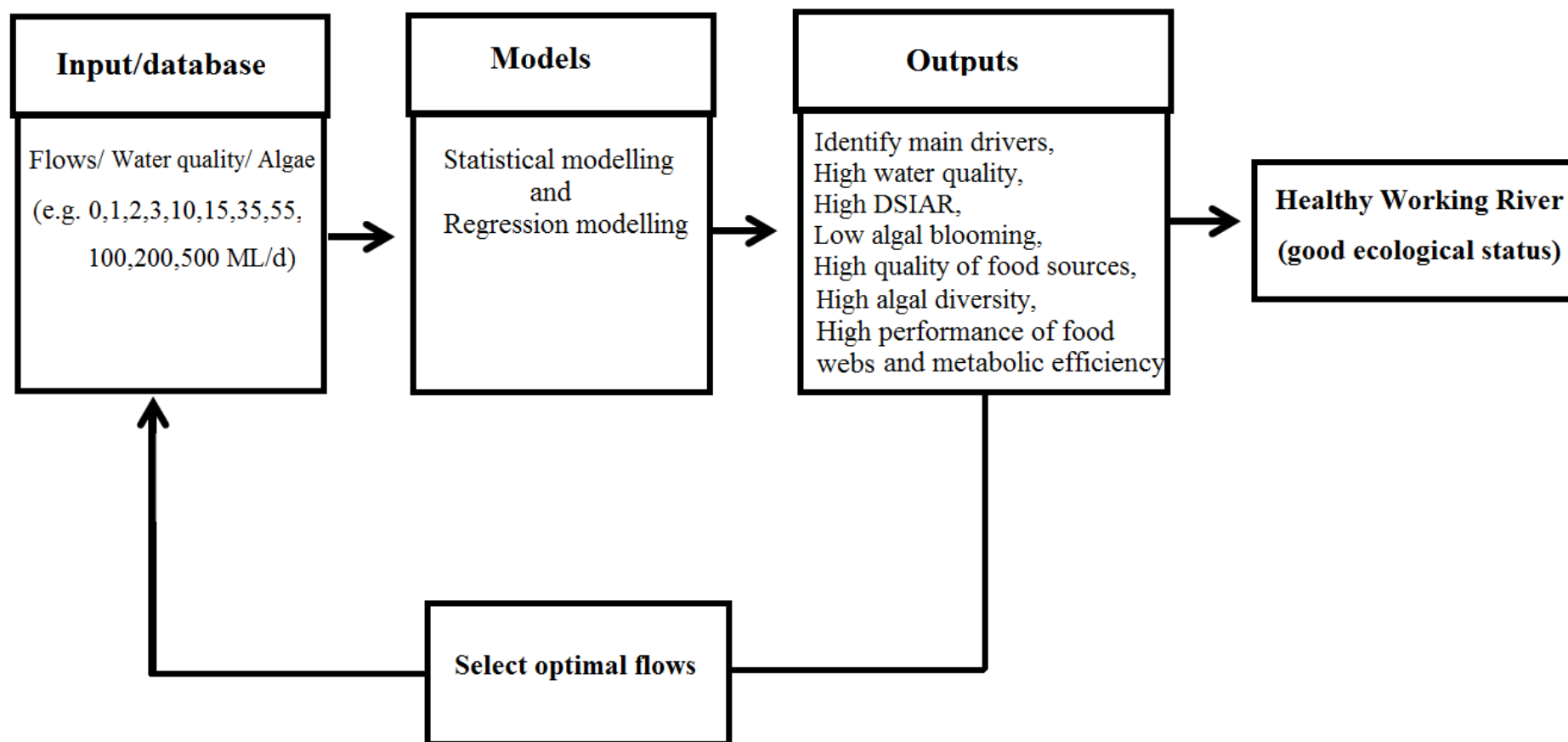
Site	Condition	Action
S1	Good	Conserve as good
S2	Good	Conserve as good
S3	Good	Conserve as good
S4	Good	Conserve as good
S5	Moderate	Need to improve
S6	Moderate	Need to improve
S7	Moderate	Need to improve
S8	Moderate	Need to improve
S9	Poor	Need to improve
S10	Poor	Need to improve

The conceptual model was based on environmental variables, base flow and water quality which influence river health indicators including algal species composition, productivity, food webs and metabolism to explain the underlying elements and mechanisms of river health in the MacKenzie River (Figure 6.8). The model shows environmental variables (e.g. climate), flow regimes (e.g. base flows) and water quality affect the algal species composition, DSIAR, algal productivity, food webs and metabolism of the benthic community. Based on water quality measurements and biological properties, it can be concluded that river health is at risk in the middle and lower part of the MacKenzie River under base flow due to poor water quality and low ecological integrity (low ability to support biological processes and functions). However, the stream condition and river health improved under freshes (see section 6.3).

It has been assessed whether flows moderate a tendency towards simplified algal communities by removing 'invasive' flora and allowing opportunities for a greater diversity of taxa to colonise and flourish. The biomass/metabolism studies have examined the role of algae in providing energy to the food web and the influence of flow in promoting this; or mitigating over production. The relationship between input, model and output, and their components for a healthy working river, are depicted in Figure 6.9.



**Figure 6.9** Conceptual model for the MacKenzie River (e.g. under base flow 10-15 ML/day).



**Figure 6.10:** The relationship of input, model and output and their components for a healthy working river



The success in river protection and restoration depends on the understanding and accurate modelling of the regulated system (Christensen et al. 1996). Acreman and Ferguson (2010) discussed that the water body in rivers will be in good condition when the biology, water quality and hydrology meet the reference condition.

It seems the ecological impacts of the water release events are not consistent across waterways either. The ecological models based on algal responses to different flow regimes reveal that, under low flows, the downstream sections of the MacKenzie River are stressed and so require further water release events to sustain an enhanced ecosystem health. The models show that flow affects algal community patterns and ecosystem function and so water release events can play a major role to improve the ecosystem processes in the MacKenzie River. The models infer the fluctuation of algal species composition and algal biomass under different flow regimes. The models illustrated that water release events (freshes) represent clear opportunities to improve the ecological conditions of the MacKenzie River despite limited water availability, particularly in the middle and lower parts of the river.

### **6.5 Adaptive management**

The configuration of consumptive flows in the MacKenzie River system (one of the main tributaries of the Wimmera River) fall within the Wimmera-Glenelg Bulk and Environmental Entitlements, for which Grampians Wimmera Mallee Water (GWMWater) is the storage manager. Although coordinated use of entitlements is implied within their administrative arrangements, cooperation still proves difficult, particularly during times of water shortage when entitlement holders become focused on their own individual requirements. Storage managers have, however, a duty of care to the environment in the way they operate reservoir systems and manage water delivery to both consumptive and environmental entitlement holders. Biological indices and

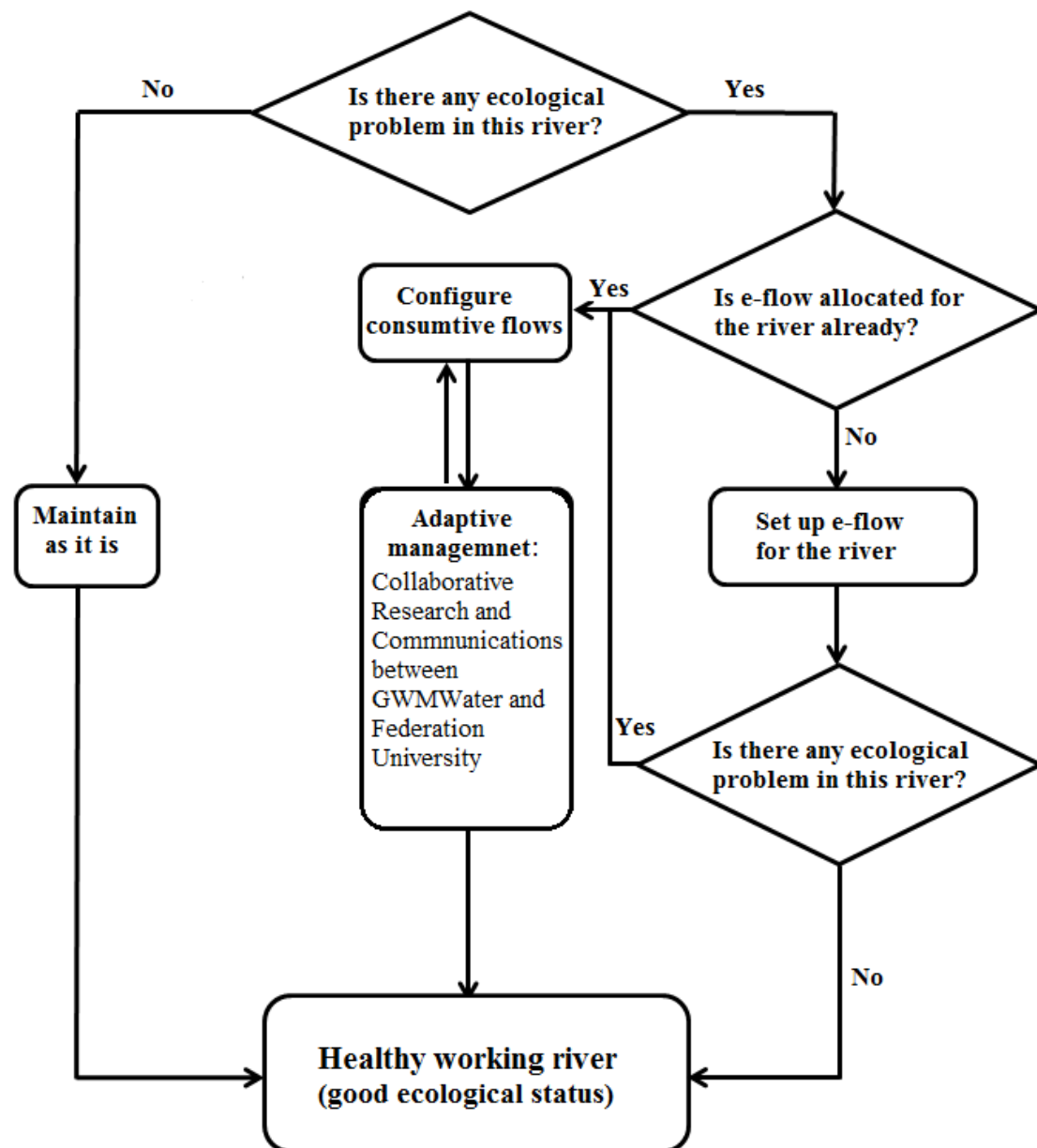
ecological models can be useful as tools (see Chapter 2 and Chapter 5) for water resource managers in their assessment of water quality, stream condition and their decision making with regards to water sharing amongst the consumptive users. These models can be utilised to improve environmental benefits and river health.

This research provides the means to enhance stream condition by gearing consumptive flows to complement environmental flows. The passage of time has shown that many historical water sharing arrangements have not adequately protected aquatic environments (MDBA 2014b). The extraction of water has proven to be unsustainable for many rivers within arid or semi-arid regions which are particularly vulnerable to the over exploitation of vulnerable water resources due to their more variable climate.

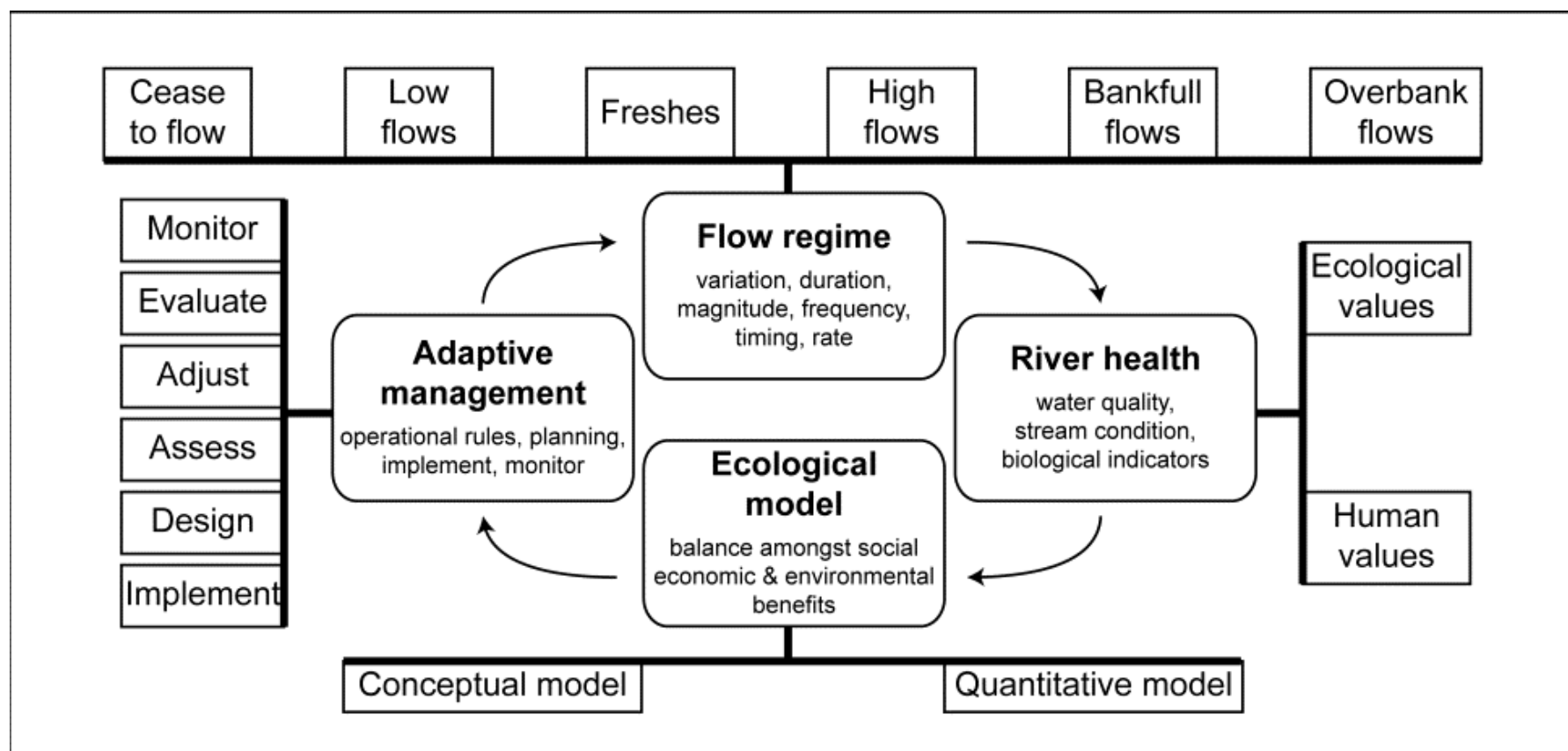
Implementing ecological response models in regulated systems as an aid to decision making therefore, also holds great potential for a more adaptive approach to water resource management. Adaptive management creates an efficient framework from within which trade-offs occur between human uses and the environment, to the benefit of both (Watts et al. 2009a). Furthermore, adaptive management can play a significant role to decrease tension in an extremely complicated system associated with social, technological, political, economic and environmental issues, as trial and error approaches can be more readily employed with the aim of improving arrangements over time. A conceptual flow chart was developed to summarise the absence and presence of ecological problems, allocation of environmental flows, and potential of consumptive flows and adaptive management to underpin a healthy working river systems (Figure 6.10). Hence equitable and effective sharing of the water resource between consumptive users and the environment is critical.

The relationship of adaptive management, ecological models, flow regimes and river health were depicted in Figure 6.11. As mentioned earlier, water resource

management is an ongoing challenge for water managers and river scientists. Therefore, new ecological models based on changes in water resources is essential in terms of adaptive management. For example, the results of the present study can be used by water managers (e.g. GWMWater) to tailor the duration and discharge of freshes used to deliver consumptive water to improve the condition of the stream thereby supplementing the flows dedicated to environmental outcomes.



