Phytoplankton Dynamics of Riverine Water Holes in the Dry Tropics of North Queensland, Australia

Thesis submitted by
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Abstract

Microalgae are key components of aquatic food webs and biodiversity, yet dynamics of algal assemblages in tropical systems are largely unknown. This study investigated algal assemblages and physico-chemical properties of remnant riverine water holes in the Burdekin River catchment, which is located in the seasonal Australian tropics. A seasonal survey was conducted of three sites in three rivers, followed by a more detailed study on one site at each river. Principle component analyses revealed significant differences in physico-chemical parameters between rivers and seasons. Non-metric multi-dimensional scaling demonstrated compositional differences in phytoplankton assemblages between seasons and rivers, and showed that conductivity and temperature were major physico-chemical correlates contributing to the observed differences. Since sites differed substantially in turbidity, it was expected that turbidity would be important in determining phytoplankton assemblages. Mulitvariate statistics confirmed that turbidity had a significant effect on phytoplankton assemblages. Although phytoplankton assemblages differed between season and river, the differences were not systematic. At a fine-scale within a single water hole, season had a large influence on phytoplankton assemblages at each site. Season, in the dry tropics, encompasses not only a change in temperature, but also levels of irradiance and flow regimes. Phytoplankton assemblages are largely governed by a rather obvious suite of physical and chemical variables, especially relating to seasonal change in, flow and temperature, and such factors as turbidity, pH and nutrient concentrations, which relate to lithology, soils and land use. A majority of the study sites showed dominance of Chlorophyta, followed by Cyanobacteria and diatoms. Overall, 138 phytoplankton species were identified; of these Chlorophyta were most species rich and Cyanobacteria were most abundant.

Major drivers of the phytoplankton assemblages identified from the field analyses – temperature and conductivity – were tested under a controlled environment on three monoclonal species of *Scenedesmus – S. quadricauda, S. dimorphus,* and *S. ellipticus* – cultured from field collections,. The conductivity experiments established significant differences in growth rates between the species. The temperature experiments revealed significant differences in growth rates between the species, although this was mainly due to the large growth rate changes of *S. ellipticus* at extreme temperatures (below 16° C and above 30°C). The laboratory experiments were designed to validate the identified drivers of phytoplankton community composition in the field. However, growth responses of *Scenedesmus* spp. to either temperature or conductivity changes alone under laboratory conditions did not show the same strong correlations as seen in the field, but in combination with main effect ANOVAs of field data the main drivers identified by multivariate statistics were confirmed.

The results provide an improved understanding of phytoplankton dynamics in seasonal tropical rivers. They are patchy systems, driven by both predictable (seasonal) change and stochastic events (flood, drought). Water holes develop their own characteristic assemblages, according to local factors, but those within rivers are more similar than those between rivers, as a result of underlying differences in catchment characteristic. The influences of water quality and other variables therefore operate at catchment scales for part of the annual cycle, but at a local scale for the majority of it. The tolerance of phytoplankton species to environmental parameters, especially those that can be linked to river health, needs further investigation. Currently, the use of algal assemblages for regular or routine assessment of tropical river health is not practical because of their patchy and variable distribution.

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This thesis is dedicated to and in memory of my mother
Diana Ruth Hunter (1950-2006).

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Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Carrie K. Preite	May 2009
	Date

Chapter 1 . Introduction

1.1 Introduction

Australian dry tropical rivers are highly active hydrologically, being flushed by wet-season floods, then typically receding into lentic water holes during the long dry season. They represent the major aquatic habitat over much of Australia's dry tropics, and the most important source of free water for terrestrial animals, both wild and domestic. However, we have only limited knowledge of the ecology of these systems, especially of their plant assemblages. This study compared algal assemblages in relation to physico-chemical properties in three tributaries of the Burdekin River, and investigated their dynamics over a 2.5 year period. Following extensive and intensive field studies, it sought to link explicitly key physico-chemical variables with selected algal species, by means of laboratory experiments. It also aimed to assess the likely utility of algal assemblages as monitors of ecosystem health of the water holes. Because of the flooding-drying cycle, it is expected that physico-chemical parameters will vary substantially over the seasons; it is also expected that water holes in tributaries with different catchment characteristics will have different water quality characteristic and algal assemblages. Further, it is expected that as algal assemblages will reflect physico-chemical conditions, they might be of great utility in assessing ecosystem health. These predictions were investigated through detailed examination of the algae and their biophysical environment.

1.2 Dryland rivers

Streams and rivers in dry tropical inland Australia are extremely variable in flow regimes. Therefore most of these rivers produce a lotic environment in the wet season followed by a lentic environment in the dry season. Seasonal change between flowing rivers and riverine water holes may impact on the water quality and phytoplankton communities. Flow condition is important for suspension and transport of organisms, nutrients and solids, and it can also destratify the water course, which may decrease light transmission under some circumstances (Harris 1986). No flow conditions during the dry season results in stratification of water holes. Stratification divides the water column into distinct strata based on light, temperature and dissolved oxygen. In a stratified body of water the epilimnion is characterized by higher levels of dissolved oxygen and light, but decreasing nutrients, while the hypolimnion normally receives less light, is lower in dissolved oxygen, but nutrient concentrations are higher. Therefore stratification affects phytoplankton communities and water quality (Reynolds 1984). While Reynolds (1984) refers mainly to lakes, stratification can occur in much smaller water bodies in the tropics (Pearson 2007).

Land masses of the southern hemisphere are characteristic in having variable precipitation (Simpson et al. 1993). Australian rivers tend to have low discharge rates compared to other global

regions (Boulton and Brock 1999). They are also extremely variable in flow. Hydrological variability is a feature of dryland rivers. In dryland or arid regions, such as the dry tropics of Australia, hydrological variability is often associated with highly variable rainfall and low runoff (Finlayson and McMahon 1988, Thoms and Sheldon 2000). Dryland rivers and temperate rivers respond differently to hydrological changes, both physically and biologically (Davies et al. 1994).

Few studies have analyzed phytoplankton communities of dry tropical inland rivers in Australia and most regional water quality monitoring has been restricted to reservoirs. The use of phytoplankton for monitoring these environments is uncommon. However, it has been pointed out that phytoplankton are a useful tool for assessing long-term changes in rivers such as those associated with eutrophication, river management, changes in land use at the scale of watershed, and climate changes (Prygiel et al. 1999). Studies using phytoplankton for water quality monitoring have shown that changes in composition reflect not only variations in water quality, but also changes in physical variables and biotic interactions (O'Farrell et al. 2002). Ibelings et al. (1998) emphasized that phytoplankton seems a useful biological indicator because it responds quickly to changes in environmental conditions thus enabling a quick assessment of water quality. While structures and seasonal successions of river phytoplankton communities and interacting water quality parameters are well known from other regions (particularly temperate), such interactions are largely unknown for dry tropical inland rivers in Australia.

1.3 Australian river phytoplankton

The majority of Australia's dry tropical regions adhere to a 'boom and bust' ecology model (Walker et al. 1997). The patterns and processes in these systems are governed by extremes in season and favor opportunistic species which are able to endure the harsh climate and adapt quickly. In contrast, regions that obtain higher rainfall tend to support a higher biodiversity of flora and fauna and their interactions such as competition and predation determine the patterns of distribution and abundance more so than climatic changes (Walker et al. 1997).

Phytoplankton research in Australia has focused on temperate regions, especially the Murray-Darling River basin, the largest in Australia. As the Murray-Darling Rivers occur in a temperate region, these rivers do not succumb to the 'boom and bust' ecology put forth by Walker et al. (1997). The Murray-Darling River does experience cyanobacteria blooms, such as the toxic bloom of *Anabaena circinalis* in the Darling and Barwon Rivers in 1991/92 (Bowling and Baker 1996). Shiel et al. (1982) found the flows of the Murray River are closely regulated and are dominated by blooms of cyanobacteria (*Anabaena* and *Microcystis* spp.) in the summer and diatoms (*Melosira* and *Cyclotella* spp.) in winter and spring. Phytoplankton communities of the River Murray are dominated by *Aulocoseira granulata*, which was distributed along the length of

the river for the majority of the year, followed closely by small centric diatoms, e.g. *Cyclotella* spp. (Sullivan et al. 1988). Over 150 phytoplankton species were recorded from 1980-1985. The most common green algae belonged to the Chlorococcales – *Ankistrodesmus falcatus*, *Actinastrum* sp. and *Scenedesmus* spp. – and the most common cyanobacteria genera observed were *Anabaena*, *Aphanizomenon*, *Anabaenopsis* and *Microcystis.* Few phytoplankton species occurred in high numbers along the entire length of the river. The majority of the 14 taxa exhibited higher or lower diversities in different sections of the river, all sections having the highest diversity in the winter (Sullivan et al. 1988). Gell et al. (2002) established that the input into wetlands from the Murray River was dominated by *Aulocoseira* species. However, evapo-concentration shifted the diatom assemblages to be dominated by salinity tolerant species.

A phytoplankton monitoring program within the Darling River between 1980-1992, in connection with water quality and flow monitoring (Hotzel and Croome 1994), established that Chlorophytes were most abundant (particularly *Scenedesmus, Ankistrodesmus, Chlamydomonas, Planctonema, Didymocystis* and *Oocystis*), followed by diatoms (*Aulocoseira granulata, M. distans,* and *Nitzschia*), then cyanobacteria (principally *Anabaena, Nostoc, Aphanocapsa, Merismopedia* and *Microcystis*). Cell density and community composition seemed to be more related to flow and turbidity rather than temperature cycles and nutrient availability (Hotzel and Croome 1994). The occurrence of peaks in cyanobacteria abundance appeared to be an opportunistic response to conditions of lower flow, turbulence and turbidity in water with a high nutrient status. The phytoplankton community dominated by chlorophytes and diatoms with opportunistic cyanobacterial peaks of the Darling River is similar to that reported for the Murray River from 1980-85 (Sullivan et al. 1988).

In comparison, phytoplankton assemblages in Australian arid regions have received little attention (Costelloe et al. 2005). In central Australian river systems aquatic macrophytes tend to be species-poor (Roberts 1988), but as in other aquatic systems, algae are a fundamental part of the food chain and play a predominant role in carbon fixation in these systems (Bunn et al. 2003). A total of 237 phytoplankton species were reported in rivers of the Lake Eyre Basin, each river had similar numbers of genera and similar assemblages (Costelloe et al. 2005). Phytoplankton diversity in these rivers was found to be highest during or immediately following flood events, which might be generated by germination of phytoplankton cysts from channel and floodplain sediments, transport from aquatic refuges and coupled to nutrient influx and seasonal temperature increases (Costelloe et al. 2005).

1.4 Tropical river water quality and phytoplankton

1.4.1 Tropical river water quality

River water contains dissolved and suspended inorganic matter including micro-and macro-nutrients, heavy metals and other trace elements, dissolved organic matter, dissolved ions (e.g., Ca^{2+}, Na^{+}, HCO_3^{-}, Cl^{-}) and dissolved gasses. Given the short residence time and small volume in the hydrological cycle, river water plays a disproportionately large role interacting with dissolved ions, sediments, nutrients, gases and other materials, which renders the water prone to pollution from material washed in from the catchment or entering via groundwater or direct deposition from the air (Boulton and Brock 1999). However, many tropical rivers are characteristically non-flowing at certain times of the year, therefore the residence time of the water can be long, and local factors rather than upstream factors can have greater influence on the parameters mentioned. Bicarbonate and carbonate are important in aquatic ecosystems because they represent the carbon needed for photosynthesis. Tropical rivers tend to have lower total concentrations of bicarbonate and carbonate than temperate rivers (Payne 1986). The ratio of carbon dioxide and carbonate to bicarbonate in water is a function of pH (Wetzel and Likens 2000). Tropical rivers tend to have low salt content, more sodium than calcium, high silica and iron contents, and a pH within the range 4.3-7.5 (Hart and McKelvie 1986). However, factors such as rainfall, rock, soil and vegetation, which determine these characteristics, vary between tropical regions, as well as cause significant differences within the same river system (e.g., the Amazon River – (Payne 1986)). The concentrations of ions in tropical rivers of Australia do not follow the same patterns as other tropical rivers around the world. Sodium, carbonate and chloride ions tend to dominate (Boulton and Brock 1999) and sulfate tends to be higher in inland rivers of tropical Queensland (McNeil 1998).

Visser (1961) estimated that rain water contributed all of the sodium, ammonium and nitrate, 75% of the chloride and about 70% of the potassium in rivers of Kampala, Uganda. Estimates for some tributaries of the Amazon River indicate that precipitation contributes 81% of the ions as opposed to 19% from rocks (Gibbs 1970). In some tropical rivers, atmospherically transported dust plays a large role by contributing up to 90% of Ca^{2+} and HCO_3^{-}, 80% of Mg^{2+}, K^{+} and SO_4^{2-} and only 30% of the Na^{+} ions (Boulton and Brock 1999). Floods are another key factor in the distribution and transport of ions, especially in tropical rivers, which are subject to monsoonal flooding. Hart and McKelvie (1986) found a marked difference in conductivity in rivers of northern Australia between small floods early in the wet season and a large flushing flood. They attributed the difference to changes in the ionic composition influenced by the composition and volumes of surface water, interflow and groundwater.

Floods transport the majority of the nutrients in most rivers (Boulton and Brock 1999). Consequently, the movement of water, sediments and nutrients is seasonal and very often sporadic (Brodie and Mitchell 2005). Nutrients are assumed to travel in three forms: dissolved, fine particulate and coarse particulate (Boulton and Brock 1999). River systems in South America running through forested regions export considerable concentrations of dissolved organic nitrogen and phosphorus during flood events (Seitzinger and Sanders 1997), but very low concentrations of inorganic nitrogen and phosphorus (Lewis et al. 1999). Studies of tropical regions found that runoff and subsurface drainage (from pristine systems) generally have low concentrations of nitrogen (50-400 μgL^{-1}), mainly dissolved organic nitrogen (DON), with low concentrations of particulate nitrogen (PN) and dissolved inorganic nitrogen (DIN), where most of the DON is unavailable for algal utilization in the short term (Seitzinger and Sanders 1997, Aitkenhead and McDowell 2000). As in temperate fresh waters, Townsend (2000) found that phosphorus was limiting phytoplankton biomass in tropical Australia. In contrast to this, Lewis (1996) and Talling (1998) found that nitrogen limitation of phytoplankton biomass is probably more common in tropical systems than in temperate. Since undisturbed tropical streams and rivers have lower concentrations of nitrogen for a given concentration of phosphorus than tropical lakes or temperate lakes and rivers, then nitrogen is lost from aquatic systems more rapidly in the tropics than the temperate zone (e.g. through denitrification) and phosphorus erosion from tropical landscapes and phosphorus mobilization in aquatic environments are more rapid (Downing et al. 1999). The above description gives more support for nitrogen being the most frequent limiter of production in tropical fresh waters (Saijo et al. 1997).

1.4.2 Tropical river phytoplankton

Since phytoplankton reacts to variables such as rainfall, water flow, ions, pH, and nutrients, and tropical regions differ from temperate areas in these variables, it is expected that tropical phytoplankton assemblages would differ from those of the temperate zone. Thomas (1983) found 160 taxa from 32 genera of diatoms in the Alligator Rivers region of the Northern Territory, Australia. The most abundant genera were *Eunotia, Navicula, Nitzschia,* and *Pinnularia*. In the same Alligator Rivers region Ling and Taylor (1986) recorded over 530 taxa (excluding diatoms) in which the majority were desmids (Chlorophyceae) with the most abundant genera being *Staurastrum, Cosmarium, Staurodesmus,* and *Closterium*.

A study by Fabbro and Duivenvoorden (2000) in the large tropical riverine impoundment of the Fitzroy River found algal assemblages normally contained cyanoprokaryotes (Oscillatoriales), euglenophytes or non-flagellated chlorophytes during flows followed by flagellated chlorophytes and cyanoprokaryotes (Nostocales) during the dry season. Species present during the period of reduced flow occupied conditions similar to those presented in the literature for temperate and/or

tropical lakes. Common species included *Pandorina morum, Eudorina elegans, Anabaena circinalis, Aphanizomenon issatschenkoi, Aphanizomenon aphanizomenioides* and *Cylindrospermopsis raciborskii.*

There are many conspicuous species that are found both in temperate and tropical regions, such as *Staurastrum sagittarium*, *Micrasterias mahabuleshwarensis* (Thomasson 1986), *Scenedesmus quadricauda* and *S. obliquus* (Trainor 1991a). However, a study by Thomasson (1986) found some desmids that seem to be endemic for tropical north Australia, among them *Xanthidium multicorne, Cosmarium securiforme, Triploceras gracile, T. verticillatum, Pleurotaenuim australianum* and *Staurastrum elegans*. All these taxa were originally described by Borge (1897). Other endemic taxa more recently described include *Micrasterias alata, Staurastrum tyleri*, and *Peridinium lingii* (Thomasson 1986).

An investigation by O'Farrell (1994) of 15 rivers and streams of the River Plate Basin in Argentina found that the phytoplankton grouped into three main groups. Rivers with a high discharge and a large floodplain were dominated by *Aulocoseira* sp. Smaller rivers with high conductivity, low transparency and variation in discharge rates were typified by eutrophic taxa such as *Cyclotella meneghiniana, Synedra ulna* and several green algae. Rivers with a reduced floodplain and moderate temperatures and conductivity were characterized by several pennate diatoms and flagellates, *Amphipleura pellucida, Surirella tenera, Terpsinoe musica, Navicula cuspidate, Eudorina elegans, Pandorina morum* and *Peridinium gatunense*. The differences in phytoplankton composition were contributed to a decreasing gradient of conductivity, pH and solids and in increasing minimum temperature.

In another river from tropical South America, phytoplankton concentrations were higher during spring and summer with a couple of small peaks in autumn in small tributary streams (O'Farrell et al. 2002). Diatoms dominated the phytoplankton representing more than 50% of the total density mainly due to *Cyclotella meneghiniana, Nitzschia palea* and *N. stagnorum.* Green algae were subdominant usually just over 10% of the community (e.g. *Chlorella vulgaris, Monoraphidium contortoum*). Cyanobacteria usually constituted less than 10% of phytoplankton density, as did the euglenoids belonging to the genera *Euglena, Lepocinclis* and *Phacus*.

A study on the Paraguay River during two periods of its hydrological cycle, winter and summer, reported 332 phytoplankton taxa in total, 298 in the upper section of the river and 143 in the middle and lower sections of the river belonging to chlorophytes (45%), euglenoids (23%), diatoms (20%), cyanobacteria (5%), xanthophytes (3%), cryptophytes (2%), dinoflagellates (1%) and chrysophytes (1%) (Zalocar de Domitrovic 2002). The upper reaches of the river were

dominated by small chlorophytes (*Chloromonas gracilis, Crucigenia quadrata, Scenedesmus ecornis* and *Monoraphidium contortum*) and Cryptophyta (*Cryptomonas marssonii, C. ovata* and *Rhodomonas minuta*), while diatoms (genera *Aulocoseira* and *Cyclotella*) dominated in the lower reaches of the river. Diatoms were the only group to show significant differences in density and biomass between the two sections of the river. There was no significant variation in the total number of taxa along the river. However, variation in community composition of phytoplankton groups was significant.

1.5 Use of phytoplankton as indicators in river monitoring

The assessment of river water quality has historically been based on the measurement of physical and chemical characteristics; however, use of the aquatic biota to identify structural or functional integrity of ecosystems, commenced in the mid-20th century (Hynes 1970) and has since been developed substantially (Norris and Thoms 1999). Biological measures tend to focus on assemblage structure of broad taxonomic groups such as diatoms (Rott et al. 1998, Chessman et al. 1999, Wu 1999) cyanobacteria (Burton 2004), macroinvertebrates (Chessman 1995, Kay et al. 1999) and water birds (Kingsford 1999).

Phytoplankton is highly responsive to excess nutrient input and can be problematic in long stretches of rivers, especially those that are affected by agriculture or industry. Structural and functional responses of phytoplankton may provide a similar role as do other indicator groups, such as macroinvertebrates used to assess stream health. Furthermore, phytoplankton may surpass macroinvertebrates as indicators because of their quicker growth rates and turnover times, ease of collection and ubiquity, and structural and functional attributes that can be linked to specific environmental sites of the ecosystem (Steinman 1998).

Although phytoplankton can show direct change in a river's chemical and physical parameters, Wehr and Thorp (1997) found that river plankton dynamics respond primarily to physical factors in large rivers and may fluctuate considerably in time and space. Changes in phytoplankton composition reflect not only variations in physical, chemical and biological factors, but also interactions among them; it could therefore be difficult to ascertain which are the most important determinants of compositional change (Wehr and Descy 1998). This could make using phytoplankton as indicators in large river monitoring problematic.

Diatoms are the most frequently used phytoplankton group for biological assessment of rivers. Diatoms have been used in paleolimnological investigations of sediments to indicate pre-disturbance conditions (Dixit et al. 1992) and as bioindicators of pollution. In the Grand River in

Ontario, diatom composition clearly reflected the level of eutrophication and pollution with biodegradable organic compounds (Rott et al. 1998). Diatoms in the Keelung River in Taiwan were found to be more suitable for presuming the level of eutrophication rather than pollution (Wu 1999). Chessman et al. (1999) used multivariate statistical models based on diatom genera to predict human impact on rivers of coastal south-eastern Australia. The increase in diatom genus richness was attributed to an enrichment of alkaline salts, the main effect of human activity.

No matter which taxonomic group is used to assess the quality of rivers, it is important that the ecological processes that determine occurrence and dynamics of members of that taxon are properly elucidated, and the causes of the problems are addressed, rather than the symptoms (Boulton and Brock 1999).

1.6 Tropical riverine water holes

Tropical riverine water holes occur in Australia, South America, Central America, Southern Africa and Asia. Their occurrence is governed by discrete wet and dry seasons. However, the majority of research conducted in tropical water holes in these areas has been focused on wildlife uses (Herrera and Macdonald 1989, Paltridge et al. 1997, Winemiller and Jepsen 1998, Germaine et al. 2000, Scholz and Kappeler 2004, O'Farrill et al. 2006, Scholte et al. 2007). There has been very little of research on phytoplankton in these biomes. Most of the tropical freshwater phytoplankton research has been conducted in lakes and large rivers, with only a handful of studies conducted in tropical riverine water holes. These studies have been conducted in central and north Australia where these habitats are common. Bunn et al. (2003) studied the primary productivity of algae in Cooper Creek of the Lake Eyre Basin in central Australia. However, this study did not elucidate the phytoplankton assemblages that occurred in Cooper Creek or its water holes. There have been two studies that have identified phytoplankton assemblages in riverine water holes. Townsend (2006) described phytoplankton in the Mary River in the Northern Territory and Costelloe et al. (2005) identified phytoplankton from three rivers in the Lake Eyre Basin in central Australia.

A total of 237 species were identified from the three rivers in the Lake Eyre Basin, with the highest diversity corresponding to the wet season during flood events (Costelloe et al. 2005). Multivariate analyses in this study found that salinity was a significant driver of assemblage composition but only explained a small portion of the variance in genera richness. In the Mary River, water quality (conductivity, dissolved oxygen, pH, silica and water clarity) and phytoplankton assemblage of the channel lake was primarily driven by its hydraulics (Townsend 2006). The most common phytoplankton identified throughout the study were dominated by dinoflagellates, euglenoids, cyrptomonads and small chlorophytes during the lotic phase and by

diatoms and cyanobacteria during the lentic phase; there was no clear evidence of domination by any single phytoplankton group.

1.7 Aims of this study

The community structure of river phytoplankton and their responses to biophysical processes and water chemistry have been well studied in temperate regions worldwide. In Australia, investigations of phytoplankton dynamics have focused on temperate lakes and reservoirs and to a lesser extent in large temperate rivers, especially with regard to bloom-forming cyanobacteria. Conversely, there have been few studies of tropical river phytoplankton in Australia. Within the Burdekin River catchment very little is known about the algal assemblages and how they respond to water quality parameters both spatially and seasonally. This study identified phytoplankton species in selected tributaries of the Burdekin River and the bio-physical parameters that act upon these species. This information was used to investigate any natural effects that may cause changes in the river ecosystem.

The aims of this study were to:

1. Identify the phytoplankton species and assemblages within selected rivers of the Burdekin River Catchment.
2. Determine the spatial and seasonal distribution of phytoplankton species and assemblages.
3. Ascertain the relationship between phytoplankton communities and the physical and chemical properties of the water.
4. Identify species or species assemblages that might be used as indicators of water quality.
5. Test the responses of selected phytoplankton species to changes in selected water quality parameters.

Broad descriptions of the study region and of the study sites are presented in Chapter 2. Chapter 3 presents the results of seasonal surveys of three sites in each of three tributaries, characterizing their physico-chemical and algal characteristics. In Chapter 4, one site in each river system is investigated in more detail. In Chapter 5, laboratory experiments to elucidate direct effects of selected variables on selected species are described. The background to this work is presented in Chapter 5 to reduce unnecessary repetition. Chapter 6 discusses the main results of these studies, and addresses the issue of the utility of algae in assessments of ecosystem condition.

This study generated a large amount of data, which is represented by summaries in this thesis. The full raw dataset is contained in Appendix 1 on the attached cd.

Chapter 2 . Study region and sites

2.1 Burdekin River Catchment

The Burdekin River catchment is the second largest on the east coast of Queensland, after the Fitzroy River catchment. Lying between 18° and 25° South, and 144° and 149° East, the catchment covers approximately 136,000 km^2, or about 8% of Queensland (Figure 2.1). It encompasses semi-arid woodlands, wooded and open grasslands, tropical rainforests, coastal plains, mangroves and wetlands, and discharges into the Great Barrier Reef lagoon. Rural industries in the catchment include agriculture, aquaculture, forestry, mining, and fishing. The main agricultural industries are sugarcane farming and horticulture on the coastal floodplain and cattle in the interior (Figure 2.2) (ABARE 2003).

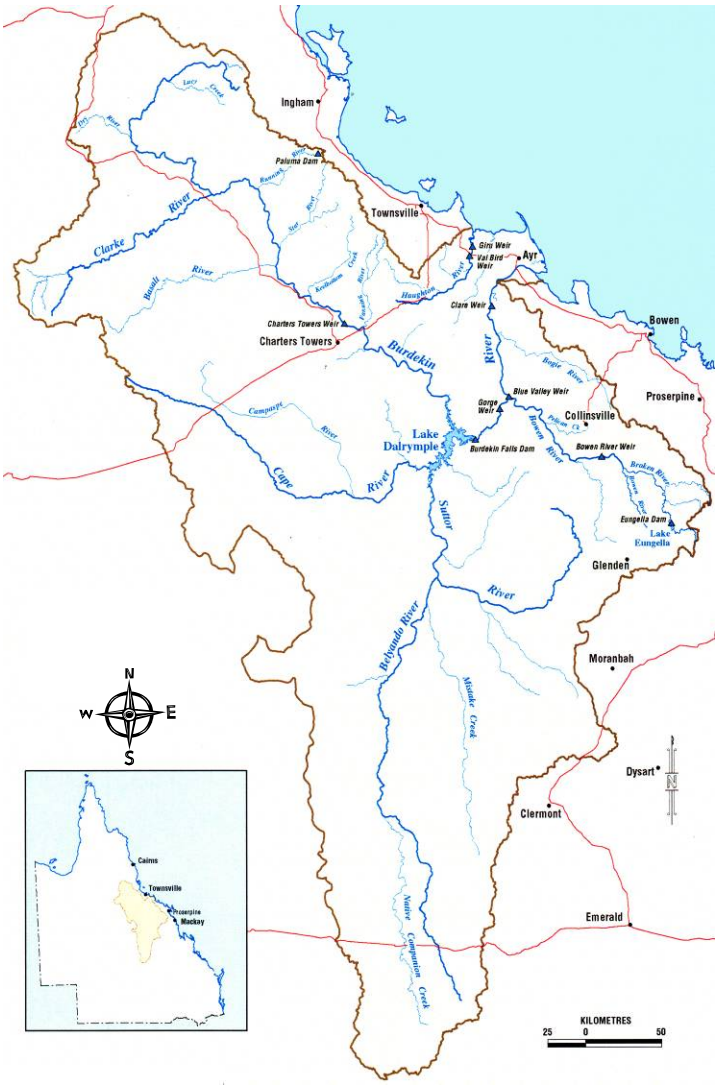


Figure 2.1 - Map of the Burdekin catchment in Queensland, Australia, showing major tributaries (NRM 2001).

The maintenance of ground and surface water infrastructure (bores, dams, weirs) is critical to rural industries and to urban water supplies. The major water storages within the Burdekin catchment are the Burdekin Falls dam, Eungella dam, and Clare, Gorge and Blue Valley Weirs. The largest of these is the Burdekin Falls dam, which has a storage capacity of 1.86 million megalitres and supplies water for irrigation to farms in the Lower Burdekin as well as being a back-up water supply for the city of Townsville (NRM 2002). Much of the water supplied to cattle is pumped from riverine water holes. Water use is dominated by irrigation and less by urban use and industrial purposes (Figure 2.3).

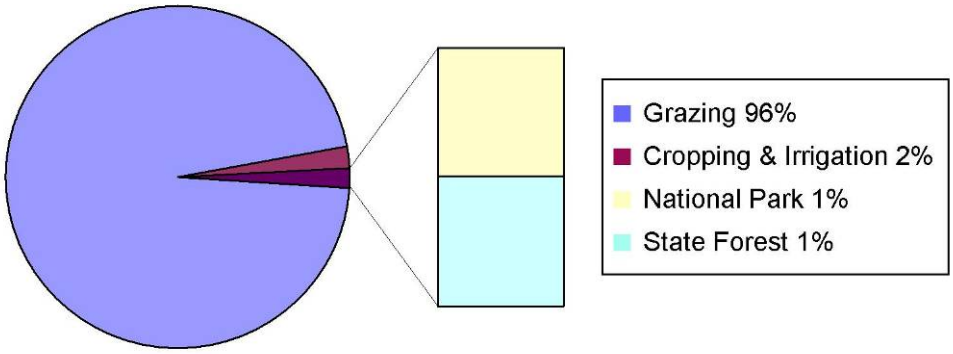


Figure 2.2 - Land use within the Burdekin River catchment (NRM 2002).

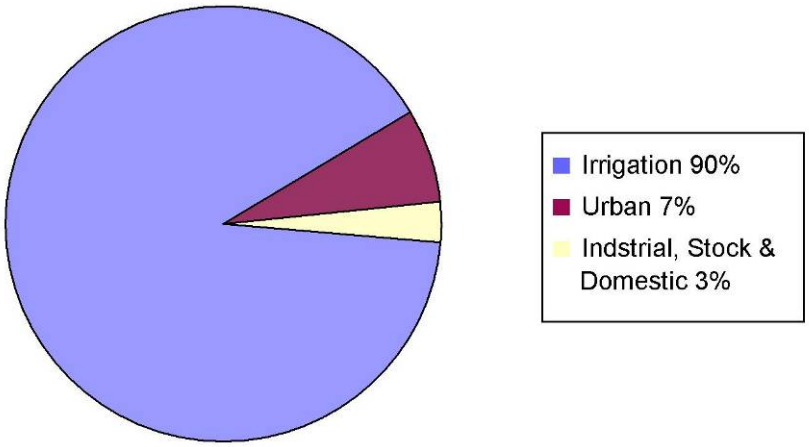


Figure 2.3 - Water use within the Burdekin River catchment (NRM 2002).

The catchment is divided into four sub-catchments: the Upper Burdekin in the north west (36,200 km^2), the Lower Burdekin and Coastal Plains centrally (16,600 km^2), the Bowen-Broken in the east (9,400 km^2), and the Belyando-Suttor in the south (73,800 km^2) (ABARE 2003) (Figure 2.4).

2.1.1 Hydrology and Rainfall

Major rivers within each sub-catchment include the Basalt, Broughton, Burdekin, Clarke, Fanning, Running and Star Rivers in the Upper Burdekin; the Bogie, Burdekin, Don, Elliot and Haughton Rivers in the Lower Burdekin and Coastal Plains; the Bowen and Broken Rivers in the Bowen-Broken; and the Belyando, Cape, Rolleston and Suttor Rivers in the Belyando-Suttor. At Clare weir, more than half the total river discharge within the basin comes from the Upper Burdekin sub-catchment, although it represents only 28% of the basin area (Table 2.1) (Roth et al. 2002).

Table 2.1 - Catchment Contributions to the Burdekin River at Clare weir (Roth et al. 2002).

Sub-catchment	Area (km^2)	Area of Burdekin Basin (at Clare weir) (%)	Sub-catchment annual contribution (ML/a)	% of total flow
Upper Burdekin	36,181	28	4,067,000	52
Belyando-Suttor	73,828	57	2,554,500	33
Bowen-Broken	9,413	7	1,021,760	13
Lower Burdekin	10,028	8	132,700	2
Total (at Clare weir)	129,450	100	7,775,960	100

Despite the size of the catchment, the air temperature varies very little. The western regions experience slightly higher average temperature maxima and lower minima than the coastal regions. Higher elevations experience lower temperatures and more rainfall. Relative humidity is fairly constant across the regions in the morning and lower in the afternoon in the western regions (Table 2.2) (Roth et al. 2002).

A characteristic feature of the Burdekin catchment is the variability in water flow between and within years (Figure 2.5 and Figure 2.6). The Burdekin River shows extreme variability in annual water flow, with a 25-fold range; thus it had flows of 1,000 gigaliters in 1969 and 25,000 gigaliters in 1974 (Figure 2.5). From the mid 20^{th} century to the present, however, below-mean flows have been recorded for the majority of the period. A typical year (e.g., 1998) has a large input of rainfall during the wet season, which increases the stream flow, followed by intermittent smaller amounts of rainfall during the year (Figure 2.6). With a climate dominated by hot wet summers and dry warm winters, peak flows occur between December and April with no or negligible flow from May to November (Roth et al. 2002). Many of the smaller streams cease to flow or dry out completely during the dry season.

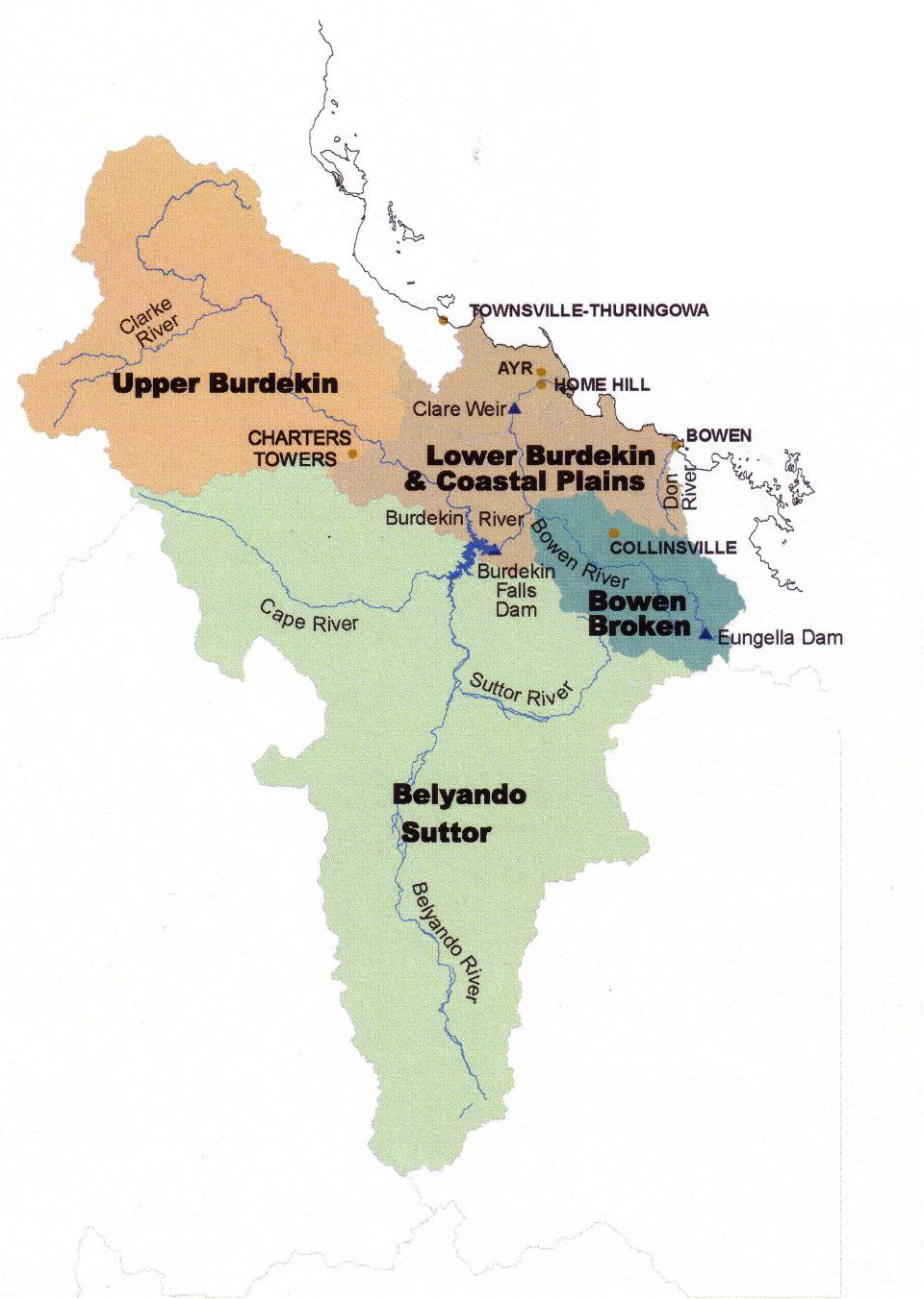


Figure 2.4 - Map of the Burdekin catchment showing the four sub-catchments: Upper Burdekin, Lower Burdekin and Coastal Plains, Bowen-Broken, and Belyando-Suttor (ABARE 2003)

Table 2.2- Rainfall, temperatures and humidity of the Burdekin River sub-catchments (Roth et al. 2002).