**Figure 6.11:** Guidelines for evaluation of river health by configuration of consumptive flows in for rivers (e.g. the MacKenzie River).



**Figure 6.12:** Conceptual model based on the relationship among flow regime, river health, ecological models, and adaptive management.

### **6.5.1 Constraints in water management efforts**

Generally, waterway managers face three constraints in water management efforts; physical, operational and policy constraints. As mentioned in Chapter 3, the MacKenzie River has been modified and engineered in several locations including the construction of Wartook Reservoir in 1887, water diversion in Mt Zero Channel, the commissioning of the Wimmera pipeline in 2010, water storages and water plant treatment. However, there are some operational and policy constraints in the Wimmera–Glenelg system (including MacKenzie River) due to scarce water availability. Those constraints are common particularly in arid and semi-arid regions. Overall, the allocation of water between consumptive users and the environment is dependent on the availability of water and ultimately the decision to release. The operational constraints are related to the range and manner of operation protocols and strategic rules used by water resource management. The policy constraints are related to the water sharing agreements at regional, state and federal government levels. The policy constraints are very complicated in comparison with other constraints.

### **6.5.2 Best available science**

In order to solve the barriers and obstacles in water resource management, water managers and environmental scientists use different methods to evaluate the ecological responses during hydrologic alteration in freshwater ecosystems (Norris et al. 2011, Webb et al. 2011). However ecologists and water managers have recently begun turning from experience-based (expert opinion) methods to evidence-based methods (Webb et al. 2012, Klaar et al. 2014). This project brought new evidence by monitoring and empirical data based on algae (bioindicators) under different flow regimes along the MacKenzie River. The modelling was performed using collected data. According to

Nichols et al. (2013), water managers are required to employ the best available science to deliver the best approach and techniques to freshwater management efforts. Therefore, managers are expected to apply the best available science for the management of the MacKenzie River. This project brought new recommendations and suggestions for local water managers (GWMWater) to configure the consumptive flows for environment benefits. Presently river scientists commend water managers and stakeholders to employ the “best available science” now rather than waiting for future science discoveries that might provide better outcomes (Ryder et al. 2010, Nichols et al. 2013). As previously discussed conflict pertaining to the use of freshwater resources has increased as a result of population growth; as well as resulting from socioeconomic developments, industrialisation, global warming and climate changes across the world (Rosenberg et al. 2000, Poff et al. 2003, Millennium Ecosystem Assessment 2005, Stevenson and Sabater 2010, Wilby et al. 2010, Arthington 2012). Such developments have proven detrimental to freshwater ecosystems globally. As a result, the quality and quantity of water has decreased tremendously, particularly in arid and semi-arid regions of the world. Therefore, water managers have tried to implement the best available science to deal with this problem.

Ecological response models (e.g. algae-based models) have a high potential to diagnose the causes and threats to freshwater systems (Yoder and Rankin 1998, Stevenson 2014). Using ecological response models for flow configuration and water allocation is one of the best available scientific tools that water managers can rely on. As previously mentioned river scientists, water managers and stakeholders are looking to sustain and improve freshwater ecosystems in order to provide goods and services for society and the environment (Baron et al. 2003, Arthington et al. 2006). Ecological

models can be used for predicting ecological response from particular operations (Stevenson 2014).

### **6.5.3 Reservoir operation**

Flow magnitude, duration, timing, frequency are the main aspects of the flow regimes (Poff et al. 1997, Arthington 2012) as well as the rate and character of rising and falling flows. Operational protocols and recommendation have been developed based on these flow attributes and are intended to improve the ecological condition for each of the three reaches of the MacKenzie River. As outlined in Chapter 5, the measured base flow regime in the MacKenzie River was 10-15 ML/day with freshes measured at about 35-40 ML/day and a high flow of 55ML/day. The suggested magnitude and duration of flow to be considered each season to maximise ecological outcomes for the MacKenzie River.

Recommendations provided here on configuring consumptive flows consider maximising the DSIAR score, water quality and stream condition, food sources, and nutrients dynamics to improve overall ecosystem health. The current study has revealed the connection between different flow types (low flows, freshes and high flows,) and ecological responses. Factors such as longitudinal connectivity of the river, flushing and transferring sediments along the river, and improving water quality, habitat for fish community and recruitment of aquatic biota, dispersal of platypus population and the interface with the larger Wimmera River can all be considered.

The management of water supply systems are constrained by a range of competing objectives such as balancing water between storages, maximising the efficiency of the system, delivering water in a timely manner, and physical constraints



such as flow rates that do not exceed valve or weir capacities. These objectives may not be always compatible with facilitating or maximising environmental outcomes.

The results in chapter 5 showed that stream condition based on algae response can improve for at least one week after water release events, including both freshes (35-40 ML/day) and high flows (55 ML/day). The data and models showed the optimal release for the MacKenzie River is 35 ML/day which is supplementary to the environmental flows of the river. Therefore, if the reservoir operator needs to transfer a high volume of water (e.g. 1000 ML) from Wartook Reservoir to Taylors Lake, it would be preferable to transfer this water at a rate of 35 ML/day which would bring greater benefits to the stream (Table 6.7). Operating hydrologic scenarios and their ecological advantages and disadvantages were explained for the configuration of consumptive flows along the MacKenzie River. The hydrologic scenarios were developed based on observation, empirical data and models (Table 6.8).

**Table 6.5:** Transfers between reservoirs and environment benefit through MacKenzie

River

Lake Wartook		(e.g.1000 ML) → Taylors Lake		
Discharge (ML/day)	Climatic scenario	Duration (days)	Benefits after water release (days)	Total benefits (days)
5	Dry	200	0	0
5	Wet	200	0	0
10	Dry	100	0	0
10	Wet	100	0	0
15	Dry	66	0	0
15	Wet	66	0	0
25	Dry	40	0	0
25	Wet	40	0	0
35	Dry	28	7	35
35	Wet	28	14	42
45	Dry	22	7	29
45	Wet	22	14	36
55	Dry	18	7	25
55	Wet	18	14	32
65	Dry	15	7	22
65	Wet	15	14	29
100	Dry	10	7	17
100	Wet	10	14	24

**Table 6.6:** Hydrologic scenarios and expected ecological response in the Mackenzie River

Hydrologic scenarios	Algal responses	Ecological pros and cons
Low flow ( $f < 15$ ML/day)	According to the models, under this scenario dry mass, AFDM, Chl- <i>a</i> increase along the river (three reaches), algal blooms occur, green algae and cyanobacteria are more dominant in the river, low DSIAR scores, particularly in lower part of the river	Water quality is low, anoxia may occur, salinity is higher, lateral connectivity and there is longitudinal disconnection along the river, aquatic biota are under stress, river is intermittent (some pools appear along the river particularly in Reach 2
Low flow ( $f = 15$ ML/day)	Under this scenario dry mass, AFDM and Chl- <i>a</i> increase in the river, algal blooms occur, green algae and cyanobacteria are more dominant and common in the river. Low DSIAR scores	Water quality is low particularly in a dry season, anoxia occurs occasionally, lateral connectivity and longitudinal disconnection may occur along the river, eutrophication occurs.
Small freshes ( $15 < f < 35$ ML/day)	dry mass is less while AFDM and Chl- <i>a</i> increase in the river, algal blooming occurs rarely, algal community is shifting from green algae and cyanobacteria to diatoms where diatoms are more dominant in the river, moderate DSIAR score in the river.	Water quality increases, salinity decrease, longitudinal connection occurs occasionally, it controls ecological function and hydrological process such as maintenance of habitat, aquatic biota,
Freshes ( $f = 35$ ML/day)	under this scenario also dry mass decreases while AFDM and Chl- <i>a</i> increase in the river, algal blooming disappear in upstream, but algal blooming can be seen in lower part of the river, algal community is shifting to diatoms where diatoms are dominant in the river,	Water quality improves, river health and stream condition improve, salinity decreases, longitudinal connection occur sometimes along the river, sediments transfer along the river, and eutrophication disappear.

**Table 6.4 (continued):** Hydrologic scenarios and expected ecological response in the Mackenzie River.

Large Freshes ( $35 < f < 55$ ML/day)	According to the models, under this scenario dry mass decrease while AFDM and Chl- <i>a</i> decrease in the river, algal blooming disappear in upstream, algal community is shifting to diatoms where diatoms are dominant in the river, high DSIAR score in the river.	Water quality improves substantially, river health and stream condition improve, longitudinal connection occur sometimes along the river, sediments transfer along the river, habitat ameliorate for fish community and recruitment of aquatic biota and eutrophication disappear.
High flows ( $f=55$ ML/day)	The models shows under this scenario dry mass decreases AFDM increase and Chl- <i>a</i> decrease in the river, algal blooming disappears in upstream, diatoms are dominant in the river, high DSIAR score in the river.	It is important for the hydrological process and ecological function along the river. The high flows not only maintain the habitats but also create further habitats for the aquatic biota, lateral and longitudinal connections, improve habitat for fish species breeding in the river.
High flows ( $55 < f < 100$ ML/day)		
Bankfull flows ( $100 < f < 500$ ML/day)		

#### **6.5.4 Operating recommendations for the MacKenzie River**

Wartook Reservoir and the MacKenzie River are both integral parts of the water supply system for the region and so there are a range of operational and strategic considerations that need to be made in determining the release of water. One of these considerations is the availability of environmental allocation and the constraints this brings to maximising environmental outcomes. There remain opportunities, however, within the consumptive allocation to enhance stream condition. Operational protocols, therefore, aim to consider how water allocations can be used to maximise outcomes for both environmental and consumptive users and have been formulated as strategic (high level) and operational (day-to-day) rules. A new flow allocation was recommended for the MacKenzie River by amalgamating allocated environmental flows and consumptive flows (Table 6.9). A total of 10,000 ML/year of water is released from Lake Wartook into the MacKenzie River. Approximately 4,000 ML/year (about one third) was released explicitly for environmental purposes as so called environmental flows. The remaining 6,000 ML (about two thirds) was released to meet consumptive demands and to transfer water to downstream reservoirs. The findings of this study showed that configuration of consumptive flows brings more benefits to the river. This study showed the river health will improve if local water agencies (GMWWater and WCMA) return 35 ML/day for three days (Table 6.9).

**Table 6.7:** Recommendations for configuration of flow regimes by amalgamation of allocated environmental flows and consumptive flows in the MacKenzie River

Amalgamation of allocated environmental flows and consumptive flows				Description
Timing	Flow type	Magnitude	Duration	
January	Cease to flows	0 ML/d	22-25 day	Return from consumptive flows as a top-up for environmental flows
	Base flows	2 ML/d		
	Freshes	5-50 ML/d	4-7 days	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days fortnightly</b>	
	High flows	No high flows	0	
	Bankfull	No bankfull	0	
	overbank	No overbank	0	
February	Cease to flows	0 ML/d	22-24 day	Return from consumptive flows as a top-up for environmental flows
	Base flows	2 ML/d		
	Freshes	5-50 ML/d	3-4 days	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days fortnightly</b>	
	High flows	No high flows	0	
	Bankfull	No bankfull	0	
	Overbank	No overbank	0	
March	Cease to flows	0 ML/d	22-26 day	Return from consumptive flows as a top-up for environmental flows
	Base flows	2 ML/d		
	Freshes	5-50 ML/d	4-7 days	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days fortnightly</b>	
	High flows	No high flows	0	
	Bankfull	No bankfull	0	
	Overbank	No overbank	0	
April	Cease to flows	0 ML/d	22-25 day	Return from consumptive flows as a top-up for environmental flows
	Base flows	2 ML/d		
	Freshes	5-50ML/d	4-7 days	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days fortnightly</b>	
	High flows	No high flows	0	
	Bankfull	No bankfull	0	
	Overbank	No overbank	0	
May	Cease to flows	0 ML/d	22-26 day	
	Base flows	2 ML/d		

	Freshes	5-50 ML/d	4-7 days	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days fortnightly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
	High flows	No high flows	0	
	Bankfull	No bankfull	0	
<b>June</b>	Overbank	No overbank	0	
	Cease to flows	N/A	N/A	
	Base flows	7 ML/d	Continuous	
	Freshes	N/A	0	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days monthly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
	High flows	130 ML/d	15 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
<b>July</b>	Cease to flows	N/A	N/A	
	Base flows	7 ML/d	Continuous	
	Freshes	N/A	0	
	<b>No need additional freshes</b>	<b>-</b>	<b>-</b>	<b>-</b>
	High flows	130 ML/d	22 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
	Cease to flows	N/A	N/A	
<b>August</b>	Base flows	7 ML/d	Continuous	
	Freshes	N/A	0	
	<b>No need additional freshes</b>	<b>-</b>	<b>-</b>	<b>-</b>
	High flows	130 ML/d	22 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
	Cease to flows	N/A	N/A	
	Base flows	7 ML/d	Continuous	
<b>September</b>	Freshes	N/A	0	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days monthly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
	High flows	130 ML/d	15 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
	Cease to flows	N/A	N/A	
	Base flows	7 ML/d	Continuous	
	Freshes	N/A	0	
<b>October</b>	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days monthly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
	High flows	130 ML/d	15 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
	Cease to flows	N/A	N/A	
	Base flows	7 ML/d	Continuous	
	Freshes	N/A	0	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days monthly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
<b>November</b>	High flows	130 ML/d	15 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
	Cease to flows	N/A	N/A	
	Base flows	7 ML/d	Continuous	
	Freshes	N/A	0	

	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days monthly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
	High flows	130 ML/d	15 days	
	Bankfull	500 ML/d	Any	
	Overbank	900 ML/d	1 day	
<b>December</b>	Cease to flows	0 ML/d	22-25 day	
	Base flows	2 ML/d		
	Freshes	5-50 ML/d	4-7 days	
	<b>Recommended additional freshes</b>	<b>35 ML/d</b>	<b>3 days fortnightly</b>	<b>Return from consumptive flows as a top-up for environmental flows</b>
	High flows	No high flows	0	
	Bankfull	No bankfull	0	
	overbank	No overbank	0	

#### 6.5.4.1 Recommendations of Strategic Rules

The identification of optimum flow releases to enhance the condition of the MacKenzie River allows for the formulation of recommendations of general rules or guidelines that can be followed by the operator, and understood by the community.

Management Framework: Storage management frameworks, or any form of governing or legislative documentation, should be amended or created to enable adequate flexibility for the storage operator to configure consumptive flow deliveries to provide environmental benefit in the Wimmera-Glenelg system (including The MacKenzie River).

Communications: An agreement should be in place between the storage operator (GWMwater) and environmental flow planners (WCMA, VEWH and DELWP) to mandate that discussions will take place around all flows (both consumptive and environmental) and to look for opportunities for water use efficiency and the optimisation of benefits around each flow.



Mapping environmental values in the MacKenzie River system: Values should be mapped (based on the best available science) in both a spatial and temporal context so that when flow enhancement opportunities arise, flows can be quickly configured to match the spatial/temporal environmental requirements at the time.

#### **6.5.4.2 Operational Rules**

Allocation of Water: Water sharing frameworks vary from region to region, however the allocation or availability of water for various uses will present a constraint on how water can be configured for environmental benefit. The configuration of consumptive flows in the MacKenzie River for environmental benefit must fully regard the volume of consumptive water for use and work within this constraint.

Best Available Science: Only the best available science should be used to guide the development of flow plans. This science should include consideration of all aspects of the flow regime and provide the detail for how flows should be configured based on the environmental values identified from the strategic rules, such as cease to flows, freshes and so on.

System Operating Rules: Flows can only be configured within the physical and operational capacities of the supply system. Flow rates, the rate at which flows rates can be changed, target filling or drawdown curves, system monitoring and availability of personnel to make operational changes will all constrain the ability to deliver desired flows and particular flow regimes. These constraints should be documented and be adhered to in the configuration of any flows.

Site Specific Rules: Some reservoirs and rivers will have particular and unique operating rules associated with them. This might be due to flood management, water quality management, provision of recreational values and so on. This site specific

information usually presents as an additional set of constraints to how flows can be configured, but are not universal and so should be considered separately.

These operational rules are derived from the main facets of the flow regimes including magnitude, duration, variation, timing, frequency and rate of change. The magnitude, timing and duration of the water release should be optimised to meet all ecological requirements and management objectives. This applies in particular to the main water release events which can be planned by either the responsible environmental flows planner or the storage operator, to ensure flows can be manipulated to provide benefits to stream ecosystems.

## **6.6 Chapter summary**

Algae-based response models were developed for base flows, freshes and high flows for between 15 ML/d to 55 ML/d in the MacKenzie River. Algal communities were observed and found to respond to different flow regimes which demonstrated that appropriate flow manipulations can be used to improve river health. Under the right circumstances, consumptive flows can be used to improve river health and this presents an interesting challenge for river scientists and water engineers given the additional constraints that consumptive flows have over dedicated environmental flows. The results were also used to tailor the duration and discharge of consumptive water to improve the condition of the stream thereby supplementing the flows dedicated to environmental outcomes.

This chapter has provided a worked example of how a consumptive flow transfer can be configured to enhance stream condition. In addition, this research forecasts that benefits can accrue when management moves from a contest between

volumes for allocations and configurations to a coordinated approach to bring environmental benefits without compromising consumptive needs. A number of conceptual diagrams and a flow chart were developed to summarise the steps for the configuration of consumptive flows in working river systems.

## **Chapter 7: Conclusions and future research**

### **7.1 Use of algae to evaluate stream condition in the MacKenzie River**

Human activities in western Victoria have altered the ecological condition of the MacKenzie River. As a consequence of this modification, the algae community in many parts of the river reflect a degraded state. The observations presented here show that water quality, algal community structure and biological properties respond to different flow regimes in different seasons.

The findings of this study have revealed the upstream reaches of the MacKenzie River to have higher DSIAR scores, relatively few filamentous algae and high water quality, and so can be considered to be in good condition. However, the mid-stream and downstream sections are under stress because of low flows and the poorer quality of the water. Indeed, the health and condition of the river shifted from moderate to poor condition classes towards the lowermost parts of the river, particularly in dry seasons.

High diversity within the algal community along the river indicates a healthy river with good ecological status. Under base flow, diatoms were more abundant upstream and green algae and cyanobacteria are more abundant downstream. Green algae and cyanobacteria gradually increase downstream under base flow conditions, and before water releases, whereas diatoms decrease in relative abundance downstream. However, the algal composition shifted downstream after water-release events, as a result of the reduction in the abundance of green algae and cyanobacteria in downstream reaches.

The biological properties (dry mass, AFDM and chl-*a*) of the algal periphyton communities, and the species composition, varied between sites under different flow regimes. The accumulation of dry mass decreased downstream during freshes.

However, the accumulation of AFDM gradually increased from upstream to downstream. In contrast, the concentration of chlorophyll-*a* decreased from upstream to downstream under high-flow events (water releases and natural high flows) due to reduction of algal assemblages by scouring and burial.

Whilst the water quality does vary during different seasons, the main changes in water quality are observed during the water release events. The river health and condition shifts from moderate to poor condition towards the lower most parts of the river in dry season.

## **7.2 Ecological response models to configure consumptive flows in the MacKenzie River**

In the present study, ecological response models were developed using freshwater algal assemblages to configure consumptive flows to achieve greater ecological benefit in the MacKenzie River. The quantitative and conceptual ecosystem response models, based on the functional relationships between stream hydrology, water quality, algal species composition, biomass, chlorophyll-*a* concentration and ecosystem function (food webs and river benthic metabolism), were developed as the tool to assist in the future configuration of flows in this river. The models built are based on data (empirical evidence) which was collected under different seasons and different flow regimes.

Although the base flows of the MacKenzie River support the ecosystem function, aquatic biota, physicochemical processes, biological processes and ecological attributes, anthropogenic modifications have profoundly changed the river since the construction of Wartook Reservoir in 1887. The data and models show that the structure and function of the algal communities, as primary producers, were strongly influenced by the various flow regimes. The study demonstrates that consumptive flows can be used

to improve river health under the right circumstances and this presents an interesting challenge for the river scientists and water engineers given the constraints (physical, operational and policy) that consumptive flows have relative to those of dedicated environmental flows. Indeed, the models predict that appropriate flow manipulation can be used to improve river health. The most important findings pertain to the lower reaches of the river which are shown to be in poor condition under low flows, but also show improvement under flows of 35 ML/day, as indicated by the reduction in green algae and cyanobacteria and an increase in DSIAR scores. The outcomes of this study can be used to improve the condition of the stream by guiding the duration and discharge of freshes used to deliver consumptive water, and thereby to supplement the flows dedicated to environmental outcomes.

Overall, the lower parts of the MacKenzie River were in poor conditions in terms of water quality, stream condition and river health. However, environmental flows changed the water quality and stream condition, and these elements improved. On the basis of this if 35ML/day can be released then the condition of the system will be improved. However, this improvement is only temporary and poor conditions return after about 15 days.

### **7.3 River management and water sharing**

Rivers bring many socio-economic, geopolitical and cultural values for society worldwide such as political power, social services, cultural initiatives, health care, sanitation, power generation, irrigation, fisheries, industries, transportations, urban and domestic water, scenic and recreational values. Indeed, rivers make a significant contribution in providing goods and services for human well-being. Meanwhile, rivers play a pivotal role in the maintenance of aquatic wildlife biodiversity, habitat diversity and ecosystem

function (Assessment Millennium Ecosystem 2005). Unfortunately, most of the rivers globally are modified and regulated. In concert, rivers are both incredibly valuable and highly threatened. Therefore, the conservation of rivers is absolutely imperative, and given the incremental increase in river knowledge, this is best achieved through adaptive management (Arthington 2012) because waterway managers need to update the water allocation and abstraction under adaptive management processes including monitoring, operational rules, implement, assess and planning.

While management usually focuses on environmental flow allocation as the means of improving stream condition, this study highlights the benefits that can arise from flows tied to consumptive water transfers and opens the way to address the ongoing challenge for river scientists and water engineers in providing ecological benefits from scarce water. This study shows that benefits can accrue when management moves from a contest between volumes to allocations and abstraction. Considerable environmental benefits can be gained from the configuration of consumptive flows by rigorous adaptive management in engineered and modified rivers. In essence, this study demonstrates how to measure and assess responses to inform the manipulation of water releases, with an in-built capacity for fine-tuning, reflecting an adaptive approach.

Evaluation of river health, stream condition and water quality identify the causes and threats of the ecological disturbances and flows alteration in riverine ecosystems which is very important for river management and water sharing. Suggested operational protocols, strategic rules and recommendations are provided in chapter 6 and are based on the water-release events to improve ecological condition in three different reaches of the MacKenzie River. These operational rules are derived by the amalgamation of

environmental flows and consumptive flows from the main attributes of the flow regimes including magnitude, duration, variation, timing and frequency.

At present there is only 4000 ML allocated for environmental flows for the MacKenzie River. This volume would only allow for approximately 10 ML/day in each year. This study showed the volume of allocated water for the MacKenzie River as environmental flows did not meet the ecological requirements, particularly in the lower reaches. However, good ecological condition can be achieved if consumptive flows are also released in a manner that benefits the ecology of the River. There are certain constraints on the release of this water, but where consumptive flow release operations are developed with environmental flow operations (10 ML/day for 12 months) with flow of 35 ML/day for three days every two weeks as a top-up from consumptive flows. Indeed, there are clear benefits that would accrue from integrating environmental flows and consumptive flow operations. This would be achieved by respective operators exchanging flow release plans and for this to be coordinated between water agencies (GWMwater, WCMA and DELWP). The agencies have this as a planning goal and so the way is paved for more effective use of all water releases.

Overall, evidence-based decisions and strategies in water resources management are imperative today due to scarce water availability. Therefore, the development of hydroecological models (e.g. statistical models) have been increased to bring accurate evidence for water managers in terms of an understanding of antecedent condition, hydrologic alterations, ecological responses and ecological limits for those alterations in riverine ecosystems.





### **8.3 Recommendations for future work**

Configuration of consumptive flows is a very controversial topic across the world particularly in drought prone regions. Improving the present conceptual and quantitative ecological models towards returning water to the environment represents an ongoing challenge for water managers and river scientists. Therefore, additional work and research configuring consumptive flows for ecological benefits is needed. In particular this could include the use of a broader range of bio-indicators (e.g. fish).

The model that underpins this is based on three experimental release flows and could be refined by continuing to monitor across a greater range of flow discharges. In the early stages of this release protocol it would be beneficial to monitor other aspects of the river ecosystem to assess if higher organisms are responding positively, and to assess the utility of algal monitoring in reflecting whole of system changes.

Although flow regime is the main driver for ecological response modelling in the MacKenzie River, investigation of secondary drivers such as physical topography, vegetation canopy, sediment movement and other environmental variables are needed to comprehensively evaluate the function of the system.

Additional work is required to address the operational rules of water management with respect to social and economic objectives. This is because the allocation of water in the MacKenzie River is multilateral in character and must support social and economic aspirations.

Lastly, the MacKenzie River system was greatly affected by the fire in 2014 and this is likely to have compromised the stream's water quality. It is important to monitor the flow-water quality-algae relationships after fire to assess the impact of bushfires, and to modify the response model to accommodate post fire conditions to ensure the ecological benefits that accrue from flow releases for both environmental and

consumptive purposes. Therefore, additional work on the impact of bushfires on MacKenzie River, and other stream systems, deserves further attention.

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## **Appendix A:** List of publications and presentations from this thesis.

### **Papers**

1. **Atazadeh E**, Gell P., Barton A., Mills K., Newall P. 2015. Using algae-based models to configure consumptive flows for ecological benefits. *River Research and Application* (under revision)
2. **Atazadeh E**, Edlund M, Van de Vijver B., Mills K., Spaulding S., Gell P., Crawford S., Barton A., Lee S., Smiths K. Newall P., Potapova M. (2014). Morphology, ecology and biogeography of *Stauroneis pachycephala* P.T. Cleve (Bacillariophyta) and its transfer to the genus *Envekadea*. *Diatom Research* 29(4): 455-464
3. **Atazadeh E**, Barton A., Mills K., Gell P., Newall P. (2014). Using an algal response model to inform water resource system operations HWRS 2014: 78-85.
4. **Atazadeh E**, Mills K., Barton A., Gell P., 2012. Configuring consumptive water transfers for ecological benefit: An algal response model for water resource operations. HWRS 2012: 848-855.

### **Conference presentations**

1. **Atazadeh E.**, Mills K., Barton A., Gell P., 2012. Configuring consumptive water transfers for ecological benefit: An algal response model for water resource operations. (Presented at UB research annual conference 2012).
2. **Atazadeh E.** Confirmation report; Configuring water transfers for ecological benefits: An algal response model for improved water resource operations in the MacKenzie River, 76 pages (University of Ballarat).
3. **Atazadeh E.**, Mills K., Barton A., Gell P., 2013. Development of an algal response model to evaluate of water quality and stream condition and configuring consumptive flow in the MacKenzie River, south-east Victoria, Australia (ASL conference).
4. **Atazadeh E.**, Edlund M, Spaulding S., Gell P., Mills K., Barton A., 2013. The transfer of *Stauroneis pachycephala* to the genus *Envekadea* (UB research annual conference 2013).
5. **Atazadeh E.** Barton A. and Gell P., Milestone 2 & 3; Configuring Water Transfers for Ecological Benefits: An algal response model for improved water resource operations in the MacKenzie.

6. **Atazadeh E.** Attendance in Ramsar wetlands workshop; detecting change in ecological character 5-8 Nov 2013, Queenscliff, Vic, Australia.
7. **Atazadeh E.** Invited talk at the International Union for Quaternary Science (INQUA) Early Career Researcher inter-congress meeting, December 2nd - 6th, 2013, Wollongong University, Australia
8. **Atazadeh E.**, Barton A., Mills K., Gell P., Newall P. R. Schrieke 2014. Developing a tool to assist in the configuration of flows for a regulated river: using diatom assemblages as an indicator of river health. 23rd International Diatom Symposium, Nanjing- China
9. **Atazadeh E.** 2014. 23<sup>rd</sup> International Diatom Symposium (IDS2014) Grant Award. Nanjing Institute of Geography and Limnology, Chinese Academy of Science, Nanjing, China.
10. **Atazadeh E.**, Gell P., Barton A., Mills K., Newall P. 2016. Using algae-based models to configure consumptive flows for ecological benefit in the highly regulated MacKenzie River, south-east Australia. ASL 2016

## **Appendix B:**

### **Development of an Algal Response Model to Inform Water Resource System Operations**

**Atazadeh E, Barton A., Mills K., Gell P., Newall P. (2014). HWRS 2014: 78-85.**

#### **Abstract**

An algal response model is being developed to inform the operational characteristics of a water supply system. The intent of this research is to refocus approaches to water allocations from a contest over volume, towards a cooperative approach between all users with multiple socioeconomic and environmental benefits.

The algal response model will ultimately be used to aid in the development of a framework, and operational principles, to configure consumptive water transfers to complement dedicated environmental flows. A constraint imposed within this framework will be that whilst providing beneficial environmental outcomes, any flow configuration identified will not compromise consumptive water users.

Field trials have begun in the MacKenzie River in western Victoria, Australia. This River has experienced a highly modified flow regime since the construction of Wartook Reservoir in 1887. Water released from the reservoir is regulated at several locations for water supply and also, more recently, for the specific provision of environmental flows.

With baseline monitoring of the waterway now completed, preliminary results are available to commence evaluating the complex environmental response patterns and benefits that may accrue from flows dedicated to consumptive use.

#### **1. INTRODUCTION**

Historically, the management of rivers and streams has focused on extracting water for consumptive use for agriculture, industry and urban water supply (Acreman & Dunbar, 2004). The main factors of concern have been the amount of water available and the quality of water with respect to its suitability for agricultural, industrial, domestic or recreational uses (Norris & Thoms, 1999). However, water managers have increasingly realised that the protection of natural ecological processes in rivers and streams also helps to protect some of their utilisation value.

Today, there is an increasing requirement, supported by international, national and regional legislation, to conserve and restore the ecological and biological health of rivers and their associated aquatic ecosystems (Acreman & Dunbar, 2004). Typically, these flow requirements specify a flow regime to support the structure and function of aquatic ecosystems within streams and rivers. Current scientific understanding of hydrologic controls on riverine ecosystems, and evidence obtained from river studies, support the development of environmental flow standards at regional scales (Arthington *et al*, 2006).

The challenge of maintaining healthy working rivers is in balancing the environmental requirements against the broader social and economic elements which sustain productive industries and communities. There is no better contemporary example of the difficulty of this challenge than the Murray-Darling Basin Plan (MDBA, 2012). The uptake of flow management recommendations by agencies and water managers, and their acceptance by regional communities, is dependent on an understanding of the interdependencies among management actions and ecosystem health.

The MacKenzie River in western Victoria has been chosen as the case study because this river has been substantially modified since the construction of Wartook Reservoir in 1887. The project is supported by Grampians Wimmera Mallee Water (GWMWater) as the water corporation who owns and operates the dams, weirs and other assets associated with the water supply system. This close relationship with GWMWater has enabled the coordination of releases of water for the benefit of this project.

Work has started with the collection of baseline information from the MacKenzie River. An ecological response model, using an algal-based index, will be developed to assist with the formation of a framework to enable the storage manager to configure water transfers to provide positive environmental benefits. Such actions will close the loop on the overall water balance so that the environment is considered at all stages of managing the water balance.

Although the allocation of water for consumptive users (industry, agriculture, fisheries, urban, recreational and domestic usage) is based mainly on engineering and mathematical models, the use of ecological models, incorporating biological indices, has great potential to improve the way water supply systems are operated and transfers between reservoirs are made.