Sub-catchment (town)	Average Rainfall (mm per annum)	Average Temperature (Jan and July) (°C)	Average Humidity at 9am (Jan and July) (%)
Upper Burdekin (Charters Towers)	661	27.0 – 17.8	68 – 65
Belyando-Suttor (Twin Hills)	615	28.6 – 15.4	63 – 64
Bowen-Broken (Collinsville)	715	27.6 – 17.1	68 – 71
Lower Burdekin (Millaroo)	875	28.0 – 17.7	66 – 64

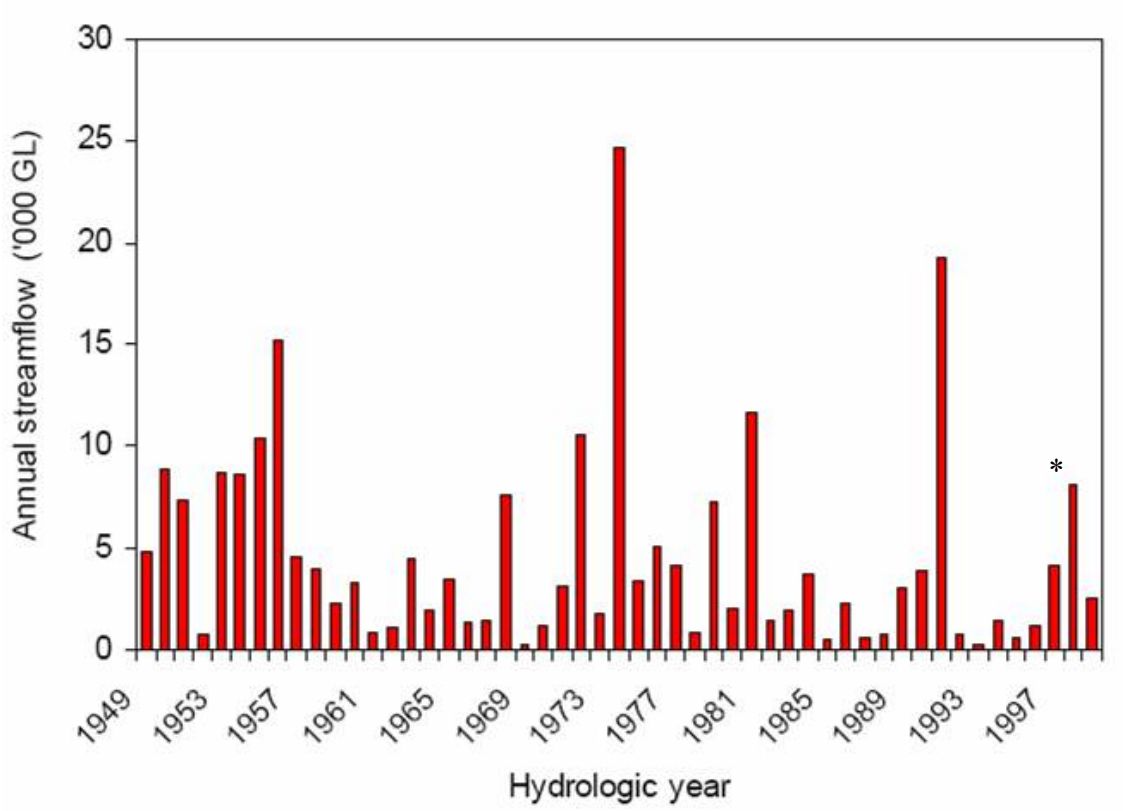


Figure 2.5 - Annual stream flow (in thousands of gigaliters) recorded for the period 1949 to 1999 in the Upper Burdekin catchment, at Sellheim Bridge (Roth et al. 2002).
*** indicates the year 1998.**

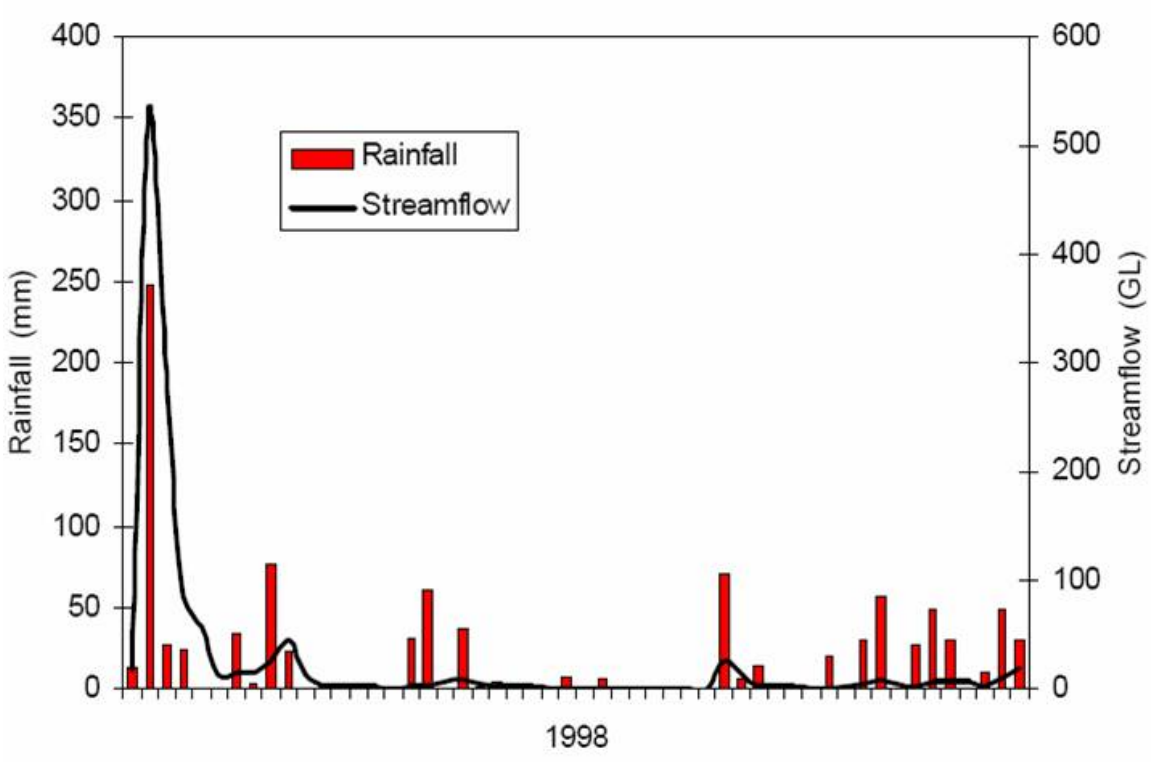


Figure 2.6 - Weekly stream flow (in gigaliters, 1 gigaliter = 10^9 liters) recorded for a flood year (1998) in the Upper Burdekin catchment, at Sellheim Bridge (Roth et al. 2002).

The groundwater system in the Burdekin basin is one of the largest alluvial aquifer systems in Australia (Arunakumaren et al. 2000). Inflows to recharge ground water include rainfall, flooding, irrigation and seepage from artificial recharge pits and diversion channels (ABARE 2003). Annual recharge in the Burdekin basin varies between 430,000 and 850,000 megalitres (Arunakumaren et al. 2000). However, rainfall is less than the requirements of the vegetation in most of the catchment, so very little water enters the soil to recharge aquifers, except during monsoonal rainfall in the wet season (Roth et al. 2002).

2.1.2 Geology and Soils

Soil types vary widely in accordance with geology, topography and rainfall in the Burdekin catchment. The Upper Burdekin sub-catchment is characterized by volcanic and sedimentary rocks. The areas to the north-west are of basalt (1-5 myo), while the eastern areas have older basalt formations. Large areas of granite and granodiorite are present in the north and eastern sections (Roth et al. 2002). Upper Burdekin soil types include: red duplex soils; black and red soils on basalt; fertile sands; sodic duplex soils; and red and yellow soils. Most soils in the Upper Burdekin have low organic matter and nitrogen content due to severe weathering and leaching (Roth et al. 2002).

The Lower Burdekin and Coastal Plains sub-catchment consists mainly of volcanic and sedimentary rock. The Bowen coastal area contain plains of sand, gravel, silt and mud, whereas the Bowen Basin consists of tablelands of basalt (Roth et al. 2002). Many of the soils in this region have been subjected to intense weathering and leaching processes, which reduce nutrient content. The floodplain soils are derived from fluvial deposits and comprise black cracking clays, sands and a range of duplex soils (Roth et al. 2002).

The Bowen-Broken sub-catchment is characterized by sandstones, mudstone, siltstone and conglomerates (Roth et al. 2002), and it includes limestone formations originating from old marine reefs within the basins. The soils vary widely in this sub-catchment with red/brown earths in the east, to yellow soils in the west, to black soils in the north (Roth et al. 2002).

The southern regions of the Belyando-Suttor sub-catchment are mainly composed of alluvial deposits consisting of sand, gravel and soil. The central region is characterized by volcanic products, while the northern areas are predominantly sedimentary formations and isolated granite outcrops (Roth et al. 2002). Cracking clay soils predominate throughout the sub-catchment due to igneous rocks, argillaceous sedimentary rocks and fine textured alluvium and colluvium (Roth et al. 2002). These soils include grey/brown clays and red/yellow earths.

2.1.3. Vegetation and Bioregions

Bioregions are broad landscape patterns that reflect structural geology, geomorphology and climate as well as flora and fauna. Sattler and Williams (1999) described three principal bioregions within the Burdekin River Catchment (Figure 2.7):

- The Brigalow belt comprises about 47% of the catchment and covers half of the Belyando-Suttor sub-catchment, most of the Bowen-Broken sub-catchment, and some of the coastal plain. This bioregion is characterized by *Acacia harpophylla* (Brigalow), which forms woodland communities on clay soils.
- The Einasleigh Uplands comprise about 32% of the catchment and cover most of the Upper Burdekin sub-catchment. This bioregion consists of ranges and plateaus. It is dominated mainly by ironbark woodlands. Because of its generally shallow soils, increases in grazing intensities, pasture development and clearing can have a significant impact on biodiversity and the river systems.
- The Desert Uplands cover about 18% of the catchment and extend along the western edge of the Belyando-Suttor sub-catchment. This region has a semi-arid climate and soils of poor structure and low fertility. Parts of the region are an important part of the catchment of the Great Artesian Basin.

In addition to these three bioregions, the catchment also contains small areas of the Wet Tropics, Gulf Plains, and the Central Queensland Coast bioregions.

Wetlands within the lower Burdekin that form part of the Townsville-Burdekin wetland aggregation are recognized as one of the largest concentration of wetlands in eastern Australia (ABARE 2003). These wetlands include floodplain sedge swamps, *Melaleuca* paperbark forests and coastal, estuarine, mangrove-salt flat complexes. These environments support nursery habitats that form the basis for commercial and recreational fisheries, host regionally significant breeding aggregations of water birds and are a major stopover point for migratory wading birds on the Asian flyway (NRM 2002).

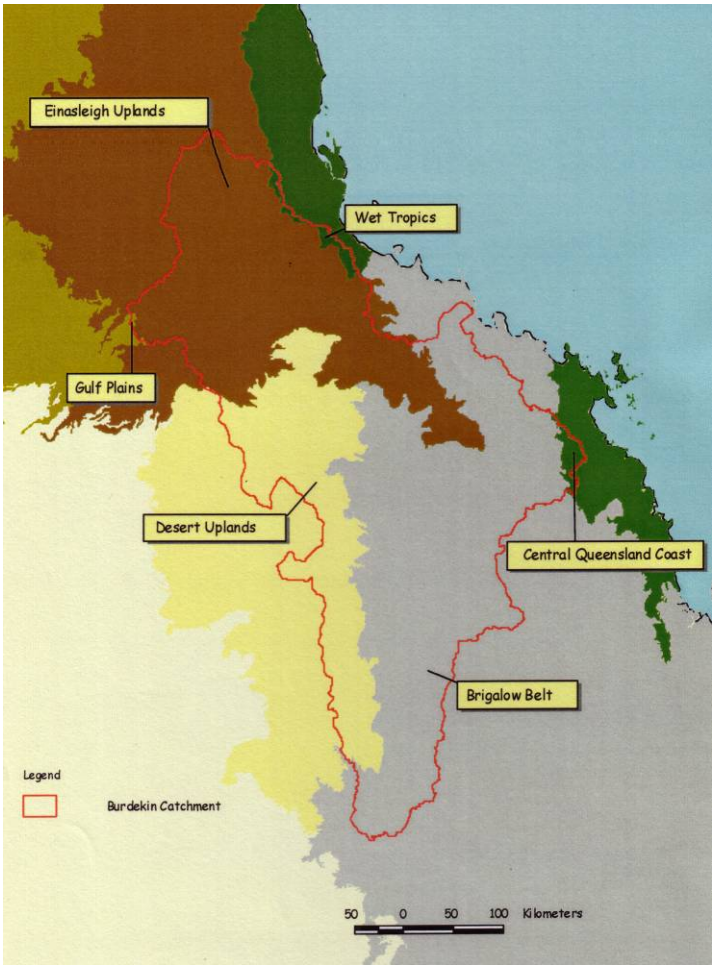


Figure 2.7- Bioregions of the Burdekin River catchment (QEPA 2002)

2.1.4 Water Quality

Many government agencies use a conservative approach to monitoring water quality within water basins. Individual sites are evaluated and described in terms of conditional rating for five parameters: domestic, irrigation, stock, recreation and aquaculture (NRM 2002). Most of these data (i.e., hydrology, floral surveys, faunal surveys, bedload deposition, and suspended sediment load) are sufficient to describe general physical factors, such as flow, temperature, turbidity, light availability, types of suspended sediments, but may not provide comprehensive water quality analysis. Specifically, data for levels of flow, nutrients, suspended solids, metals and dissolved oxygen would have to be monitored more regularly to improve reliability (DNR 2000) .

Within the Burdekin catchment most sites were rated poor to moderate for domestic use; moderate to good for irrigation and stock; poor for aquaculture; and variable for recreational use (NRM 2002). Flow regime and soil type directly affect the turbidity of a river. Turbidity in the catchment has a significant impact on water quality in storages (ACTFR 1999). The highest levels of turbidity occur in the Belyando-Suttor sub-catchment, which is dominated by clay soils and fine alluvium, which is easily suspended into the water column. Lower levels of turbidity are

associated with the Upper Burdekin sub-catchment mainly because much of it is composed of basalt and granite rock formations. Although the Belyando-Suttor sub-catchment appears to be a major problem area, the other sub-catchments can also have high turbidity during flood events (NRM 2002). Turbidity in this sub-catchment frequently exceeds guideline limits for drinking water (1 NTU) and aquatic ecology (50 NTU) (NRM 2002). This high turbidity is evident in historic records of the region and supports the Burdekin River's classification as a naturally turbid watercourse (DNR 2000).

2.2 Study Sites

The intent of this study was to gain an understanding of the dynamics of the limnology of representative water holes in the Burdekin catchment. Given the size of the catchment, full representation was impossible; instead, I concentrated on three major tributaries that were perceived to demonstrate the range of characteristics of rivers in the catchment. In each of these tributaries, three sites were selected yielding a total of nine sampling sites. Sites located in the Basalt sub-catchment area were Hillgrove Station and Bluff Downs Station on the Basalt River and Emu Valley Station on Allingham Creek, a tributary of the Basalt River; sites located in the Keelbottom sub-catchment area were Dotswood Station, Mirambeena Station, and Keelbottom Bridge, all located along Keelbottom Creek; and sites located in the Belyando-Suttor sub-catchment were Mt. Douglas Station on the Belyando River, Disney Station on Mistake Creek, a tributary of the Belyando River, and Mt. Hope Station on the Suttor River (Figure 2.8). Seven of these sites were on privately owned cattle stations. The Australian Army owns Dotswood Station and Keelbottom Bridge is on public land. The sites within each of the three tributaries were similar in biophysical characteristics (e.g., riparian vegetation, water clarity, macrophytes, and soils). The three tributaries, however, had contrasting characteristics, described below, and indicated in Table 2.3.

2.2.1 Hydrology of the study rivers

The selected rivers follow the Burdekin River's hydrographic pattern fairly closely with a large input of water into the systems from rainfall during the wet season and little to no rainfall, and so little or no flow, during the dry season (Figure 2.9). However, the Burdekin catchment is very large and while one sub-catchment may receive rainfall, others may not. During the study year 2003, the Suttor River sites received comparatively substantial rainfall but the sites of the Upper Burdekin did not. Not only is there variability between sub-catchments, but also within sub-catchments. For the study years 2003 and 2004, the Belyando River sites received very low rainfall, whereas the Suttor River sites in the same sub-catchment were well flushed. The same

pattern was seen in 2004 in the Basalt River and Keelbottom Creek sites in the Upper Burdekin sub-catchment.

All of the rivers studied except the Basalt River ceased to flow during the dry seasons. This is illustrated by Figure 2.10, where the Keelbottom Creek and the Suttor and Belyando Rivers have no flow during the dry seasons (b, c, d), but the Basalt River has continuous flow throughout the year (a).

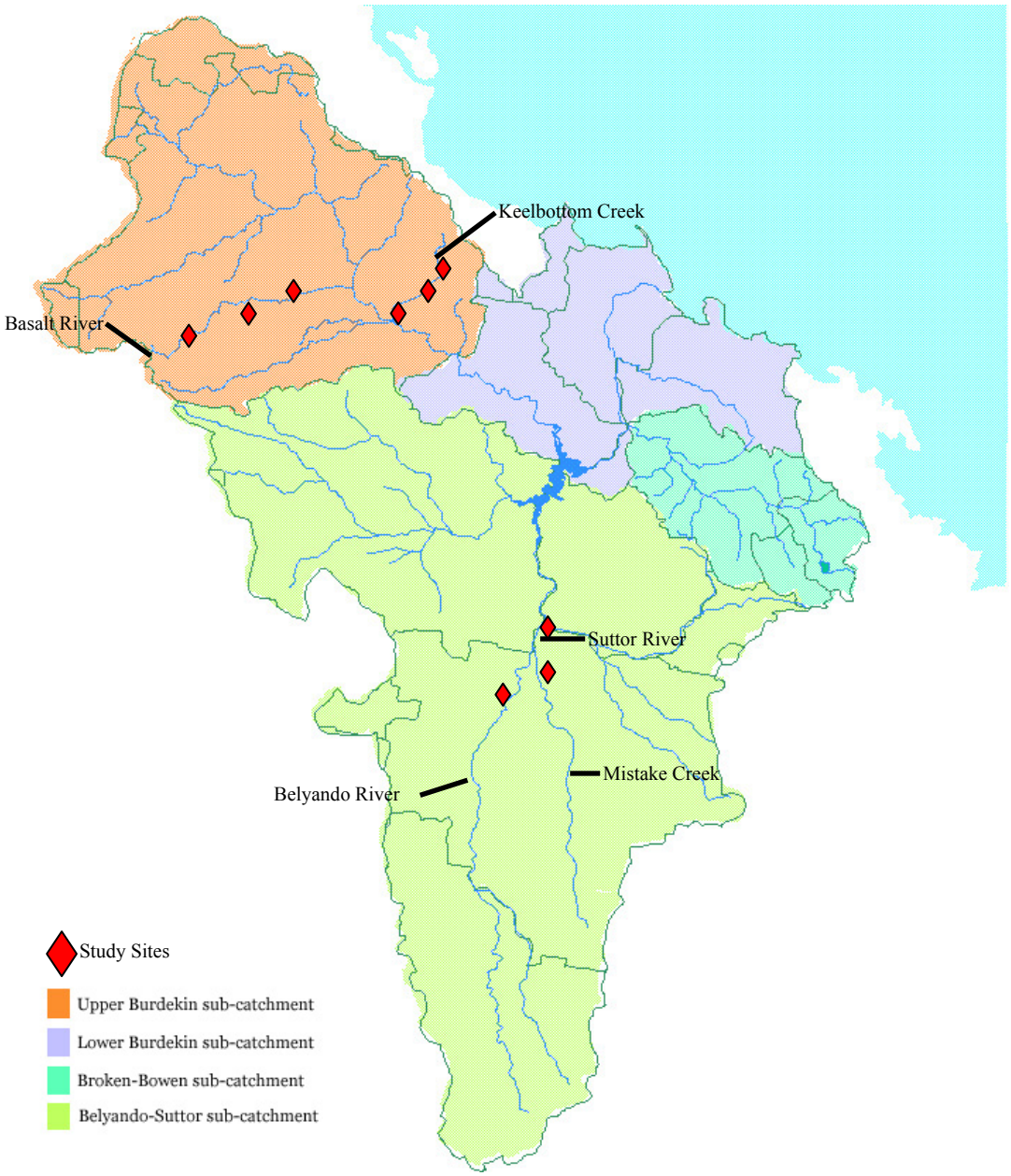


Figure 2.8 - Burdekin River catchment showing sub-catchments and study sites.

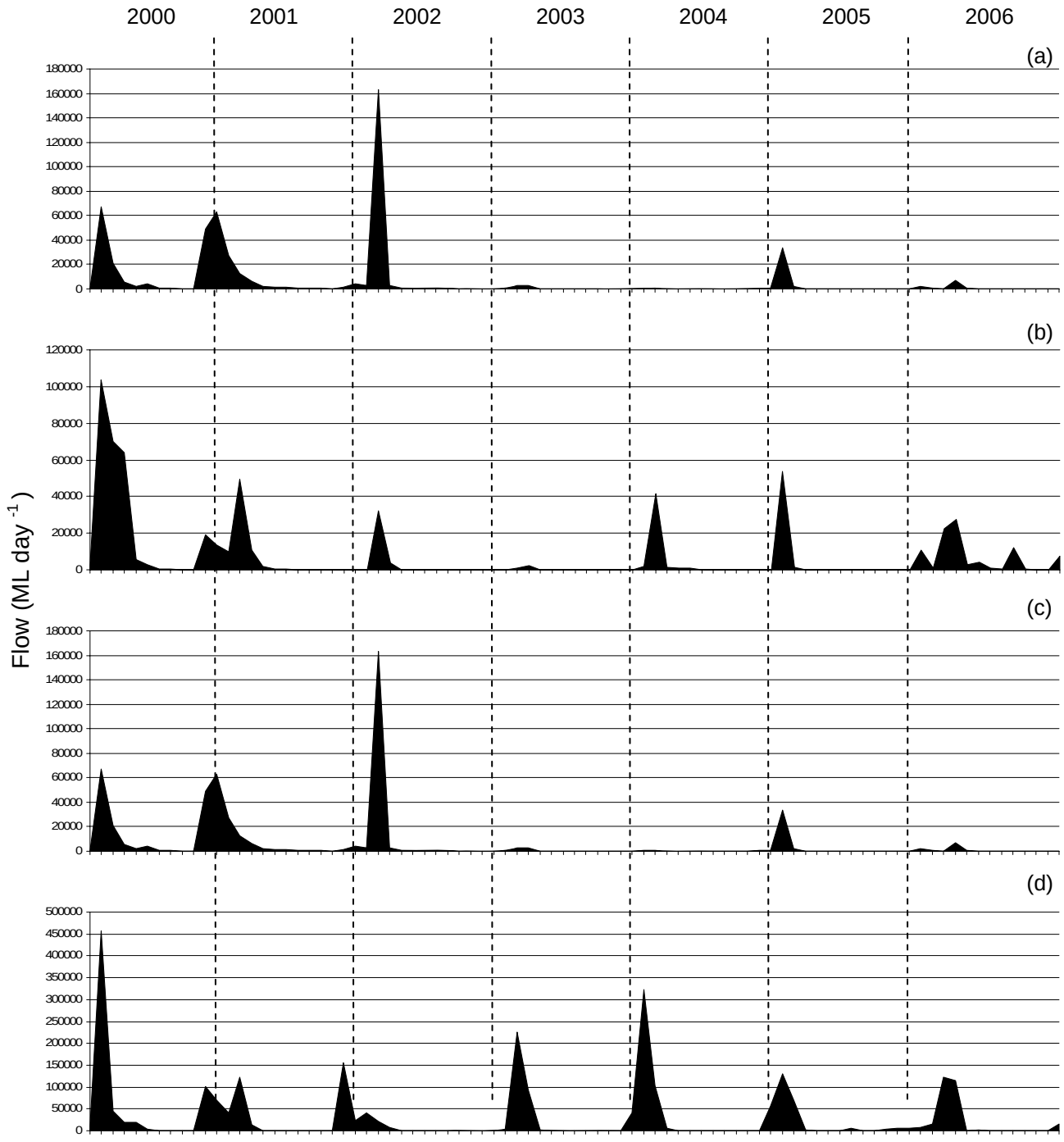


Figure 2.9 – Hydrographs showing monthly flow data in megaliters for 2000-2006 for (a) Basalt River at Bluff Downs Station, (b) Keelbottom Creek at Hervey's Range Rd, (c) Belyando River at Gregory's Developmental Rd and (d) Suttor River at St. Ann's Station. Data supplied by the Queensland Department of Natural Resources and Water, 2008.

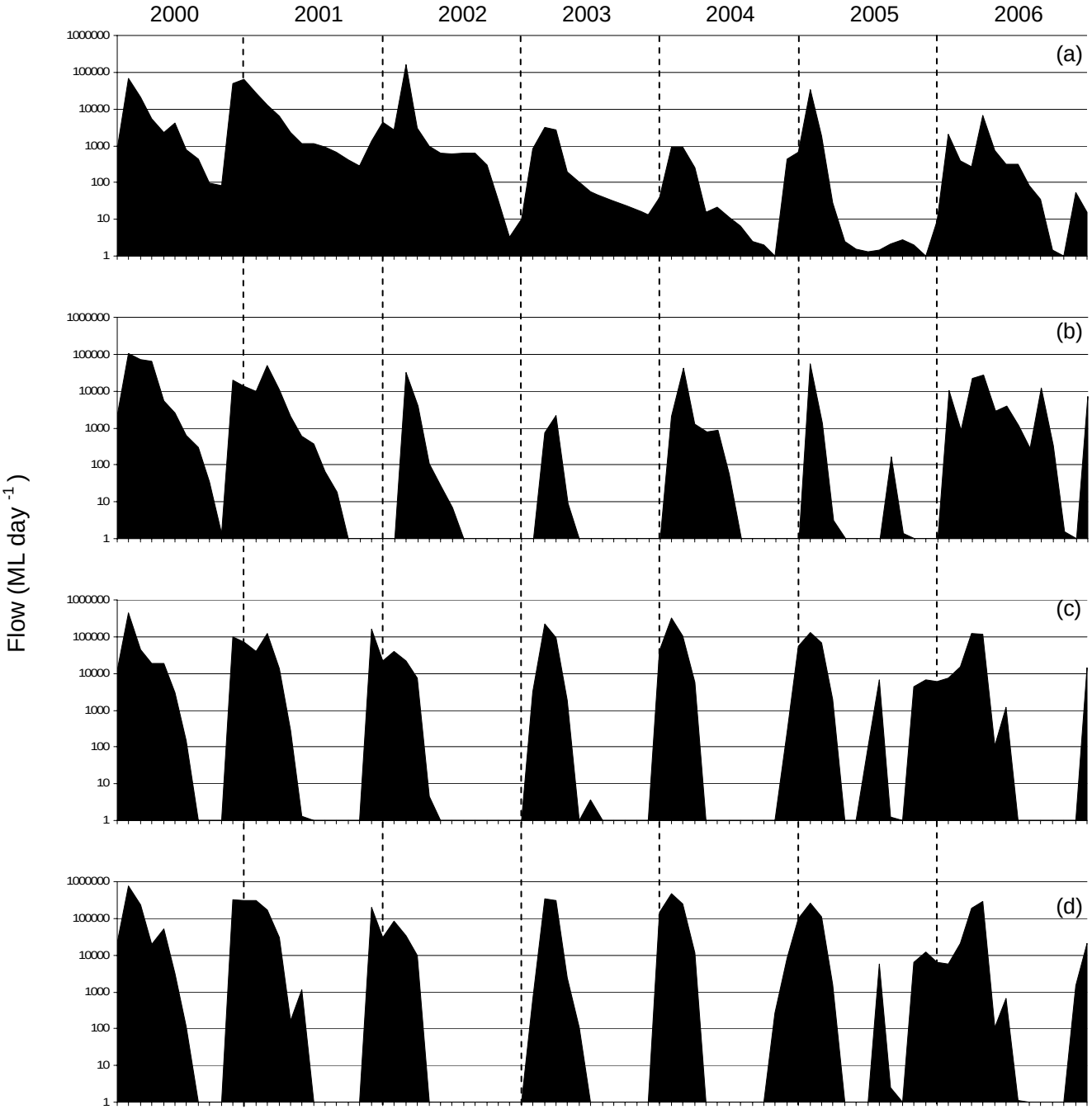


Figure 2.10 – Hydrographs showing monthly flow data for 2000-2006 using a logarithmic scale for (a) Basalt River at Bluff Downs Station, (b) Keelbottom Creek at Hervey's Range Rd, (c) Belyando River at Gregory's Developmental Rd and (d) Suttor River at St. Ann's Station. Data supplied by the Queensland Department of Natural Resources and Water, 2008.

2.2.2 Basalt River

The Basalt River (Figure 2.11) is characterized by large basalt rocks and cobbles on the sides and within the river bed. The river is fed by ground water filtering through basalt, resulting in good water clarity. For the years of 2003-2006, during this study, there was little rainfall during the wet season (Figure 2.9a), but flow was maintained by ground water. *Casuarina (*she-oaks) and various *Eucalyptus* allies characterized the riparian vegetation, which was not dense and did not grow immediately at the water's edge. Various aquatic macrophyte species were present along the water's edge. Cattle were present at the sites on the Basalt River; however, while they had access to the sites, there were no great densities in and around the water holes. Their presence was apparent by the hoof marks and manure along the water's edge.

Figure 2.11 - Photograph of Basalt River at Bluff Downs Station.

2.2.3 Keelbottom Creek

Keelbottom Creek (Figure 2.12) is characterized by fine loose soil on the bank and sand in the stream bed. The stream has no surface flow during the dry season, but water holes are permanent. This stream is highly affected by seasonal water input. During the dry season the water ceases to flow. During the study years 2003-2006 the input decreased from previous years and the months of no flow increased (Figure 2.9b). The riparian vegetation consisted mainly of *Melaleuca* spp., which grew fairly densely along the water's edge. Aquatic macrophytes were in high abundance

(≥ 50% cover) in the water holes. Turbidity was higher than for the Basalt River. Dotswood and Bridge sites had no cattle present, as they were not located on cattle stations. The Mirambeena site, however, was accessible to cattle. They were not in high numbers, but their presence was apparent by hoof prints and manure at the water's edge.

Figure 2.12 - Keelbottom Creek at Mirambeena Station. (a) Wet Season (April). (b) Dry Season (November).

2.2.4 Belyando and Suttor Rivers

The Belyando/Suttor River area (Figure 2.13) is characterized by extremely turbid water. The soils are clay/silt on the bank and in the river bed. No aquatic macrophytes were found at these sites. Various eucalypts and shrubs grew fairly densely along the water's edge. The study sites on these rivers have persistent water holes through the dry season. The rivers show the very characteristic water flow in the dry tropics of Australia (Figure 2.9 c, d) – regular influxes of water from seasonal rains, followed by no flow during the dry season. These sites were accessible to cattle, small groups of which were seen and hoof prints and manure were found at the water's edge.

Figure 2.13 - Suttor River at Mt. Hope Station.

Table 2.3 - Summary of characteristics of the nine study sites.

River and Site	Latitude and Longitude	Waterhole Length		Waterhole Width		Maximum Depth		Turbidity		Substrate	Dominant Riparian Vegetation
		Max	Min	Max	Min	Wet	Dry	Max	Min		
Basalt River											
Emu Valley	S 19° 42' . 29" E 145° 39' . 32"	>100 m	>100 m	45 m	45 m	5.5 m	5.0 m	18.5 NTU	1.88 NTU	Sand, cobbles, basalt rocks	*Casuarina littoralis*., *Eucalyptus* spp., *Acacia* spp.
Bluff Downs	S 19° 40' . 595" E 145° 32' . 730"	>100 m	>100 m	105 m	105 m	8.0 m	8.0 m	5.55 NTU	2.37 NTU	Sand, basalt rocks	*Casuarina littoralis*., *Eucalyptus* spp., *Acacia* spp.
Hillgrove	S 19° 37' . 462" E 145° 50' . 890"	>100 m	>100 m	90 m	90 m	6.0 m	6.0 m	14.3 NTU	3.1 NTU	Sand, cobbles, basalt rocks	*Casuarina littoralis*., *Melaleuca viridiflora* *Eucalyptus* spp., *Acacia* spp.
Keelbottom Creek											
Dotswood	S 19° 39' . 556" E 146° 14' . 795"	>100 m	>100 m	50 m	50 m	2.0 m	1.0 m	17.2 NTU	3.57 NTU	Sand, silt, loose gravel	*Cryptostegia grandiflora, Brachiaria mutica, Melaleuca quinquenervia*, *Eucalyptus* spp., *Acacia* spp.
Mirambeena	S 19° 43' . 319" E 146° 08' . 653"	>100 m	30 m	50 m	10 m	4.0 m	0.5 m	12.0 NTU	2.33 NTU	Sand, black soil	*Cryptostegia grandiflora, Melaleuca quinquenervia*, *Eucalyptus* spp., *Acacia* spp.
Bridge	S 19° 29' . 318" E 146° 20' . 045"	>100 m	20 m	40 m	10 m	1.2 m	0.3 m	22.3 NTU	4.98 NTU	Sand, black soil, silt	*Brachiaria mutica, Melaleuca quinquenervia*, *Eucalyptus* spp., *Acacia* spp.
Belyando / Suttor Rivers											
Mt. Douglas	S 21° 32' . 023" E 146° 51' . 575"	>100 m	20 m	30 m	10 m	1.0 m	0.2 m	1,000 NTU	86.6 NTU	Silt, clay	*Melaleuca quinquenervia*, *Eucalyptus* spp., *Acacia* spp
Disney	S 21° 48' . 16" E 146° 52' . 51"	>100 m	>100 m	30 m	30 m	2.5 m	2.5 m	405 NTU	18.1 NTU	Silt, clay	*Melaleuca quinquenervia*, *Eucalyptus* spp., *Acacia* spp
Mt. Hope	S 21° 23' . 777" E 146° 51' . 885"	>100 m	>100 m	70 m	70 m	3.0 m	2.3 m	181 NTU	13.6 NTU	Silt, clay	*Melaleuca quinquenervia*, *Eucalyptus* spp., *Acacia* spp

Chapter 3 . Biophysical characteristics of water holes in the Burdekin Catchment

3.1 Introduction

Rivers and streams of the Australian dry and semi-arid tropics typically have a highly seasonal hydrological regime with turbid flows during the wet season (December to April) followed by periods of low flow or, more commonly, no flow during the dry season (May to November). These rivers therefore switch between riverine and lacustrine environments. Phytoplankton living in these rivers therefore must either be able to adapt to highly variable irradiation (Reynolds et al. 1994), nutrient availability, stratification and mixing (Reynolds 1980) or these changes are accompanied by seasonal changes of the phytoplankton community.