This research aims to develop an ecosystem response model to consider changes in algal periphyton communities in response to water chemistry and associated impacts on biofilm biodiversity, biological structure or general ecosystem function.

## **2. METHODS**

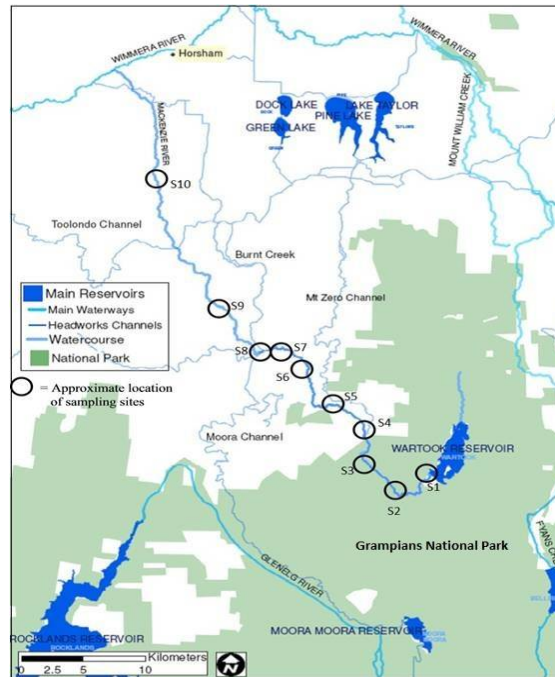
The MacKenzie River is located on the northern slopes of the Grampians National Park in Victoria, south-east Australia. The Mackenzie River is one of the main tributaries of the Wimmera River and flows approximately 50 km from Wartook Reservoir before joining the Wimmera River. Ultimately it discharges into Lakes Hindmarsh and

Albacutya (Figure 1). The catchment lies to the south of the city of Horsham and covers an area of approximately 597 km<sup>2</sup> (WCMA, 2004). The River has had a substantially modified and engineered flow regime since the construction of Wartook Reservoir in 1887 (SKM, 2002). Water released from Wartook Reservoir is regulated at several locations for water supply and also, more recently, for the provision of environmental flows. The environmental watering of the MacKenzie River often includes pulses to mimic part of the flow regime to generate specific ecological responses and ‘top up’ critical refuge pools (VEWH, 2012). The MacKenzie River is ephemeral in nature. The discontinuous sections are mostly in the middle and lower parts of the River where flows vary greatly and are often dependent on transfers of water to consumptive users. Field sites have been established to reflect this variable and ephemeral nature. Commencing at Wartook Reservoir, an additional ten sites are located on the River proper. Further sites are located on the Mt Zero Channel which takes water from the upstream reach of the River for consumptive purposes. This channel is being used as a control site where flows can be manipulated with greater precision.

Monitoring is undertaken regularly across the whole year, at all sites, to understand the effect of water release events on water quality and algal periphyton communities. Samples for water quality analyses were collected coincident with the algal samples. *In situ* measurements of temperature, pH, conductivity and dissolved oxygen were obtained using an Horiba multimeter. Other measurements, including total suspended solids (TSS), total nitrogen (TN) and phosphates (PO<sub>4</sub>-P), were undertaken in the laboratory using a HachDR 2800 and following standard methods.

Algae were collected from five cobbles, pebbles or rocks, selected at random at each sampling station where velocity was relatively low at each of the established sampling sites. The periphyton was scraped from an area of 20-30 cm<sup>2</sup> using a soft toothbrush and the algal suspension rinsed into a collection bottle. Separate samples were collected for estimation of biomass (dry mass, ash-free dry mass [AFDM] and Chlorophyll-*a*) and for algal identification and counting. Soft algae and diatoms were identified in the laboratory according to selected texts (Krammer & Lange-Bertalot 1986, 1988, 1991a, 1991b; Sonneman *et al*, 2000; Ling & Tyler 2000).





**Figure 1 Location of the ten sampling stations along the MacKenzie River system in the Wimmera catchment**

Streamflow information is available from a number of sites along the length of the MacKenzie River, and for this paper the data were obtained from GWMWater operational data. Other information (such as site information, flow regime variations and water quality data) can be obtained from the Victorian Water Resources Data Warehouse as quality controlled data (<http://www.vicwaterdata.net/vicwaterdata/home.aspx>).

The algae-based ecological model, incorporating biological indices, is developed in this project, and is based on ecological conditions which define a healthy working river. The sensitivity values of species to anthropogenic stressors will be used to calculate algae-based index scores for each sample in the datasets. Using the algae-based indices, every site of the MacKenzie River can be categorised as in bad, poor, moderate, good or excellent condition.

The conceptual ecological model will be configured so that the influence of flow regimes on water quality, algal biomass, chlorophyll-*a* concentration, species composition and ecosystem function can be understood. This information will be used to inform the overall health implications for the River and, hence, assist the storage manager in configuring the consumptive flow for the MacKenzie River for greater ecological benefit.

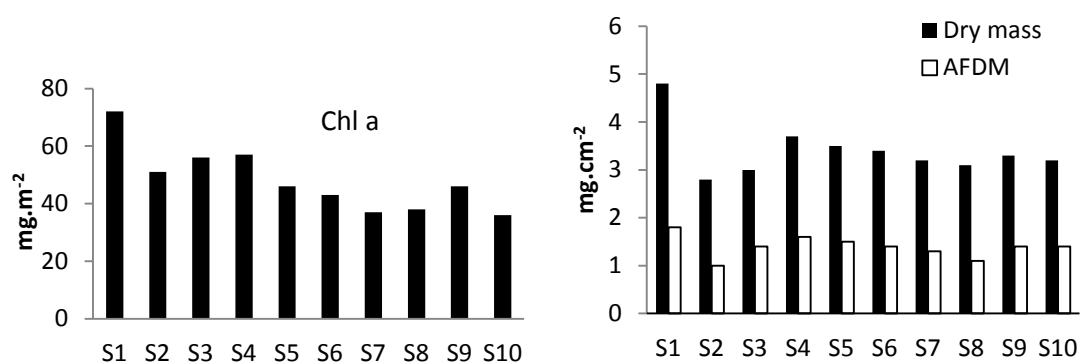
### 3. RESULTS

The water quality results show the up-stream sections of the MacKenzie River has low pH (acidic), low conductivity and low nutrients. Down-stream however, the waters have higher pH (alkaline), higher conductivity and higher levels of nutrients (Table 1). The

results of the analyses of biomass showed changes between sampling sites. Chlorophyll-*a* concentrations were highest at sites S1 and S3 (upstream). The accumulation of AFDM and dry mass were highest at the first sampling station (Figure 2) and results show there to be a 100% variation in values between site 1 and site 10.

**Table 1 Average annual physical and chemical water quality characteristics at the sampling stations (S1-S10) on the MacKenzie River in 2012 (Temp. = temperature, Cond. = conductivity, DO = Dissolved Oxygen). The data are an average of three repeat samples along the river during different seasons.**

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
pH	-	6.5	6.8	6.7	6.6	6.9	7.2	7.4	7.3	7.5	7.5
Temp.	°C	14	13	12	13	15	16	15	15	13	14
Cond.	$\mu\text{S cm}^{-1}$	70	75	78	71	75	140	150	120	110	150
DO	$\text{mg l}^{-1}$	8.1	7.5	9.5	8.2	8.2	8.5	8.52	8.46	9.2	8.5
TSS	$\text{mg l}^{-1}$	4	5.2	6	7.1	9.8	9.1	8.5	17	11.5	14
TN	$\text{mg l}^{-1}$	0.6	0.7	0.9	1.2	0.9	1.3	1.4	1.6	1.8	1.6
PO <sub>4</sub> -P	$\text{mg l}^{-1}$	0.06	0.07	0.08	0.7	0.07	0.09	0.08	0.09	0.07	0.9

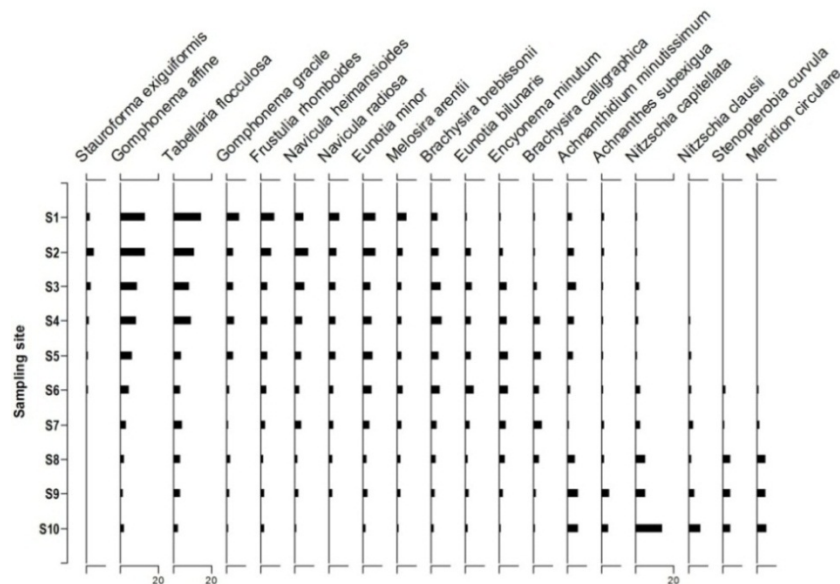


**Figure 2 Average annual concentration of chlorophyll-*a* and accumulation of dry mass and ash-free dry mass (AFDM) at each of the sampling stations along the MacKenzie River in 2012 (Based on an average of three readings from different sampling seasons).**

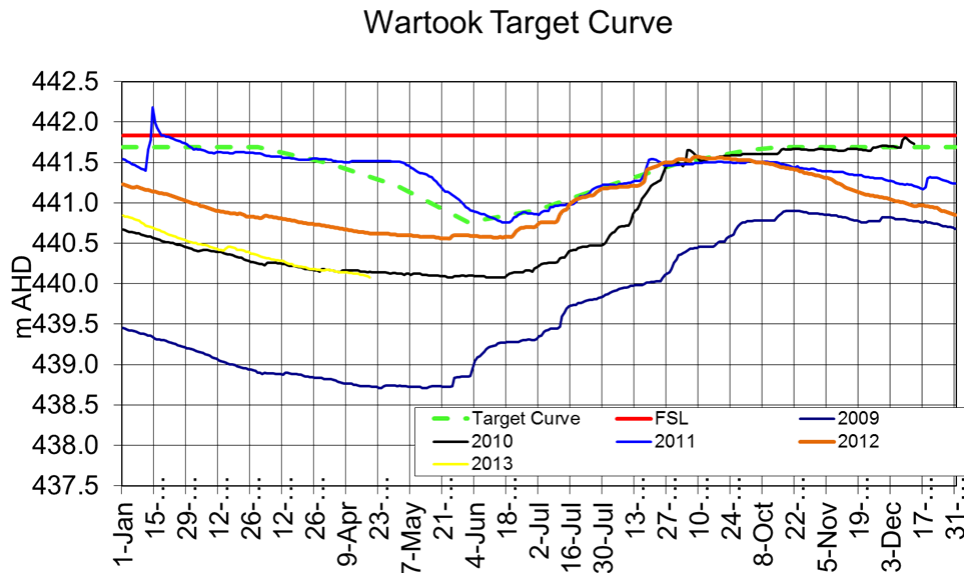
The species composition of diatoms and soft algae assemblages showed significant changes along the river. The most common diatom species in the upstream sites were *Tabellaria flocculosa*, *Gomphonema affine*, *Navicula radiosa*, *Frustulia rhomboides*, *Melosira arentii*, and *Eunotia minor*. However, this community changed downstream to include high numbers of *Nitzschia capitellata* and *Meridion circulare* (Figure 3). Here, common species in the soft algae community were *Scenedesmus gracile* and *Cosmarium circulare*.

The lower, discontinuous part of the MacKenzie River is ephemeral in nature. Here flows depend largely on the transfer of water to consumptive users. However it is one of the main tributaries of the Wimmera River and so plays an important role ecologically. Figure 4 compares the volume of the water in Wartook Reservoir and the head gauge of MacKenzie River, showing that water levels have varied seasonally in recent years.

Figure 5 presents the flow hydrograph from Wartook Reservoir over a several months of 2012. Flow varies significantly over the course of a year and is dependent on both consumptive and environmental demands from the reservoir. Note that the maximum flow rate which can be discharged from Wartook is about 500 ML/d. Additional inflows can occur downstream of the reservoir from adjacent catchments and minor waterways. Sampling sites 6 to 10 would be most impacted by these other sources of inflow.

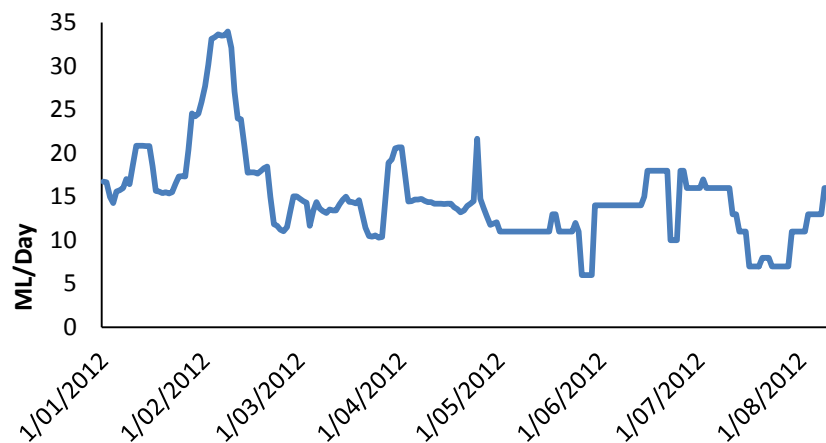


**Figure 3 Results of diatom analysis along the MacKenzie River sampling stations in July 2012. The length of each bar indicates the population size found for each species at each site.**

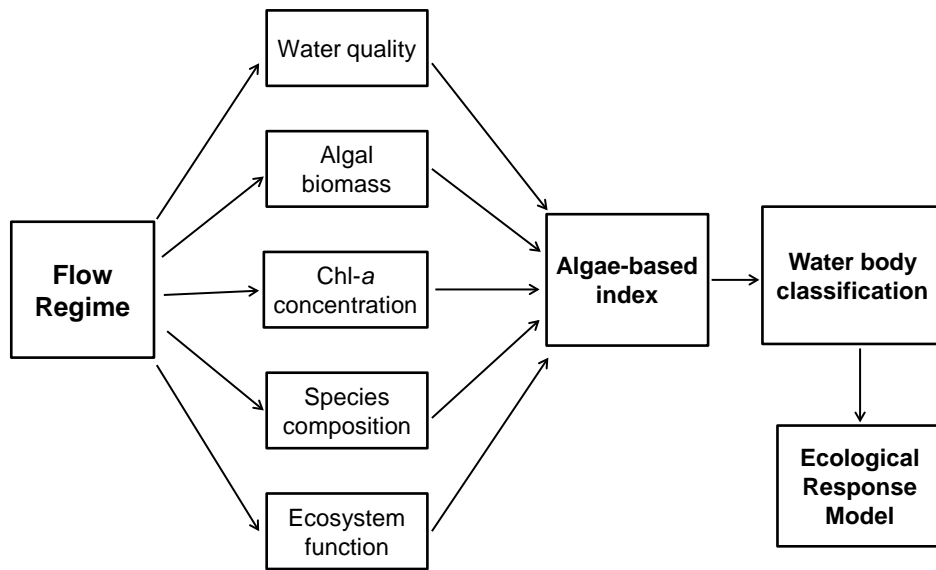


**Figure 4 Hydrographs of Wartook Reservoir measured at the head gauge for the past several years. Wartook Reservoir is located at the headwaters of the MacKenzie River.**

The flow regime of the MacKenzie River directly impacts on the water quality, algal biomass, chlorophyll-a concentration, species composition and ecosystem functions. The conceptual model developed for the flow regime, and its impacts on water quality and biological properties of algal communities, is shown in Figure 6. The algae-based index will be developed for the Mackenzie River to understand stream condition and assist in configuring consumptive flows. Applied across the whole of the MacKenzie River system the index will allow sections to be classified as excellent, good, moderate, poor or bad condition. The conceptual model connects, multiple elements of the flows regime, the algae-based index and subsequent water body classification along the MacKenzie River to enhance the ecological performance of consumptive flows.