Seasonal succession of phytoplankton species typically reflects changes in physical and chemical characteristics of the water body (Reynolds 1989, Billen et al. 1994, Bormans and Condie 1998). The low number of studies of phytoplankton assemblages in tropical systems, especially rivers, serves as a point of reference (Table 3.1). For example, in the Lake Eyre Basin of Australia, algal diversity was found to be at its highest during, or directly after, flood events (Costelloe et al. 2005). Turbulence and stratification had a significant effect on the phytoplankton of a small tropical reservoir on Palm Island (Hawkins and Griffiths 1993). Hawkins and Griffiths (1993) found that an artificially destratified reservoir was largely dominated by diatoms and, upon silica depletion, cyanobacteria replaced the diatom community, whereas the unmanaged, stratified reservoir was dominated by *Cylindrospermopsis raciborskii* and *Anabaena tenericaulis* during the warmer months, but replaced by chlorophytes and euglenoids after periods of heavy rainfall. Along the River Plate Basin in Argentina, O'Farrell (1994) found that phytoplankton species grouped depending upon the physical, chemical and hydrological parameters of the rivers. These studies show that tropical freshwater phytoplankton assemblages are, not surprisingly, closely linked to physico-chemical conditions.

In the tropics, light and temperature conditions are favorable for year-round growth such that high phytoplankton concentrations are likely to create the most acute environmental problems. In these systems, periodicity of the phytoplankton community is likely to be governed by a complex of local and short-term environmental factors (Hawkins and Griffiths 1993), such as nutrient limitation (Hecky and Kling 1981, Talling 1986a), flow regime (Fabbro and Duivenvoorden 2000, Castillo et al. 2004), salinization (Fazio and O'Farrell 2005), light availability (O'Farrell et al. 2003, Izaguirre et al. 2004) and stratification (McGregor and Fabbro 2000, Bormans et al. 2004).

Table 3.1 – Studies of phytoplankton in tropical regions

Site	Site Character	Water Quality influences on phytoplankton	Phytoplankton	Reference
Lake Victoria, Kenya, Africa	Large permanent lake			(Talling 1966)
Blue Nile, Africa	Large river; highly seasonal	Seasonal changes in light, turbidity, discharge, & flow	*Melosira* spp., *Synedra acus*, *Anabaena* spp., *Lyngbya limnetica*, *Anabaenopsis* spp., *Raphidiopsis curvata* – low algal densities occur during flooding; diatoms are first to develop then succeeded by blue-greens	(Talling and Rzoska 1967)
Lake George, Uganda, Africa	Large permanent lake	Diurnal cycles and water flow.	58 spp. – cyanobacteria most abundant, followed by diatoms, then chlorophytes; maximum Chlorophyll *a* values occurred in towards center of lake	(Ganf 1974b)
Various lakes, Africa	Large / medium permanent lakes	Hydrological (water input/output) or hydrographic (water column structure or circulation) features	Focused mainly on diatoms and cyanobacteria; hydrological features tend to have diatoms dominant then succeeded by cyanobacteria; hydrographic features can have similar patterns as well as having other patterns of succession between diatoms, cyanobacteria and green algae	(Talling 1986b)
Various lakes, Africa	Large / medium permanent lakes	Si:P ratios decrease as lake levels increase	*Stephanodiscus* sp. physiology is known and the species dominate when the supply ratio of Si:P is relatively low	(Kilham and Kilham 1990)
Lake Victoria, Kenya, Africa	Large permanent lake	Phytoplankton correlated with turbidity during dry season and SiO_2 during wet season	103 spp. - Cyanobacteria most diverse, followed by diatoms, chlorophytes, & dinoflagellates	(Lung'Ayia et al. 2000)
Lake Lanao, Philippines	Large permanent lake	Growth controlled by light availability and nutrients	70 spp. – Cryptomonads and diatoms occur during low light and high nutrient periods; green, blue-green and dinoflagellates occur at opposite periods	(Lewis 1978)
Man made lakes, India	Large / medium permanent lakes	Composition affected mainly by rains and temperature	120 spp. – cyanobacteria dominated, followed by diatoms and/or Chlorococcales and then euglenoids	(Zafar 1986)
Solomon Dam, Palm Island, Australia	Small permanent reservoir	Communities correlated with stratification	Stratified reservoir dominated by cyanobacteria, destratified reservoir dominated by diatoms	(Hawkins and Griffiths 1993)
Various reservoirs, Queensland, Australia	Large to small permanent reservoirs	Phytoplankton were influenced by temperature and stratification	Monitoring cyanobacteria, *Cylindrospermopsis raciborskii*	(McGregor and Fabbro 2000)
Fitzroy River, Australia	Large impounded river, seasonal	Phytoplankton were separated into groups by water clarity, temperature and water chemistry	Dominated by Oscillatoriales and euglenoids during flowing periods and Nosctocales and flagellated chlorophytes during dry periods	(Fabbro and Duivenvoorden 2000)
Weirs of Fitzroy River, Australia	Permanent reservoirs	Stratification, flow and turbidity influenced cyanobacteria growth	Cyanobactera	(Bormans and Ford 2002)
Fitzroy River, Australia	Large impounded river, seasonal	Light, turbidity, flow and stratification influenced cyanobacteria growth	Cyanobacteria (i.e., *Cylindrospermopsis*, *Planktolyngbya*, *Limnothrix*)	(Bormans et al. 2004)

Lake Erye Basin, Australia	Large lake, highly seasonal	Algal diversity highest during flood events and salinity correlated with composition	237 spp. – chlorophytes and euglenoids negatively correlated with salinity and cyanobacteria and diatoms no correlation	(Costelloe et al. 2005)
Lakes of Salado River Basin, Argentina, S. America	Medium shallow permanent lakes, seasonal	Phytoplankton were influenced by water levels and macrophytes	Low macrophytes were dominated by cyanobacteria and coccal greens, while high macrophytes were dominated by periphyton and benthos	(Izaguirre and Vinocur 1994)
River Plate Basin, Argentina, S. America	Small to large rivers, seasonal	Phytoplankton grouped on the basis of discharge, conductivity, water clarity	Large rivers w/ large floodplains were dominated by *Aulocoseira* spp., smaller rivers w/ high conductivity were dominated by chlorophyte spp.	(O'Farrell 1994)
Reservoirs of Paraná Basin, Brazil, S. America	Permanent reservoirs	Biomass influenced by discharge, P, N and conductivity	Chlorophyll *a* increased w/ N, P and conductivity, while decreasing w/ discharge	(Gomes and Miranda 2001)
Floodplain of Paraná River, Argentina, S. America	Large river transitioning into a permanent floodplain (water-water ecotone)	Discharge, transparency, nitrate influenced phytoplankton	Temporal fluctuations were more in river than lake, mainly diatoms	(Izaguirre et al. 2001)
Paraguay River, Paraguay, S. America	Large river	Seasonal differences in temperature, pH, nitrate and nitrite influenced phytoplankton	Small chlorophytes and cryptophytes dominated, followed by diatoms (*Aulocoseira* and *Cyclotella*)	(Zalocar de Domitrovic 2002)
Barra Bonita Reservoir, Brazil, S. America	Large permanent reservoir	Light availability, season, mixing and P influenced phytoplankton	131 spp., winter had higher species' numbers, cyanobacteria had the greatest relative abundance (*Microcystis aeruginosa*), diatoms contributed most biovolume	(Calijuri et al. 2002)
Luján River, Argentina, S. America	Medium river, seasonal	Transparency, discharge, temperature, P and conductivity were correlated with phytoplankton groups	Diatoms dominated (*Cyclotella* and *Nitzschia*), followed by chlorophytes, cyanobacteria and euglenoids; higher concentrations during summer	(O'Farrell et al. 2002)
Oxbow lakes of Paraná River, Argentina, S. America	Oxbow lakes, small ponds and semi-permanent lakes	Phytoplankton were influenced by light levels and macrophytes	Species diversity was higher in the shallow lakes than in the relict oxbow lakes	(O'Farrell et al. 2003)
Wetlands of Paraná River, Argentina, S. America	Oxbow lakes, small ponds and semi-permanent lakes	Phytoplankton correlated with light availability, macrophytes, anoxic conditions, N, P and K	Oxbow lakes dominated by cyanobacteria and diatoms; shallow lakes dominated by cryptophytes, euglenoids and dinoflagellates	(Izaguirre et al. 2004)
Los Coipos Lake, Argentina, S. America	Large shallow lakes, seasonal	Conductivity and dissolved salts influenced phytoplankton assemblages	153 spp.; number of genera decreased as conductivity increased; different phytoplankton groups were assigned to different salt concentrations	(Fazio and O'Farrell 2005)
Floodplain of Paraná River, Argentina, S. America	Large river transitioning into a permanent floodplain (water-water ecotone)	Eugleophytes differences depended on hydraulic condition of each aquatic habitat	88 spp. Euglenoids (*Trachelomonas*, *Euglena*, *Lepocinclis*, *Phacus*) increased in species richness towards the lake; *Strombomonas* was constant across all habitats	(Tell et al. 2005)

The pulse of river discharge is a major factor controlling the biota in seasonal river floodplains (Junk et al. 1989) and is expected to have a major influence on the composition of the biota within the river itself. In South America, a clear pattern of phytoplankton species richness and abundance has been discerned correlating with transitions from riverine to a lacustrine environments (O'Farrell 1994, Izaguirre et al. 2001, O'Farrell et al. 2002, Tell et al. 2005). In Australia, phytoplankton assemblages occurring at particular times in the annual cycle incorporate assemblages from both temperate and tropical systems in addition to those occurring in lakes and rivers (Hotzel and Croome 1996, Sherman et al. 1998, Fabbro and Duivenvoorden 2000).

Phytoplankton of tropical rivers received little attention until the 1990s. Much of the recent literature has focused on phytoplankton of reservoirs and lakes, and problem species such as toxin-producing cyanobacteria. The rivers of inland, dry tropical Australia have a highly seasonal hydrologic regime with turbid flows followed by periods of low-flow or impoundment of water (i.e. water holes). Literature on these seasonal rivers and the effect of seasonality and corresponding water quality on phytoplankton assemblages is sparse and factors that determine phytoplankton composition and succession in these dry tropical inland rivers are largely unknown. This study was conducted in selected water holes in the Burdekin system, to provide a description of phytoplankton seasonal and spatial dynamics and influencing physico-chemical parameters. The study involved broad surveys of three sites on each of the three rivers over three seasons. More detailed investigations of one site on each river are described in Chapter 4.

3.2 Methods

Water quality measurements, water samples and phytoplankton samples were taken in July and November 2003 and April 2004, corresponding to the mid-dry, late dry and post-wet seasons, at each of the nine study sites across the three rivers (Figure 3.1). Measurements and samples were collected at dawn, mid-day, and afternoon (approximately 0600, 1130 and 1600 respectively) for a single day. Collections were taken from an open water habitat and an aquatic macrophyte habitat at each site and time.

3.2.1 Water quality

In-situ measurements

Water temperature (°C), conductivity (μS cm^{-1}), dissolved oxygen (% and mg L^{-1}) and pH were measured in the water column at 25 cm depth and then at one-meter intervals to the bottom of the water body. These measurements were taken with an YSI 556 Multi-Probe System with a 10 m cable. Photoactive radiation (PAR) (μmol) was measured with a Li-Cor LI-250 Light meter with

a 10 m cable at the same depths. At the same time, air temperature (°C) and wind speed (m s^{-1}) were recorded with an Extech Mini Thermo-Anemometer.

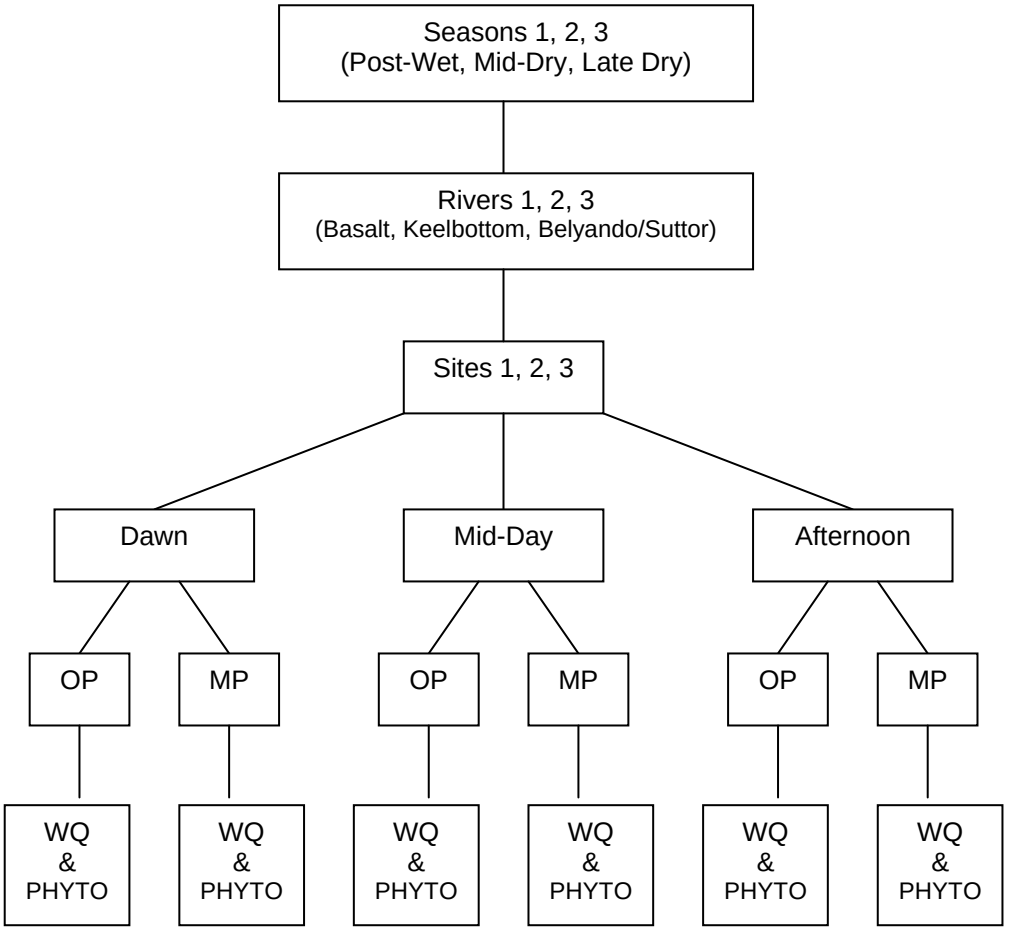


Figure 3.1 - Survey sampling design. OP = Open water habitat; MP = Macrophyte habitat; WQ = Water quality measurements (Temperature, Photosynthetic Active Radiation (PAR), pH, Dissolved Oxygen, Conductivity, Turbidity, Chlorophylls *a, b, c*); PHYTO = P

Water sampling

To determine Chlorophyll *a, b,* and *c*, phaeophytin *a* (μg L^{-1}) and turbidity (NTU), 1-L water samples were taken from each habitat at each site at each time. Water samples of 30 mL to measure turbidity were taken at the same times. Composite water samples were collected by pumping (peristaltic pump, Solinst 410) 10 L of water from close to the bottom to the surface of the water column and sub-sampling 1 L for pigment analysis or 30 mL for turbidity analysis. Samples were labeled with appropriate field information and immediately placed on ice in the dark. Water samples were taken to the Australian Centre for Tropical Freshwater Research laboratory at James Cook University for analysis.

Water analysis

Pigment analysis was done by filtering each 1 L water sample on a Whatman GF/B 47 mm glass microfibre filter using a vacuum filter setup. Filters were wrapped in aluminum foil, labeled with the field information and placed at -20 °C for a minimum of three days. Filters were then removed from the freezer and ground in a glass test tube with 10 mL of 90% acetone using a motor-driven grinder. The filter/sample slurries were placed in centrifuge tubes. Pigments of the samples were extracted at 4 °C for 24 hours in the dark. Extracted samples were centrifuged for 15 minutes and 10 minutes at 100 g in a Hettich Universal centrifuge fitted with a four bucket swing-out rotor. Supernatants were transferred into a 3 mL with a 1 cm light path spectrophotometer cuevette and samples were read on a PU 8670 VIS/NIR spectrophotometer at wavelengths 630nm, 647nm, 664nm, 665nm and 750nm. To determine the amounts of Chlorophyll *a*, *b*, and *c*, and phaeophytin *a* in the sample the equations of Jeffery and Humphrey (1975) were used. Samples were acidified with 1N HCl to determine phaeophytin at wavelengths 665nm and 750nm.

Turbidity samples were transferred into a calibrated glass vial and readings were taken with a HACH 2100P Turbidimeter.

3.2.2 Phytoplankton

Sampling

Phytoplankton was sampled by a vertical haul through the water column (bottom to surface) with a 25 μm and 50 μm phytoplankton net. Samples were collected in 100 mL jars connected at the base of the phytoplankton net. Samples were immediately preserved by adding Lugol's solution at a ration of 1:100 (Vollenweider 1969). Samples of living phytoplankton were also collected and immediately placed on ice in the dark. The preserved samples were returned to the laboratory for identification. The live samples were returned to the laboratory and cultured in a culture cabinet at 24 °C for use in laboratory experiments.

Identification

The preserved phytoplankton samples were shaken for 30 seconds and placed into a 100 mL graduated cylinder. The samples were then allowed to settle by gravity. The sedimentation chambers were covered with parafilm and placed in a fume hood. A settlement time of four days was used to allow for settlement of any small centric diatoms that may have been present (Furet and Benson-Evans 1982), then the top 90 mL of the sample was removed without disturbing the settled cells. The remaining 10 mL sample was placed in a sample jar and labeled with appropriate field information and stored in the dark.

Algae were identified to species level where possible using Playfair (1915, 1916, 1918, 1919), Smith (1950), Prescott (1970, 1978), Croasdale and Scott (1976), Thomas (1983) , Ling and Tyler (1986) , Thomasson (1986), Round et al. (1990), Entwisle et al. (1997), Gell et al. (1999), John et al. (2002), Wehr and Sheath (2003). Higher order classification was determined from Graham and Wilcox (2000). Counts of cell density were made with a calibrated glass Sedgwick-Rafter chamber. It is recommended that 30 fields be counted within the chamber so as to include 90 to 95% of the species present (McAlice 1971). In this study, 40 fields were counted for each sample. For filaments and colonies of cyanobacteria in which the individual cells were easy to recognize and of regular length, the number of cells per filament for the first 50 filaments observed was counted and the mean number of cells per filament for the sample was calculated. For filaments in which cells were not distinguishable, the average length of 30 cells at 1,000× magnification was determined. That length was used to determine cell counts in the sample.

3.2.3 Analyses

Water Quality

Patterns in water quality parameters between seasons, rivers, times and habitats were analyzed by Principal Component Analysis (PCA) using the PC-ORD package (McCune and Mefford 1999). PCA is a multivariate analysis that ordinates data and is an ideal method for data with approximately linear relationships among variables. PCA is not the best technique for community structure data as it tries to fit nonlinear data to a linear model; however, community analysts have found PCA a useful tool for relating environmental data to community data (McCune and Grace 2002). It has been widely used in the study of water quality and algal assemblages (Fabbro and Duivenvoorden 2000, Haag and Westrich 2002, Zalocar de Domitrovic 2002, Ouyang 2005). Multi-response Permutation Procedures (MRPP), using PC-ORD, were conducted to determine if there were significant differences between *a priori* groups. MRPP is a powerful multivariate hypothesis-testing technique that makes no assumptions about the distribution of data, such as multivariate normality and homogeneity of variances (McCune and Grace 2002).

Phytoplankton

Patterns in phytoplankton between seasons, rivers, times and habitats and corresponding to water quality parameters were analyzed using Non-metric Multidimensional Scaling (NMS) with a Sorensen (Bray-Curtis) distance measure in PC-ORD. NMS is an ordination method well suited to data that are non-normal or are on arbitrary, discontinuous, or otherwise questionable scales (McCune and Grace 2002), as is the case with most species community data. An advantage of NMS is that it is based on ranked distances, so it linearizes the relationship between environmental distance and community distance (Beals 1984). NMS performs well when applied

to community data (Fasham 1977, Kenkel and Orloci 1986, Minchin 1987, Clarke 1993, McCune 1994). Table 3.6 lists only the phytoplankton species with a significant r-value, however, all species present in the study were included in the analyses.

3.3 Results

3.3.1 Physico-chemical data

Depth profiles of temperature, dissolved oxygen, pH, conductivity and PAR showed differences within rivers across seasons, as well as differences between rivers. Diel ranges of these parameters also exhibited observable variation across season and river. Unfortunately, due to equipment malfunctions in the field, some readings were not recorded for the Mirambeena and Bridge sites on the Keelbottom Creek for the mid dry and late dry seasons and for the sites on the Basalt River during the late dry season. For Figures 3.2-3.6, I used the daily minimum and maximum record for each parameter for clarity and to show diel variation.

Temperature

Thermal stratification of a water body is usually based on temperature gradients. It is suggested that the existence of a stable thermocline is connected with the presence of a vertical temperature gradient of 1 ºC per meter depth (Heitmann 1973). The Basalt River showed thermal stratification during the mid dry and late dry seasons, the Keelbottom Creek showed thermal stratification during the late dry and post wet seasons and the Bleyando/Suttor Rivers showed thermal stratification during all three seasons (Table 3.2). Figure 3.2 shows daily temperature ranges across rivers, sites and seasons. Highest temperatures were recorded during the late dry season across all sites, with the highest reading, 33 °C, recorded at Mt. Douglas on the Belyando River. Lowest temperatures were recorded during the mid dry season across all sites, with the lowest reading, 17 °C , recorded at Mt. Douglas on the Belyando River. Temperatures and temperature ranges remained fairly consistent across depth and season in sites of the same river. The sites of the Basalt River were deep in comparison to the other river sites, ranging from 4 – 8 m, therefore the diel variation was low. The greatest diel variation was during the late dry season, with the largest variation being 3 °C at the surface of the Hillgrove site. Although there was little diel variation at the Basalt sites, there was a distinct stratification gradient with depth, especially during the mid dry season. Sites on the Basalt River showed consistent temperatures, ranging around 27 °C, across depths during the post wet season. Sites on the Keelbottom Creek ranged in depth from 1 – 4 m. Due to the more shallow nature of these sites, there was no apparent temperature gradient with depth, except at the Dotswood site, which showed a 1.5-2 °C temperature gradient per meter water column in the mid and late dry seasons. All three sites had a noticeable diel variation for the mid and late dry seasons, with the highest variation, 7 °C, being

recorded at the Bridge site at the surface. Although these shallow sites showed diel variation in temperatures, temperatures in the post-wet season remained constant. The sites on the Belyando/Suttor River were shallow (maximum depth from 1 – 3 m) and showed diel variation, especially in the late dry season. The Mt. Douglas site showed the greatest daily variation of all sites across all rivers, with a range of 11 °C at the surface. Temperature for the mid-dry (winter) season was consistently lower than late dry and post wet seasons for all rivers and sites. These sites also showed constant temperatures during the post wet season as observed for the other river sites. The Disney and Mt. Hope sites showed a slight temperature gradient with depth during the mid dry season. The sites on the Basalt River were much deeper, hence daily temperatures in the Belyando/Suttor River and Keelbottom Creeks fluctuated more than in the Basalt River. The daily temperature variation across all rivers and sites was highest near the surface.

Table 3.2 - Presence or absence of thermal stratification within the nine study sites.

Site	River	Thermal Stratification (+ / -) Season		
		Mid-Dry	Late Dry	Post Wet
Emu Valley	Basalt River	+	+	-
Bluff Downs	Basalt River	+	- *	-
Hillgrove	Basalt River	+	- *	-
Dotswood	Keelbottom Creek	-	+	+
Mirambeena	Keelbottom Creek	-	+	-
Keelbottom Bridge	Keelbottom Creek	-	+	-
Mt. Douglas	Belyando / Suttor River	+	+	+
Disney	Belyando / Suttor River	+	+	+
Mt. Hope	Belyando / Suttor River	+	+	+

* - YSI meter malfunctioned in the field during this field trip only allowing measurements to 0.5m

Dissolved Oxygen

With the exception of shallow sites (the Bridge site on Keelbottom Creek and Mt. Douglas site on the Belyando/Suttor River), dissolved oxygen decreased with increasing depth, with a steady gradient across all sites and seasons (Figure 3.3), there were no distinct boundaries between strata. Average daily ranges within rivers varied with season. The sites in the Basalt River had the highest mean daily range in the post wet season, with the highest range at Hillgrove (56%). Keelbottom Creek sites showed the highest mean daily range in the late dry, with the highest range at the Bridge site (100%). Belyando/Suttor River sites showed the highest mean daily range in the mid and late dry seasons, with the highest mean daily range at the Mt. Douglas site (25% in mid dry and 20% in late dry). Bluff Downs and Hillgrove on the Basalt River showed the lowest oxygen levels during the post wet season, whereas oxygen values at Emu Valley were similar across seasons. During the late dry season at Emu Valley some stratification was evident towards the deeper strata. The Keelbottom Creek sites showed large diel variation in the late dry season.

The late dry season was also the only season in which oxygen reached over 100% saturation, with the highest recording at Mirambeena site (115%). Dotswood and Mirambeena sites showed the lowest oxygen levels during the post-wet season, whereas the Bridge site, due to its shallow nature, had very large diel variation. In the Belyando/Suttor River, the Mt. Douglas site had a similar pattern to the Bridge site on Keelbottom Creek. Due to its shallow depths the diel variation was large, so values were very variable and no difference between seasons was discernible. The Disney and Mt. Hope sites, on the other hand, showed lower oxygen readings in the late dry season.

pH

Figure 3.4 shows daily pH ranges across rivers, sites and seasons. There was a contrast between seasons across all rivers with pH being highest during the late dry season, ranging from 9.0 to 11.0. This season also showed the highest daily range among rivers and sites. The only exception was Bluff Downs on the Basalt River, which had the highest daily range in the mid dry season. The Basalt River and the Keelbottom Creek had similar pH measurements for the late dry season, ranging between 9.0 and 10.0, whereas the Belyando/Suttor River was slightly lower at 8.5 – 9.5. During the post wet season the Keelbottom Creek and Belyando/Suttor River were more similar with measurements ranging between 7.0 and 8.0, whereas the Basalt River was higher, 8.0 – 9.0. The lowest mean diel variation across rivers and sites was in the post wet season. The Emu Valley site on the Basalt River had a large diel variation during the late dry season, small variation during the mid dry season and little to no diel variation during the post wet season, although there were differences in the diel variations across season the pH measurements remained constant with depth. The Hillgrove site had similar characteristics as Emu Valley, although there was a slight pH gradient with depth during the mid dry season. The Bluff Downs site was different in that it had its highest diel variation during the mid dry season. This season also had an interesting depth gradient: as depth increased pH decreased until 6 m where there was little to no diel variation and the pH began to increase with depth. All sites on the Keelbottom Creek had a large contrast between measurements from the late dry season and the other seasons. pH measurements remained constant with depth across all three sites. The Disney site on the Belyando/Suttor River was similar to the Keelbottom sites in having a large contrast between the measurements of the late dry and other seasons, and having constant readings with depth. The Mt. Douglas and Mt. Hope sites had less contrast between seasons, although pH was higher during the late dry season. Measurements for these sites remained constant across depth, except for Mt. Hope during the mid dry season where there was a slight gradient.

Conductivity

Conductivity, unlike the other physico-chemical measurements, had very little diel variation across seasons, rivers and sites (Figure 3.5). Conductivity was highest during the late dry season for all sites, except for Mt. Douglas on the Belyando/Suttor River, where late dry and post wet seasons were similar. The post-wet season tended to have the lowest conductivity readings for most sites, except for Bluff Downs on the Basalt River and Mt. Douglas on the Belyando/Suttor River, which had the lowest recordings during the mid-dry season. Emu Valley and Bluff Downs on the Basalt River ranged between 800 and 960 μS cm^{-1}, with clear but not large differences between the seasons. The Hillgrove site on the Basalt River had lower conductivity than the other two sites (550 – 740 μS cm^{-1}) with contrast between the seasons, but little variation with depth. The three sites on the Keelbottom Creek showed contrast between the seasons with small variations, except the Bridge site, which had a large difference (approximately 350 μS cm^{-1}) between the late-dry and post-wet seasons. Sites on the Belyando/Suttor River also showed contrast between the seasons with little variation, except for Mt. Douglas site, which had a large contrast of 150 μS $cm^{-1}$for this river between the late dry/post-wet and mid-dry seasons. This site was also unique in that measurements for the post-wet were higher than for the other seasons, whereas for other sites of this river and other rivers, the post-wet measurements were the lowest across season. Among all seasons, rivers and sites conductivity remained constant across depth.

Photosynthetically active radiation

Photosynthetically active radiation (PAR) decreased exponentially with depth (Figure 3.6). This pattern was consistent across all seasons, rivers and sites. The highest mean daily variation among most sites and rivers was recorded during the late dry season, with the highest variation (610 μmol) recorded from Hillgrove on the Basalt River at a depth of 0.25 m. The Dotswood site on the Keelbottom Creek had the highest diel variation (145 μmol) during the post west season. Water clarity had a high impact on the amount of PAR entering the water body. As the Basalt River had good clarity, PAR values were recorded above zero to a depth of 4 m, except at Hillgrove where measurements of zero were recorded by 3 m. The Keelbottom Creek had fair water clarity, with PAR values recorded above zero to 2 m at all sites. The Belyando/Suttor River had poor water clarity, with PAR being zero at a depth greater than 1 m. The Keelbottom Creek and the Belyando/Suttor River, the more shaded rivers, had the lowest diel variation. The Basalt River, being the least shaded and turbid river, had the highest diel variation, especially above 1 m depth.

Water Quality Ordinations

Ordination by physico-chemical parameters, grouped samples by season, river, time of day and habitat type with highly significant MRPP groupings for all variables (Figures 3.7 & 3.8).

Correlations between physico-chemical variables and the ordination axes (Table 3.3) established that important variables were: for Axis 1, dissolved oxygen and conductivity; for Axis 2, dissolved oxygen, temperature, pH, PAR and conductivity; and for Axis 3, dissolved oxygen and temperature. MRPP analyses (Table 3.4) showed that rivers were significantly different from one another; and seasons were significantly different from each other, except for the marginally non-significant late-dry and pos-wet comparison ($p < 0.066$); all times of day were significantly different from one another; and habitats were significantly different.

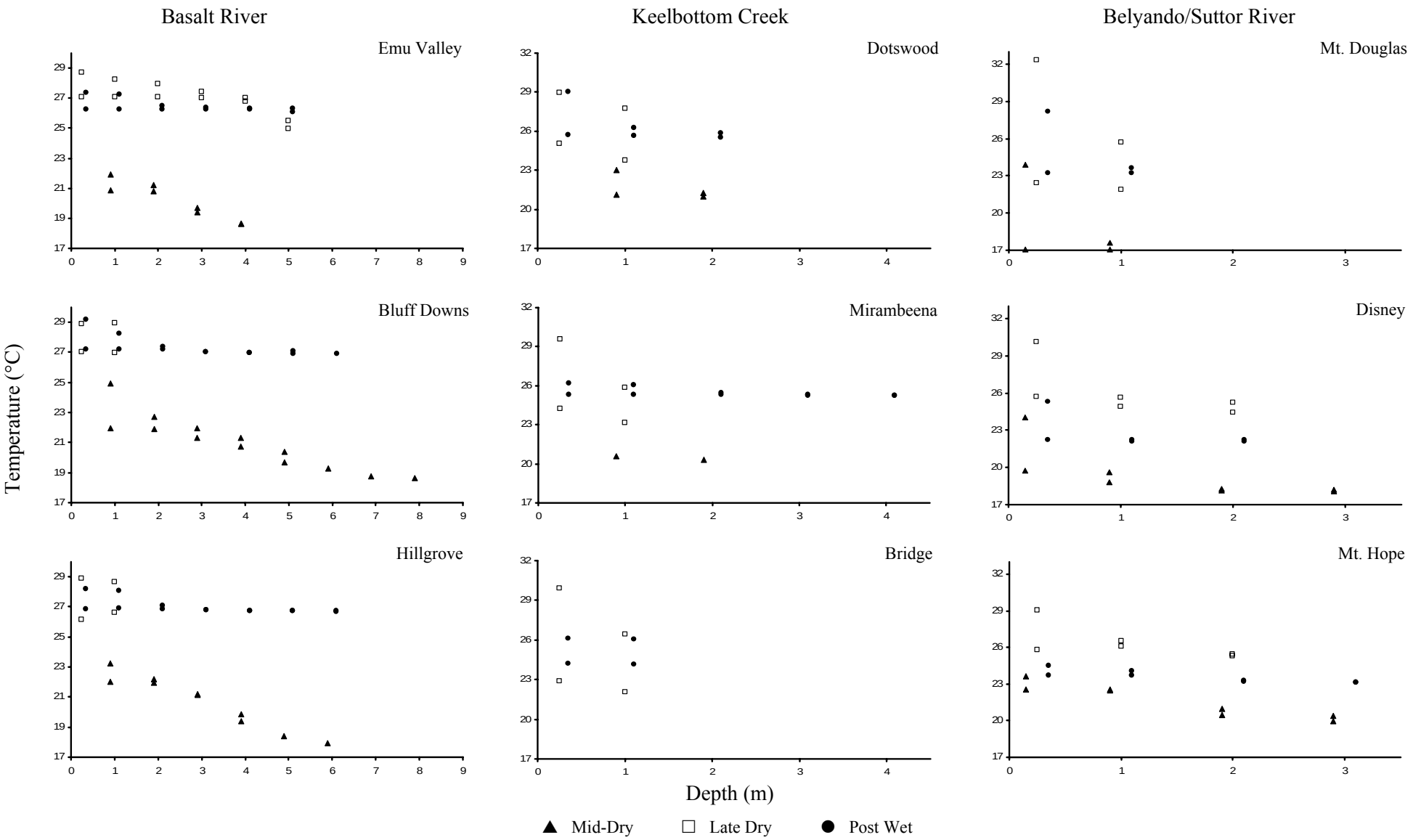


Figure 3.2 - Temperature records at specific depths at nine sites over three seasons among three rivers. Paired symbols for each depth represent daily range.

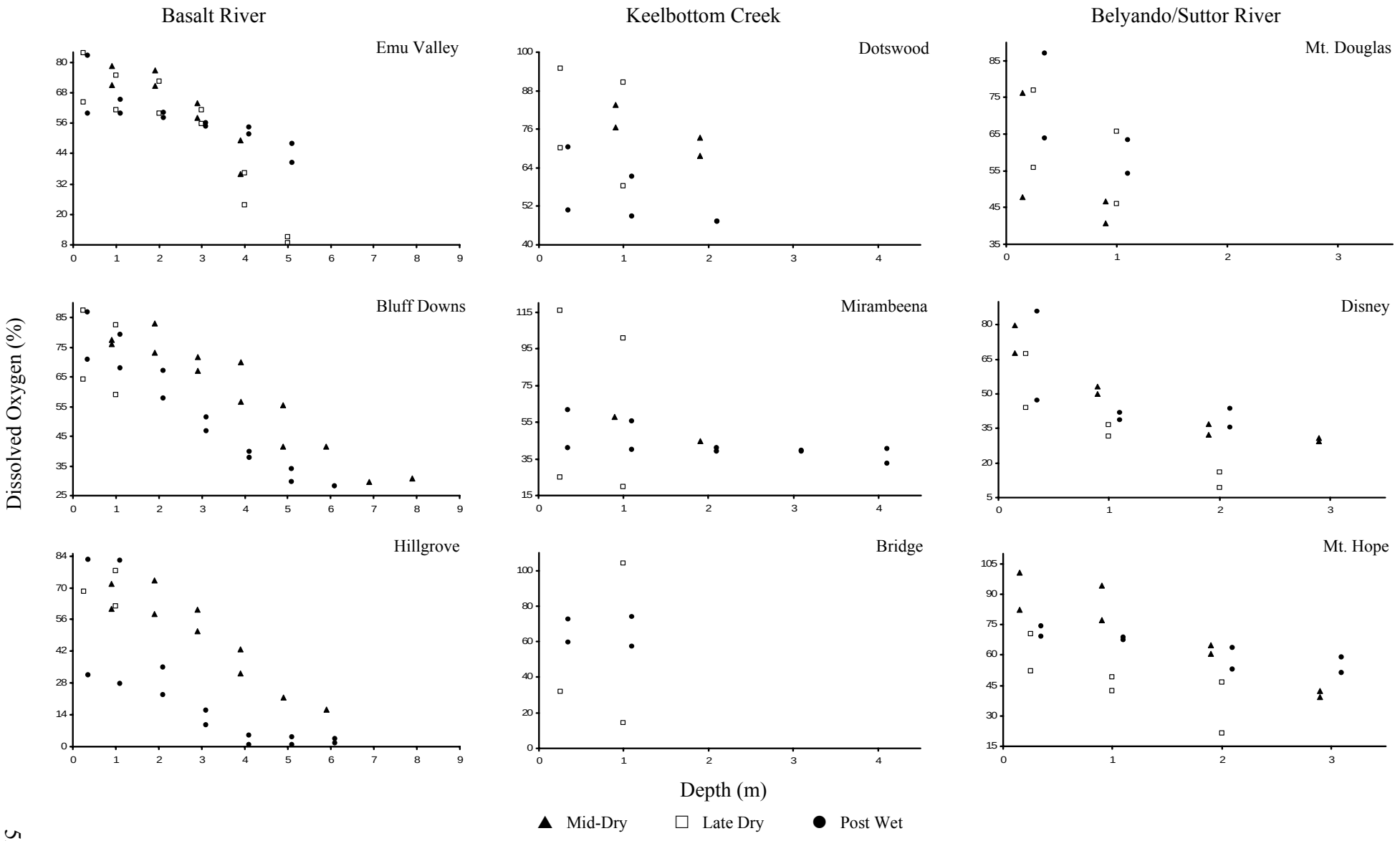


Figure 3.3 - Dissolved oxygen records at specific depths at nine sites over three seasons among three rivers. Paired symbols for each depth represent daily range.

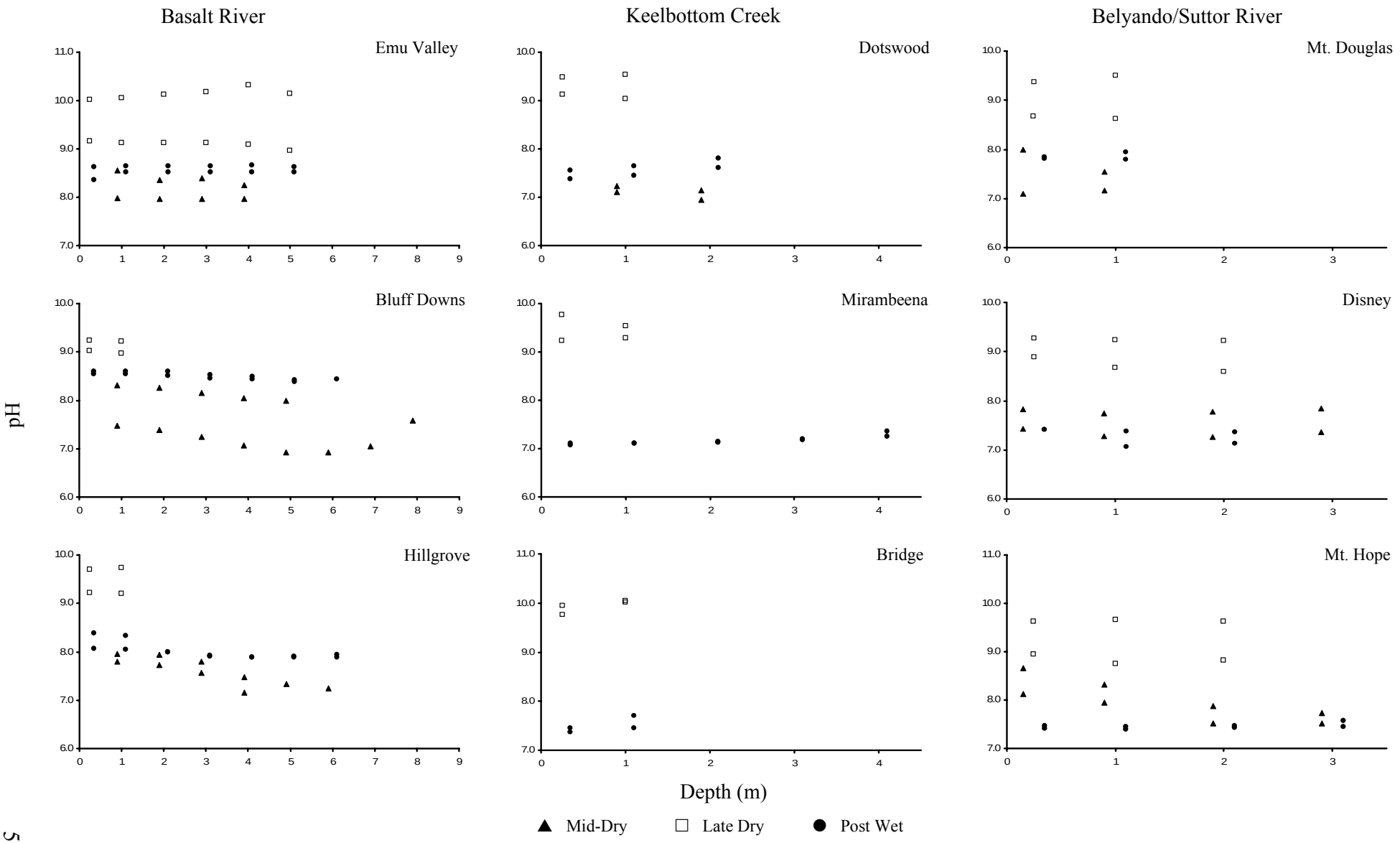


Figure 3.4 - pH records at specific depths at nine sites over three seasons among three rivers. Paired symbols for each depth represent daily range.

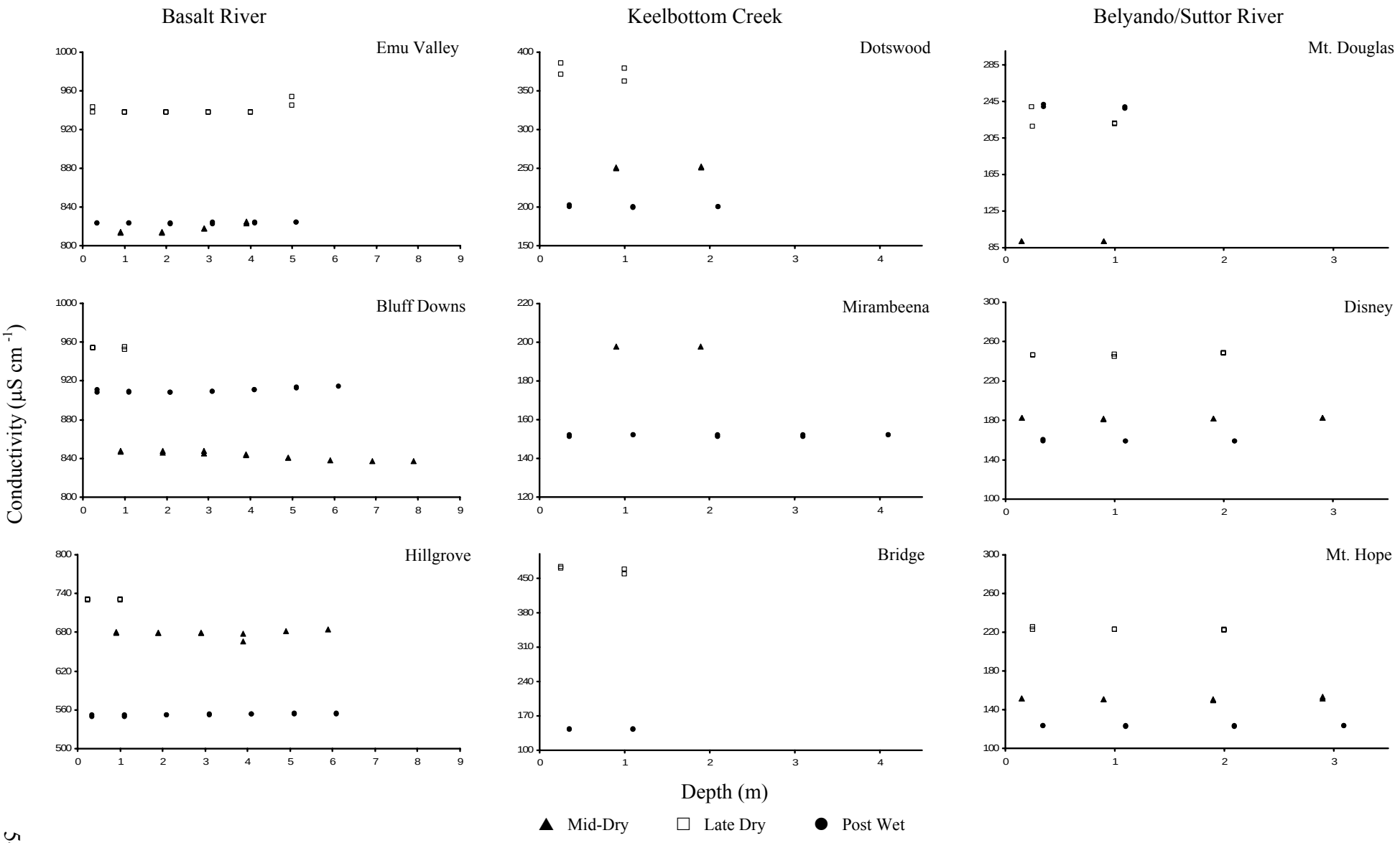


Figure 3.5 - Conductivity records at specific depths at nine sites over three seasons among three rivers. Paired symbols for each depth represent daily range.

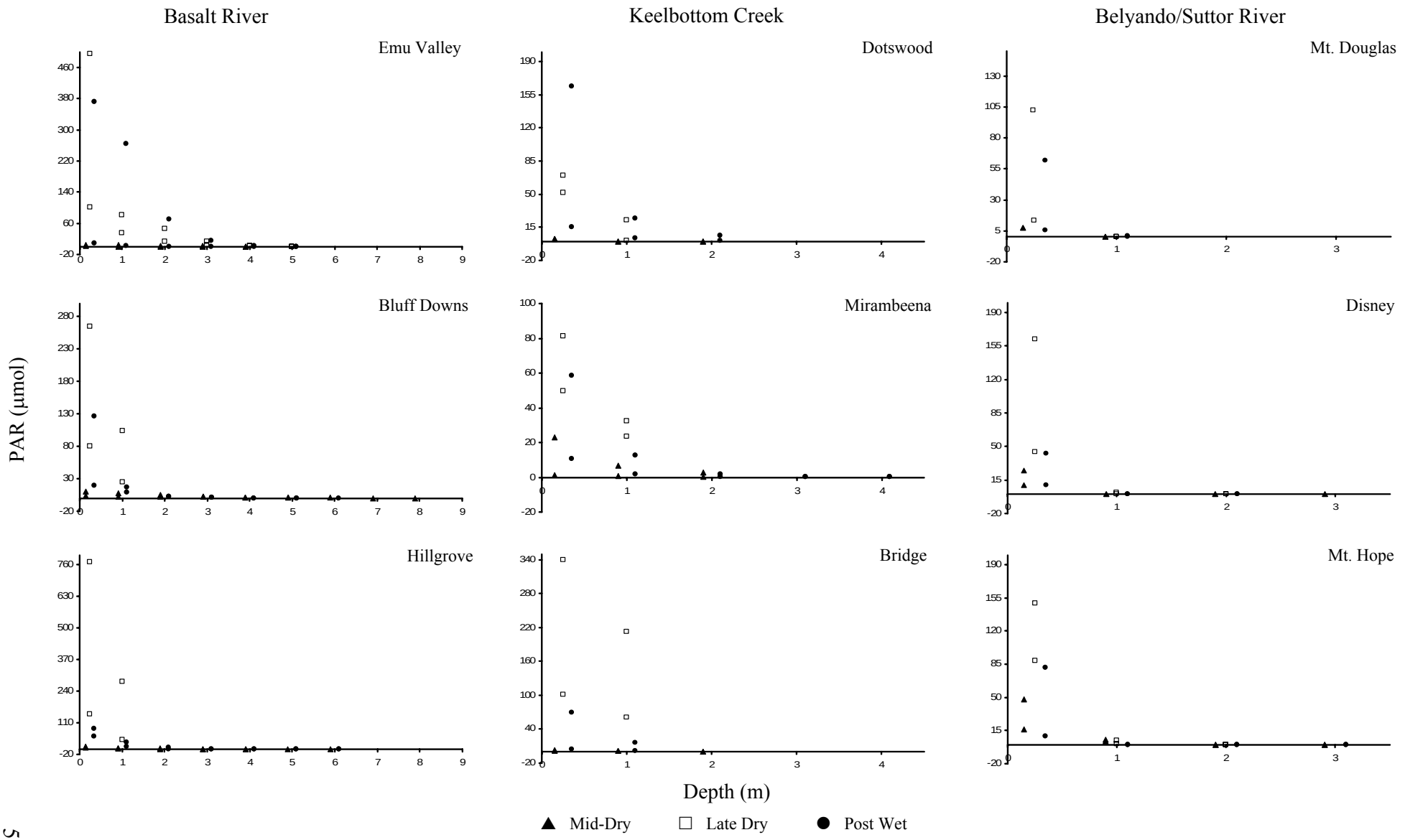
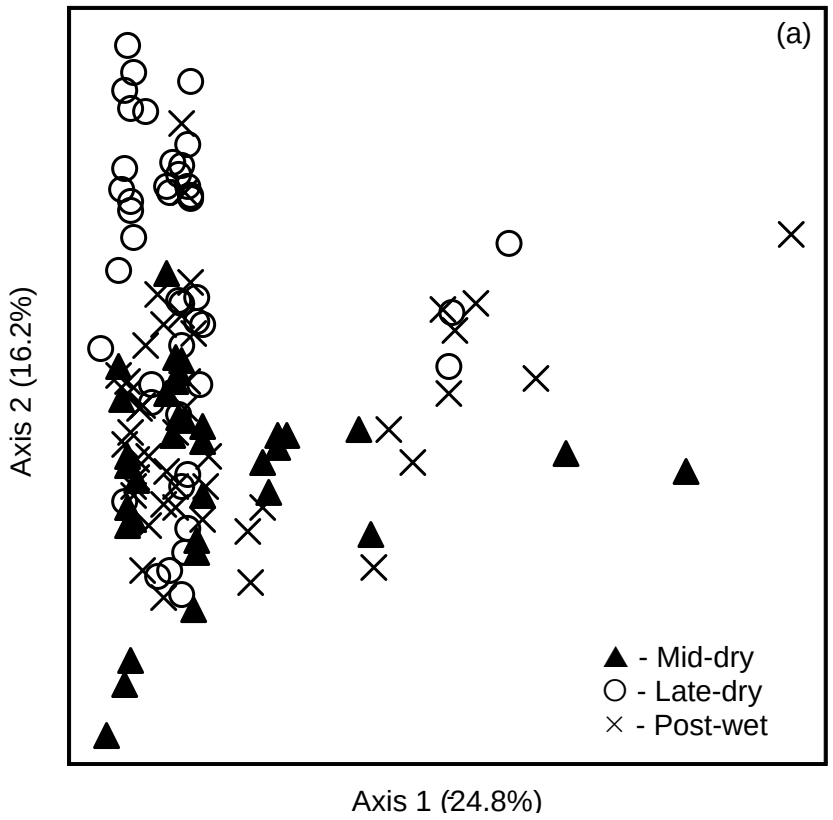


Figure 3.6 – PAR records at specific depths at nine sites over three seasons among three rivers. Paired symbols for each depth represent daily range.



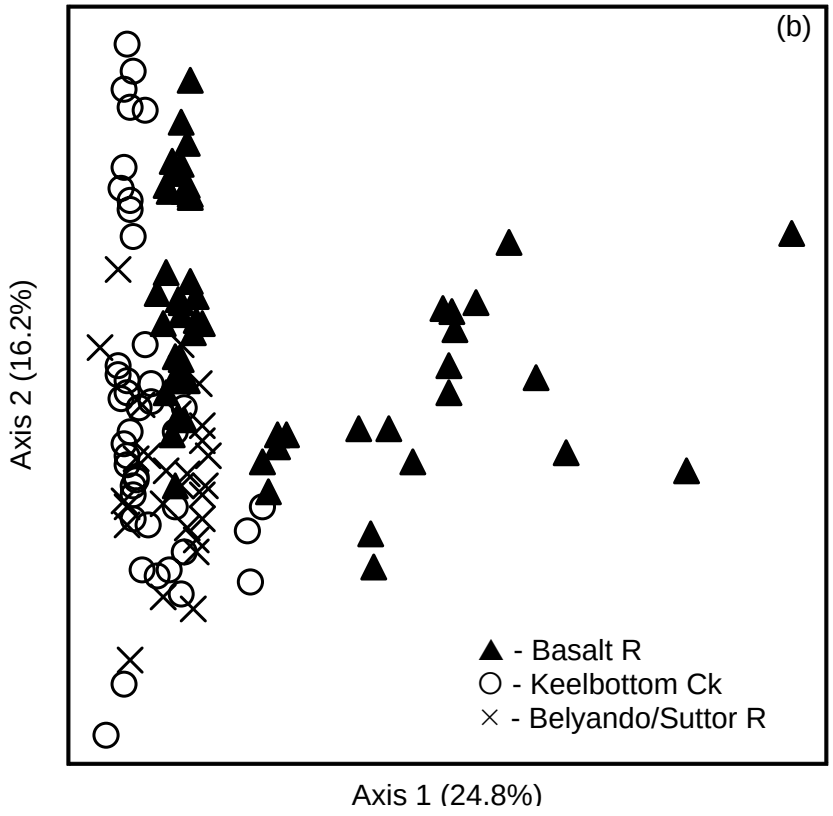
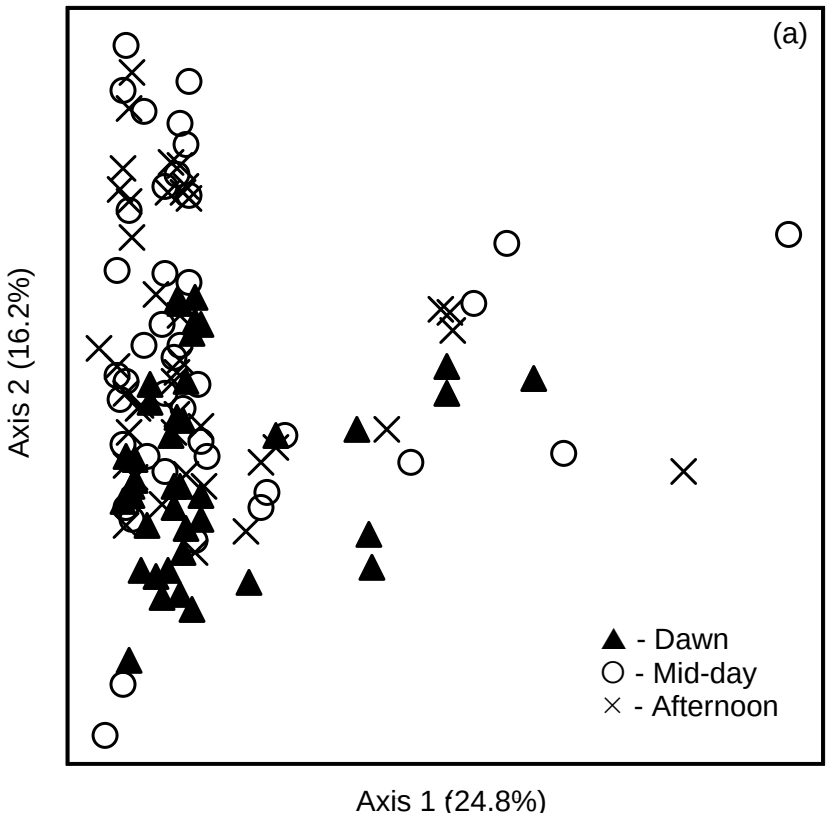


Figure 3.7 - PCA ordination of samples using physico-chemical data, with overlays of (a) season and (b) river; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p = 0.00001217 and river, p < 0.000000



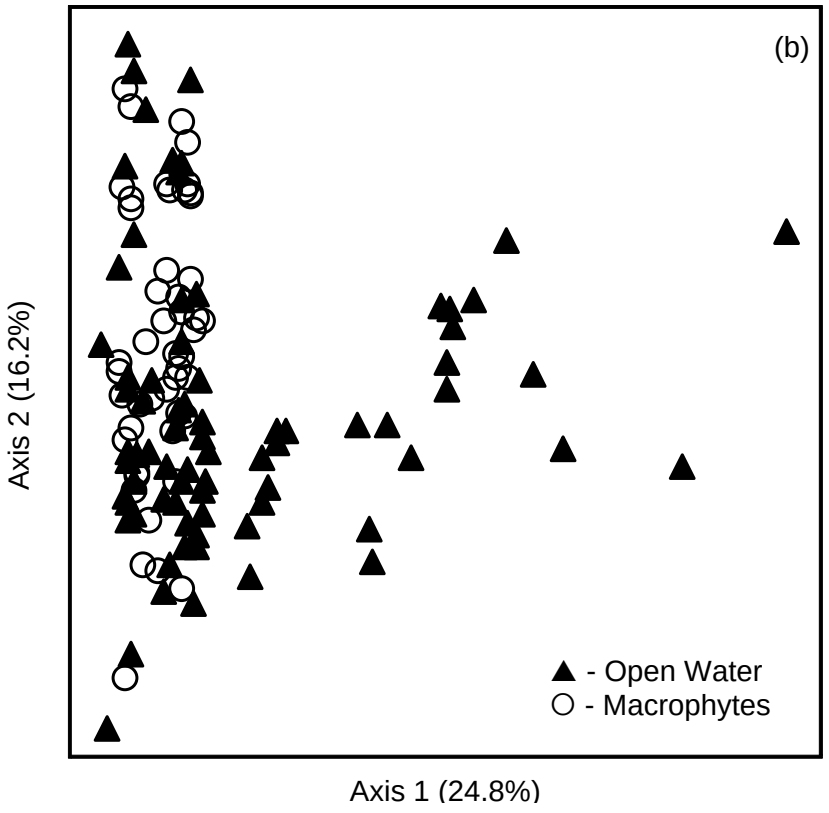


Figure 3.8 - PCA ordination of samples using physico-chemical data, with overlays of (a) time and (b) habitat; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for time, p < 0.00000001 and habitat, p < 0.00000001

Table 3.3 - Correlation table showing r values for physico-chemical parameters corresponding to different axes of a PCA analysis. Significant r values shown in bold ($r_{(0.01,121)} = 0.232$).

Parameter	Axis 1 (24.8%)	Axis 2 (16.2%)	Axis 3 (10.5%)
Bottom Dissolved Oxygen	**-0.519**	**0.641**	**-0.373**
Minimum Dissolved Oxygen	**-0.504**	**0.652**	**-0.379**
Chlorophyll *a*	**-0.337**	0.133	0.150
Chlorophyll *b*	**-0.289**	-0.002	0.100
Mean Dissolved Oxygen	**-0.286**	**0.748**	**-0.570**
Phaeophytin	**-0.238**	-0.206	**-0.283**
Chlorophyll *c*	-0.222	0.009	0.014
Turbidity	-0.207	-0.131	0.015
Middle Dissolved Oxygen	-0.194	**0.700**	**-0.580**
Bottom PAR	-0.149	**0.464**	**0.242**
Minimum PAR	-0.145	**0.455**	**0.250**
Surface pH	-0.083	0.026	0.009
Minimum Temperature	-0.071	**0.721**	**0.448**
Mean PAR	-0.051	**0.524**	**0.361**
Maximum Dissolved Oxygen	-0.029	**0.680**	**-0.629**
Middle PAR	-0.029	**0.477**	**0.290**
Mean Temperature	-0.024	**0.739**	**0.402**
Minimum pH	-0.024	-0.016	0.063
Surface Dissolved Oxygen	-0.011	**0.633**	**-0.616**
Middle Temperature	-0.006	**0.732**	**0.366**
Bottom Temperature	-0.006	-0.083	-0.070
Maximum Temperature	0.021	**0.690**	**0.330**
Surface Temperature	0.072	**0.673**	**0.356**
Middle pH	0.080	**0.582**	**0.414**
Bottom pH	0.081	**0.577**	**0.397**
Maximum pH	0.108	**0.570**	**0.376**
Surface PAR	0.121	**0.427**	**0.366**
Maximum PAR	0.139	**0.472**	**0.373**
Mean pH	**0.407**	**0.416**	**0.281**
Surface Conductivity	**0.489**	**0.531**	0.111
Mean Conductivity	**0.490**	**0.527**	0.114
Maximum Conductivity	**0.490**	**0.530**	0.113
Middle Conductivity	**0.490**	**0.525**	0.116
Minimum Conductivity	**0.491**	**0.525**	0.116
Bottom Conductivity	**0.492**	**0.525**	0.116

Table 3.4- MRPP table showing A-statistic and p-value comparing physico-chemical parameters among sites between groups.

Comparison Group	A-statistic	p-value
All Rivers (Keelbottom Creek, Basalt River & Belyando/Suttor River)	0.08266258	0.00000001
Keelbottom Creek vs Basalt River	0.05138717	0.00001138
Basalt River vs Belyando/Suttor River	0.08537036	0.00000001
Belyando/Suttor River vs Keelbottom Creek	0.05378161	0.00015190
All Seasons (Mid-Dry, Late Dry & Post Wet)	0.04256145	0.00001217
Mid-Dry vs Late Dry	0.06192609	0.00000215
Late Dry vs Post Wet	0.00951884	0.06619108
Post Wet vs Mid-Dry	0.03324389	0.00043445
All Times (Dawn, Mid-Day & Afternoon)	0.12469988	0.00000001
Dawn vs Mid-Day	0.15775472	0.00000001
Mid-Day vs Afternoon	0.09946751	0.00000001
Afternoon vs Dawn	0.00996609	0.04679711
Habitat (Open water & Macrophytes)	0.06208104	0.00000001

3.3.2 Phytoplankton

Algal Assemblages

138 species from six divisions were identified (Appendix 2). Chlorophytes were the most species rich with 56 species, followed by diatoms, euglenoids, cyanobacteria, dinoflagellates and other ochrophytes, with 18, 16, 12, 3 and 3 species, respectively (Table 3.5). Cyanobacteria had the highest abundance, in terms of cells per milliliter. Keelbottom Creek had the greatest presence of the species identified (e.g., 89 species at the Bridge site). The Belyando/Suttor River sites showed more prevalence of the euglenoids than the other divisions. The Basalt River had a higher prevalence of cyanobacteria than the other rivers (Appendix 2).

Table 3.5 – Abundance and species richness of phytoplankton identified from the general study.

	Chlorophyta	Ochrophyta	Euglenoids	Cyanobacteria	Dinophyta	Other Ochrophyta
August 2003						
Abundance (cells mL^{-1})	61,025	6,650	24,475	99,525	23,100	150
Species richness	30	12	8	8	2	1
November 2003						
Abundance (cells mL^{-1})	67,236	4,700	21,050	3,588,350	17,075	250
Species richness	36	13	15	12	3	1
April 2004						
Abundance (cells mL^{-1})	122,525	14,775	17,725	346,475	5,550	33,650
Species richness	56	18	16	12	3	3

Algal Assemblage Ordinations

NMS and MRPP analyses showed that phytoplankton assemblages grouped by season, river and habitat (Figures 3.9 and 3.10, Table 3.8). There were many phytoplankton species that significantly correlated with the different axes of the NMS analyses (Table 3.6). Correlations of physico-chemical variables with the algal ordination axes show strong correlations between conductivity on Axis 1, and conductivity and turbidity with Axis 2 (Table 3.7). There is some correlation with dissolved oxygen on Axis 3.

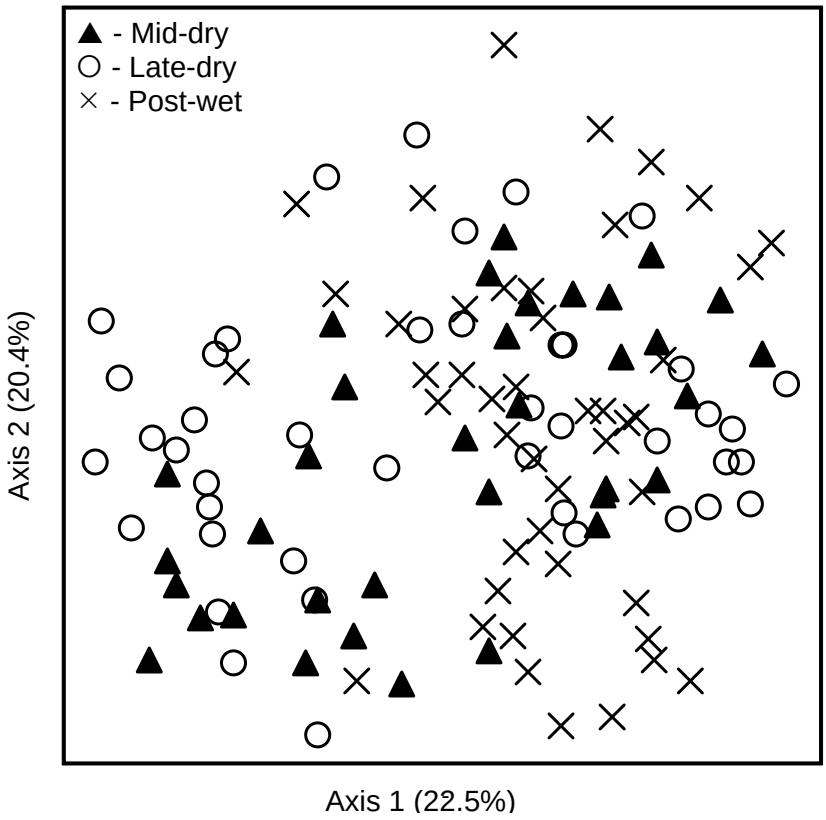
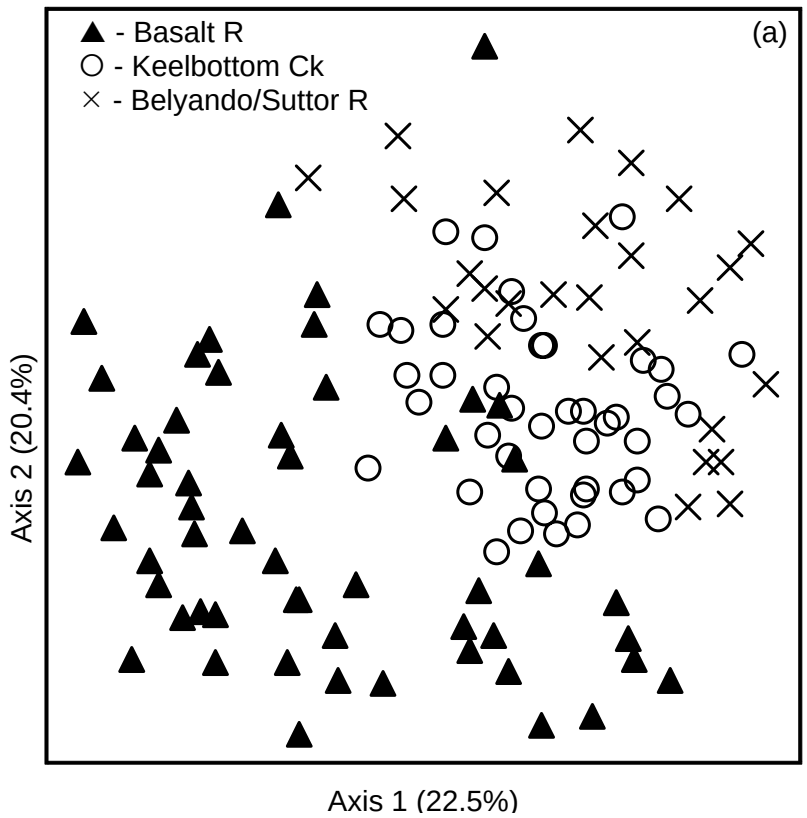


Figure 3.9 - NMS ordination of samples using algal data, with overlay of season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p = 0.00000001. Stress = 30.356.



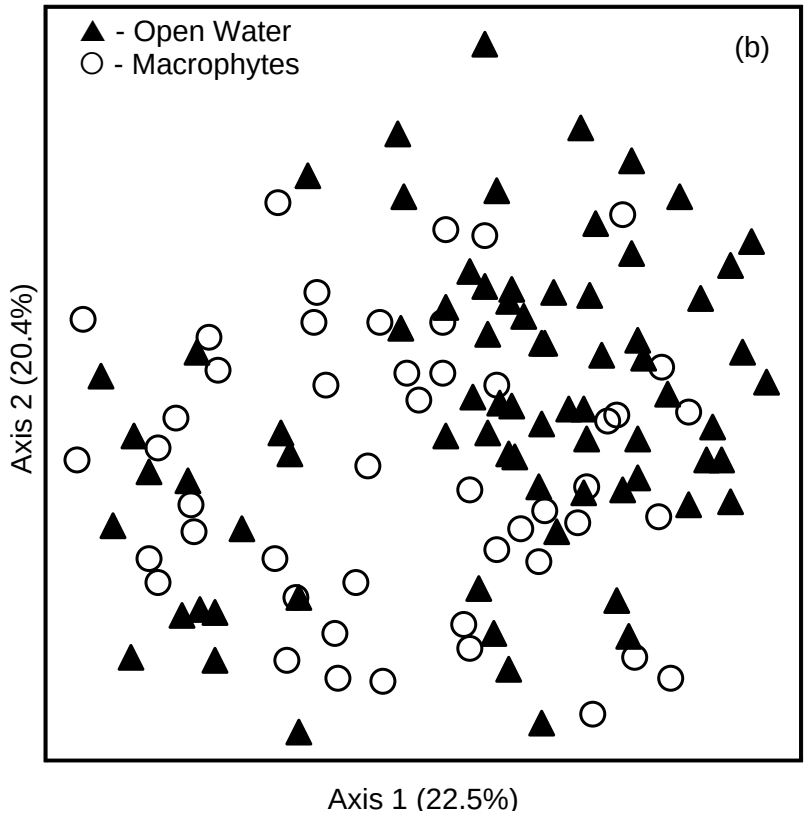


Figure 3.10 - NMS ordination of samples using algal data, with overlays of (a) river and (b) habitat; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for river, p = 0.00000001 and habitat, p = 0.00000450. Stress = 30.356.

Table 3.6 - Correlation table showing r values for algal species corresponding to different axes of a NMS analysis. Significant r values shown in bold ($r_{(0.01, 121)}$ = 0.232), including Log transformed total abundance.