**Figure 5 Streamflow fluctuations at Wartook measured at the outlet structure of the Reservoir in 2012. These flows represent the water being released from the Reservoir into the River.**



**Figure 6 Conceptual model based on algae response for the MacKenzie River**

#### **4. DISCUSSION**

The flow regime is a significant feature that directly influences the physical river environment (Gordon *et al*, 2004). According to Acreman & Dunbar (2004), all environmental flow methods for the allocation of water can be divided into four categories: look-up tables, desk top analysis, functional analysis and hydraulic habitat modelling. Each method has advantages and disadvantages. The flow regime has a direct impact on physical form, streamside zone, habitat structure, water quality and the aquatic biota of a river (Ladson *et al*, 1999). Deviation from the natural flow regime must be considered when trying to understand water quality and the allocation of water for environmental benefits (Norris & Thoms 1999). There are six types of flows (flow components) that describe the full flow regime of the rivers including cease to flow, low flows, freshes, high flows, bankfull flows and overbank flows (VEWH 2012). Each type of flow component has five ecological characteristics including magnitude, frequency, timing, duration and rate of change of hydrological conditions (Richter *et al*, 1996; Poff *et al*, 1997; Arthington, 2012).

Algae are sensitive to changes in environmental conditions and respond rapidly to disturbance (Stevenson *et al.*, 2010). In addition to this, they are abundant and cosmopolitan in their distribution, can be sampled rapidly and have a wide range of structural (biomass, composition) and functional (metabolism) attributes (Burns & Ryder, 2001; EPA, 2003). Flow variation in rivers has been shown to affect biofilm structure (e.g. Ryder *et al.*, 2006) and ecosystem processes (Ryder & Miller, 2005). Within the biofilm, diatom algae assemblages are highly responsive to shifts in water quality (Reid *et al.*, 1995) and so their identification can reveal ecological responses to flow-driven changes in stream water quality.

The results of this research have provided base line data for the MacKenzie River. They suggest that the algal communities, especially diatoms, are indeed sensitive to changes in water quality and different flow regimes. It is evident that assemblages vary with season suggesting that their response to flow will vary depending on the season and characteristics of water releases. Further analyses are being undertaken to assess the statistical relationship between the diatom results, water quality data and the antecedent conditions (e.g. rainfall and temperature in the lead up to the sampling period), and to changes in the primary productivity and algal community structure along the MacKenzie River.

The MacKenzie River has been substantially regulated to allow for the manipulation of water transfers to generate multiple benefits for consumptive and environmental users. The project aims to advance the means in which water supply systems are operated and allocations to entitlement holders are made. The method employed to date has focussed on the use of biofilms and algal indicators for understanding in-stream ecology, water quality and their relation to the allocation of water for environmental benefits. However, more sampling is required and an opportunity exists to examine changes under field-based, experimental conditions by designing a pilot experiment at Mt Zero channel to understand the relationships among water quality, algal community structure, algal biomass and ecosystem functions. This will further enhance the development of an ecological response model to guide consumptive, and environmental, water transfers.

## 5. CONCLUSION

This paper has described a research project which aims to use the MacKenzie River in Victoria, Australia, to develop an ecosystem response model to help improve the operational decisions around transferring consumptive water for environmental benefits. At this stage of the research project, it is concluded that:

- The establishment of ten field sites provides sufficient resolution of information along the length of the River to help create an ecosystem response model;
- The use of water quality is useful in creating an ecosystem response model;
- The monitoring of algal periphyton communities is useful in developing an ecosystem response model;
- Having support of the operator of the upstream reservoir, and associated in-stream infrastructure assets, is useful for the purpose of designing specific flows to help create an ecosystem response model;
- The conceptual framework creates a good platform to develop an ecosystem response model;

- Preliminary results indicated that water quality and algal periphyton communities are sensitive to streamflow changes along the River.

## **6. ACKNOWLEDGEMENTS**

This project was funded by Grampians Wimmera Mallee Water (GWMWater) and University of Ballarat. The authors would like to thank GWMWater and School of Science, IT and Engineering (SITE), University of Ballarat for the all supports.

## Appendix C:

### **Morphology, ecology and biogeography of *Stauroneis pachycephala* P.T. Cleve (Bacillariophyta) and its transfer to the genus *Envekadea***

**Atazadeh E**, Edlund M, Van de Vijver B., Mills K., Spaulding S., Gell P., Crawford S., Barton A., Lee S., Smiths K. Newall P., Potapova M. (2014. **Diatom Research** 29(4): 455-464

#### **Abstract**

*Stauroneis pachycephala* was described in 1881 from the Baakens River, Port Elizabeth, South Africa. Recently, it was found during surveys of the MacKenzie River (Victoria, Australia), the Florida Everglades (USA), and coastal marshes of Louisiana (USA). The morphology, ecology and geographic distribution of this species are described in the present paper. This naviculoid species is characterised by lanceolate valves with a gibbous centre, a sigmoid raphe, an axial area narrowing toward the valve ends, and capitate valve apices. The central area is a distinct stauros that is slightly widened near the valve margin. The raphe is straight and filiform, and the terminal raphe fissures are strongly deflected in opposite directions. Striae are fine and radiate in the middle of the valve, becoming parallel and eventually convergent toward the valve ends. The external surface of the valves and copulae is smooth and lacks ornamentation. We also examined the type material of *S. pachycephala*. Our observations show this



species has morphological characteristics that fit within the genus *Envekadea*.

Therefore, the transfer of *S. pachycephala* to *Envekadea* is proposed and a lectotype is designated.

**Keywords:** diatoms, morphology, *Stauroneis pachycephala*, ecology, *Envekadea*, biogeography, taxonomy, lectotype

Recent revisionary efforts on the classification and phylogeny of diatoms have targeted the naviculoid diatoms and recognised the importance of valve ultrastructure, protoplast organisation, molecular sequences, ecological and geological ranges, sexual compatibility and biogeography in defining relationships and diversity within this heterogeneous group (Round and Sims 1980, Round et al. 1990, Mann 1999, Spaulding et al. 1999). Revisions have resulted in the resurrection of old or description of numerous new genera, split off from the catch-all genus *Navicula* s.l. Bory de St. Vincent (Round et al. 1990). The genus *Envekadea* Van de Vijver et al. was described to include naviculoid diatoms with a sigmoid raphe, non-porous copulae, and large, rectangular to polygonal areolae closed by external hymenes (Gligora et al. 2009). Members of this genus are distributed across a broad ecological spectrum from marine to oligotrophic freshwaters (Gligora et al. 2009). To date, five species of *Envekadea* have been recognized (Gligora et al. 2009, Graeff et al. 2013, Lee et al. 2013b), including *Envekadea pseudocrassirostris* (Hustedt) Van de Vijver, Gligora, Hinz, Kralj & Cocquyt, *E. hedinii* (Hustedt) Van de Vijver, Gligora, Hinz, Kralj & Cocquyt, *E. metzeltinii* Lee, Tobias & Van de Vijver, *E. palestinae* (Gerloff, Natour & Rivera) Lee, Tobias & Van de Vijver and *E. vanlandinghamii* Graeff, Kociolek & S.R. Rushforth.

*Stauroneis pachycephala* P.T. Cleve has morphological features that conform to *Envekadea*. *Stauroneis pachycephala* was described in 1881 from the Baakens River, Port Elizabeth, South Africa, and subsequently reported from Australia (Foged 1978, John 1983, 1993, Gell and Gasse 1994, Hodgson 1995, Vyverman et al. 1995, Hodgson et al. 1997b, Haynes et al. 2007, Taukulis and John 2009), New Zealand (Foged 1979), Sweden (Cleve-Euler 1953), Sri Lanka, Cuba, and Papua New Guinea (Foged et al. 1976, Foged 1984, Vyverman 1991).

In the present paper, we provide morphological, ecological, and biogeographical analyses of *S. pachycephala* based on new collections from Victoria (Australia), Florida (USA), Louisiana (USA), and compare these with type material from South Africa. The transfer of *S. pachycephala* to *Envekadea* is proposed and a lectotype is designated.

The MacKenzie River is located on the northern slopes of the Grampians National Park in Victoria, south-east Australia. The river receives a substantially modified and engineered flow regime since the construction of the Wartook Reservoir in 1887 (SKM 2002b, Atazadeh et al. 2012). The river is one of the main tributaries of the Wimmera River and flows approximately 50 km from the Wartook Reservoir before joining the Wimmera River. The catchment lies to the south of the city of Horsham and covers an area of approximately 597 km<sup>2</sup> (WCMA 2004b). The upstream section of the river receives water for most of the year, providing a water supply for the city of Horsham. However, the lower part of the river is ephemeral and flows vary depending on withdrawals for consumption (WCMA 2004b). Despite the irregular flow regime, riparian vegetation is present along the length of the river and the river supports a range of aquatic fauna, such as brown trout (*Salmo trutta* Linnaeus), eastern gambusia

(*Gambusia holbrooki* Girard), and platypus (*Ornithorhynchus anatinus* Shaw) (WCMA 2004b).

The second site studied was the Florida Everglades, USA. This large wetland extends from Lake Okeechobee in the north to Florida Bay in the south, and includes both freshwater marsh in the interior and saline marsh along the coast. The Everglades has a distinct wet and dry season controlled by the subtropical climate and hydrologic management. Anthropogenic modification for water storage and flood control to support agricultural activity and urban populations beginning in the early 1900s have altered the broad, slow-moving sheet flow of water across the Everglades landscape into a complex network of channelised and controlled flow into distinct compartments, including Water Conservation Areas (WCAs) and the Everglades National Park (Light and Dineen 1994). Much of the Everglades has a shallow peat layer that allows biogeochemical interactions between the limestone bedrock, groundwater, and biota, producing hard-water conditions and thick, calcareous periphyton assemblages (Gaiser et al. 2011, Hagerthey et al. 2011). In WCA 1 (the Arthur R. Marshall Loxahatchee National Wildlife Refuge). However, a much deeper peat layer produces soft-water conditions and loose, flocculent assemblages with a distinct species composition (Harvey and McCormick 2009, Gaiser et al. 2011). Common Everglades wetland vegetation includes sawgrass (*Cladium jamaicense*), spikerush (*Eleocharis* spp.) and water lily (*Nymphaea odorata*), and common aquatic fauna include mosquitofish (*Gambusia holbrooki*), largemouth bass (*Micropterus salmoides*) and American alligator (*Alligator mississippiensis*) (Davis and Ogden 1994).

The third region studied was the coastal marsh in the Chenier Plain of southwest Louisiana, USA. The Louisiana coastal marsh can be divided into two geomorphic zones: the Mississippi delta plain on the southeast coast and the Chenier plain of the

southwest with the dividing line located near Vermillion Bay (29°43'11" N, 91°58'34" W). The Chenier plain extends from Vermillion Bay to the Texas state border and includes over 6,000 km<sup>2</sup> of coastal marshes. It was formed by deposits of fine-grained sediments of the Mississippi River during the Middle to Late Holocene. A series of regressive-transgressive phases created relict beach ridges (called 'cheniers') within a 30-km wide coastal plain of low-energy fresh, brackish, and saline marshes (McBride et al. 2007). The cheniers act as barriers, reducing tidal flow to some areas of the marsh. The sediments are largely fine-grained silts and clays, with large amounts of organics and peat. Vegetation follows a general north-south salinity gradient of fresh to brackish to saline marsh. Diatom samples were collected from a network of permanent wetland monitoring stations located throughout coastal Louisiana, the Coastwide Reference Monitoring System (Steyer 2010, Folse et al. 2012). The CRMS was designed to monitor coastal habitats in Louisiana and evaluate the effectiveness of wetland restoration strategies (Steyer 2010, Smith 2012).

Benthic diatom samples from Australia were collected using standard methods (Stevenson and Bahls 1999). Physical and chemical characteristics of the water, including temperature, pH, specific conductivity and dissolved oxygen were measured *in situ* using an Horiba multimeter (Water checker U-10). Total nitrogen (TN), dissolved phosphorus (PO<sub>4</sub>-P) and total suspended solids (TSS) were measured in the laboratory using a Hach DR 2800 spectrophotometer. Diatom samples were digested in 10% H<sub>2</sub>O<sub>2</sub> at 90°C on a hotplate for 2 hours, after which 2 drops of 10% HCl were added. Samples were topped up with distilled water and left to settle overnight, the supernatant was discarded, and this process repeated at least four times (Battarbee 1986). Permanent slides were prepared using the mountant Naphrax. Diatoms were identified using a Nikon Eclipse 80i microscope equipped with differential interference

contrast (DIC). For scanning electron microscopy (SEM), the rinsed samples were resuspended in a solution of deionised water and household bleach (50:1) for 30 minutes and rinsed three times in distilled water. Diatom suspensions were dried directly on 22 mm aluminium stubs and gold coated with a Dynavac Xenosput sputter coater. Frustules were imaged in a Philips XL30 field-emission scanning electron microscope, with a working voltage of 2.0 kV and spot size 2. Material and samples from Florida and Louisiana were collected and prepared as noted in (Smith 2012, Lee et al. 2013a), respectively, and studied using an Olympus BX51 light microscope equipped with full immersion DIC optics capable of 1000x magnification. Permanent slides (or duplicates) of all collections that were microscopically analysed have been deposited at the Academy of Natural Sciences of Drexel University in Philadelphia (ANSP), the National Botanic Garden of Belgium (BR; Meise) and the Canadian Museum of Nature (CANA).

Additional material examined for this study included exsiccatum Nr. 197 for *Stauroneis pachycephala* Cleve (Cleve & Møller 1879; South Africa (Cape of Good Hope) Baakens River, Port Elizabeth) from the Diatom Herbarium, Academy of Natural Sciences of Drexel University in Philadelphia (ANSP) and the isotype slide (ANSP GC64419) of *Stauroneis pachycephala* var. *alaskana* (Foged 1981) from Kuzitrin Lake, Alaska.

***Envekadea pachycephala* (P.T. Cleve) I. Atazadeh & M.B. Edlund comb. nov.** (Figs 1-39)

**Basionym:** *Stauroneis pachycephala* P.T. Cleve 1881, p. 15; pl. 3, fig. 43.

**Lectotype:** Here designated as the specimen (Fig. 13) located 9.4 mm E x 5.8 mm S from the origin marked on slide ANSP Cleve & Møller 197.

**Remarks:** *Stauroneis pachycephala* was included in Cleve & Møller's (1878, and not 1879 as erroneously reported several times by various authors) list of species present in exsiccatum 197. However, this record must be considered as a *nomen nudum*, since the name was published without a valid description or diagnosis (ICBN Art. 38, (McNeill et al. 2012)). Therefore the valid description in (Cleve 1881) is taken as the basionym.

**Australian material:** Cells solitary, frustules rectangular in girdle view with rounded capitate apices (Figs 1-11). Valves linear-lanceolate with convex margins, gradually narrowing towards capitate, broadly rounded valve apices. Valve dimensions (n=30): length 43.1-58.0  $\mu\text{m}$ , breadth 7.0-9.0  $\mu\text{m}$ , length:breadth 5.2:6.5, breadth of apex 5.4-6.7  $\mu\text{m}$ , breadth of constriction 4.6-6.0  $\mu\text{m}$ . Axial area very narrow, linear. Central area forming a large and distinct stauros, slightly widening towards the valve margin. Raphe straight and filiform. Proximal raphe ends slightly expanded. Distal raphe fissures hooked towards opposite sides and widening at their ends. Striae radiate in the middle becoming convergent near the apices, 27.0-30.3 in 10  $\mu\text{m}$ .