Species	Total Abundance (Log Transformed)	Axis 1 (22.5%)	Axis 2 (20.4%)	Axis 3 (15.7%)
Anaphanizomenon gracile	6.54860	**-0.698**	-0.182	0.179
Mougoetia sp.	4.54900	**-0.405**	**-0.298**	0.019
Anabaena spiroides	4.64665	**-0.375**	**-0.334**	0.111
Pediastrum simplex	4.01599	**-0.353**	**-0.324**	0.098
Merismopedia glauca	3.99564	**-0.336**	**-0.319**	-0.047
Pediastrum boryanum	4.25828	**-0.317**	-0.178	0.064
Gomphonema sp. 1	3.10551	**-0.304**	0.027	0.183
Navicula sp. 1	3.83569	**-0.296**	0.079	**-0.341**
Cladophora sp.	3.61805	**-0.272**	0.026	0.036
Glenodinium sp.	3.49136	**-0.269**	0.134	**0.265**
Cosmarium pseudopyramidatum	3.57692	**-0.235**	-0.128	-0.207
Spirulina sp.	3.20412	**-0.234**	-0.147	0.048
Peridinium bipes	4.55509	-0.117	**-0.393**	**0.333**
Cymbella sp. 1	2.62839	-0.098	**-0.246**	0.081
Cymbella sp. 2	2.60206	-0.071	**0.246**	0.057
Anabaenopsis elenkinii	3.79414	-0.064	**-0.303**	-0.068
Gomphonema sp. 2	2.47712	0.005	-0.060	**-0.262**
Gomphonema sp. 3	2.60206	0.035	0.050	**-0.252**
Nitzschia sp. 2	2.60206	0.036	0.078	**-0.278**
Peridinium inconspicuum	3.19033	0.036	-0.060	**-0.342**
Scenedesmus quadrucauda	4.23558	0.053	**-0.434**	**0.354**
Oscillatoria sp.	3.94817	0.089	-0.008	**-0.315**
Synedra ulna	3.43537	0.104	-0.214	**-0.306**
Microspora sp.	4.45599	0.109	**0.264**	-0.184
Fragilaria sp. 1	3.52504	0.115	-0.151	**-0.281**
Tracholemonas planctonica	2.35218	0.117	0.135	**-0.312**
Closterium acerosum	3.01072	0.137	**-0.250**	-0.172
Koliella spiculiformis	3.51521	0.145	-0.063	**-0.315**
Gloeocystis sp.	3.87938	0.146	0.038	**-0.376**
Navicula sp. 3	3.45864	0.149	0.011	**-0.356**
Monoraphidium contortum	3.72222	0.154	-0.119	**-0.477**
Tracholemonas granulata	3.29003	0.156	**0.245**	**-0.288**
Scenedesmus ellipticus	3.72428	0.160	-0.226	**-0.325**
Monoraphidium arcuatum	3.09691	0.162	-0.106	**-0.299**
Tribonema affine	4.59824	0.165	**-0.325**	-0.192
Limnothrix redekei	5.56644	0.203	-0.165	**-0.412**
Amorpha sp. 1	2.43933	0.227	**0.306**	-0.175
Peridinium lomnickii	3.92814	**0.242**	-0.018	-0.062
Scenedesmus bernardii	3.57403	**0.245**	-0.034	0.178
Phacus longicauda	3.04139	**0.249**	0.009	0.054
Scenedesmus dimorphus	3.82607	**0.251**	-0.035	0.020
Treubaria triappendiculata	2.98900	**0.253**	0.228	**-0.276**
Strombomonas acuminatus	3.08814	**0.259**	0.214	**-0.236**
Trachelamonas armata	3.09691	**0.262**	0.069	0.033
Euglena ganulata	3.85884	**0.270**	0.011	0.041
Euglena acus	3.69020	**0.294**	0.126	**-0.233**
Trachelamonas oblonga	3.53466	**0.297**	**0.238**	**0.253**
Centritractus belanophorus	3.46613	**0.319**	-0.048	-0.143
Crucigenia quadrata var *secta*	3.51851	**0.334**	-0.081	**-0.267**
Euglena limnophila	3.38917	**0.343**	-0.148	**-0.356**
Strombomonas tambowika	3.31175	**0.387**	0.070	**-0.281**
Phacus caudatus	3.55931	**0.430**	-0.088	-0.031
Pediastrum duplex	4.09237	**0.437**	-0.184	-0.035
Trachelamonas hispida	3.88508	**0.603**	**0.253**	0.280
Trachelamonas volvocina	4.40226	**0.604**	**0.568**	0.212

Table 3.7 - Correlation table showing r values for physico-chemical parameters corresponding to different axes of a NMS analysis for algal species. Significant r values shown in bold ($r_{(0.01, 121)} = 0.232$).

Parameter	Axis 1 (22.5%)	Axis 2 (20.4%)	Axis 3 (15.7%)
Mean Conductivity	**-0.711**	**-0.474**	0.082
Minimum Conductivity	**-0.711**	**-0.474**	0.079
Middle Conductivity	**-0.711**	**-0.475**	0.080
Maximum Conductivity	**-0.710**	**-0.474**	0.084
Bottom Conductivity	**-0.710**	**-0.475**	0.081
Surface Conductivity	**-0.709**	**-0.472**	0.082
Middle Dissolved Oxygen	**-0.258**	0.039	**0.337**
Middle pH	-0.222	-0.118	0.120
Mean pH	-0.204	-0.117	0.139
Maximum pH	-0.198	-0.116	0.148
Bottom pH	-0.193	-0.098	0.130
Middle Temperature	-0.176	-0.125	-0.143
Mean Dissolved Oxygen	-0.167	0.020	**0.305**
Minimum Dissolved Oxygen	-0.137	0.092	0.202
Bottom Dissolved Oxygen	-0.120	0.098	0.204
Minimum Temperature	-0.111	-0.144	-0.196
Mean Temperature	-0.107	-0.115	-0.184
Surface pH	-0.076	0.159	0.041
Surface Dissolved Oxygen	-0.065	0.017	**0.317**
Maximum Dissolved Oxygen	-0.062	-0.018	**0.308**
Mean PAR	-0.045	0.032	-0.005
Bottom PAR	-0.031	0.040	0.110
Minimum PAR	-0.030	0.033	0.108
Middle PAR	-0.016	0.023	0.083
Maximum Temperature	-0.010	-0.045	-0.158
Surface Temperature	-0.009	-0.100	-0.226
Maximum PAR	0.009	0.010	-0.082
Surface PAR	0.026	0.013	-0.087
Bottom Temperature	0.062	0.188	-0.121
Turbidity	0.108	**0.470**	0.046
Chlorophyll *c*	0.112	**0.353**	0.182
Minimum pH	0.126	-0.017	-0.078
Phaeophytin	0.143	**0.244**	**0.333**
Chlorophyll *b*	0.205	**0.351**	0.221
Chlorophyll *a*	**0.298**	0.201	**0.259**

Table 3.8 - MRPP table showing A-statistic and p-value comparing algal species among sites between groups.

Comparison Group	A-statistic	p-value
All Rivers (Keelbottom Creek, Basalt River & Belyando/Suttor River)	0.07826835	0.00000001
Keelbottom Creek vs Basalt River	0.05751613	0.00000001
Basalt River vs Belyando/Suttor River	0.07548143	0.00000001
Belyando/Suttor River vs Keelbottom Creek	0.03616384	0.00000001
All Seasons (Mid-Dry, Late Dry & Post Wet)	0.05412326	0.00000001
Mid-Dry vs Late Dry	0.02929807	0.00000217
Late Dry vs Post Wet	0.03715601	0.00000001
Post Wet vs Mid-Dry	0.05703915	0.00000001
Habitat (Open water & Macrophytes)	0.01225678	0.00000450

3.4 Discussion

3.4.1 Physical characteristics

All study sites selected for this study were permanent water holes which held water even through the dry season, when there was no incoming surface water into the river system, and were therefore sustained by groundwater input. Some of the sites varied more substantially in size and depth between the wet and dry seasons than others (Table 2.3). The sites of the Basalt River showed consistent depth across seasons. Sites on the Belyando/Suttor Rivers and Keelbottom Creek were more variable: mostly they were much shallower and smaller during the dry season, but the Mt. Hope and Disney sites of the Belyando/Suttor Rivers remained similar between the seasons. All rivers showed an increase in flow during the wet season (November – February) due to rainfall in their catchments (Figures 2.9 and 2.10). For the year 2003, there was very little rainfall input into the Basalt and Keelbottom systems. There was also very little input into the Basalt system in 2004. However, the width and depth of the sites on the Basalt River were largely unaffected since the Basalt River continued to have low flow during the dry season. Keelbottom Creek, however, was more variable.

Wet-season flooding is critical for many streams in the arid tropics. The sites in this study can be considered seasonal, if there is a wet and dry season, or intermittent, if there are no distinct wet and dry periods (Paijmans et al. 1985). Flood behavior in Australian rivers is more variable than for rivers of other tropical and temperate regions – for example, rivers of other continents that have a 100-year flood recurrence interval are in the order of 2 to 3 times the size of the mean annual flood, whereas in Australia they are 5 to 30 times the size of the mean annual flood (Finlayson and McMahon 1988). Floods are normally associated with increased sediment loads and decreased conductivity (Cosser 1989, Griffiths and Faithful 1996), a pattern also observed for

the study sites. The flush of water during the post wet season was accompanied by an increase in turbidity, a decrease in PAR, and decreased conductivity.

3.4.2 Water quality

The Basalt River, Keelbottom Creek and Belyando/Suttor River were quite distinct in their physico-chemical parameters, but, there was little variation between sites within rivers. Nevertheless, the different rivers did show similar patterns, such as dissolved oxygen, PAR, and temperature gradients with depth. Due to the clarity of the water in the Basalt River, there was a more prevalent temperature and PAR gradient with depth since more light was able to enter the water body, but less diel variation. Also, the depth of these water holes (up to 10m) in the Basalt River contributed to gradients in these parameters.

Thermal stratification in a water column is based on the vertical differences of water density. A warmer water body with a thermal stratification of 2 ºC is equivalent to a colder water body with a thermal stratification of 8 ºC (Heitmann 1973). This consept suggests that a 1 ºC thermocline in tropical waters is similar to a 4 ºC thermocline in temperate waters; therefore a smaller variation in temperature gradients could have a substantial effect on phytoplankton in tropical waters. The Basalt River showed a stable thermal straitification during the mid dry and late dry seasons; the Keelbottom Creek showed a stable thermal stratification during the late dry season and the Belyando/Suttor Rivers showed a stable thermal stratification in all seasons, mid dry, late dry and post wet (Table 3.2). Multivariate statistics showed that temperature did not have a significant correlation with phytoplankton assemblages (Table 3.7), suggesting that temperature may not have a direct effect or that potential impact of thermal stratification on phytoplankton assemblages cannot be resolved due to the collection technique employed. However dissolved oxygen (Table 3.7) did have a significant correlation, which is strongly linked to temperature. Therefore, thermal stratification may have an indirect effect through other physico-chemical parameters on phytoplankton composition.

There were significant differences in physico-chemical parameters between seasons. Temperature was much lower in the mid-dry season (June - August) for all rivers. The Basalt River showed little diel variation, with the exception of pH and PAR for the late-dry season, which can be explained by stratification. This pattern also occurred in the other rivers, suggesting that suspended solids (influencing PAR) or nutrients and ions (pH) were concentrated within certain strata. The Keelbottom Creek and Belyando/Suttor River were shallower than the Basalt River, which would also contribute to larger diel variation. Rises in pH occur with the removal of CO_2 by photosynthesis (Shapiro 1997). The photosynthetic activity of freshwater phytoplankton can

be determined largely by CO_2 (Talling 1985). Carbon dioxide in fresh waters occurs as carbon dioxide gas (dissolved CO_2), carbonic acid (H_2CO_3), carbonate ions (CO_3^{2-}) and bicarbonate ions (HCO_3^-). Inorganic carbon as dissolved CO_2 and the bicarbonate ion are the primary source of carbon for photosynthesis by algae and aquatic macrophytes (Wetzel and Likens 2000). The sites in this study showed a higher pH during the late dry season, which corresponded to no flow and stratification of the water column allowing more PAR to enter the water, therefore more light availability for photosynthesis. A higher pH typically means that bicarbonate ion becomes more available to phytoplankton for photosynthesis.

Conductivity at all sites varied very little during the day. Conductivity was one of the main parameters explaining much of the variation in the PCA of physico-chemical parameters (Table 3.3) between rivers. Season, described by temperature, also explained much of the variation in the PCA. This suggests that conductivity and temperature might be major determinants of phytoplankton assemblages within these water holes.

Much of the difference between the physico-chemical parameters of these rivers can be explained by the physical nature of each river. The Basalt River has high clarity and appears to have continuous inflow of ground water. The high conductivity is due to the soluble nature of minerals in the basalt substrate. Water temperature was lower due to groundwater input and the greater depth of the water holes. The high turbidity encountered at the Belyando/Suttor Rivers is due to the clay/silt substrate, as these particles are small and stay suspended in the water column even during times of no flow, therefore reducing PAR values significantly. Although Keelbottom Creek sites had relatively good clarity, PAR values were low, similar to the Belyando/Suttor River sites, due mainly to shading by riparian vegetation. Shading also affected the surface temperature at these sites. Even though different physical characteristics explained much of the differences in water quality, one parameter, water flow, affected the sites in a similar way. Increased water flow or flooding during the wet season tended to decrease dissolved oxygen, pH and conductivity in the sites of all rivers.

The study sites in the Burdekin catchment have many similarities to other tropical rivers. For example, they are greatly affected by the amount and variability of rainfall received (Boulton and Brock 1999); they cease to flow during the dry season or have minimal groundwater flow and they have similar temperature and dissolved oxygen dynamics (Table 3.9). However, the rivers studied have higher conductivities than other tropical rivers. Hart and McKelvie (1986) found that tropical rivers have a pH within the range 4.3-7.5, whereas the Burdekin sites had much greater pH values.

3.4.3 Phytoplankton

This study identified 138 species from six phyla. This was within the range recorded in other studies of sub-tropical and tropical rivers (Table 3.10). Chlorophytes and diatoms were most species rich in the three rivers. However, cyanobacteria were most abundant in terms of cells per milliliter. Phytoplankton species composition varied significantly between sites. Multivariate analyses showed that phytoplankton assemblages significantly reacted to season and river. Multivariate analyses also showed that the majority of chlorophytes, cyanobacteria and euglenoids significantly correlated with Axis 1, which represented conductivity. Multivariate analyses also found that diatoms, chlorophytes and a number of euglenoid species significantly correlated with Axis 3, which represented dissolved oxygen, suggesting that oxygen levels, directly or indirectly, affected species composition. Some species correlated with Axis 2, which was represented by conductivity and turbidity. Although temperature was not significant on its own, it is an important part of seasonal changes, which was significant to species composition. Phytoplankton occurring in the Basalt River tended to correlate with Axis 1 representing conductivity, whereas phytoplankton of the Keelbottom Creek and Belyando/Suttor Rivers tended to correlate equally with Axes 1 and 3 (dissolved oxygen). A possible reason for this is turbidity, which was significant on Axis 2, and shading. The Basalt River had very low turbidity and little shading by riparian vegetation, therefore allowing a large amount of PAR to enter the water column, and hence causing a fairly deep surface stratum (1.5-3 m) in which the dissolved oxygen was constant. Keelbottom Creek and the Belyando/Suttor Rivers showed medium to extremely high turbidity, respectively, which allowed limited PAR to enter the water, therefore causing a shallower surface stratum (0.5-1 m).

These results are similar to the findings in other studies of rivers in the tropics, in which chlorophytes, diatoms and euglenoids tend to dominate (Izaguirre et al. 2001, Zalocar de Domitrovic 2002, Costelloe et al. 2005, Tell et al. 2005). In contrast to these results, tropical lakes and reservoirs tend to be dominated by cyanobacteria (Ganf 1974b, Zafar 1986, Hawkins and Griffiths 1993, Lung'Ayia et al. 2000). A study by Townsend (2006) found that chlorophytes and diatoms tended to dominate during the lotic period of an arid river system and cyanobacteria tended to dominate during the lentic period. This study also found a significant increase in species richness of chlorophytes in the wet season and an abundance of cyanobacteria during the dry season (Table 3.4), which may suggest that chlorophytes can tolerate higher turbidity and lower light availability due to mixing than epiphytic or benthic diatoms. Phytoplankton at the study sites was dominated by planktonic species of chlorophytes and cyanobacteria, rather than benthic diatoms; a pattern also observed in the San Joaquin River, California (Leland et al. 2001, Leland 2003).

Table 3.9 – Physical and chemical characteristics in the upper stratum (0.0 – 2.0 m) of tropical rivers / water holes.

Site	Flow Regime	Temp (C °)	Cond (µS cm $^{-1}$)	pH	Dissolved Oxygen (%)	Turbidity (NTU)	Reference
Basalt R (Australia)	Perennial	18.2 – 29.2	653 – 954	7.2 – 10.32	55.8 – 93.1	2.36 – 18.2	This Study
Keelbottom C (Australia)	Ephemeral & perennial	20.1 – 31.4	141 – 474	6.51 – 9.98	19.7 – 130	2.33 – 20.4	This Study
Belyando/Suttor R (Australia)	Ephemeral & perennial	17.2 – 31.7	92 - 248	7.09 – 9.77	43.9- 112	18.7 - >1000	This Study
Mary R (Australia)	Ephemeral & perennial	22 – 30	35 - 92	6.2 – 7.6	70 - 91	5 – 15.5	(Schultz et al. 2002, Townsend 2006)
Elizabeth R (Australia)	Ephemeral & perennial	20.9 – 23.9	18 – 42	6.85 – 7.21	80 -93	3 – 4.8	(Dostine 2002)
Howard R (Australia)	Ephemeral & perennial	19.3 – 24.2	14 - 102	6.48 – 7.01	66 - 76	2.3 – 4.9	(Dostine 2002)
Elechi Creek (Africa)	Ephemeral & perennial	28 - 30	123 - 3230	6.3 – 7.7	4.16 – 6.62		(Obire et al. 2003)
Bugamba R. (Africa)	Perennial	22 - 23	44.8 – 47.2	7.7	115.9 – 117.1		(Bellinger et al. 2006)
Mkenke R. (Africa)	Perennial	18.8 – 19.0	13.6 – 14.0	6.8 – 7.0	113 - 115		(Bellinger et al. 2006)
Wadi Bani Habib (Asia)	Perennial		415 - 530	8.2 – 8.8	4.3 – 6.2		(Victor and Al-Mahrouqi 1996)
Paraná R (S.America)	Ephemeral & perennial	9.2 - 27	32 - 115	6.26 – 7.92	46.6 - 115		(Depetris and Paolini 1985, Izaguirre et al. 2001)
Orinoco R (S.America)	Ephemeral & perennial		15.7 – 21.5	6.35 – 6.7		47 – 52	(Depetris and Paolini 1985)
Sura R. (Meso America)	Perennial	25 – 27	145 – 268	5.5 – 6.8			(Ramirez et al. 2006)
Piper R. (Meso America)	Perennial	25 - 26	21 - 24	4.0 – 6.0			(Ramirez et al. 2006)

3.4.4 Correlations between water quality and phytoplankton

Species richness increased in all divisions of phytoplankton identified during the wet season, which may be due to an increase in nutrients from runoff from surrounding lands and an increase in temperature to more optimal temperatures for phytoplankton photosynthesis and growth. Leland (2003) also found seasonally varying factors such as temperature that influence algal growth rates contributed significantly to species selection in rivers. Nutrient availability will be discussed further in Chapter 4. Wet-season-correlated increases in species richness observed in this study are also similar to those from other tropical studies (O'Farrell 1994, O'Farrell et al. 2002, Zalocar de Domitrovic 2002, Costelloe et al. 2005, Fazio and O'Farrell 2005, Townsend 2006). Multivariate statistics found that conductivity and turbidity were highly significant in driving the phytoplankton species assemblages (Tables 3.7). This study found that low conductivity sites (Belyando/Suttor Rivers) tended to be dominated by euglenoids, as conductivity increased (Keelbottom Creek and Basalt River) so did the number of chlorophyte and cyanobacteria species. These results are supported by studies conducted in Argentina (Fazio and O'Farrell 2005). In contrast, at the landscape scale McGregor et al. (2006) found that

phytoplankton communities showed significant differences between catchments, as was seen in this study, but the general assemblage patterns were poorly correlated with the measured waterhole environmental parameters, which is in contrast to the finding of this study.

Table 3.10 – Number of phytoplankton species identified from subtropical and tropical field studies

Site	Site Character	# of spp.	Dominate spp.	Reference
Lake George, Uganda, Africa	Large permanent lake, Tropical	58	Cyanobacteria most abundant, followed by chlorophytes, then diatoms	(Ganf 1974b)
Lake Victoria, Kenya, Africa	Large permanent lake, Tropical	103	Cyanobacteria most diverse, followed by diatoms, chlorophytes, & dinoflagellates	(Lung'Ayia et al. 2000)
Lake Lanao, Philippines	Large permanent lake, Tropical	70		(Lewis 1978)
Man made lakes, India	Large / medium permanent lakes, Tropical	120	Cyanobacteria dominated, followed by diatoms and/or Chlorococcales and then euglenoids	(Zafar 1986)
Lake Erye Basin, Australia	Large lake, highly seasonal, Sub-tropical	237	Chlorophytes and diatoms	(Costelloe et al. 2005)
Barra Bonita Reservoir, Brazil, S. America	Large permanent reservoir, Tropical	131	Cyanobacteria had the greatest relative abundance (*Microcystis aeruginosa*), diatoms contributed most biovolume	(Calijuri et al. 2002)
Los Coipos Lake, Argentina, S. America	Large shallow lakes, seasonal, Tropical	153		(Fazio and O'Farrell 2005)
Floodplain of Paraná R, Argentina, S. America	Large river / floodplain, Tropical	88	*Trachelomonas*, *Euglena*, *Lepocinclis*, *Phacus* increased in species richness towards the lake; *Strombomonas* was constant across all habitats	(Tell et al. 2005)
Paraguay R, Argentina, S. America	Large river, Tropical	332	*Chloromonas gracilis, Choricystis minor, Crucigenia quadrata, Scenedesmus ecornis, Monoraphidium contortum, M. minutum* (chlorophytes), *Crypotmonas marssonii, C. ovata*, and *Rhodomonas minuta* (cryptophytes)	(Zalocar de Domitrovic 2002)
Parana R, Argentina, S. America	Large river, Tropical	290	Diatoms and chlorophytes	(Izaguirre et al. 2001)
Basalt, Keelbottom, Belyando/Suttor R, Australia	Rivers / water holes, Tropical	138	Chlorophytes	This study

The broad survey described in this chapter has shown variability in water quality and phytoplankton assemblages between rivers, consistency between sites within rivers, and a substantial variation between seasons at all sites. Questions regarding detailed responses of phytoplankton to water quality within sites and with regard to putative major drivers of phytoplankton assemblages are addressed in the next chapter.

Chapter 4 . Dynamics of phytoplankton communities at fine temporal and spatial scales

4.1 Introduction

Chapter 3 demonstrated contrast in water quality and phytoplankton communities between rivers and seasons, but showed that sites within rivers and seasons were quite consistent in their biophysical variables. The number of sites in the general survey precluded examination of some of the details of water quality and algal assemblages. A more detailed examination of one waterhole per river was therefore conducted in each of two subsequent seasons. This more intensive study allowed replication of daily and within-daily sampling at each waterhole, and the inclusion of sampling for major nutrients, which had not been possible in the general survey. The extra detail was intended to provide information on the small-scale dynamics of water quality and algal communities, especially with regard to the design of monitoring programs. Nutrients were included in this study because of their importance in algal dynamics, and because they are generally considered to be major anthropological contaminants of water bodies. Relevant background to this further study is provided in section 3.1.

4.2 Methods

Physico-chemical measurements, water samples and phytoplankton samples were taken in November 2004 and April 2005, corresponding to the late-dry and post-wet seasons. The three water holes selected were Bluff Downs on the Basalt River, the Bridge on the Keelbottom Creek, and Mt. Hope on the Suttor River. Measurements were recorded for three days at dawn, mid-day, and afternoon (approximately 0600, 1130 and 1600 respectively) from six replicate locations with a minimal distance of 10m at Bluff Downs and Mt. Hope sites, and 5 m at Keelbottom Bridge site between replicates and multiple depths within the waterhole. Water samples and phytoplankton samples were collected on day 3 at dawn and afternoon (approximately 0600 and 1300) from all replicate sites and strata, except for nutrients, which were collected at only three of the six replicate locations (Figure 4.1). Water quality did not differ between strata, therefore these data were pooled.

4.2.1 Water quality

In-situ measurements

Water temperature, conductivity, dissolved oxygen and pH were measured within the water column at 25 cm depth and then at every stratum within the water column to the depth of the site. Different water column strata were determined by dissolved oxygen measurements: a change in

dissolved oxygen of 20% or more was regarded as representing a new stratum. These measurements were taken with an YSI 556 Multi-Probe System with a 10 m cable. Photosynthetically active radiation (PAR) was measured with a Li-Cor LI-250 Light meter with a 10 m cable at the same depths. At the same time, air temperature and wind speed were recorded with an Extech Mini Thermo-Anemometer.

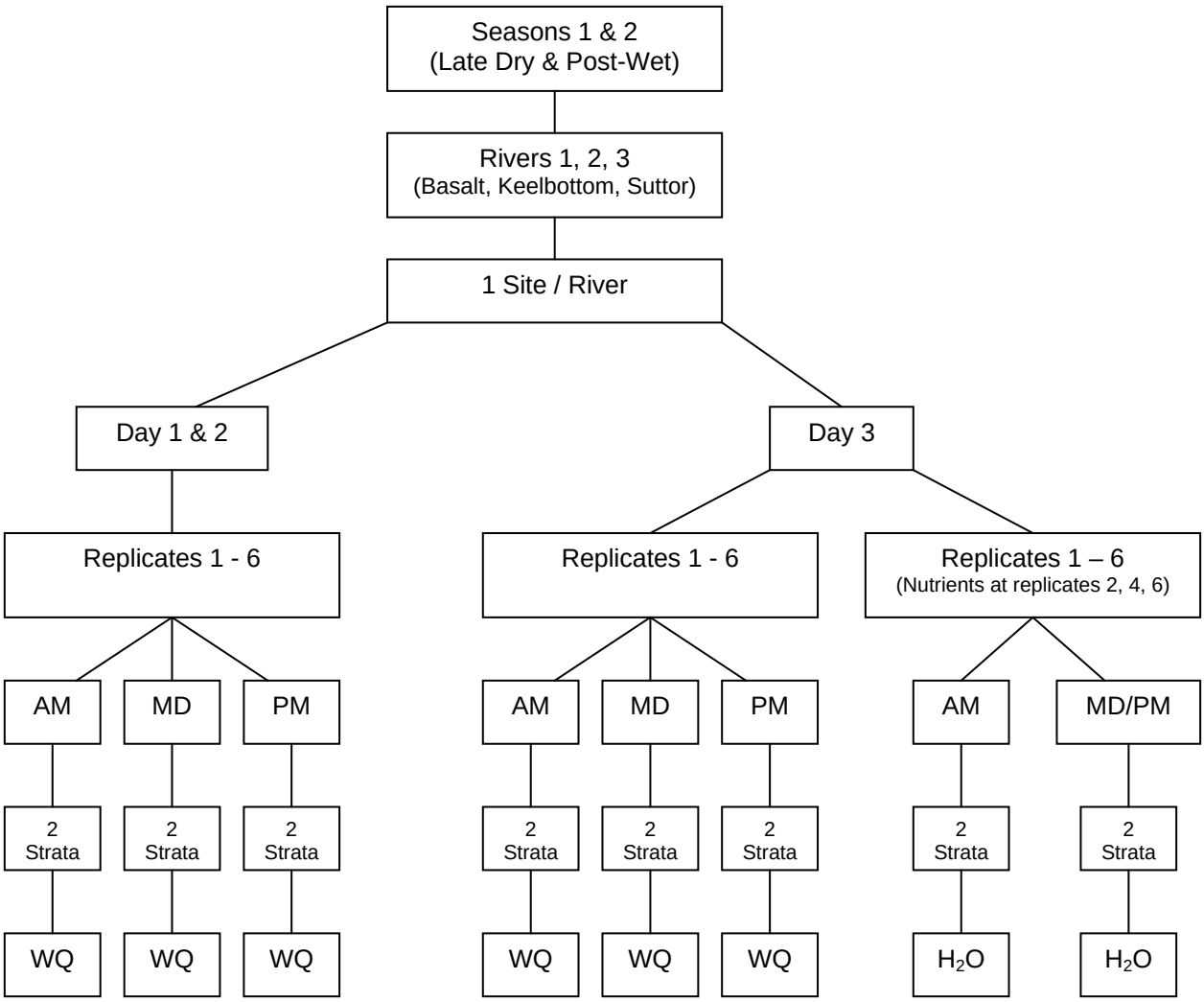


Figure 4.1 - Intensive study sampling design. AM = Dawn; MD – Mid-day; PM = Afternoon; WQ = in situ water quality measurements (Temperature, PAR, pH, Dissolved Oxygen, Conductivity); H_2O = Water samples (Phytoplankton, Turbidity, Chlorophylls *a, b, c*, Nutrients).

Water sampling

To determine Chlorophyll *a, b,* and *c*, phaeophytin *a* and turbidity, 1-L water samples were taken from each stratum at each replicate location at each time. Water samples of 30 mL to measure turbidity and nutrients were taken at the same times. Composite water samples were collected by pumping 10 L of water from each stratum of the water column and sub-sampling 1 L for pigment

analysis, 30 mL for turbidity analysis or 30 mL for nutrient analysis. Samples were labeled with appropriate field information and immediately placed on ice in the dark. Water samples were taken to the Australian Centre for Tropical Freshwater Research (ACTFR) laboratory at James Cook University for analysis.

Water analysis

Pigment analysis and turbidity analysis were conducted as described in Chapter 3. Nutrient samples were analyzed by the ACTFR to determine the amount of: total nitrogen, particulate nitrogen, total filterable nitrogen, filterable organic nitrogen, ammonia, nitrate, nitrite, total phosphorus, particulate phosphorus, total filterable phosphorus, filterable organic phosphorus and filterable reactive phosphorus, representing key species of N and P that might be expected to be important for the growth of phytoplankton. These analyses were conducted on an OI Analytical Alpkem Segmented Flow Analyzer.

4.2.2 Phytoplankton

Sampling

Phytoplankton was sampled by taking 1 L water samples from each stratum at each replicate site at dawn and afternoon on the third day. Composite water samples were collected by pumping 10 L of water from each stratum of the water column and sub-sampling 1 L for phytoplankton identification. Samples were immediately preserved by adding Lugol's solution at a ratio of 1:100 (Vollenweider 1969). The preserved samples were returned to the laboratory for identification.

Identification

Phytoplankton identification and counting was conducted by the same methods as described in Chapter 3.

4.2.3 Statistics

Water quality

Patterns in water quality parameters between seasons, rivers, days and times were analyzed as in Chapter 3, by Principal Component Analysis (PCA) and Multiple Response Permutation Procedures (MRPP), using the PC-ORD package (McCune and Mefford 1999). MRPP was chosen over Multivariate Analysis of Variance (MANOVA) because MRPP does not require distributional assumptions, such as multivariate normality and homogeneity of variances (McCune and Grace 2002). Ordination coordinates were exported and graphs were created in Sigmaplot version 7. Patterns in major variables were also plotted individually.

Phytoplankton

As in Chapter 3, patterns in phytoplankton between seasons, rivers, days, times and strata and corresponding to water quality parameters were analyzed using Non-metric Multidimensional Scaling (NMS) with a Sorensen (Bray-Curtis) distance measure in PC-ORD. NMS is an ordination method well suited to data that are non-normal or are on arbitrary, discontinuous, or otherwise questionable scales (McCune and Grace 2002), as is the case with most species community data. An advantage of NMS is that it is based on ranked distances, so it linearizes the relationship between environmental distance and community distance (Beals 1984). NMS performs well when applied to community data (Fasham 1977, Kenkel and Orloci 1986, Minchin 1987, Clarke 1993, McCune 1994). MRPP was used to determine significance between *a priori* groups. Canonical Correlation Analysis (CCA) was undertaken using the PC-ORD package to explicitly seek physico-chemical explanations of patterns in the algal assemblages. While NMS provides a good representation of the distribution of sites in species space (independent of the physico-chemical variables), CCA seeks the best relationships between physico-chemical and species matrices, by means of multiple regression analyses. It therefore gives an independent and more robust indication of the important environmental variables than the simple correlations of NMS. Ordination coordinates were exported and graphs were created in Sigmaplot version 7.

The relative importance of each species in characterizing the samples, and the characteristic assemblage for each water hole, were determined using Indicator Species Analysis in the PC-ORD package.

4.3 Results

4.3.1 Physico-chemical parameters of rivers studied

Physico-chemical measurements were taken from each study site with 6 replicates at different strata (if possible). Measurements presented are from day 3 of a 3-day study, as there were no differences between physico-chemical parameters between days, and because day 3 measurements included nutrient concentrations. The results re-iterated the findings in Chapter 3, that there were major differences in physico-chemical parameters among rivers between seasons. Temperature in the Basalt River was higher than the other rivers during both seasons, whereas Keelbottom Creek showed much larger variation in temperature during both seasons (Figure 4.2a). There was no considerable difference in dissolved oxygen between river and season, but the rivers showed a much larger range of dissolved oxygen in the late dry season (Figure 4.2b). pH and conductivity measurements showed a similar pattern for rivers with the Basalt River having significantly higher readings than the other rivers. These measurements also showed similar patterns for season, where the late dry season had higher readings than the post wet season (Figure 4.3). The Suttor

River showed significantly higher turbidity during both seasons than the other rivers (Figure 4.4a). The Chlorophyll *a* content in Keelbottom Creek was significantly lower in the late dry season than the post wet, whereas the Suttor River showed the opposite seasonal pattern. The Basalt River showed no difference between seasons in Chlorophyll *a* (Figure 4.4b). Total phosphorus and nitrogen was significantly higher in the Suttor River than in Keelbottom Creek and the Basalt River. Total nitrogen significantly decreased from the late-dry to the post-wet season in all rivers. Total phosphorus significantly increased from the late-dry to the post-wet season in Keelbottom Creek, whereas it significantly decreased in the Suttor River. The Basalt River showed no change in total phosphorus between the seasons (Figure 4.5).

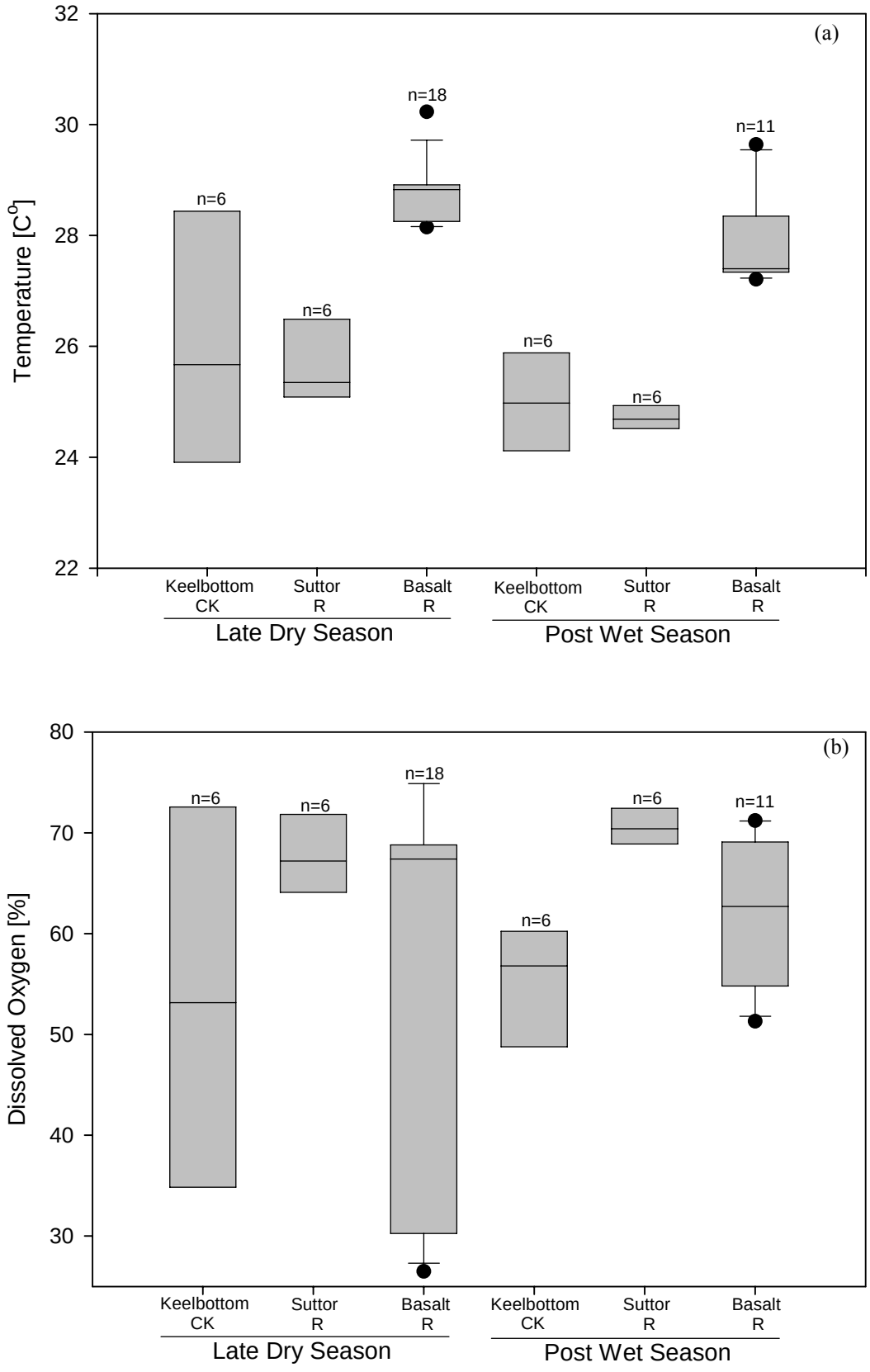


Figure 4.2 – Summaries of (a) Temperature and (b) Dissolved oxygen from two seasons from three rivers studied. The horizontal line with in the box plots represents the median. The whiskers represent the 10th and 90th percentiles. The black circles represent the outliers.

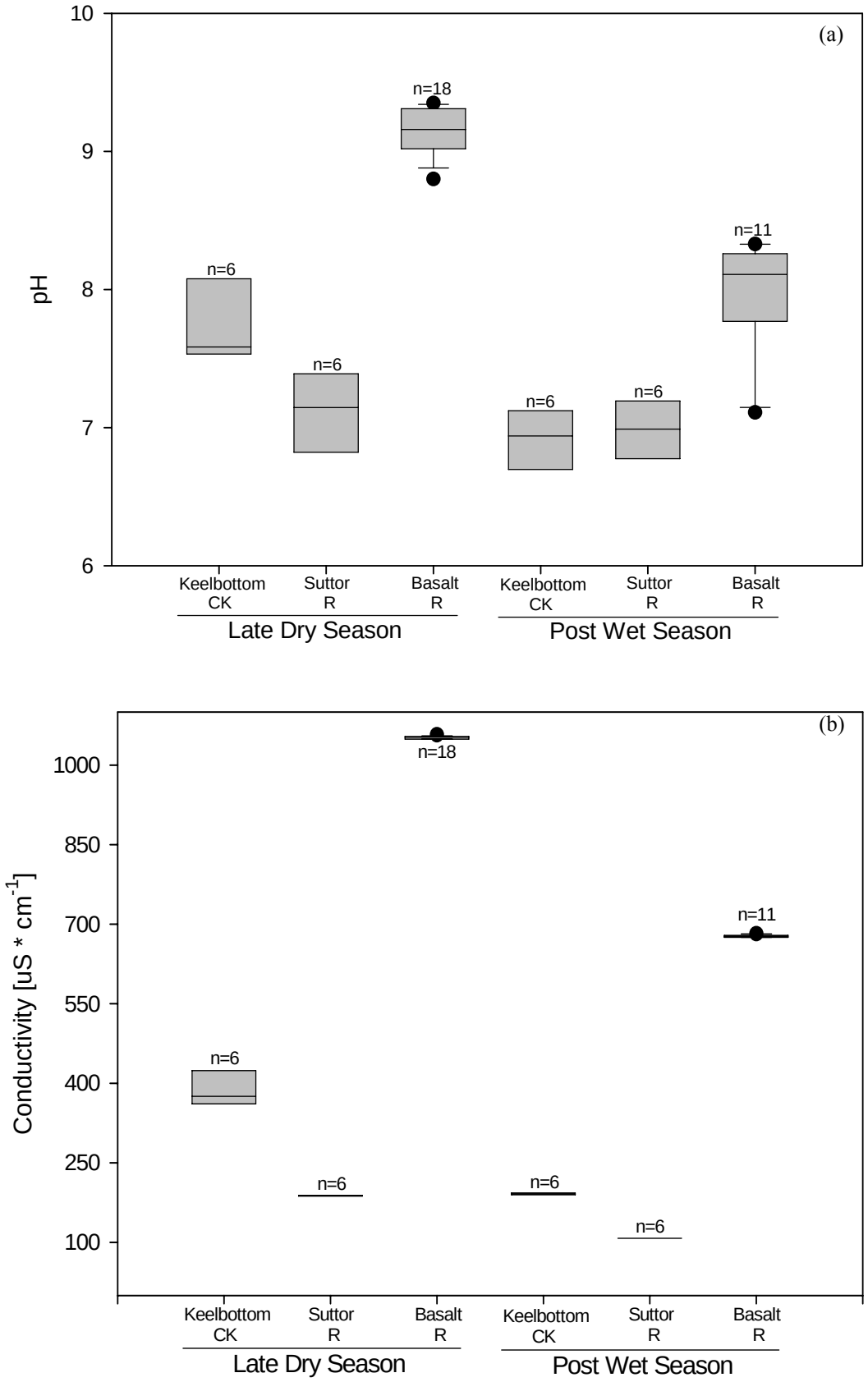


Figure 4.3 – Summaries of (a) pH and (b) Conductivity from two seasons from three rivers studied. Explanation of symbols as for Figure 4.2.

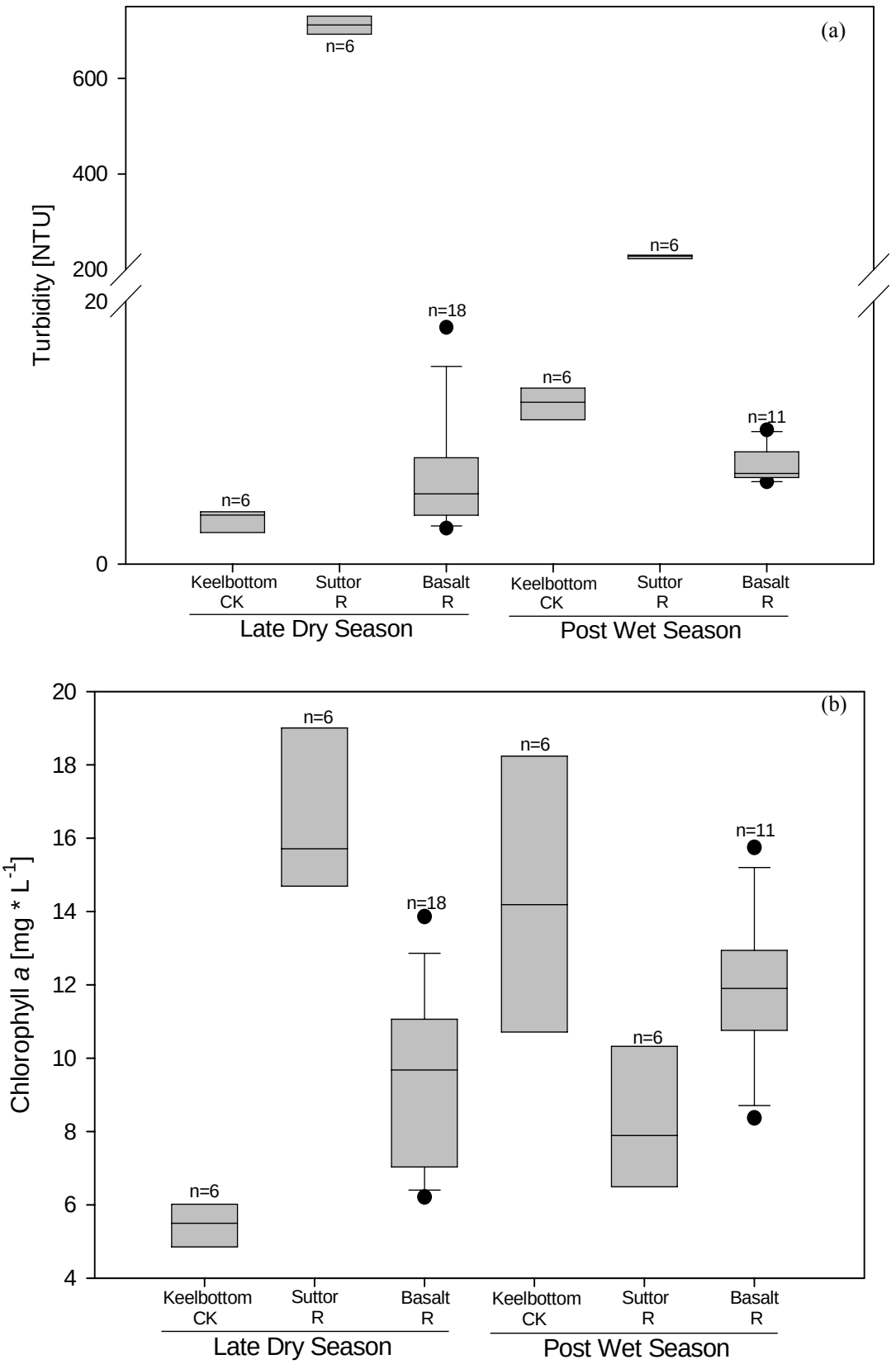


Figure 4.4 – Summaries of (a) Turbidity and (b) Chlorophyll *a* from two seasons from three rivers studied. Explanation of symbols as for Figure 4.2.

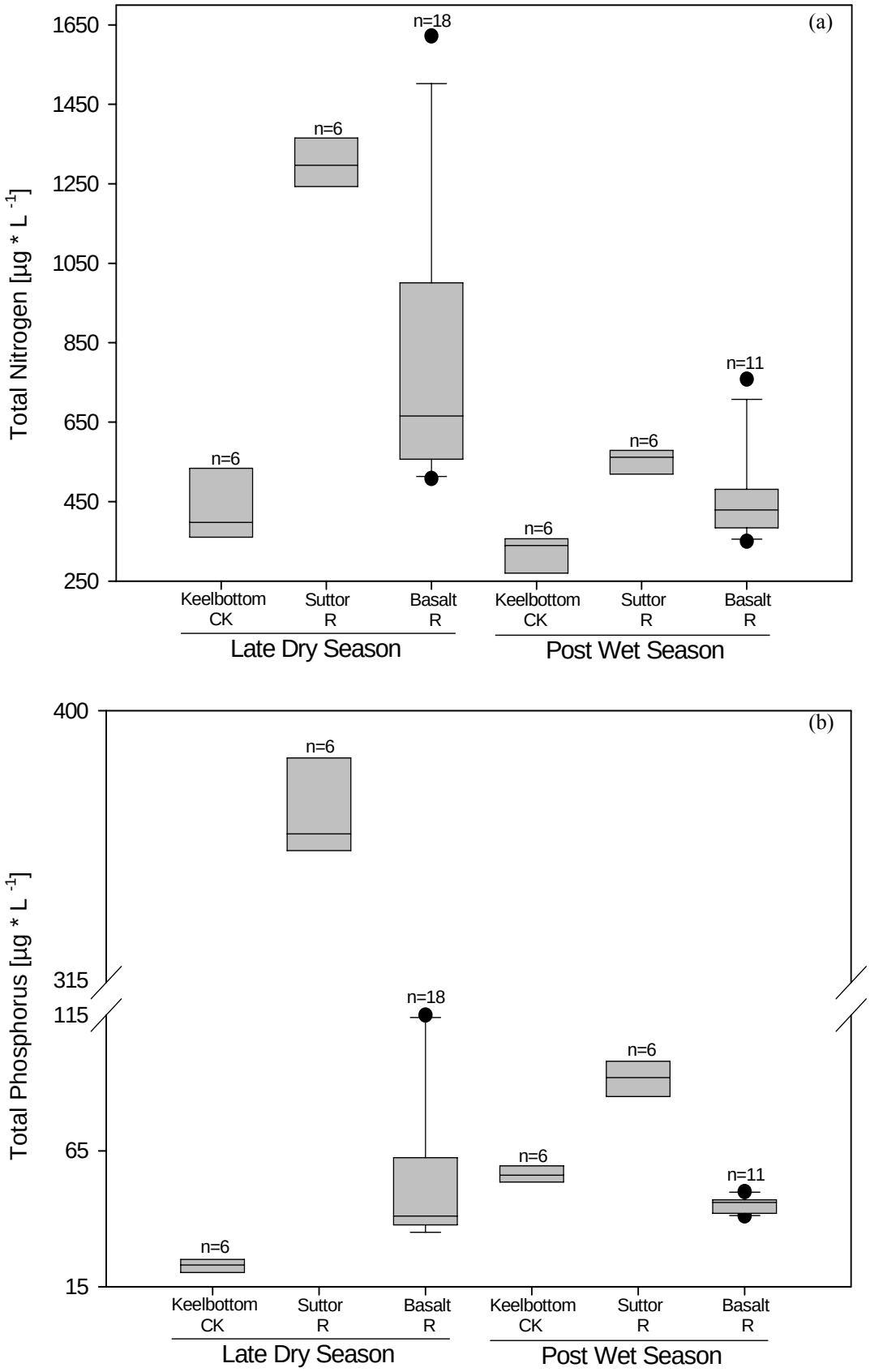


Figure 4.5 – Summaries of (a) Total Nitrogen and (b) Total Phosphorus from two seasons from three rivers studied. Explanation of symbols as for Figure 4.2.

4.3.2 All rivers physico-chemical data sample days 1, 2 & 3 – PCA analysis

Figure 4.6 shows that sites of the Basalt River group quite separately from the other river sites. The sites of the Suttor River are tightly grouped, showing little variation by site or season. The Keelbottom Creek sites show substantial variation on axis 2 and overlap with the Suttor River sites on both axis 1 and 2. The difference in physico-chemical parameters between rivers is highly significant (Table 4.2). Both Figure 4.7 and Table 4.1 show the expected correlates that are driving the pattern of the ordination (Figure 4.6), mainly seasonal factors and the basic chemical differences reflected by conductivity and pH. Table 4.2 confirms that both river and season were significantly different, as expected. These results confirm the results from chapter 3, that the Keelbottom Creek, Suttor River and Basalt River represent systems in the Burdekin system with very different physico-chemical conditions.

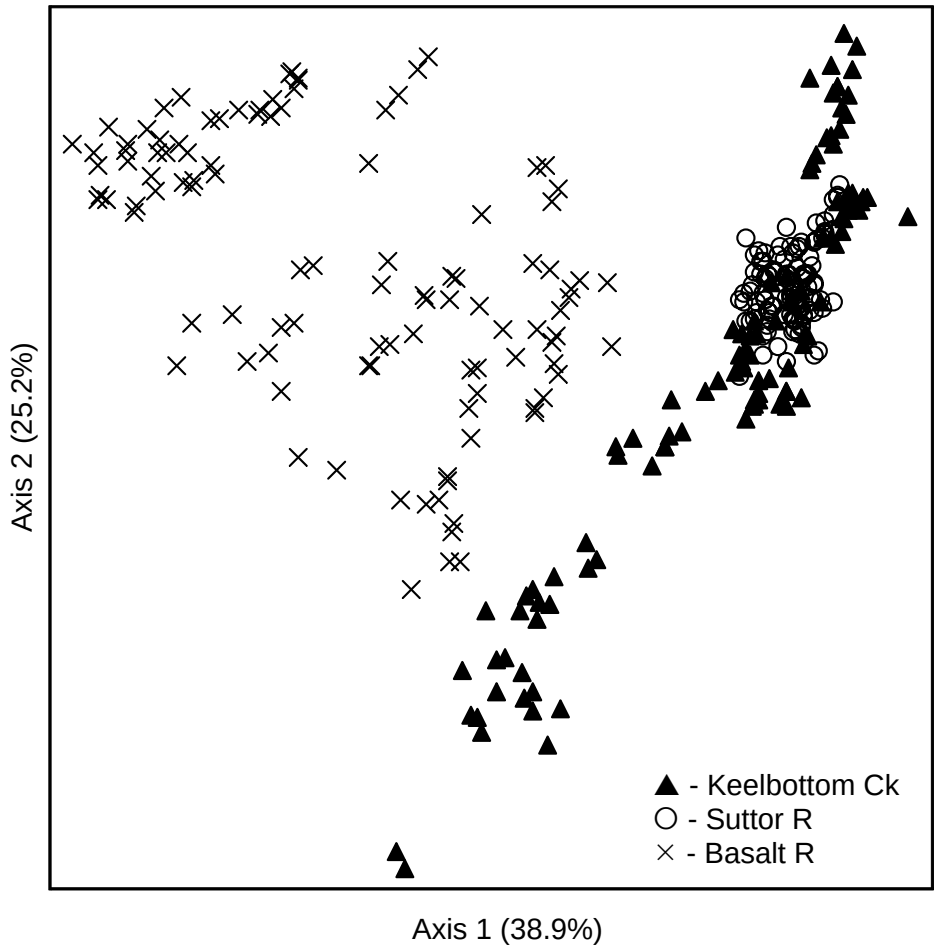


Figure 4.6 - PCA ordination of samples using physico-chemical data with overlay of river; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for river, p < 0.0001

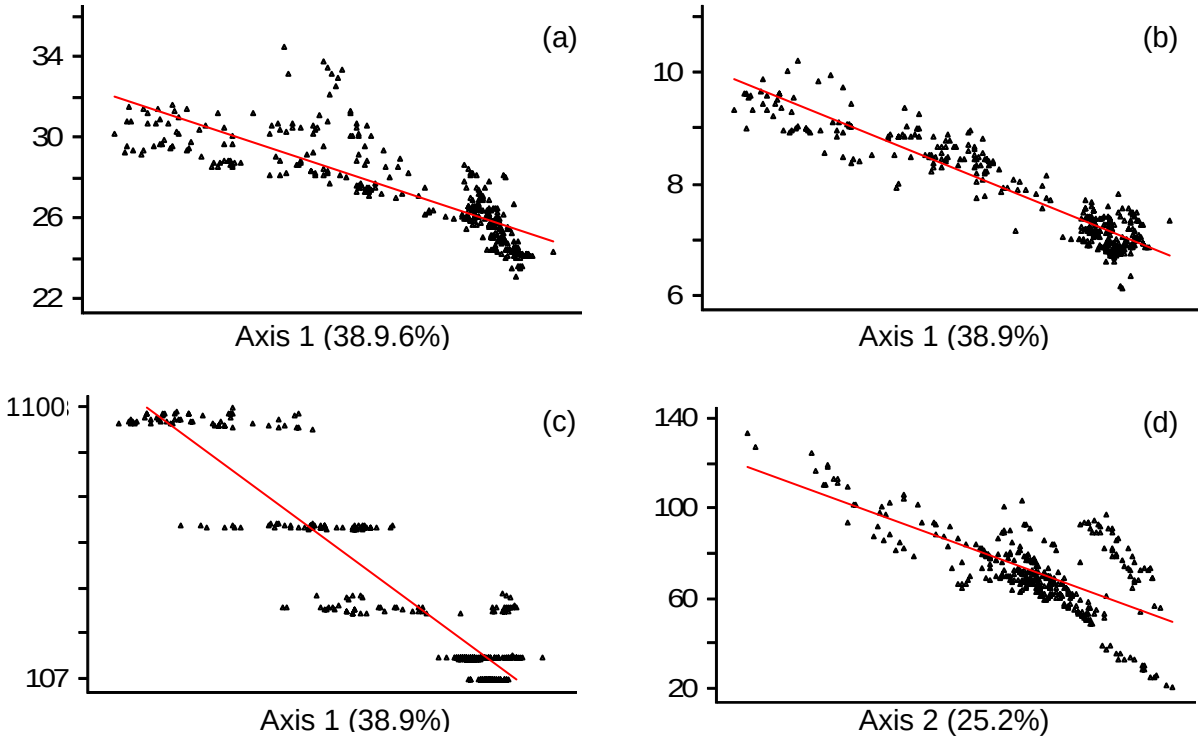


Figure 4.7 – Relationships between physico-chemical variables and PCA axis scores of (a) Temperature [°C]; r=-0.824; (b) pH; r=-0.935; (c) Conductivity [µS cm^{-1}]; r=-0.924; (d) Dissolved Oxygen [%]; r=-0.696; from three study rivers. The proportion of the variance explained by each axis is indicated (%) on the axes.

Table 4.1- Correlation table showing r values for physico-chemical parameters corresponding to first two axes of a PCA analysis. Significant r values shown in bold ($r_{(0.01, 322)}$ = 0.230).

Parameter	Axis 1 (38.9%)	Axis 2 (25.2%)
Temperature	**-.824**	**-.443**
pH	**-.935**	-.062
Conductivity	**-.924**	.229
Dissolved Oxygen	**-.548**	**-.696**
PAR	-.031	**-.466**

Table 4.2 - MRPP table showing T-statistic, A-statistic and p-value comparing physico-chemical parameters among seasons, rivers and sites within rivers.

Comparison Group	T-statistic	A-statistic	p-value
Season	-26.296379	0.05377157	0.00000000
River	-148.27526	0.42945319	0.00000000
Keelbottom sites	1.9716584	-0.03060194	0.99928836
Basalt sites	-1.4284205	0.02094895	0.08854854
Suttor sites	2.0642603	-0.03777117	1.00000000

4.3.3 All rivers physico-chemical data and phytoplankton data sample day 3 – PCA and NMS analyses

Physico-chemical PCA analysis

The physico-chemical data reported here include samples from day three only to investigate the influence of nutrients on the various relationships determined in section 4.3.2. There is a clear separation of sites according to nutrients on axis 1 and season on axis 2 (Figures 4.8 and 4.9; table 4.3), indicating nutrient and season drivers for phytoplankton productivity. Table 4.4 indicates the expected differences by season and river, but also shows differences in some parameters according to sites and times within rivers.

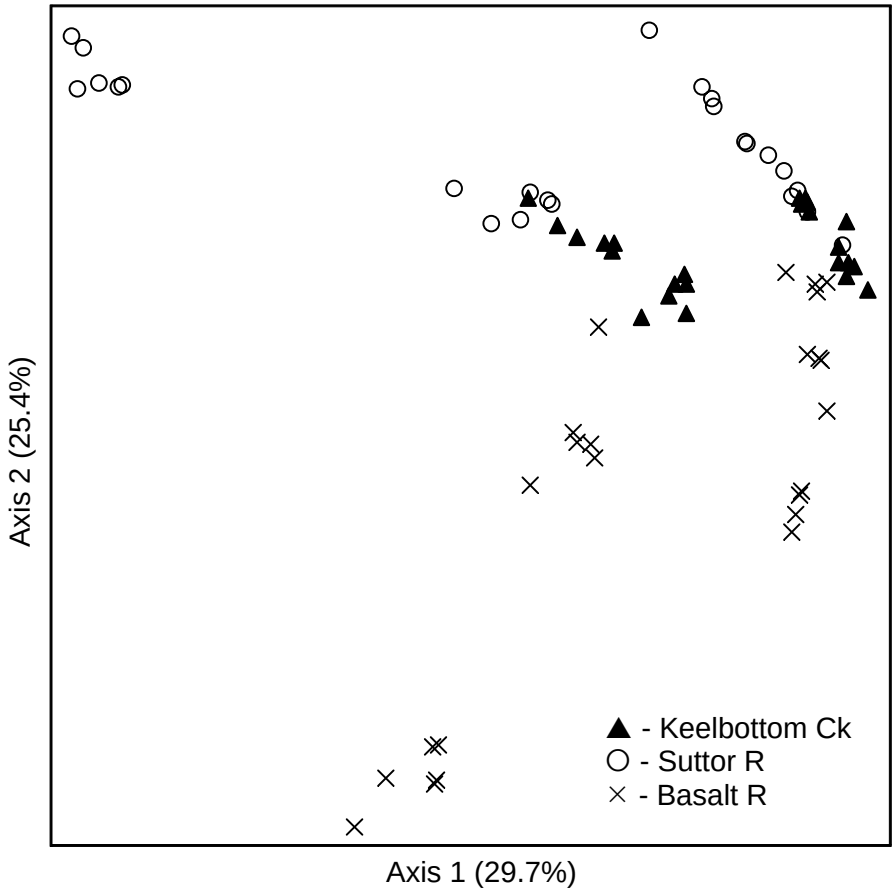


Figure 4.8 - PCA ordination of samples using physico-chemical data with overlay of river; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for river, p < 0.0001

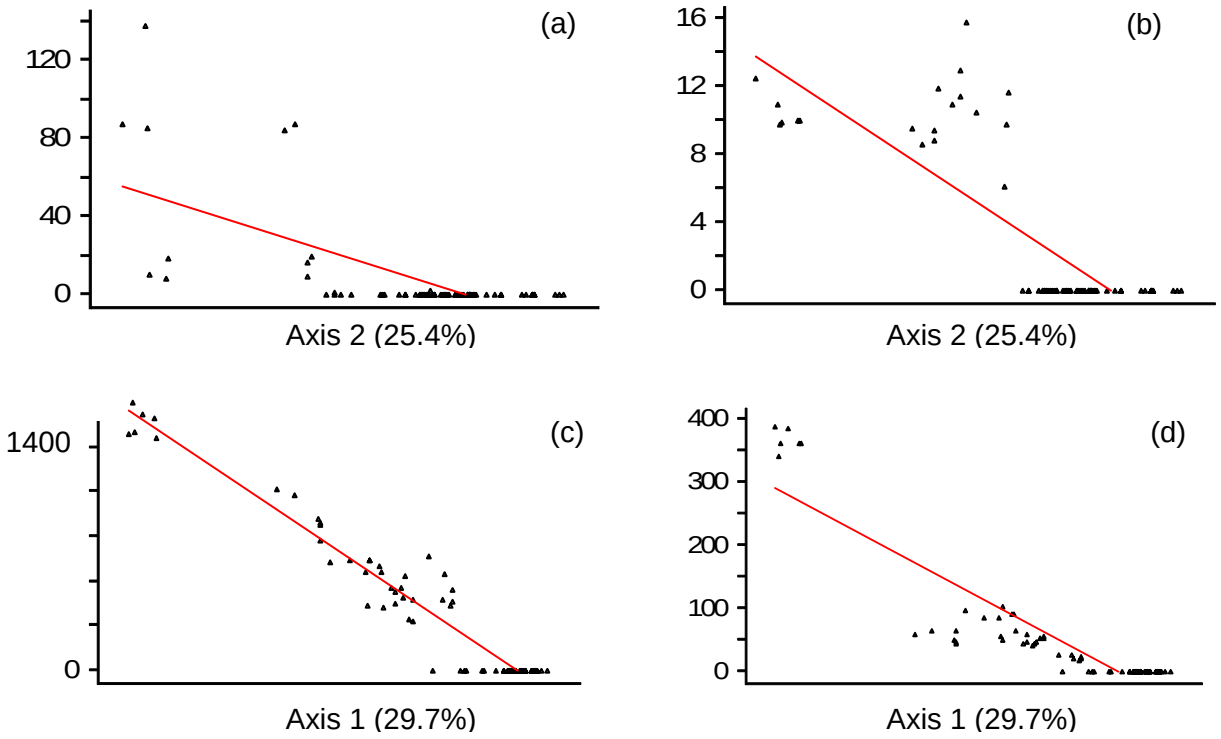


Figure 4.9 – Relationships between physico-chemical variables and PCA axis scores: (a) PAR [µmol]; r=-0.656; (b) Chlorophyll *a* [µg L^{-1}]; r=-0.802; (c) Total Nitrogen [µg L^{-1}]; r=-0.969; (d) Total Phosphorous [µg L^{-1}]; r=-0.901. The proportion of the variance explained by each axis is indicated (%) on the axes.

Table 4.3- Correlation table showing r values for physico-chemical parameters corresponding to PCA axes. Significant r values shown in bold ($r_{(0.01, 70)}$ = 0.303).

Parameter	Axis 1 (29.7%)	Axis 2 (25.4%)
Temperature	-0.009	**-0.726**
Dissolved Oxygen	0.038	**0.758**
pH	0.070	**-0.839**
Conductivity	0.060	**-0.872**
PAR	-0.126	**-0.656**
Turbidity	-0.014	**-0.702**
Chlorophyll *a*	-0.078	**-0.802**
Chlorophyll *b*	**-0.316**	**0.309**
Chlorophyll *c*	**-0.395**	**0.377**
Phaeophytin	-0.294	-0.275
Total Nitrogen	**-0.969**	-0.104
Particulate Nitrogen	**-0.806**	-0.262
Total Filterable Nitrogen	**-0.935**	-0.042
Ammonia	**-0.825**	0.287
Nitrite	**-0.840**	0.083
Nitrate	**-0.822**	**0.368**
Total Phosphorus	**-0.901**	0.283
Particulate Phosphorus	**-0.865**	**0.324**
Total Filterable Phosphorus	**-0.680**	-0.270
Filterable Reactive Phosphorus	**-0.809**	**-0.330**

Table 4.4 - MRPP table showing T-statistic, A-statistic and p-value comparing physico-chemical parameters among seasons, rivers and sites within rivers.

Comparison Group	T-statistic	A-statistic	p-value
Season	-5.5815980	0.03768286	0.00060396
River	-23.120370	0.22234099	0.00000000
Keelbottom Creek sites	-3.4502527	0.22685576	0.00235438
Suttor River sites	-4.6598970	0.38736973	0.00029119
Basalt River sites	-4.4216430	0.29752081	0.00033764
Time	-6.6623447	0.04497927	0.00015429

Phytoplankton NMS analysis

As predicted from the PCA analysis, season and nutrients correlate strongly with the phytoplankton-derived axes, indicating that these are strong drivers of phytoplankton community composition (Figure 4.10 and Table 4.5). Table 4.6 confirms these results and also indicates that there are strong differences in communities within rivers and even within strata.

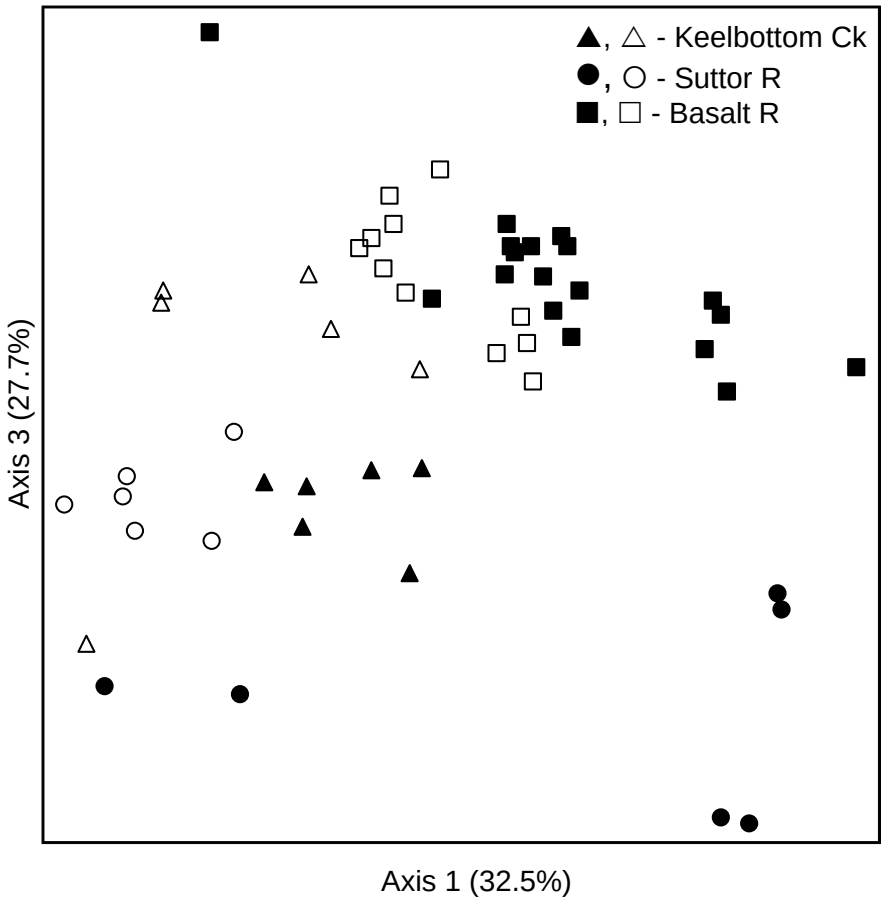


Figure 4.10 - NMS ordination of sites using phytoplankton data. Solid symbols represent the late dry season and open symbols represent post-wet season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p < 0.0001 and river, p < 0.0001. Stress = 26.78895.

Table 4.5- r values for physico-chemical parameters corresponding to different axes of a NMS analysis for algal species. Significant r values shown in bold ($r_{(0.01, 51)}$ = 0.354).

Parameter	Axis 1 (32.5%)	Axis 2 (14.1%)	Axis 3 (27.7%)
Temperature	**0.498**	**0.539**	**0.567**
Dissolved Oxygen	-0.140	0.186	-0.188
pH	**0.527**	**0.487**	**0.569**
Conductivity	**0.397**	**0.453**	**0.657**
PAR	-0.193	0.131	0.168
Turbidity	0.066	0.059	**-0.761**
Chlorophyll *a*	0.023	-0.041	-0.240
Chlorophyll *b*	0.168	0.023	**-0.448**
Chlorophyll *c*	0.01	0.123	**-0.463**
Phaeophytin	0.343	-0.155	-0.052
Total Nitrogen	**0.504**	0.234	-0.349
Particulate Nitrogen	**0.401**	0.213	-0.015
Total Filterable Nitrogen	**0.467**	0.203	**-0.461**
Filterable Organic Nitrogen	**0.395**	0.286	**-0.589**
Ammonia	0.289	-0.007	0.152
Nitrite	-0.195	-0.315	**-0.666**
Nitrate	0.152	0.111	**-0.732**
Total Phosphorus	0.217	0.098	**-0.697**
Particulate Phosphorus	0.212	0.111	**-0.709**
Total Filterable Phosphorus	0.064	-0.286	**0.391**
Filterable Organic Phosphorus	-0.208	**-0.499**	0.220
Filterable Reactive Phosphorus	**0.513**	**0.444**	0.287

Table 4.6 - MRPP table showing T-statistic, A-statistic and p-value comparing algal species among season, river, sites and strata.

Comparison Group	T-statistic	A-statistic	p-value
Season	-12.516925	0.07141798	0.00000002
River	-15.587808	0.12803862	0.00000000
Sites within rivers	-6.9893834	0.12443974	0.00000022
Strata	-6.2914879	0.05296752	0.00003890

Figure 4.11 and Table 4.7 confirm the strong determining effect of physico-chemical variables on phytoplankton assemblages, summarized in terms of season and river. The main drivers appear to be temperature, conductivity and pH (Figure 4.11). Phytoplankton species that determine these assemblage patterns are listed in Table 4.8, according to the significance of their relationship with site patterns. There is a large suite of species, mainly chlorophytes and euglenoids, corresponding with Keelbottom Creek. The Basalt River has representatives from the cyanobacteria as well as the chlorophytes. Dinoflagellates and diatoms play a minor roll according to these rivers. Unexpectedly, some benthic species were identified (e.g. *Amphora* sp.), possible due to disruption of the sediment while sampling. The Suttor River has only two representative indicator species, both euglenoids. It could be assumed that these species are specially adapted to the very different conditions (e.g. turbidity, conductivity, nutrients) of the Suttor River.