**South African material (lectotype; ANSP Cleve & Møller Nr. 197):** The lectotype slide of *S. pachycephala* was examined during this study. Cleve described *S. pachycephala* in 1881 from the Baakens River, Port Elizabeth, South Africa: "(valves) Linear, gibbous in the middle and at the ends, which are broadly rounded and capitate. Striae oblique, very fine, about 29 in 0.01 mm. (29 in 10  $\mu\text{m}$ ), reaching the median line. Stauros reaching the margin. Median line straight. Terminal nodules turned opposite direction. Length 0.055 mm (55  $\mu\text{m}$ ). Breadth 0.009 mm (9  $\mu\text{m}$ )."

Valves lanceolate with the valve outline narrowing abruptly from the valve center to the subparallel sides, then expanding to the capitate valve apices (Figs 12-15). Valve dimensions (n=23): length 44.8-60.0  $\mu\text{m}$ , breadth 8.0-10.0  $\mu\text{m}$ , length:breadth 5.2-6.5, breadth of apex 5.4-6.7  $\mu\text{m}$ , breadth of valve constriction 4.5-6.0  $\mu\text{m}$ . Central area

forming a distinct stauros, abruptly widening near the valve margin. Raphe straight and filiform. Distal raphe fissures deflected in opposite directions. Striae fine, radiate in the valve center, becoming parallel mid-valve and convergent at the valve ends, 24.4-29.4 in 10  $\mu\text{m}$ .

African specimens show slight differences in valve outline compared to other populations, with margins that narrow more abruptly away from the valve center, with larger specimens having nearly parallel sides tapering to capitate ends. The sample from South Africa contains an alkaline or brackish diatom assemblage dominated by *Mastogloia* G.H.K. Thwaites in W. Smith, *Plagiotropis* E. Pfitzer and several brackish *Navicula* J.B.M. Bory de Saint-Vincent species.

**North American material:** In 1981, Foged described *S. pachycephala* var. *alaskana* Foged from Alaska, USA. A specimen found on the isotype slide (Fig. 16) conforming to the original illustration and description of *S. pachycephala* var. *alaskana* belongs to *Caloneis* P.T. Cleve and is not treated further in this paper. Recently, *E. pachycephala* was found during a survey in the Florida Everglades. The taxon is rare in Florida, but the highest abundances were observed in WCA-1, a distinctive, soft-water environment within the Everglades (E. Gaiser, unpublished data).

Valves lanceolate, raphe sigmoid, axial area narrowing toward the valve ends, and capitate valve apices. The central area a distinct stauros that is slightly widened near the valve margin (Figs 17-24). Valve dimensions (n=16): length 34-46.4  $\mu\text{m}$ , breadth 6.7-8.6  $\mu\text{m}$ . length:breadth 5.2-6.2, constriction breadth 3.5-4.6  $\mu\text{m}$ , apex breadth 4.9-5.7  $\mu\text{m}$ , 27.8-29.4 striae per 10  $\mu\text{m}$ .

Samples from Florida were dominated by acidophilic species such as *Brachysira brebissonii* R. Ross in Hartley and *Frustulia crassinervia* (Brébisson) Lange-Bertalot & Krammer in Lange-Bertalot & Metzeltin.

Additional North American populations of *E. pachycephala* were found in coastal marshes in Louisiana, USA (Smith 2012). Valves lanceolate with an expanded valve center that narrowed toward a constriction that subtended capitate valve apices (Figs 25-32). Valve dimensions (n=15): length 34.8-50.3  $\mu\text{m}$ , breadth 7.0-8.3  $\mu\text{m}$ , length:breadth 5.0-6.4, breadth at constriction 3.6-4.6  $\mu\text{m}$ , breadth at apex 4.8-6.2  $\mu\text{m}$ . Striae radiate at the valve center, becoming parallel and eventually convergent at the valve ends, 26.3-30.3 striae per 10  $\mu\text{m}$ .

In Louisiana, *E. pachycephala* was found in association with diverse *Eunotia* Ehrenberg and *Pinnularia* Ehrenberg species as well as *Nitzschia obtusa* W. Smith and *Nitzschia scalaris* (Ehrenberg) W. Smith in brackish sites (Smith 2012).

**SEM observations of Australian material:** Areolae are entirely covered by external hymenes, giving the valve a smooth outer surface and obscuring the external areolar features. The raphe branches are straight, located in a shallow, narrow groove, clearly widening towards the central area (Figs 33, 34). The external proximal raphe ends are slightly enlarged and terminate in expanded pore-free regions. The external distal raphe ends are widened and hooked in opposite directions (Figs 33, 35) giving the raphe a sigmoid path. Internal raphe branches are straight and located on a raised, thickened sternum (Figs 36, 37). Internal distal raphe ends terminate on short but prominent helictoglossae, situated in an asymmetrically expanded part of the raphe sternum, most likely corresponding with the external groove (Figs 36, 37). Proximal raphe ends are short but clearly unilaterally hooked (Figs 36, 39). Striae are clearly visible in LM images and internal SEM views (Figs 36, 38). Striae are clearly bent and radiate near the central area, becoming more geniculate about 1/3 of the way toward the valve apex. Striae become parallel and even convergent at the apices. Striae are uniseriate and internally open by small, rounded areolae. The striae are situated between slightly raised



virgae and the areolae separated by very narrow struts. Striae continue uninterrupted with a few areolae on the valve mantle. The valve mantle is bordered by a large, unornamented zone that becomes smaller near the apices (Figs 33, 35, 38). The central area is formed by a thickened stauros, extending from the central nodule to the valve margins, where it merges into the valve mantle. The girdle is composed of apparently open, smooth copulae lacking perforations (Figs 38, 39).

**Taxonomic remarks:** The morphological characteristics observed in *E. pachycephala*, such as the sigmoid raphe with the unilaterally deflected internal proximal ends, external distal raphe ends deflected in opposite directions and uniseriate, radiate striae occluded by external hymenes covering the entire valve, the internal structure of the areolae, and the unperforated copulae justify its transfer to *Envekadea*. The stauros, which is easily visible in the interior the valve of *E. pachycephala*, is absent in other known *Envekadea* species (Gligora et al. 2009, Graeff et al. 2013, Lee et al. 2013b).

**Ecology:** The pH in the MacKenzie River where *E. pachycephala* was reported was circumneutral to acidic and the specific conductivity, suspended solids, and nutrient concentrations were low (Table 1). During this survey in the MacKenzie River, *E. pachycephala* was found only in the upper reaches of the MacKenzie River, especially near the Wartook Reservoir. The most common diatom genera in the MacKenzie River were *Tabellaria* Ehrenberg ex F.T. Kützing, *Navicula*, *Gomphonema* Ehrenberg, *Frustulia* Rabenhorst, *Brachysira* Kützing, *Brevisira* K. Krammer, *Eunotia*, and *Neidium* E. Pfitzer. The relative abundance of *E. pachycephala* was low (3-4%). It was found in standing or slowly flowing water.

In contrast, this species was present (maximum abundance 5.8%) in the Florida Everglades under higher specific conductances (96.9-5420  $\mu\text{S cm}^{-1}$ ) with slightly higher nutrient content (total phosphorus 163-595  $\mu\text{g/g}$ ) and pH from 5.23-7.67.

In Louisiana, the salinity optimum for *E. pachycephala* was estimated at 2.43 ppt, but the taxon was found at sites with salinities from 0.02 to 10.67 ppt (Smith 2012). The type material from South Africa showed that this species co-occurs with brackish species such as *Navicula*, *Mastogloia*, and *Plagiotropis*. These results show that *E. pachycephala* has a wide ecological tolerance, from freshwater to brackish and low to high concentrations of nutrients.

In the original description by Cleve (1881), *E. pachycephala* (as *Stauroneis*) was reported from brackish water in South Africa. In 1953, it was reported by Cleve-Euler from both Sweden and the southern hemisphere, in fresh and brackish waters (Table 2). (Cleve 1894, Hustedt 1959 ) reported it in brackish water from Tasmania and South Africa. According to (Foged 1978), *E. pachycephala* is mesohalobous and alkaliphilous (Table 2). This taxon was also reported from the Swan River Estuary in Western Australia by (John 1983). In our study, *E. pachycephala* was found in freshwater in Australia, whereas populations from Florida and Louisiana were growing in fresh to brackish conditions and across a wide nutrient spectrum. Based on its wide geographic range (including a report from Cuba (Foged 1984), it is a subtropical species that is more common in the southern hemisphere (Fig. 40).

When originally described, *Envekadea* included species inhabiting a broad ecological spectrum, from brackish to fresh waters (Gligora et al. 2009). There are relatively few diatom genera that inhabit both fresh and saline waters (e.g. *Surirella* P.J.F. Turpin, *Mastogloia*, *Navicula*, *Nitzschia* A.H. Hassall; (Round and Sims 1980); most genera are restricted to fresh or saline habitats. Our study confirmed that *Envekadea* species tolerate a wide range of salinities, but in our case, we note that a single *Envekadea* species, *E. pachycephala*, is found across a wide range of salinity and nutrient conditions. For example, *E. pachycephala* has been reported from both brackish and

freshwater in Australia and adjacent areas (Foged 1978, John 1983, Gell and Gasse 1994, Vyverman et al. 1995, Hodgson et al. 1997a). Several studies suggest that *E. pachycephala* is rare in mesohalobous (Foged 1978) or brackish habitats (John 1983), which might suggest that these habitats are simply depositional areas where freshwater populations accumulate in fluvial sediments. However, Hodgson (1995) and Hodgson et al. (1997) studied both modern and fossil collections from Tasmania and noted that *E. pachycephala* has an ecological optimum in brackish waters, and that it was most abundant in Tasmania's Lake Fidler during a brackish water phase. Clearly additional work on the ecophysiology of this taxon is warranted to define its salinity tolerance, because salinity is one of the strongest ecological gradients controlling diatom distribution (Gell & Gasse 1994, Smith 2012). Further evidence for the wide tolerance of this taxon is found in its species associations. In freshwater, it was most often associated with *Frustulia*, *Brachysira*, *Brevisira*, *Eunotia*, *Tabellaria*, *Navicula*, *Gomphonema* and *Neidium* species, while in brackish water it was associated with brackish species of *Navicula*, *Nitzschia*, *Mastogloia*, and *Plagiotropis*.

There are slight morphological differences among the worldwide populations of *E. pachycephala*. We note that the MacKenzie River and type specimens from South Africa are slightly larger than those reported elsewhere, including the new populations from Florida and Louisiana, but the full range of valves encountered (length 34.8-60.0  $\mu\text{m}$ ) encompasses the typical range of sizes expected for a single species (Edlund and Bixby 2001). The African specimens also show slight differences in valve outline, with margins that narrow more abruptly away from the gibbous valve center, with larger specimens having nearly parallel sides tapering to capitate ends. However, efforts to identify non-reducible morphological groups within the populations we studied did not provide clear separation of any subset of specimens or populations to suggest that we

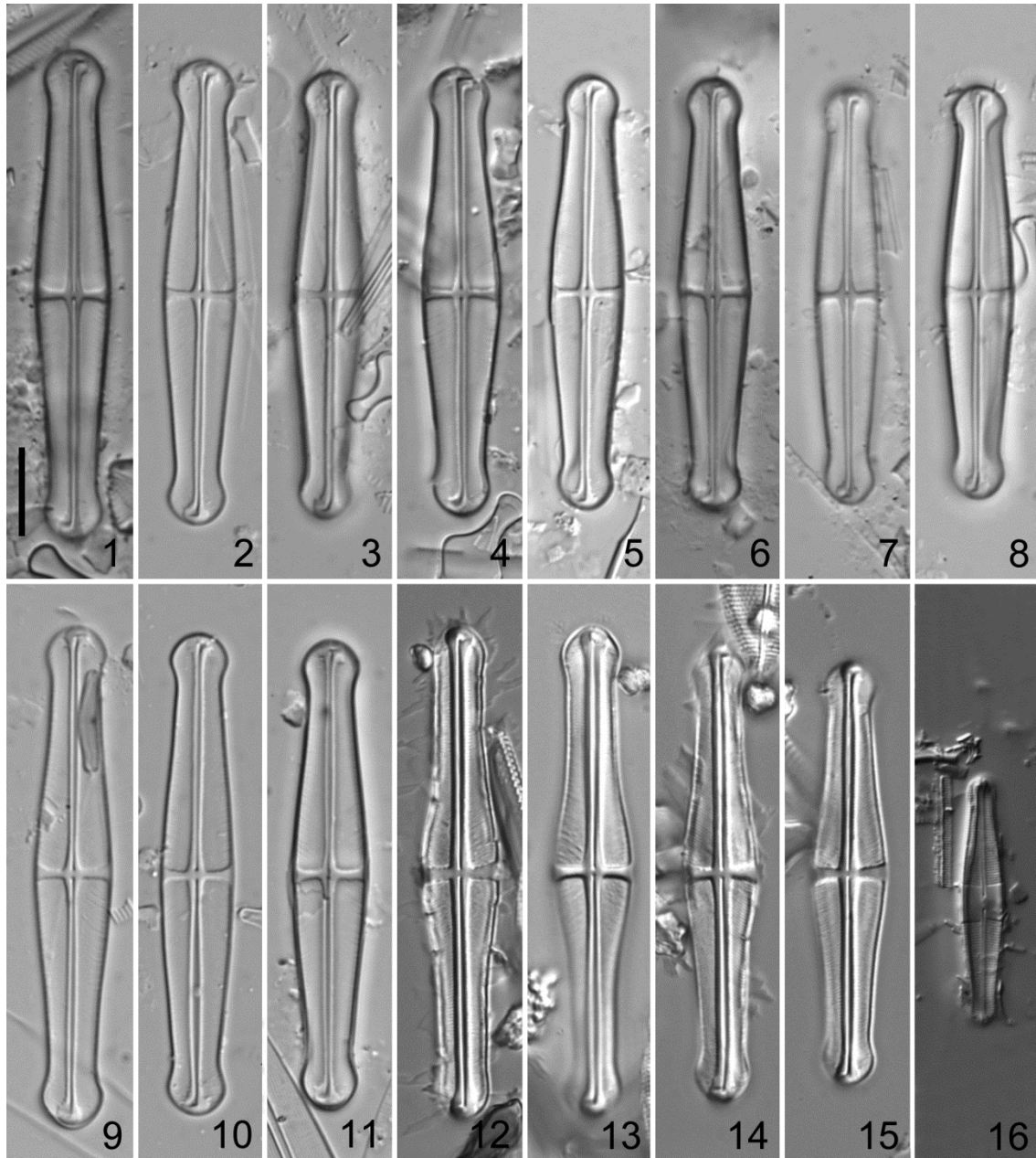
are working with more than one taxon. The broad geographic range and environmental tolerance clearly invites future efforts to consider reproductive and molecular evidence that might support the recognition of separate species within this widespread taxon. We examined the morphology, ecology and biogeography of *E. pachycephala* and demonstrated that this species has a wide geographic distribution with a broad ecological tolerance, from fresh to brackish water. However, its salinity tolerance requires further investigation. Based on its morphology, *S. pachycephala* is transferred to the genus *Envekadea* as *E. pachycephala* (P.T. Cleve) I. Atazadeh & M.B. Edlund. Until now, only five species had been included within *Envekadea* (Gligora et al. 2009, Graeff et al. 2013, Lee et al. 2013b); this taxon adds another species to the genus.

**Table 1.** Minimum and maximum values of water quality parameters measured at the Lake Wartook head gauge at the MacKenzie River from November 2011 through October (GWMWC 2012).