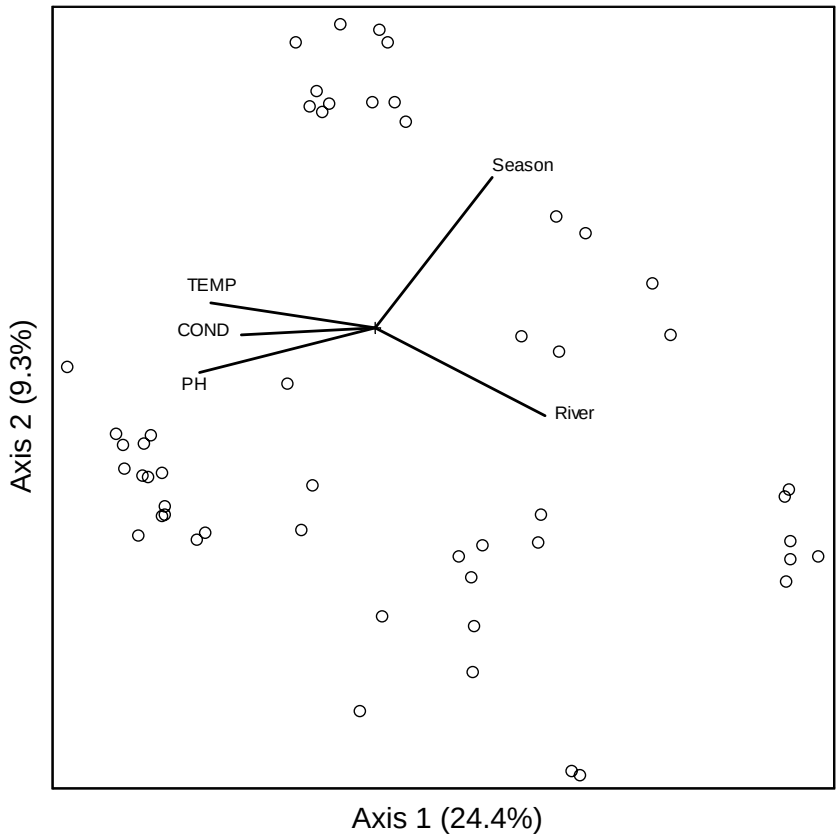


Figure 4.11 - CCA ordination of sites using algal data, with vectors indicating significant physico-chemical parameters; the proportion of the variance explained by each axis is indicated (%) on the axes. Abbreviations: TEMP = temperature; COND = conductivity.

Table 4.7- Correlation table showing r values for physico-chemical parameters corresponding to different axes of a CCA analysis for algal species. Significant r values shown in bold ($r_{(0.01, 50)} = 0.354$); Monte Carlo Species-Environment correlations were 0.941 for axis 1, 0.914 for axis 2 and 0.815 for axis 3; all axes were significant, p = 0.01.

Parameter	Axis 1 (24.4%)	Axis 2 (9.3%)
Season	**0.558**	**0.679**
River	**0.817**	**-0.399**
Temperature	**-0.786**	0.112
Dissolved Oxygen	-0.119	-0.028
pH	**-0.841**	-0.202
Conductivity	**-0.636**	-0.035
PAR	-0.006	0.113
Turbidity	**0.454**	-0.300
Chlorophyll *a*	0.156	**0.392**
Total Nitrogen	-0.339	**-0.404**
Ammonia	-0.133	-0.187
Total Phosphorus	0.268	-0.186

Table 4.8- Indicator species table for rivers studied, ordered according to p and indicator values.

Species	Division	River	Indicator value	p value
Tracholamonas curta	Euglenoids	Keelbottom Creek	54.4	0.001
Tracholamonas armata	Euglenoids	Keelbottom Creek	43.6	0.001
Phacus alatus	Euglenoids	Keelbottom Creek	37.5	0.001
Staurastrum chaetoceras	Chlorophyta	Keelbottom Creek	35.9	0.001
Mougeotia sp. 1	Chlorophyta	Keelbottom Creek	28.2	0.001
Cosmarium contractum	Chlorophyta	Keelbottom Creek	28.0	0.001
Phacus caudatus	Euglenoids	Keelbottom Creek	27.9	0.001
Synedra ulna	Ochrophyta	Keelbottom Creek	26.7	0.001
Staurastrum gracile	Chlorophyta	Keelbottom Creek	25.0	0.001
Tracholamonas oblonga	Euglenoids	Keelbottom Creek	38.3	0.002
Euglena granulate	Euglenoids	Keelbottom Creek	32.1	0.002
Gyrosigma sp. 1	Ochrophyta	Keelbottom Creek	25.0	0.002
Navicula sp. 2	Ochrophyta	Keelbottom Creek	20.8	0.002
Koliella spiculiformis	Chlorophyta	Keelbottom Creek	20.8	0.002
Treubaria triappendiculata	Chlorophyta	Keelbottom Creek	20.8	0.002
Centritractus belanophorus	Dinophyta	Keelbottom Creek	20.8	0.002
Glenodinium sp. 1	Dinophyta	Keelbottom Creek	20.8	0.003
Lagerheimia longiseta	Chlorophyta	Keelbottom Creek	16.7	0.003
Amphora sp. 1	Ochrophyta	Keelbottom Creek	28.3	0.004
Closterium kurtzingii	Chlorophyta	Keelbottom Creek	19.8	0.004
Scenedesmus ellipticus	Chlorophyta	Keelbottom Creek	32.5	0.005
Peridinium volzii	Dinophyta	Keelbottom Creek	20.8	0.005
Phacus longicauda	Euglenoids	Keelbottom Creek	16.7	0.005
Closterium praelongum	Chlorophyta	Keelbottom Creek	16.7	0.007
Pinnularia sp. 2	Ochrophyta	Keelbottom Creek	14.0	0.010
Euglena acus	Euglenoids	Keelbottom Creek	20.3	0.014
Navicula sp. 1	Ochrophyta	Keelbottom Creek	21.6	0.023
Euglena limnophila	Euglenoids	Keelbottom Creek	12.5	0.023
Eunotia sp. 2	Chlorophyta	Keelbottom Creek	12.5	0.027
Staurastrum sp. 1	Chlorophyta	Keelbottom Creek	12.5	0.030
Strombomonas acuminatus	Euglenoids	Suttor River	31.8	0.001
Strombomonas tambowika	Euglenoids	Suttor River	30.2	0.002
Anabaena spiroides	Cyanobacteria	Basalt River	64.2	0.001
Peridinium bipes	Dinophyta	Basalt River	59.3	0.001
Anabenopsis elenkinii	Cyanobacteria	Basalt River	54.8	0.001
Aphanizomenon gracile	Cyanobacteria	Basalt River	39.4	0.001
Peridinium volzii	Dinophyta	Basalt River	20.8	0.005
Scenedesmus ellipticus	Chlorophyta	Basalt River	33.6	0.008
Pediastrum simplex	Chlorophyta	Basalt River	25.7	0.008
Scenedesmus dimorphus	Chlorophyta	Basalt River	23.1	0.012
Monoraphidium contortum	Chlorophyta	Basalt River	34.1	0.016

4.3.4 Physico-chemical data for sample days 1, 2 & 3

The comparison of physico-chemical data within sites within rivers showed little difference between days, or sites within rivers, with main effects due to seasonal and diel variability (Table 4.9). Therefore, subsequent analyses can be restricted to one day, to reduce the complications of extra multiple comparisons. Day 3 is used, as it includes nutrient analyses.

Table 4.9 - MRPP table showing T-statistic, A-statistic and p-value comparing physico-chemical parameters by season, site, time and day between rivers.

Comparison Group	T-statistic	A-statistic	p-value
KeelBottom Creek			
Season	-38.22246	0.26026653	0.00000000
Site	1.9716584	-0.03060194	0.99928836
Time	-40.549232	0.39231536	0.00000000
Day	1.4026796	-0.01357098	0.99959235
Suttor River			
Season	-70.841002	0.56864716	0.00000000
Site	2.0642603	-0.03777117	1.00000000
Time	-4.3848071	0.05001289	0.00361537
Day	0.03233789	-0.00042637	0.34599977
Basalt River			
Season	-56.196350	0.36155678	0.00000000
Site	-1.4284205	0.02094895	0.08854854
Time	-27.356872	0.25009679	0.00000000
Day	1.1424452	-0.01044424	0.93311809

4.3.5 Keelbottom Creek physico-chemical data and phytoplankton data, day 3

Physico-chemical data – PCA analysis

Figure 4.12 and Table 4.10 show a very strong seasonal change in physico-chemical parameters, again pointing out the main drivers. There is also a significant difference in time of day (Figure 4.12). There is a high correlation of temperature, pH, conductivity, chlorophylls and turbidity on axis 2 and a high correlation of nitrogen on axis 1. Although axis 3 only explains 15.3% of the variation, there is a very high correlation with dissolved oxygen and PAR (Table 4.10).

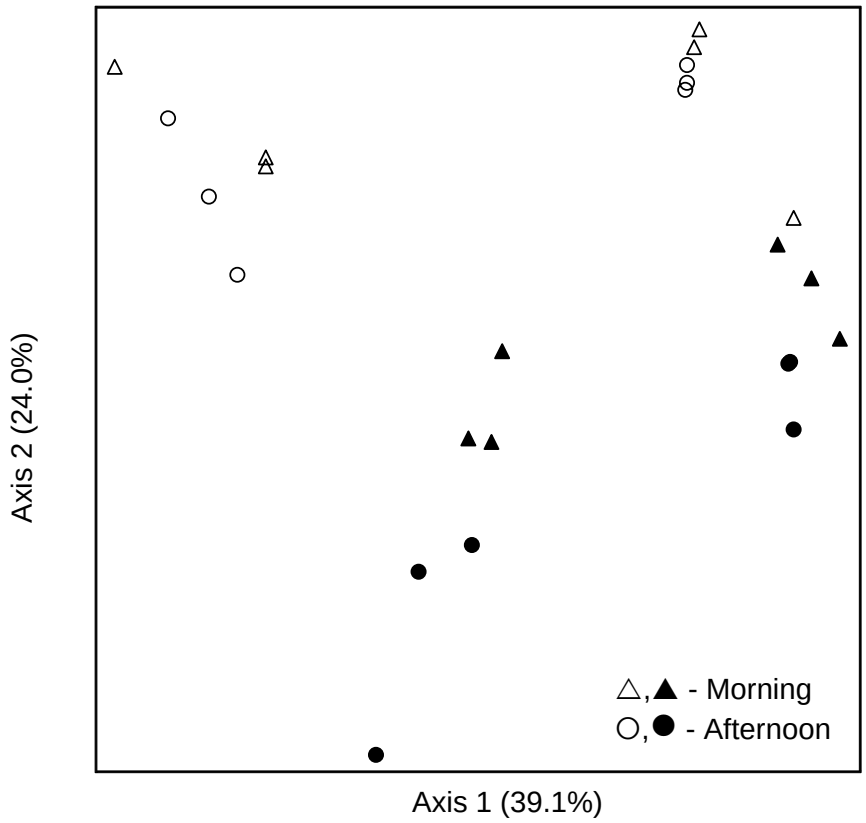


Figure 4.12 - PCA ordination of samples using physico-chemical data for Keelbottom Creek, by time. Closed symbols represent late-dry season, open symbols represent post-wet season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p < 0.0001 and time, p < 0.0001.

Table 4.10- Correlation table showing r values for physico-chemical parameters in Keelbottom Creek corresponding to different axes of a PCA analysis. Significant r values shown in bold ($r_{(0.01, 22)}$ = 0.515).

Parameter	Axis 1 (39.1%)	Axis 2 (24.0%)	Axis 3 (15.3%)
Temperature	0.069	**-0.630**	**0.693**
Dissolved Oxygen	-0.105	-0.247	**0.928**
pH	0.315	**-0.813**	0.043
Conductivity	0.318	**-0.843**	-0.262
PAR	-0.089	-0.453	**0.779**
Turbidity	-0.386	**0.753**	0.319
Chlorophyll *a*	-0.419	**0.613**	0.418
Chlorophyll *b*	-0.427	**0.572**	0.242
Chlorophyll *c*	-0.502	**0.599**	0.107
Phaeophytin	-0.195	0.436	-0.119
Total Nitrogen	-0.800	**-0.515**	-0.162
Particulate Nitrogen	**-0.729**	-0.436	0.088
Total Filterable Nitrogen	**-0.773**	-0.505	-0.213
Nitrite	**-0.908**	0.110	-0.148
Nitrate	**-0.789**	-0.257	-0.161
Total Phosphorus	**-0.966**	0042	-0.047
Total Filterable Phosphorus	**-0.960**	0.018	-0.124

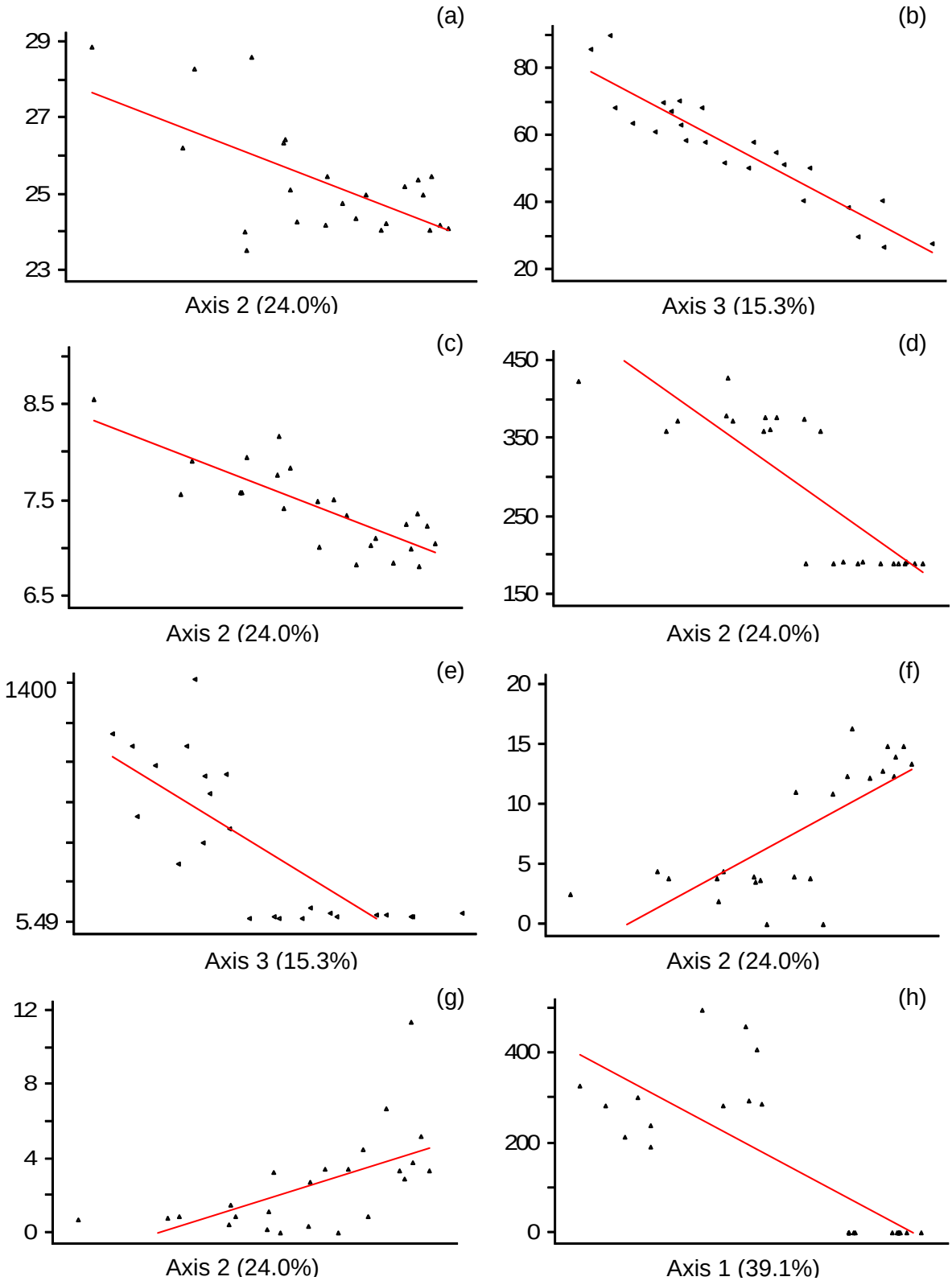


Figure 4.13 – Relationships between selected variables and PCA axis scores: (a) Temperature [°C], r=-0.630; (b) Dissolved Oxygen [%], r=0.928; (c) pH, r=-0.813; (d) Conductivity [µS cm^{-1}], r=-0.843; (e) PAR [µmol]; r=0.779; (f) Turbidity [NTU]; r=0.753; (g) Chlorophyll *c* [µg L^{-1}]; r=0.599; (h) Total Filterable Nitrogen [µg L^{-1}], r=-0.773, in Keelbottom Creek. The proportion of the variance explained by each axis is indicated (%) on the axes.

Phytoplankton data – NMS analysis

Figure 4.14 shows a strong effect of seasonality on phytoplankton species. Turbidity and phosphorus were the main drivers of these phytoplankton assemblages (Table 4.11). The significant difference in season is confirmed by Table 4.12, suggesting that there was no significant difference in phytoplankton species within sites of Keelbottom Creek.

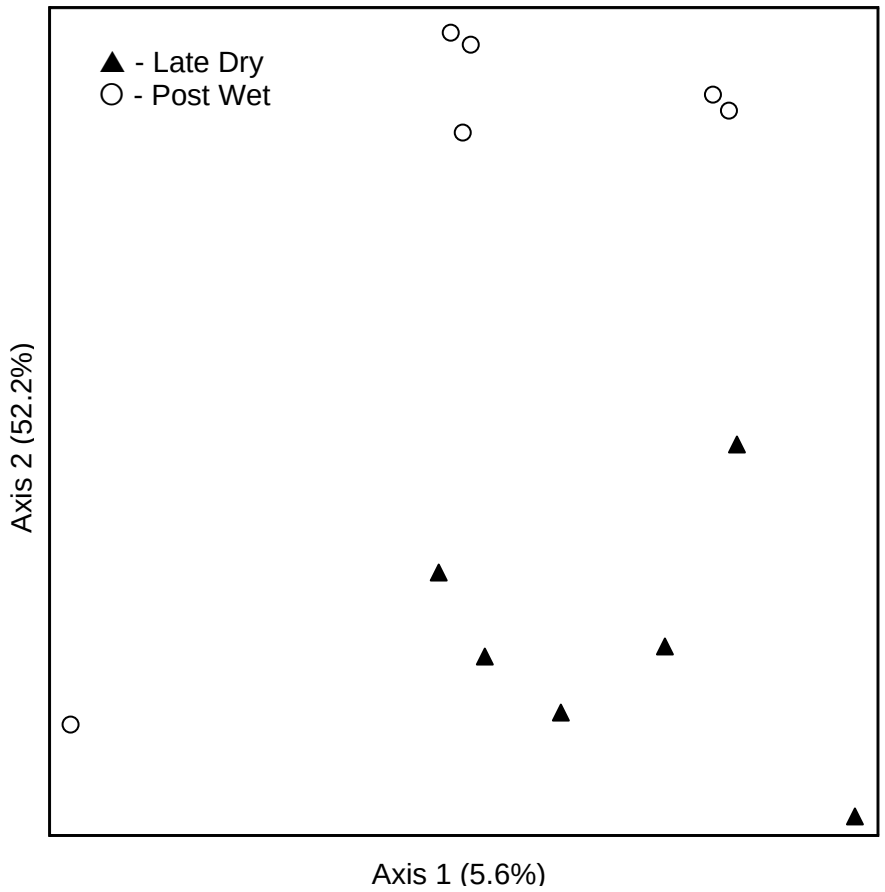


Figure 4.14 - NMS ordination of locations in Keelbottom Creek using algal data, with overlay of season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p < 0.0015. Stress = 14.806.

Table 4.11- Table showing r values for physico-chemical parameters corresponding to different axes of a NMS analysis for algal species in Keelbottom Creek. Significant r values shown in bold ($r_{(0.01, 10)} = 0.708$).

Parameter	Axis 1 (3.5%)	Axis 2 (52.2%)
Temperature	-0.067	-0.432
Dissolved Oxygen	-0.039	-0.192
pH	0.552	-0.609
Conductivity	-0.072	0.244
PAR	-0.346	-0.212
Turbidity	-0.233	**0.802**
Chlorophyll *a*	-0.652	0.550
Chlorophyll *b*	-0.430	0.538
Chlorophyll *c*	-0.343	0.589
Phaeophytin	0.173	0.494
Total Nitrogen	0.364	-0.625
Particulate Nitrogen	-0.396	-0.587
Total Filterable Nitrogen	0.518	-0.414
Filterable Organic Nitrogen	0.490	-0.393
Ammonia	0.332	-0.376
Nitrite	-0.378	0.546
Nitrate	0.463	0.376
Total Phosphorus	-0.397	**0.734**
Particulate Phosphorus	-0.394	0.628
Total Filterable Phosphorus	-0.322	**0.754**
Filterable Organic Phosphorus	-0.159	0.688
Filterable Reactive Phosphorus	-0.472	0.051

Table 4.12 - MRPP table showing T-statistic, A-statistic and p-value comparing algal species among sites and season in the Keelbottom Creek.

Comparison Group	T-statistic	A-statistic	p-value
Season	-4.6246754	0.09597707	0.00149566
Site	0.35792491	-0.01107317	0.59516486

CCA confirms the importance of nutrients, season and turbidity (Figure 4.15). The indicator species table (Table 4.14) illustrates the characteristic species from the two seasons. The late dry season is indicated by chlorophytes, dinoflagellates, and diatoms, with a majority of chlorophyte species. The shift to the post-wet season shows a shift in indicator species, with the introduction of euglenoids and ochrophyte species and the loss of vegetative dinoflagellate species from the assemblage (presumably, cysts would still be present in the sediment), but the post-wet season is still dominated by chlorophyte species. Such a strong contrast with so many species suggests a large seasonal shift rather than just a general increase or decline of species.

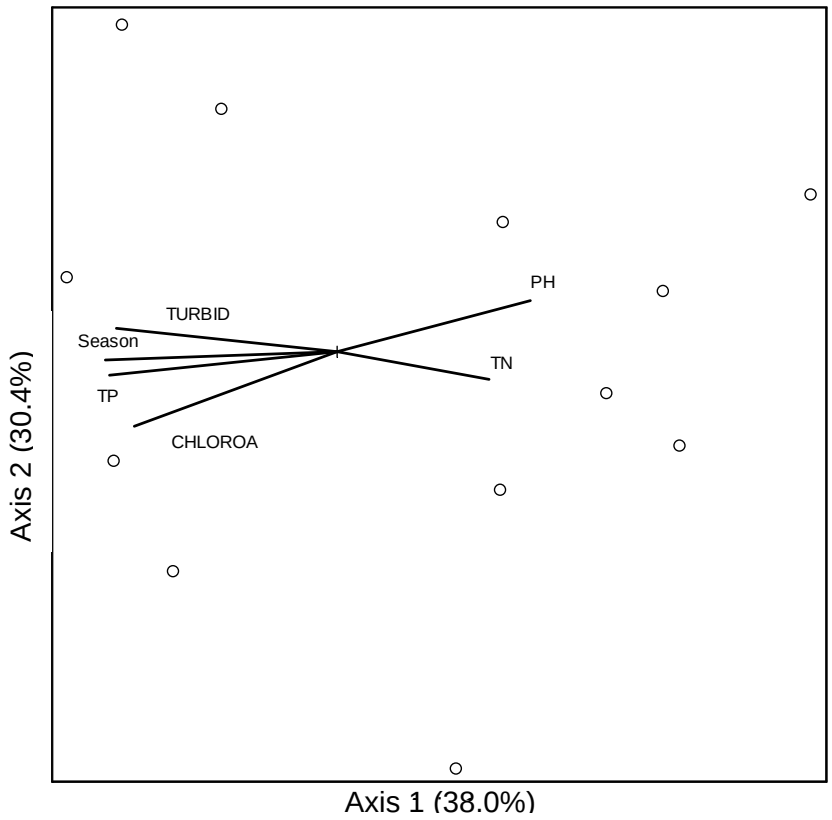


Figure 4.15 – CCA ordination of samples using algal data for Keelbottom Creek, with vectors indicating significant physico-chemical parameters; the proportion of the variance explained by each axis is indicated (%) on the axes. Abbreviations: TURBID = turbidity; TP = total phosphorus; CHLOROA = Chlorophyll *a*; TN = total nitrogen.

Table 4.13 - Correlation table showing r values for physico-chemical parameters corresponding to different axes of a CCA analysis for algal species in the Keelbottom Creek. Significant r values shown in bold ($r_{(0.01, 10)} = 0.708$); Monte Carlo Species-Environment correlations were 1.00 for all axes; all axes were significant – axis 1 & 2, p = 0.01; axis 3, p = 0.02.

Parameter	Axis 1 (38.0%)	Axis 2 (30.4%)
Season	**-0.937**	-0.046
Temperature	0.320	-0.428
Dissolved Oxygen	-0.008	-0.121
pH	**0.776**	0.283
Conductivity	-0.255	-0.263
PAR	-0.047	-0.580
Turbidity	**-0.893**	0.133
Chlorophyll *a*	**-0.821**	-0.416
Total Nitrogen	0.611	-0.154
Ammonia	0.359	0.141
Total Phosphorus	**-0.919**	-0.136

Table 4.14- Indicator species table for Keelbottom Creek, ordered according to p and indicator values.

Species	Division	Season	Indicator value	p value
Peridinium bipes	Dinophyta	Late Dry	89.3	0.001
Staurastrum chaetoceras	Chlorophyta	Late Dry	50.0	0.014
Gyrosigma sp. 1	Ochrophyta	Late Dry	50.0	0.017
Mougeotia sp. 1	Chlorophyta	Late Dry	50.4	0.022
Closterium kurtzingii	Chlorophyta	Late Dry	36.1	0.033
Glenodinium sp. 1	Dinophyta	Late Dry	41.7	0.042
Koliella spiculiformis	Chlorophyta	Late Dry	41.7	0.048
Cosmarium binum	Chlorophyta	Post Wet	75.0	0.001
Staurastrum chaetoceras	Chlorophyta	Post Wet	75.0	0.002
Euglena granulata	Euglenoids	Post Wet	58.3	0.005
Phacus caudatus	Euglenoids	Post Wet	58.3	0.006
Synedra ulna	Ochrophyta	Post Wet	58.3	0.006
Closterium kurtzingii	Chlorophyta	Post Wet	50.0	0.014
Scenedesmus ellipticus	Chlorophyta	Post Wet	51.4	0.022
Tetrastrum elegans	Chlorophyta	Post Wet	41.7	0.035
Centritractus belanophorus	Ochrophyta	Post Wet	41.7	0.037

4.3.6 Basalt River physico-chemical data and phytoplankton data sample day 3

Physico-chemical data PCA analysis

Figure 4.16 shows a distinct difference in physico-chemical parameters between season as well as time of day. The main drivers of these patterns are conductivity, PAR, turbidity, Chlorophyll *a*, nitrate and phosphorus (Figure 4.17). There is a high correlation of dissolved oxygen, Chlorophyll *a* and nutrients on axis 1 with temperature, dissolved oxygen, pH, conductivity and Chlorophyll *a* on axis 2. Although axis 3 only describes 4.7% of the variance, there is a strong correlation with PAR (Table 4.15).

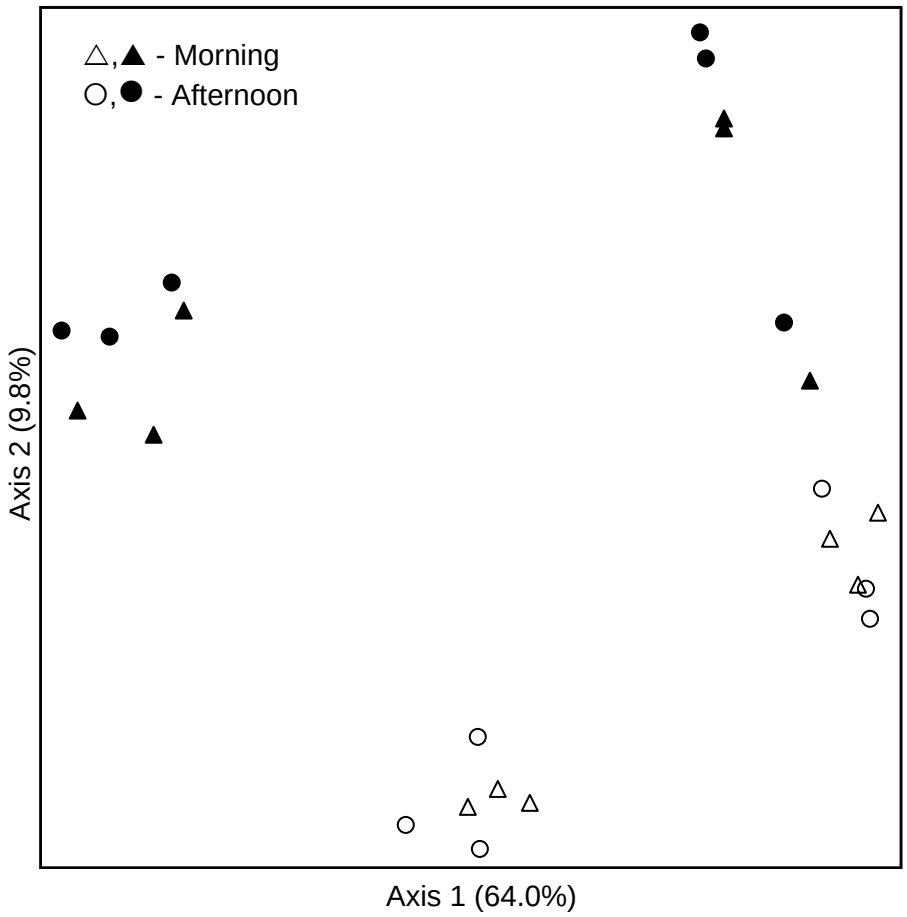


Figure 4.16 – PCA ordination of samples using algal data for the Basalt River by time. Closed symbols represent late dry season, open symbols represent post-wet season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p < 0.0001 and time, p < 0.0001

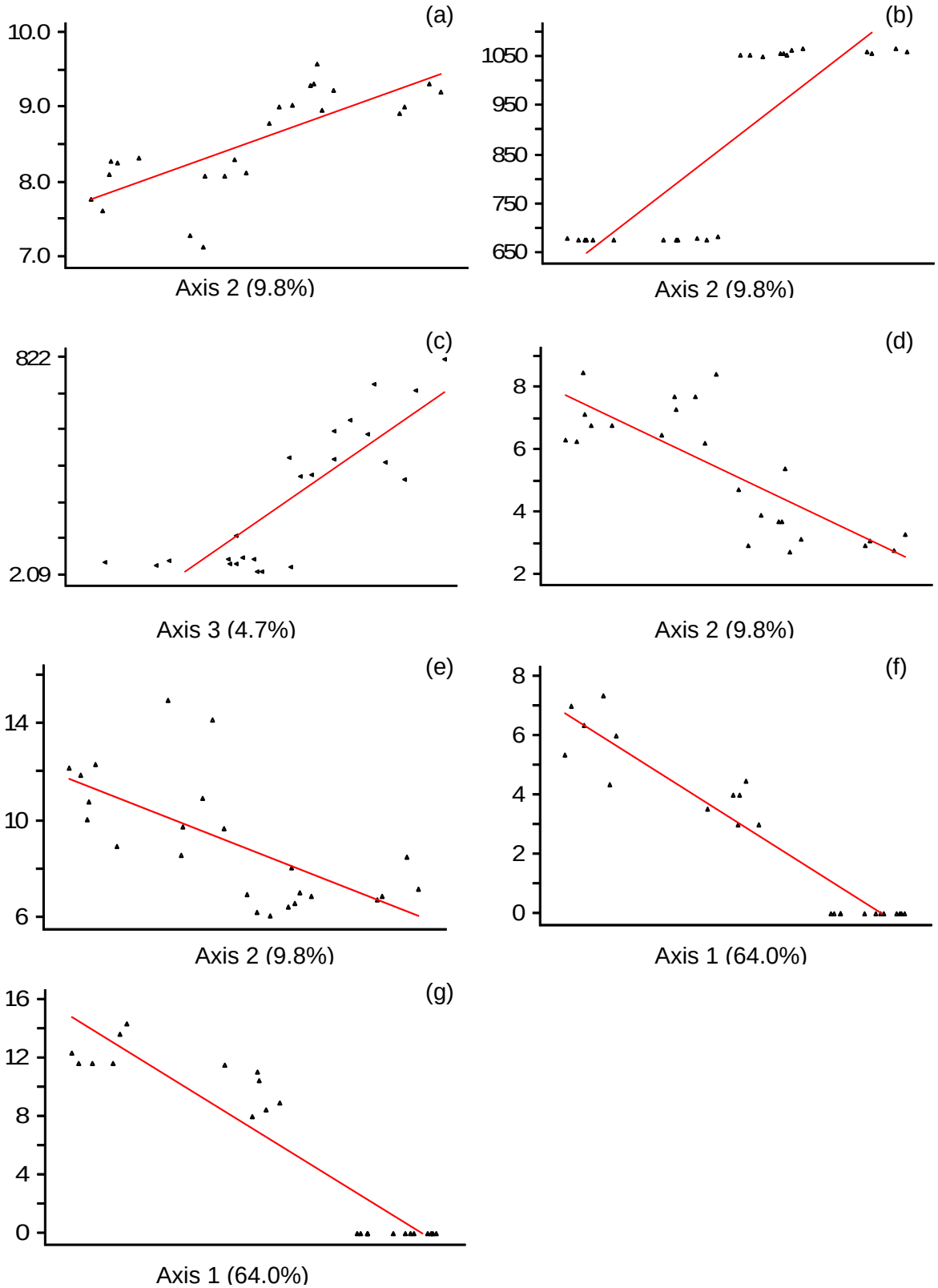


Figure 4.17 – Relationships between selected variables and PCA axes: (a) pH, r=0.754; (b) Conductivity [µS cm^{-1}], r=0.850; (c) PAR [µmol], r=-0.843; (d) Turbidity [NTU]; r=-0.796; (e) Chlorophyll *a* [µg L^{-1}]; r=-0.668; (f) Nitrate [µg L^{-1}]; r=-0.953; (g) Filterable Reactive Phosphorus [µg L^{-1}], r=-0.930, in Basalt River. The proportion of the variance explained by each axis is indicated (%) on the axes.

Table 4.15- Correlation table showing r values for physico-chemical parameters in the Basalt River corresponding to different axes of a PCA analysis. Significant r values shown in bold ($r_{(0.01, 22)} = 0.515$). * Multiple measures from different depths presented when they showed substantial differences; only a single representative measure when they did not.

Parameter	Axis 1 (64.0%)	Axis 2 (9.8%)	Axis 3 (4.7%)
Temperature	-0.424	**0.845**	-0.151
Dissolved Oxygen – 2*	-0.127	**0.668**	-0.283
Dissolved Oxygen – 4*	**-0.602**	**0.641**	0.111
Dissolved Oxygen – Max*	-0.302	**0.517**	**-0.619**
Dissolved Oxygen – Mid*	**0.532**	**-0.690**	-0.334
Dissolved Oxygen – Bot*	**0.558**	**-0.753**	-0.162
pH	-0.385	**0.754**	-0.036
Conductivity	-0.409	**0.805**	-0.055
PAR	0.041	-0.080	**-0.842**
Turbidity – Min*	0.423	**-0.796**	-0.053
Turbidity – Max*	-0.032	0.506	-0.130
Chlorophyll *a* – 1*	0.412	**-0.675**	0.178
Chlorophyll *a* – 3*	**-0.519**	**0.749**	0.112
Chlorophyll *b* – 1*	0.205	0.097	0.058
Chlorophyll *b* – 3*	-0.352	**0.797**	0.190
Chlorophyll *c* – 1*	0.360	-0.282	0.411
Chlorophyll *c* – 3*	-0.313	**0.787**	0.159
Phaeophytin – 1*	0.157	**-0.515**	0.307
Phaeophytin – 3*	-0.505	**0.770**	0.030
Total Nitrogen	**-0.974**	-0.164	0.048
Particulate Nitrogen	**-0.949**	-0.145	0.076
Total Filterable Nitrogen	**-0.969**	-0.170	0.036
Ammonia	**-0.751**	0.249	0.016
Nitrite	**-0.935**	-0.196	-0.018
Nitrate	**-0.953**	-0.211	0.077
Total Phosphorus	**-0.951**	-0.152	-0.031
Particulate Phosphorus	**-0.949**	-0.255	0.006
Total Filterable Phosphorus	**-0.859**	-0.458	-0.029

Phytoplankton data NMS analysis

Figure 4.18 strongly differentiates the seasons, as well as strata among the phytoplankton species. There is a strong correlation between the phytoplankton species and pH, conductivity, nitrogen and phosphorus on axis 1 (Table 4.16). There is a good correlation between phytoplankton species and dissolved oxygen, nitrogen and phosphorus on axis 3. Although axis 2 describes less of the variation, there is still a good correlation with temperature. MRPPs show the significant difference in phytoplankton assemblages between season and a marginally non-significant difference in sites within the Basalt River (Table 4.17). There is also a significant difference in phytoplankton species among strata.