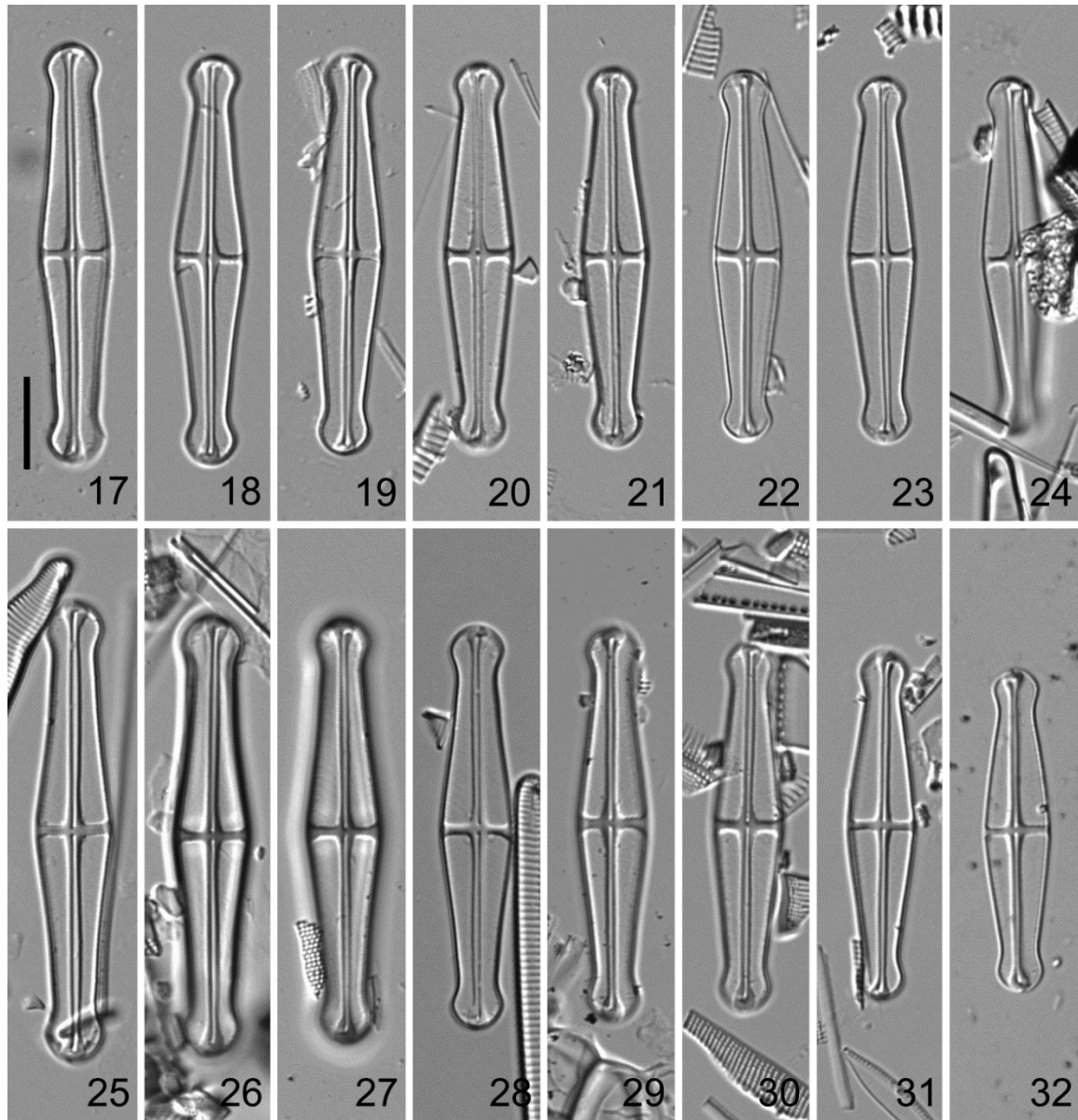
Parameter	minimum	maximum	units
pH	6.3	6.8	–
Specific conductivity	69	85	$\mu\text{S cm}^{-1}$
Ammonia, $\text{NH}_3$	0.024	0.150	$\text{mg L}^{-1}$
Soluble reactive phosphorus, SRP	<0.003		$\text{mg L}^{-1}$
Total Kjeldahl nitrogen, TKN	0.007	0.015	$\text{mg L}^{-1}$
Total phosphorus, TP	0.007	0.015	$\text{mg L}^{-1}$
Nitrate-Nitrite, $\text{NO}_x$	<0.003	0.014	$\text{mg L}^{-1}$
Chlorophyll- <i>a</i>	3.2	12	$\mu\text{g L}^{-1}$

**Table 2.** Morphological and ecological characteristics of *Envekadea pachycephala* populations. NSW = New South Wales, QLD = Queensland, PNG = Papua New Guinea

<i>Envekadea pachycephala</i>					
Author (year)	Length (µm)	Width (µm)	Stria density (#/10 µm)	Habitat	Locality
(Cleve and Moller 1877-1882)	44.8-60.0	8.0-10.0	24.4-29.43	Brackish	S. Africa
Cleve 1894	40-55	7-9	29	Brackish	Tasmania, S. Africa
Cleve-Euler 1953	40-55	7-9	29	Fresh-brackish	Southern Hemisphere, Sweden
Hustedt 1959	40-60	7-9	30	Brackish	Tasmania, S. Africa
Foged 1976	35-41	7-8	30-35	Halophilous, alkaliphilous	Sri Lanka
Foged 1978	35	6.5	30	Mesohalobous, alkaliphilous	NSW, QLD
Foged 1979	46	7	-	Oligohalobous, alkaliphilous	New Zealand
John 1983	36-40	6-7	30	Freshwater	Western Australia
Foged 1984	40	8	dense	Oligo- to mesohalobe, alkaliphile	Cuba
Vyverman 1991	44-45	7.9-8.1	28	Mesohalobous, alkaliphilous	PNG
Gell & Gasse 1994	40-60	7-9	30	Freshwater	Victoria
Hodgson 1995	52.5	7.7	-	Fresh-brackish	Tasmania
Hodgson et al. 1997	33	5	30	Brackish	Tasmania
(this study)	43.1-58.0	7.0-9.0	27.0-30.3	Freshwater	Victoria, Australia
(this study)	38.8-45.6	6.9-7.8	27.8-29.4	Freshwater, alkaline	Florida
(this study)	34.8-50.3	7.0-8.3	26.3-30.3	Fresh-brackish	Louisiana



**Figs 1-16.** *Envekadea* species, light micrographs, DIC, scale bar = 10  $\mu$ m. **Figs 1-8.** *Envekadea pachycephala* MacKenzie River, Wartook Outlet, Victoria, Australia (ANSP GC40128, CANA 87195, BR-4323). **Figs 9-11.** *Envekadea pachycephala* Wartook Reservoir, Victoria, Australia (ANSP GC40129, BR-4324). **Figs 12-15.** *Envekadea pachycephala*, Cl. & Møller exsiccatum Nr. 197 (ANSP Cleve & Moller 197), South Africa, Cape of Good Hope, Baakens River, Port Elizabeth. **Fig. 13.** Lectotype specimen. **Fig. 16.** Isotype slide of *Stauroneis pachycephala* var. *alaskana* Foged (1981) from ANSP GC64419 (Kuzitrin Lake, Alaska); this taxon belongs in the genus *Caloneis*.

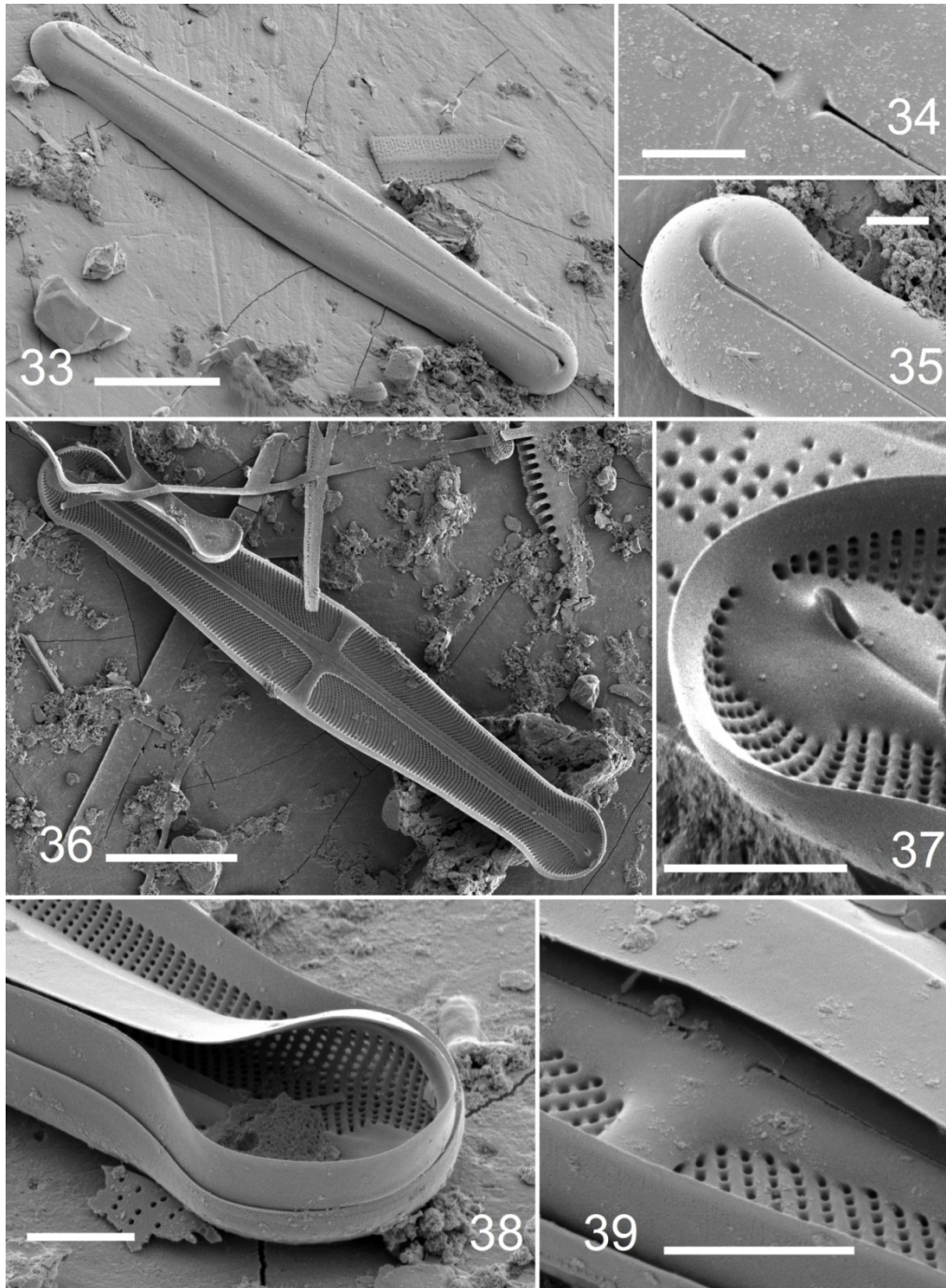


**Figs 17-32.** Size diminution series of *Envekadea pachycephala* from Florida Everglades and Louisiana, USA, light micrographs, DIC. All figures as same scale; scale bar (Fig. 17) = 10  $\mu$ m.

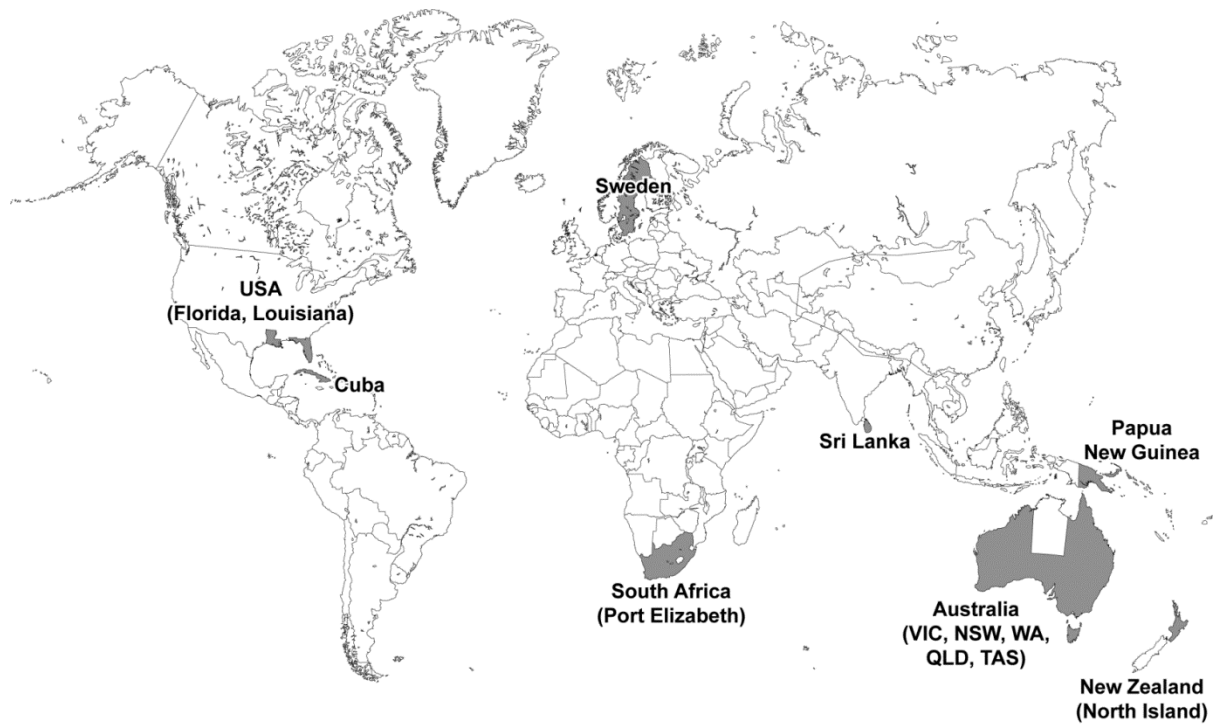
**Figs 17-24.** *Envekadea pachycephala*, Florida Everglades, USA (ANSP GC59136).

**Figs 25-32.** *Envekadea pachycephala*, Louisiana, USA (ANSP GC65210).





**Figs 33-39.** *Envekadea pachycephala*, MacKenzie River, Australia. SEM. **Fig. 33.** External valve view. **Fig. 34.** Central part of the valve with expanded proximal raphe ends, external view. **Fig. 35.** External distal raphe end. **Fig. 36.** Internal valve view. **Fig. 37.** Valve apex showing distal raphe end and helictoglossa. **Fig. 38.** Internal view of valve end showing unornamented valvocopula. **Fig. 39.** Central part of the valve with unilaterally deflected proximal raphe ends, internal view. Scale bars = 10  $\mu\text{m}$  (Figs 33, 36) and 2  $\mu\text{m}$  (Figs 34, 35, 37-39).



**Fig. 40.** World-wide distribution of the records for *Envekadea pachycephala* (shaded area). South Africa (Cleve et Moller 1881, Cleve 1894, Hustedt 1959 ), Australia (Cleve 1894, Hustedt 1959, Foged 1978, John 1983,1993, Gell & Gasse 1994, Hodgson 1995, Vyverman et al. 1995, Hodgson et al. 1997, Haynes et al. 2007, Taukulis & John 2009). New Zealand (Foged 1979), Sri Lank (Foged 1976) Cuba (Foged 1984) Papua New Guinea (Vyverman 1991), Sweden (Cleve-Euler 1953). In the present study, it is reported from Victoria (Australia) and Florida and Louisiana (USA).

**Appendix D:** List of all diatom species and their taxonomic authorities identified during this research in the MacKenzie River.

Code	Species Name	Taxonomic Authority
AcInf	<i>Achnanthes inflata</i>	(Kützing) Grunow 1868
AcMin	<i>Achnanthidium minutissimum</i>	(Kützing) Czarnecki 1994
AsFor	<i>Asterionella formosa</i>	Hassall 1850
AuAmb	<i>Aulacosira ambigua</i>	(Grunow) Simonsen 1979
AuDis	<i>Aulacoseira distans</i>	(Ehrenberg) Simonsen 1979
AuLac	<i>Aulacoseira lacustris</i>	(Grunow) Krammer 1991
AuSub	<i>Aulacoseira subarctica</i>	(Müller) Haworth 1990
BrBre	<i>Brachysira brebissonii</i>	Ross in Hartley 1986
BrCal	<i>Brachysira calligraphica</i>	Lange-Bertalot 1994
BrIra	<i>Brachysira irawanoidea</i>	Lange-Bertalot & Gerd Moser 1994
CaUnd	<i>Caloneis undosa</i>	Lange-Bertalot & Krammer 1987
CaVen	<i>Caloneis ventricosa</i>	(Ehrenberg) Meister 1912
CoPla	<i>Cocconies placentula</i>	Ehrenberg 1838
CrCus	<i>Craticula cuspidata</i>	(Kützing) D.G.Mann 1990
CsTho	<i>Cyclostephanos tholiformis</i>	Stoermer, Håkansson & Theriot 1988
CyMen	<i>Cyclotella meneghiniana</i>	Kützing 1844
CyRos	<i>Cyclotella rossii</i>	Håkansson 1990
CmAsp	<i>Cymbella aspera</i>	(Ehrenberg) Cleve 1894
CmCis	<i>Cymbella cistula</i>	(Ehrenberg) Kirchner 1878
CmLan	<i>Cymbella lanceolata</i>	(Agardh) Kirchner 1878
CbCus	<i>Cymboppleura cuspidata</i>	(Kützing) Krammer 2003
CbNav	<i>Cymboppleura naviculiformis</i>	(Auerswald) Krammer 2003
CbSpp	<i>Cymboppleura sp.</i>	(Krammer) Krammer 1997
CbSub	<i>Cymboppleura subanglica</i>	Krammer 2003
DtSpp	<i>Diatoma sp.</i>	Bory de Saint-Vincent 1824
DtTen	<i>Diatoma tenuis</i>	Agardh 1812
DpSub	<i>Diplonies subovalis</i>	(Hilse) Cleve 1891
DsSte	<i>Discostella stelligera</i>	(Cleve & Grunow) Houk & Klee 2004
EnMin	<i>Encyonema minutum</i>	(Hilse) D.G.Mann 1990
EnSpp	<i>Encyonema sp.</i>	Kützing 1834
EnPac	<i>Envekadea pachycephala</i>	(Cleve) Atazadeh & Edlund 2014
EpSor	<i>Epithemia sorex</i>	Kützing 1844
EuBig	<i>Eunotia bigibba</i>	Kützing 1849
EuBil	<i>Eunotia bilunaris</i>	(Ehrenberg) Schaarschmidt 1881
EuCar	<i>Eunotia carolina</i>	Patrick 1958
EuCur	<i>Eunotia curvata</i>	(Kützing) Lagerstedt 1884
EuDio	<i>Eunotia diodon</i>	Ehrenberg 1837
EuExi	<i>Eunotia exigua</i>	(Brébisson in Kützing) Rabenhorst 1864
EuFab	<i>Eunotia faba</i>	Ehrenberg 1837
EuFal	<i>Eunotia fallax</i>	Cleve 1895
EuFle	<i>Eunotia flexuosa</i>	(Brébisson ex Kützing) Kützing 1849
EuHex	<i>Eunotia hexaglyphis</i>	Ehrenberg 1854
EuMin	<i>Eunotia minor</i>	(Kützing) Grunow 1881
EuPec	<i>Eunotia pectinalis</i>	Kützing) Rabenhorst 1864
EuRho	<i>Eunotia rhomboidea</i>	Hustedt 1950
EuSer	<i>Eunotia serpentina</i>	Ehrenberg 1854

EuSer	<i>Eunotia serra</i>	Ehrenberg 1837
EuSud	<i>Eunotia sudetica</i>	Müller 1898
EuTri	<i>Eunotia triodon</i>	Ehrenberg 1837
FrAci	<i>Fragilaria acidobiontica</i>	(Charles) Williams and Round 1988
FrCap	<i>Fragilaria capucina</i>	Desmazières 1830
FrVau	<i>Fragilaria vaucheriae</i>	(Kützing) J.B.Petersen 1938
FsBla	<i>Frustulia blanchiana</i>	Maillard 1978
FsRho	<i>Frustulia rhomboides</i>	(Ehrenberg) De Toni 1891
FrSpp	<i>Frustulia</i> sp.	Rabenhorst 1853
FrVul	<i>Frustulia vulgaris</i>	(Thwaites) De Toni 1891
GoAcu	<i>Gomphonema acuminatum</i>	Ehrenberg 1832
GoAff	<i>Gomphonema affine</i>	Kützing 1844
GoAng	<i>Gomphonema angustatum</i>	(Kützing) Rabenhorst 1864
GoGra	<i>Gomphonema gracile</i>	Ehrenberg 1838
GoOli	<i>Gomphonema olivaceum</i>	(Hornemann) Brébisson 1838
GoSpp	<i>Gomphonema</i> sp.	Ehrenberg 1832
GoCla	<i>Gomphonema clavatum</i>	Ehrenberg 1832
GyAcu	<i>Gyrosigma acuminatum</i>	(Kützing) Rabenhorst 1853
GyAtt	<i>Gyrosigma attenuatum</i>	(Kützing) Rabenhorst 1853
HaAmp	<i>Hantzschia amphioxys</i>	(Ehrenberg) Grunow 1880
KaObl	<i>Karayevia oblongella</i>	(Østrup) Aboal 2003
LuMut	<i>Luticola mutica</i>	(Kützing) Mann 1990
MIAre	<i>Melosira arentii</i>	(Kolbe) Nagumo & Kobayashi 1977
MeCir	<i>Meridion circulare</i>	(Greville) Agardh 1831
NaCry	<i>Navicula cryptocephala</i>	Kützing 1844
NaGre	<i>Navicula gregaria</i>	Donkin 1861
NaHei	<i>Navicula heimansioides</i>	Lange-Bertalot 1993
NaInc	<i>Navicula incertata</i>	Lange-Bertalot 1985
NaLan	<i>Navicula lanceolata</i>	Ehrenberg 1838
NaRad	<i>Navicula radiosa</i>	Kützing 1844
NaRhy	<i>Navicula rhynchocephala</i>	Kützing 1844
NaVir	<i>Navicula viridula</i>	Ehrenberg 1836
NeAff	<i>Neidium affine</i>	(Ehrenberg) Pfitzer 1871
NeApi	<i>Neidium apiculatum</i>	Reimer 1959
NeBoy	<i>Neidium boyeri</i>	Reimer 1959
NeIri	<i>Neidium iridis</i>	(Ehrenberg) Cleve 1894
NiAgn	<i>Nitzschia agnita</i>	Hustedt 1957
NiCao	<i>Nitzschia capitellata</i>	Hustedt 1922
NiCla	<i>Nitzschia clausii</i>	Hantzsch 1860
NiDiss	<i>Nitzschia dissipata</i>	(Kützing) Rabenhorst 1860
NiGra	<i>Nitzschia gracilis</i>	Hantzsch 1860
PiAba	<i>Pinnularia abaujensis</i>	(Pantocsek) R.Ross 1947
PiBor	<i>Pinnularia borealis</i>	Ehrenberg 1843
PiBra	<i>Pinnularia braunii</i>	Cleve 1895
PiTab	<i>Pinnularia</i> cf. <i>tabellaria</i>	Ehrenberg 1843
PiDiv	<i>Pinnularia divergens</i>	Smith 1853
PiInt	<i>Pinnularia interrupta</i>	Smith 1853
PiSim	<i>Pinnularia similis</i>	Hustedt 1937
PiSub	<i>Pinnularia subcapitata</i>	Gregory 1856
PiBir	<i>Pinnularia viridiformis</i>	Krammer 1992
PIFre	<i>Planothidium frequentissimum</i>	(Lange-Bertalot) Lange-Bertalot 1999
PmAbu	<i>Psammothidium abundans</i>	(Manguin) Bukhtiyarova & Round 1996
PmCon	<i>Psammothidium confusum</i>	(Manguin) van de Vijver 2002

PsBre	<i>Pseudostaurosira brevistriata</i>	(Grunow) Williams & Round 1988
RhBre	<i>Rhopalodia brebissonii</i>	Krammer 1987
SePup	<i>Sellaphora pupula</i>	(Kützing) Mereschkovsky 1902
SfExi	<i>Stauroforma exiguiiformis</i>	(Lange-Bertalot) Flower, Jones & Round 1996
StAcu	<i>Stauroneis acuta</i>	Smith 1853
StAnc	<i>Stauroneis anceps</i>	Ehrenberg 1843
StKri	<i>Stauroneis kriegeri</i>	Patrick 1945
StPho	<i>Stauroneis phoenicenteron</i>	(Nitzsch) Ehrenberg 1843
StCon	<i>Staurosira contruens</i>	Ehrenberg 1843
StEll	<i>Staurosira elliptica</i>	(Schumann) Williams & Round 1987
SsPin	<i>Staurosirella pinnata</i>	(Ehrenberg) Williams & Round 1988
SpAnc	<i>Stenopterobia anceps</i>	(Lewis) Brébisson ex Van Heurck 1896
SpCur	<i>Stenopterobia curvula</i>	(Smith) Krammer 1987
SpDel	<i>Stenopterobia delicatissima</i>	(Lewis) Brébisson ex Van Heurck 1896
SuAng	<i>Surirella angusta</i>	Kützing 1844
SuEle	<i>Surirella elegans</i>	Ehrenberg 1843
SuLin	<i>Surirella linearis</i>	Smith 1853
SyAcu	<i>Synedra acus</i>	Kützing 1844
SyRum	<i>Synedra rumpens</i>	Kützing 1844
SySub	<i>Synedra subrhombica</i>	Nygaard 1954
TaFen	<i>Tabellaria fenestrata</i>	(Lyngbye) Kützing 1844
TaFlo	<i>Tabellaria flocculosa</i>	(Roth) Kützing 1844
TaVen	<i>Tabellaria ventricosa</i>	Kützing 1844

**Appendix E:** List of all soft algae species and their taxonomic authorities identified during this research in the MacKenzie River.

Code	Species Name	Taxonomic Authority
AnMac	<i>Anabaena macrospora</i>	Klebahn 1895
AnFlos	<i>Anabaena flos-aquae</i>	Brébisson ex Bornet & Flauhault 1886
AkFal	<i>Ankistrodesmus falcatus</i>	(Corda) Ralfs 1848
BaBre	<i>Bambusina brebissonii</i>	Kützing ex Kützing 1849
BuPyg	<i>Bulbochaete pygmaea</i>	Kargupta et al. 1977
CaRef	<i>Campylomonas reflexa</i>	(Marsson) Hill 1991
CeCor	<i>Ceratium cornutum</i>	(Ehrenberg) Claparède & Lachmann 1859
CeFur	<i>Ceratium furcoides</i>	(Levander) Langhans 1925
ChVul	<i>Chara vulgaris</i>	Wallroth 1815
ChSpp	<i>Chara</i> sp.	Linnaeus 1753
ClVul	<i>Chlorella vulgaris</i>	Beyerinck [Beijerinck] 1890
CrTur	<i>Chroococcus turgidus</i>	(Kützing) Nägeli 1849
CdGlo	<i>Cladophora glomerata</i>	(Linnaeus) Kützing 1843
CoSpp	<i>Closterium</i> sp.	
CoEhr	<i>Closterium ehrenbergii</i>	Meneghini ex Ralfs 1848
CoTum	<i>Closterium tumidulum</i>	Gay 1884
CsCir	<i>Cosmarium circulare</i>	Reinsch 1867
CsSpo	<i>Cosmarium spotella</i>	Brébisson ex Kützing 1849
CsDep	<i>Cosmarium depressum</i>	(Nägeli) Lundell 1871
CsJav	<i>Cosmarium javanicum</i>	Nordstedt 1880
CySpp	<i>Cryptomonas</i> sp.	Ehrenberg 1832
DiDiv	<i>Dinobryon divergens</i>	Imhof 1887
DiSer	<i>Dinobryon sertularia</i>	Ehrenberg 1834
EuAns	<i>Euastrum ansatum</i>	Ehrenberg ex Ralfs 1848
EuSpp	<i>Euastrum</i> sp.	Ralfs 1848
EuDiv	<i>Euastrum divergens</i>	Joshua 1886
EgAcu	<i>Euglena acus</i>	(Müller) Ehrenberg 1830
GyAus	<i>Gymnodinium australicum</i>	Playfair 1919
GoPec	<i>Gonium pectorale</i>	Müller 1773
LySpp	<i>Lyngbya</i> sp.	Agardh Ex Gomont 1892
LyHie	<i>Lyngbya hieronymusii</i>	Lemmermann 1905
MePun	<i>Merismopedia punctata</i>	Meyen 1839
MiAla	<i>Micrasterias alata</i>	Wallich 1860
MoSub	<i>Monoraphidium subclavatum</i>	Nygaard 1977
NiFur	<i>Nitella furcata</i>	(Roxburgh ex Bruzelius) Agardh 1824
NiSpp	<i>Nitella</i> sp.	Agardh 1824
NoSpu	<i>Nodularia spumigena</i>	Mertens ex Bornet & Flahault 1888
NsLin	<i>Nostoc</i> cf. <i>linckia</i>	Bornet ex Bornet & Flahault 1886
OeMon	<i>Oedogonium monile</i>	Braun ex Hirn 1900
OeUnd	<i>Oedogonium undulatum</i>	Braun ex Hirn 1900
OoPar	<i>Oocystis parva</i>	West & West 1898
OoPus	<i>Oocystis pusilla</i>	Hansgirg 1890
OsLim	<i>Oscillatoria limosa</i>	Agardh ex Gomont 1892
OsSpp	<i>Oscillatoria</i> sp.	Vaucher ex Gomont 1822
OsAnn	<i>Oscillatoria annae</i>	Goor 1918
OsTer	<i>Oscillatoria terebriformis</i>	Agardh ex Gomont 1892

OsIrr	<i>Oscillatoria irrigua</i>	Kützing ex Gomont 1892
PdAng	<i>Pediastrum anguslosum</i>	Ehrenberg ex Meneghini 1840
PdDup	<i>Pediastrum duplex</i>	Meyen 1829
PeLom	<i>Peridinium lomnickii</i>	Lindemann
PeRac	<i>Peridinium raciborskii</i>	Woloszynska 1912
PhAut	<i>Phormidium autumnale</i>	Gomont 1892
PhPla	<i>Phormidium playfairi</i>	
RhSpp	<i>Rhizoclonium</i> sp.	Kützing 1843
ScArm	<i>Scenedesmus armatus</i>	(Chodat) Chodat 1913
ScGra	<i>Scenedesmus gracilis</i>	Matvienko 1938
ScAcu	<i>Scenedesmus acutus</i>	Meyen 1829
ScAcu	<i>Scenedesmus acuminatus</i>	(Lagerheim) Chodat 1902
ScOpo	<i>Scenedesmus opoliensis</i>	Richter 1895
ScObl	<i>Scenedesmus obliquus</i>	(Turpin) Kützing 1833
SzSpp	<i>Schizothrix</i> sp.	Kützing ex Gomont 1892
SyInf	<i>Spirogyra inflata</i>	(Vaucher) Dumortier 1822
SdMeg	<i>Staurodesmus megacanthus</i>	(Lundell) Thunmark 1948
StJab	<i>Staurastrum javanicum</i>	Turner 1893
StSmi	<i>Staurastrum smithii</i>	Teiling 1946
StPin	<i>Staurastrum pinnatum</i>	Turner 1893
StGra	<i>Staurastrum gracile</i>	Ralfs ex Ralfs 1848
StEle	<i>Staurastrum elegans</i>	Borge 1896
SgFla	<i>Stigeoclonium flagelliferum</i>	Kützing 1849
UISpp	<i>Ulothrix</i> sp.	Kützing 1833