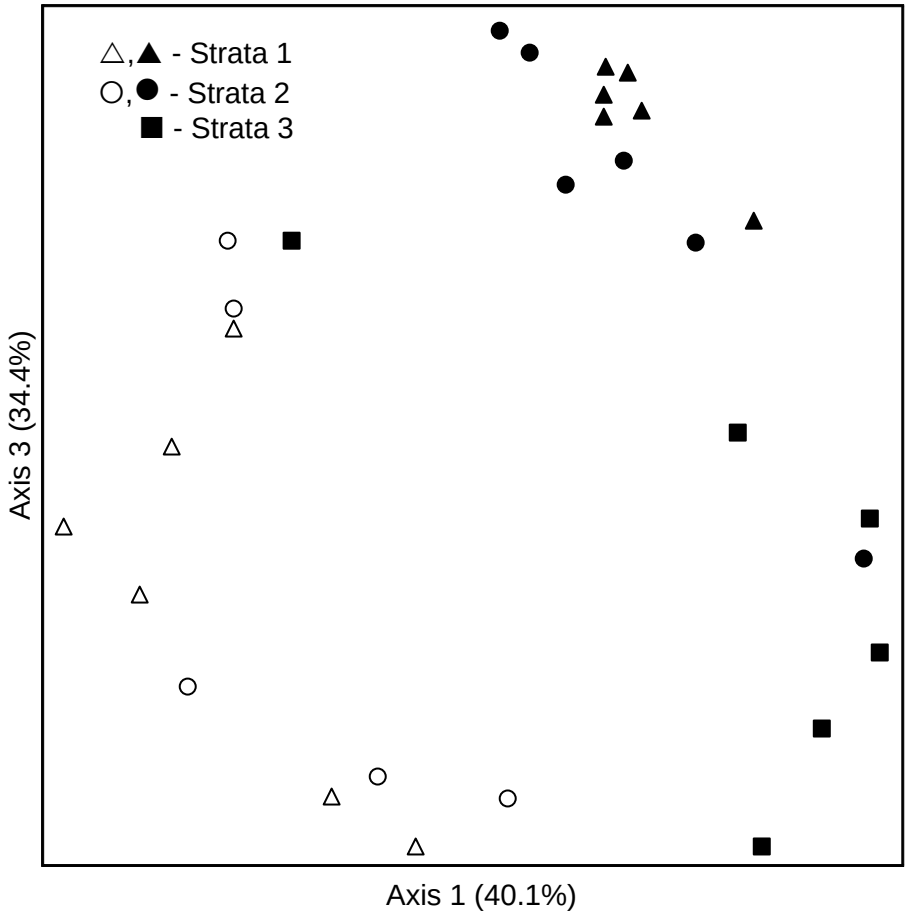


Figure 4.18 – NMS ordination of sites based on algal data for the Basalt River with overlay of season and strata. Closed symbols represent late dry season, open symbols represent post wet season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, $p < 0.0001$ and strata, $p < 0.05$. Stress = 17.037.

Table 4.16 – r values for physico-chemical parameters corresponding to different axes of a NMS analysis for algal species in the Basalt River. Significant r values shown in bold ($r_{(0.01, 27)} = 0.471$).

Parameter	Axis 1 (40.1%)	Axis 2 (19.3%)	Axis 3 (34.4%)
Temperature	0.331	**0.632**	-0.114
Dissolved Oxygen	-0.268	0.448	**0.507**
pH	**0.731**	0.412	-0.427
Conductivity	**0.815**	0.499	-0.456
PAR	-0.227	0.255	0.152
Turbidity	-0.043	**-0.472**	-0.384
Chlorophyll *a*	-0.281	-0.457	0.239
Chlorophyll *b*	**0.514**	-0.214	-0.376
Chlorophyll *c*	0.180	**-0.482**	0.063
Phaeophytin	0.078	**-0.518**	**-0.504**
Total Nitrogen	**0.650**	-0.181	**-0.507**
Particulate Nitrogen	**0.574**	-0.128	-0.300
Total Filterable Nitrogen	**0.611**	-0.190	**-0.567**
Filterable Organic Nitrogen	**0.585**	0.079	0.306
Ammonia	0.271	-0.242	**-0.758**
Nitrite	**0.479**	-0.191	-0.137
Nitrate	**0.587**	0.139	-0.351
Total Phosphorus	0.444	-0.434	-0.342
Particulate Phosphorus	**0.501**	-0.357	-0.368
Total Filterable Phosphorus	0.063	**-0.549**	-0.116
Filterable Organic Phosphorus	-0.120	**-0.621**	0.195
Filterable Reactive Phosphorus	0.379	0.179	**-0.632**

Table 4.17 - MRPP table showing T-statistic, A-statistic and p-value comparing algal species among Season, sites and strata in the Basalt River.

Comparison Group	T-statistic	A-statistic	p-value
Season	-13.296948	0.18487416	0.00000004
Site	-1.7059157	0.03402660	0.06291586
Strata	-2.9838718	0.06007681	0.01058513

Figure 4.19 and Table 4.18 confirm the interaction between phytoplankton and season, nutrients, dissolved oxygen, pH and conductivity. The indicator species table (Table 4.19) shows that many species correlate closely with the post-wet season. Species of chlorophytes, cyanobacteria, dinoflagellates, euglenoids and diatoms are indicative of the post-wet season, suggesting that all species do well in this habitat during this season. There are, however, only three species that correlate with the late dry season – *Cosmarium binum* and *Scenedesmus ellipticus*, both chlorophytes, and *Aphanizomenon gracile*, a cyanobacterium. This suggests that these species may be specially adapted to the conditions of the late dry season.

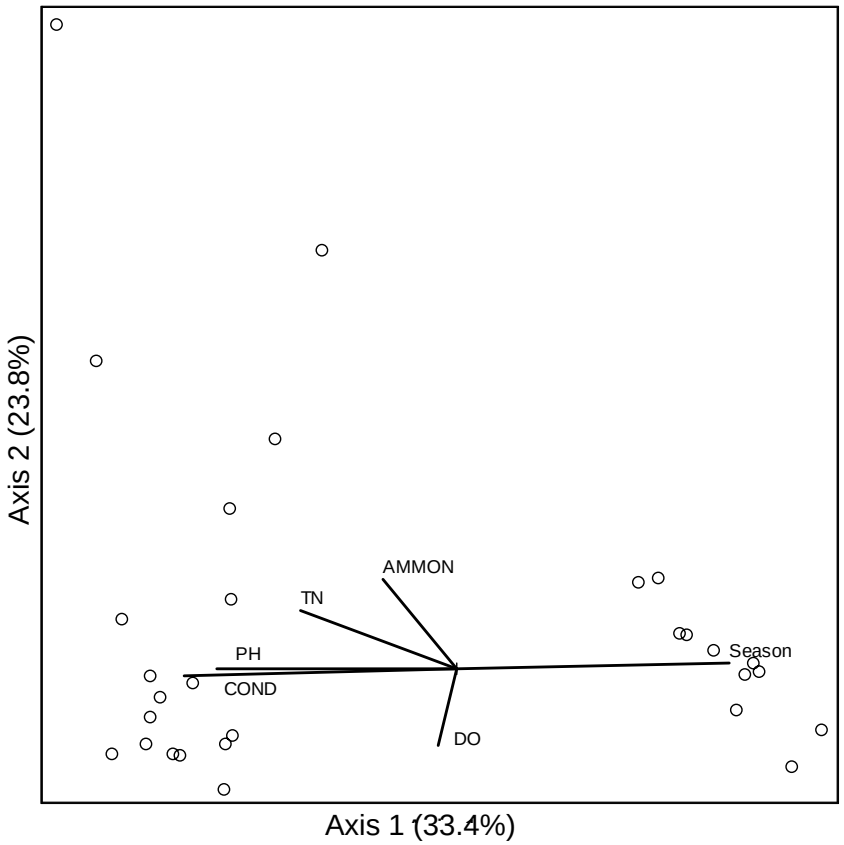


Figure 4.19 – CCA ordination of sites using algal data for the Basalt River. Vectors indicate significant physico-chemical parameters; the proportion of the variance explained by each axis is indicated (%) on the axes. Abbreviations: AMMON = ammonia; TN = total nitrogen; COND = conductivity; DO = dissolved oxygen.

Table 4.18 – Correlation table showing r values for physico-chemical parameters corresponding to different axes of a CCA analysis for algal species in the Basalt River. Significant r values shown in bold ($r_{(0.01, 27)}$ = 0.471); Monte Carlo Species-Environment correlations were 0.984 for axis 1, 0.820 for axis 2 and 0.890 for axis 3; axis 1 and 3 were significant, p = 0.01 and 0.04; axis 2 was not significant, p = 0.45

Parameter	Axis 1 (33.4%)	Axis 2 (23.8%)
Season	**0.967**	0.046
Temperature	**-0.588**	-0.294
Dissolved Oxygen	-0.067	**-0.577**
Conductivity	**-0.967**	-0.046
pH	**-0.854**	0.008
PAR	0.047	-0.258
Turbidity	0.452	0.330
Total Nitrogen	**-0.555**	0.446
Ammonia	-0.265	**0.681**

Table 4.19- Indicator species table for Basalt River, ordered according to p and indicator values.

Species	Division	Season	Indicator value	p value
Cosmarium binum	Chlorophyta	Late Dry	61.1	0.001
Scenedesmus ellipticus	Chlorophyta	Late Dry	49.6	0.015
Aphanizomenon gracile	Cyanobacteria	Late Dry	42.2	0047
Pediastrum simplex	Chlorophyta	Post Wet	94.7	0.001
Scenedesmus quadricauda	Chlorophyta	Post Wet	87.8	0.001
Anabaena spiroides	Cyanobacteria	Post Wet	84.2	0.001
Tracholamonas curta	Euglenoids	Post Wet	64.9	0.001
Crucigeniella crucifera	Chlorophyta	Post Wet	63.2	0.001
Pediastrum duplex	Chlorophyta	Post Wet	60.5	0.001
Peridinium lomnickii	Dinophyta	Post Wet	57.9	0.001
Monoraphidium contortum	Chlorophyta	Post Wet	52.6	0.001
Navicula sp. 1	Ochrophyta	Post Wet	33.2	0.001
Tetraedon regulare	Chlorophyta	Post Wet	31.6	0.001
Tetrastrum elegans	Chlorophyta	Post Wet	26.3	0.001
Monoraphidium arcuatum	Chlorophyta	Post Wet	55.5	0.009
Closterium acerosum	Chlorophyta	Post Wet	21.1	0.018
Gloeocystis sp. 1	Chlorophyta	Post Wet	23.7	0.022
Navicula sp. 2	Ochrophyta	Post Wet	15.8	0.039
Tracholamonas hispida	Euglenoids	Post Wet	15.8	0.039
Amphora sp. 1	Ochrophyta	Post Wet	15.8	0.041
Navicula sp. 3	Ochrophyta	Post Wet	15.8	0.042

4.3.7 Suttor River physico-chemical parameters and phytoplankton data

Physico-chemical data PCA analysis

The physico-chemical parameters of the Suttor River show a distinct significant difference between season and time of day (Figure 4.20). These patterns are driven by nitrogen, phosphorus, turbidity and chlorophylls (Figure 4.21). These data are correlated with temperature, dissolved oxygen, pH, conductivity, turbidity, and nutrients on axis 1 and temperature, dissolved oxygen, pH, conductivity and chlorophylls on axis 2 (Table 4.20). Correlation of these data with dissolved oxygen, pH and PAR is quite high on axis 3, even though it only describes 12.4% of the variation.

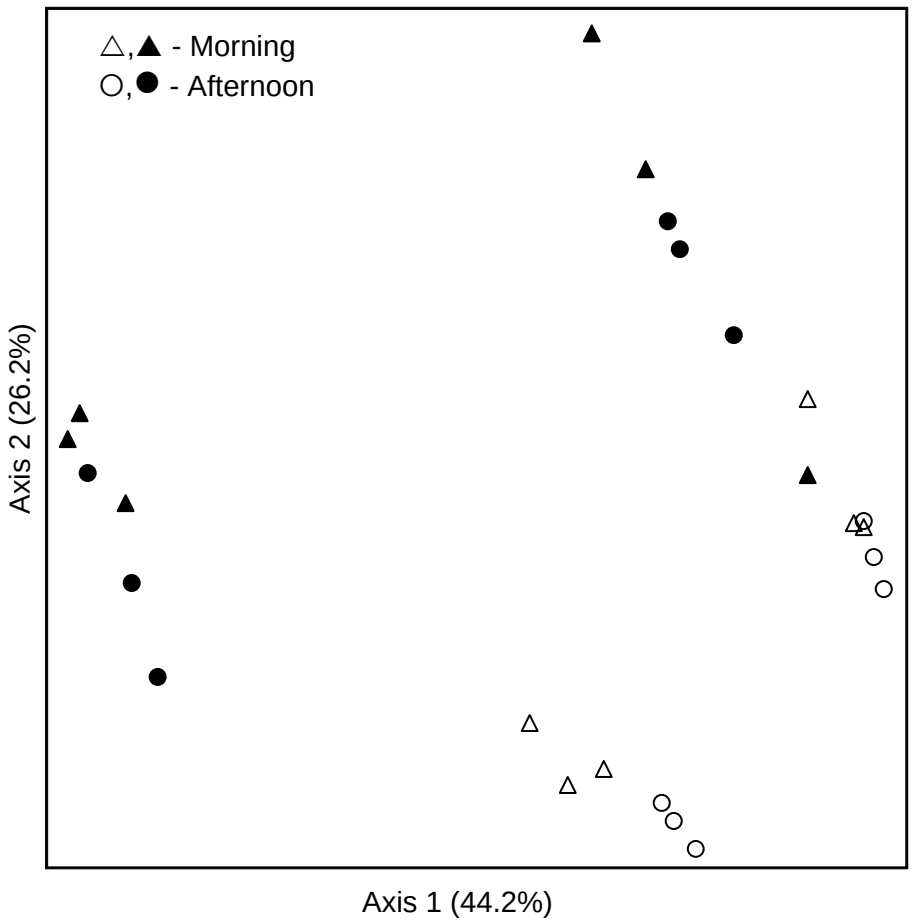


Figure 4.20 – PCA ordination of samples using physico-chemical data for the Suttor River by time and season. Closed symbols represent late dry season, open symbols represent post-wet season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, $p < 0.0001$ and time, $p < 0.0001$

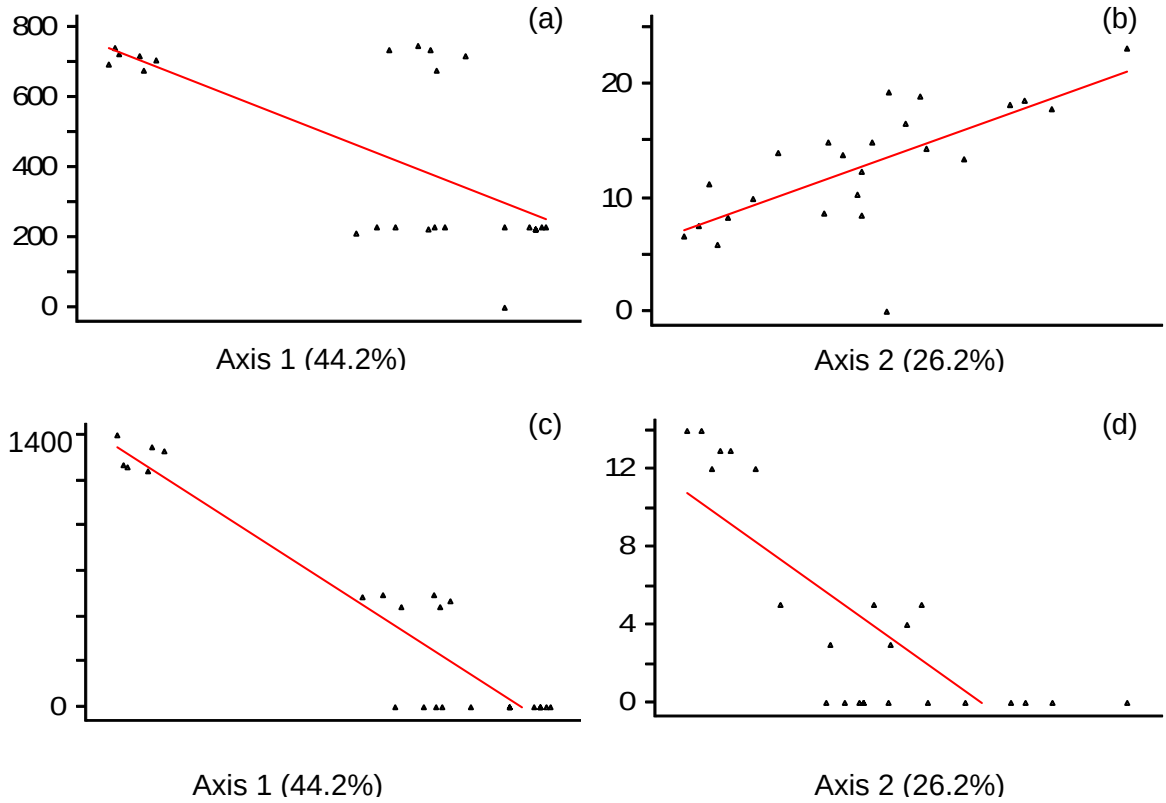


Figure 4.21 – Relationships between selected variables and PCA axes: (a) Turbidity [NTU], r=-0.660; (b) Chlorophyll *a* [µg L^{-1}], r=0.697; (c) Total Nitrogen [µg L^{-1}], r=-0.940; (d) Filterable Organic Phosphorus [µg L^{-1}], r=-0.787, in the Suttor River. The proportion of the variance explained by each axis is indicated (%) on the axes.

Table 4.20- Correlation table showing r values for physico-chemical parameters in the Suttor River corresponding to different axes of a PCA analysis. Significant r values shown in bold ($r_{(0.01, 22)}$ = 0.515). * Multiple measures from different depths presented when they showed substantial differences; only a single representative measure when they did not.

Parameter	Axis 1 (44.2%)	Axis 2 (26.2%)	Axis 3 (12.4%)
Temperature	**0.600**	**-0.649**	-0.118
Dissolved Oxygen – 3*	**0.601**	**-0.655**	0.013
Dissolved Oxygen – Mean*	0.238	-0.272	**0.867**
pH – 3*	**0.601**	**-0.638**	-0.147
pH – Mean*	-0.177	0.393	**-0.819**
Conductivity	**-0.604**	**0.645**	0.123
PAR	0.172	-0.431	**0.722**
Turbidity	**-0.660**	**0.611**	0.260
Chlorophyll *a*	-0.446	**0.697**	0.319
Chlorophyll *b*	-0.347	**0.774**	0.204
Chlorophyll *c*	-0.415	**0.716**	0.134
Phaeophytin	-0.381	**0.773**	0.131
Total Nitrogen	**-0.940**	-0.324	0.020
Total Filterable Nitrogen	**-0.902**	-0.403	0.075
Ammonia	**-0.905**	-0.230	-0.039
Nitrite	**-0.748**	**-0.535**	-0.214
Nitrate	**-0.962**	-0.127	0.033
Total Phosphorus	**-0.958**	-0.190	0.062
Particulate Phosphorus	**-0.958**	-0.157	0.068
Filterable Reactive Phosphorus	**-0.929**	-0.354	-0.049

Phytoplankton data NMS analysis

Figure 4.22 shows a significant difference in phytoplankton assemblages by season. The main drivers of these results are temperature, conductivity, turbidity, and nutrients (Table 4.21). These data again confirm the phytoplankton-physico-chemical links driven by seasonal effects (Figure 4.23). The euglenoids are much more prevalent indicators for the Suttor River than the other rivers studied (Table 4.24). The majority of euglenoids occupy a diverse range of ecological niches including acid and alkaline waters, aerobic and anaerobic conditions (John et al. 2002), which may be why only one species, *Strombomonas tambowika*, is indicative of the late dry season (Table 4.24).

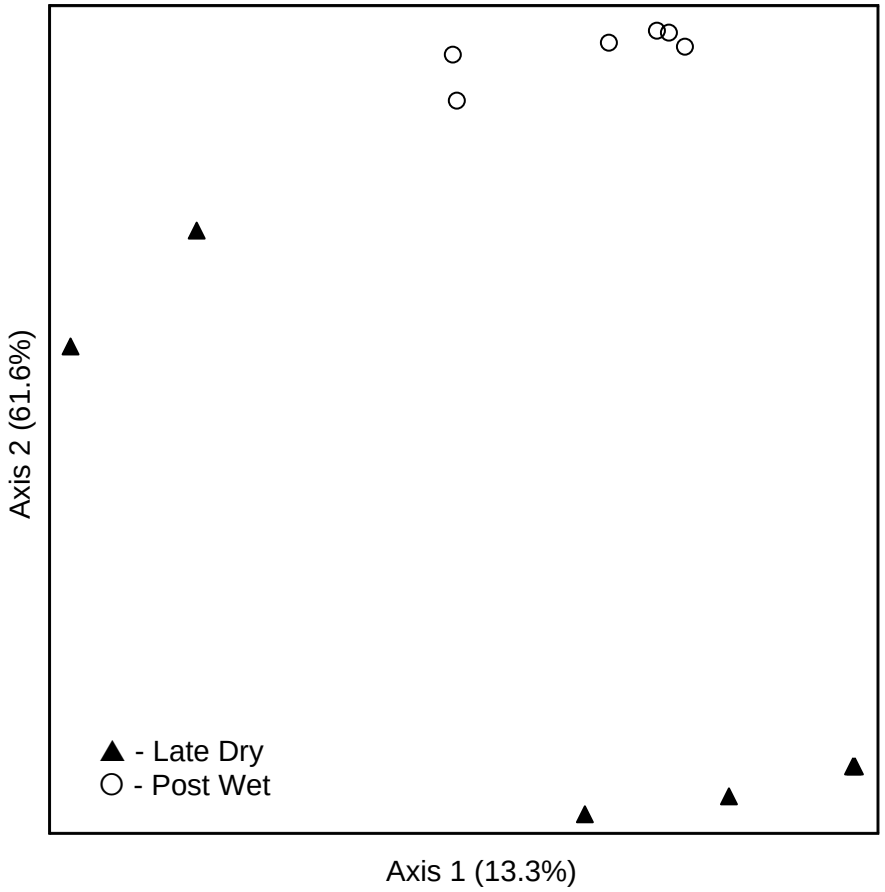


Figure 4.22 – NMS ordination of samples using algal data for the Suttor River with overlay of season; the proportion of the variance explained by each axis is indicated (%) on the axes. MRPP for season, p = 0.00208970. Stress = 10.534.

Table 4.21 – r values for physico-chemical parameters corresponding to different axes of a NMS analysis for algal species in the Suttor River. Significant r values shown in bold ($r_{(0.01, 9)} = 0.735$).

Parameter	Axis 1 (13.3%)	Axis 2 (61.6%)	Axis 3 (13.7%)
Temperature	0.255	**-0.803**	0.167
Dissolved Oxygen	0.572	0.074	0.183
pH	-0.562	0.036	-0.143
Conductivity	-0.079	**-0.865**	0.150
PAR	0.185	0.297	0.286
Turbidity	-0.105	**-0.852**	0.196
Chlorophyll *a*	-0.188	-0.711	0.248
Chlorophyll *b*	-0.092	-0.723	0.037
Chlorophyll *c*	-0.480	-0.457	0.461
Phaeophytin	-0.049	-0.567	0.028
Total Nitrogen	-0.116	**-0.825**	0.087
Particulate Nitrogen	-0.404	-0.310	-0.041
Total Filterable Nitrogen	0.082	**-0.929**	0.142
Filterable Organic Nitrogen	0.160	**-0.890**	0.150
Ammonia	0.119	**-0.806**	-0.015
Nitrite	-0.295	-0.068	-0.627
Nitrate	-0.113	**-0.847**	0.162
Total Phosphorus	-0.030	**-0.885**	0.190
Particulate Phosphorus	-0.030	**-0.884**	0.191
Total Filterable Phosphorus	0.011	0.631	-0.261
Filterable Organic Phosphorus	0.018	**0.857**	-0.094
Filterable Reactive Phosphorus	-0.019	**-0.849**	-0.015

Table 4.22 – MRPP table showing T-statistic, A-statistic and p-value comparing algal species in the Suttor River among season and sites between groups.

Comparison Group	T-statistic	A-statistic	p-value
Season	-5.0736555	0.22782618	0.00208970
Site	1.0238930	-0.06853793	0.87457100

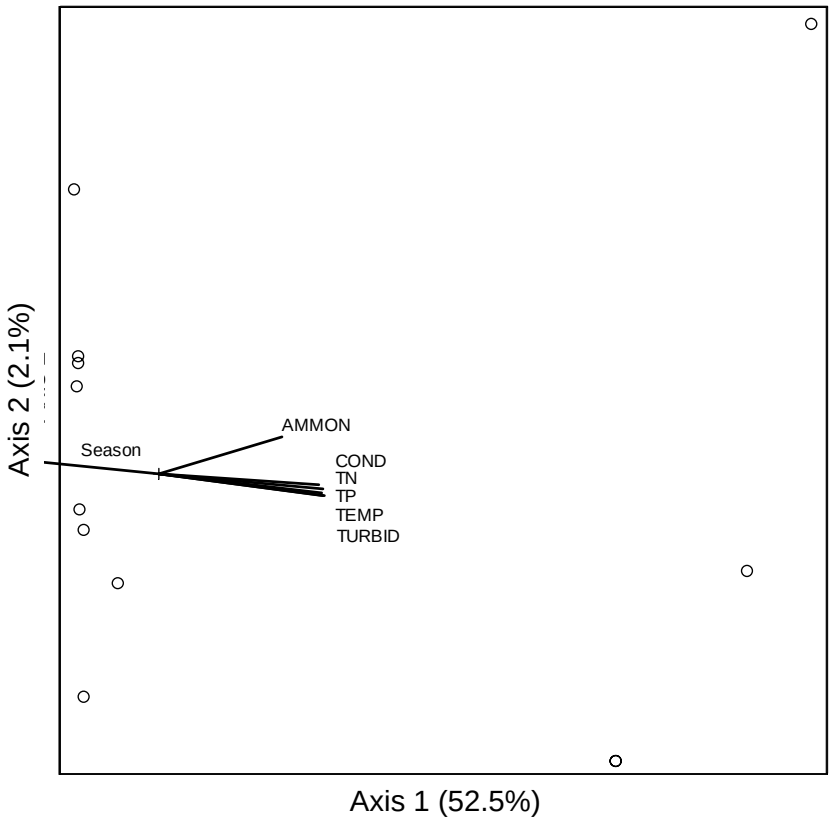


Figure 4.23 – CCA ordination of samples using algal data for the Suttor River, vectors indicating significant physico-chemical parameters; the proportion of the variance explained by each axis is indicated (%) on the axes. Abbriviations: AMMON = ammonia; COND = conductivity; TN – total nitrogen; TP = total phosphorus; TEMP = temperature; TURBID = turbidity.

Table 4.23 – Correlation table showing r values for physico-chemical parameters corresponding to different axes of a CCA analysis for algal species in the Suttor River. Significant r values shown in bold ($r_{(0.01, 9)} = 0.735$); Monte Carlo Species-Environment correlations were 1.00 for all axes; all axes were significant, p = 0.01.

Parameter	Axis 1 (52.5%)	Axis 2 (2.1%)
Season	**0.830**	0.101
Temperature	**0.831**	-0.138
Dissolved Oxygen	-0.045	-0.200
pH	-0.068	0.396
Conductivity	**0.835**	-0.096
PAR	-0.156	-0.419
Turbidity	**0.823**	-0.122
Chlorophyll *a*	0.607	-0.153
Total Nitrogen	**0.813**	-0.068
Ammonia	0.623	0.227
Total Phosphorous	**0.841**	-0.140

Table 4.24 – Indicator species table for Suttor River, ordered according to p and indicator values.

Species	Division	Season	Indicator value	p value
Strombomonas tambowika	Euglenoids	Late Dry	75.0	0.001
Strombomonas acuminatus	Euglenoids	Post Wet	91.7	0.001
Tracholamonas oblonga	Euglenoids	Post Wet	91.7	0.001
Amphora sp. 1	Ochrophyta	Post Wet	83.3	0.001
Tracholamonas curta	Euglenoids	Post Wet	77.3	0.001
Closterium moniliferum	Chlorophyta	Post Wet	41.7	0.034

4.4 Discussion

4.4.1 Water quality

Temperature was significantly different between the rivers, especially between the Basalt River and the other two rivers. The greater temperatures in the Basalt River can be explained by the constant sunlight on the water hole, whereas the Suttor River and Keelbottom Creek were shaded throughout the day. Keelbottom Creek was highly shaded explaining the variation in temperatures during both seasons. Temperature ranged from 24 – 30°C in all rivers, which is the optimal temperature range for photosynthesis and growth for all algal groups, including tropical species. Butterwick et al (2005) found that of 21 phytoplankton species tested in the laboratory, all species could grow at 25°C, but many – including most of the diatoms, some cyanobacteria, and all flagellates – persistently failed to grow at 30°C, and only *Aphanizomemom flos-aquae* survived with moderate increase at 35°C, a lethal temperature for the other species.

Dissolved oxygen was significantly different between the rivers studied. The large variations in the Basalt River and Keelbottom Creek were due to depth and shading, respectively. During the late-dry season, the Basalt river strongly stratified, therefore having significantly different levels of dissolved oxygen in each stratum, but during the post-wet season there was less variation since the flow of water and mixing reduced stratification. Keelbottom Creek showed a large variation during the late-dry season also due to the high amount of shading throughout the day. It too had less variation during the post-wet season due to water flow. Fluctuations in dissolved oxygen concentrations are driven predominantly by biological responses to the light-dark cycle (Marzolf et al. 1994).

The rivers in this study were neutral to basic in pH. The Basalt River had pH records of between 8.0 and 10.0, values much higher than the acidic to neutral pH that are more typical of tropical rivers (Hart and McKelvie 1986). Conductivity and turbidity were significantly different between

seasons and rivers, as shown in Chapter 3. Conductivity was higher in the late dry season and lower in the post-wet season, and turbidity was lower in the late-dry season and higher in the post-wet season. The observed differences were due to stratification during the late-dry and water flow (mixing) during the post-wet. Surprisingly, the Suttor River showed the opposite pattern in turbidity, perhaps because of inputs of lower turbidity water from upstream during the wet season

Chlorophyll *a* is used as a measure of phytoplankton biomass in a water body (Hotzel and Croome 1999, Townsend 2000, Webster et al. 2005). Chlorophyll *a* levels were significantly different for the Keelbottom Creek and Suttor Rivers between seasons, as well as between all rivers. Growth and biomass of phytoplankton can be controlled by nitrogen and phosphorus (Robertson 1997). In general growth-limiting concentrations of inorganic phosphorus and inorganic nitrogen are in the order of 10 mg m^{-3} and 100mg m^{-3}, respectively (Reynolds 1992). In Keelbottom Creek, total phosphorus increased and total nitrogen decreased (Figure 4.5), but Chlorophyll *a* increased from the late-dry to the post-wet season (Figure 4.4b), suggesting that phytoplankton might be limited by phosphorus. The Basalt River has continuous flow of water and therefore continuous nutrient supply; hence neither total nitrogen and phosphorus changed significantly between seasons (Figure 4.5), nor did Chlorophyll *a* (Figure 4.4b). The Suttor River showed a decrease in both total nitrogen and total phosphorus from the late-dry to the post-wet season (Figure 4.5), which corresponded with a decrease in Chlorophyll *a* (Figure 4.4b). The Suttor River is dominated by euglenoids, many of which are characteristic of eutrophic systems, therefore could explain the decline of Chlorophyll *a* as the nutrients decrease. *Trachelomonas curta* and *T. oblonga* are recognized as being indicators of moderately eutrophic waters (John et al. 2002). Zalocar de Domitrovic (2002) found that in the Paraguay River the suite of euglenoids identified were best represented in the slower moving and higher nutrient waters. The suite of euglenoids included *Trachelomonas curta* and six species of *Stromobomonas*, but none of the species found in this study. *Trachelomonas curta* and *oblonga*, and 2 species of *Strombomonas* were indicative of the Suttor River (Table 4.24), therefore suggesting that the Suttor river is eutrophic.

PCA analyses separated rivers on two axes, with temperature, conductivity, pH and dissolved oxygen defining the first axis and temperature; and dissolved oxygen and PAR defining the second axis. The analyses confirmed the strong contrast between season and rivers. The analyses also showed that replicate sites within the Keelbottom Creek and Suttor River are strongly similar in physico-chemical attributes, whereas for the Basalt River the replicate sites are neither significantly different, nor strongly similar. This is possibly due to the Basalt River having multiple strata (due to depth), resulting in differences of temperature, dissolved oxygen, pH and PAR between strata. Analyses revealed that there was much redundancy in the measurements taken from the different replicate sites within each river, therefore it would be satisfactory to

sample from 1 site in each river to obtain physico-chemical measurements. However, as some parameters differed substantially with time of day, it would be necessary to sample each site at three times a day (dawn, mid-day, afternoon) to obtain a reasonably accurate view of the physico-chemistry of the river.

The findings in the intensive study confirmed the results of chapter 3, with temperature, conductivity and turbidity again identified as the major drivers of phytoplankton assemblages (Table 4.5). Additionally, nutrients were found to be important and differed between rivers. PCA analyses showed that parameters identified in chapter 3 separated the rivers on axis 2 and nutrients separated the rivers on axis 1 (describing a larger proportion of the variance). Unlike physical parameters which were found to be consistent between replicate sites within a river, nutrients did vary significantly at replicate sites within rivers, possibly because of patchiness in distributions of macrophytes and microphytes.

4.4.2. Correlations between phytoplankton and water quality

Nutrients showed a significant relationship with phytoplankton assemblages. In total, the physical and chemical parameters explained about 74% of the variance associated with phytoplankton composition. Surprisingly, PAR and dissolved oxygen were not as strongly correlated with phytoplankton species as they were in Chapter 3. Whether this was because of different antecedent conditions between the two years is not known, but it points to a level of unpredictability in relationships between sampling times that reduces confidence in the generality of one-off survey results.

In Chapter 3 phytoplankton assemblages were found to differ between rivers and seasons, as were physico-chemical parameters, which suggested the potential of using phytoplankton species as indicators of water quality in these rivers. The intensive study supported this conclusion. For example, the indicator species for the Basalt River were mainly cyanobacteria, *Anabaena spiroides*, *Aphonizomenon gracile* and *Anabenopsis elenkinii*; and the chlorophytes, *Scenedesmus ellipticus*, *S. dimorphus* and *Pediastrum simplex*. Indicator species for the Keelbottom Creek were mainly the chlorophytes, *Staurastrum* sp., *Mougeotia* sp., *Closterium* sp., the euglenoids, *Euglena* sp. and *Phacus caudatus*, and the diatoms, *Synedra ulna* and *Gyrosigma* sp.. Indicator species for the Suttor River were mainly the euglenoids, *Strombomonas tambowika*, *S. acuminatus*, *Tracholamonas oblonga*, *T. curta* and the diatom, *Amphora* sp. O'Farrell (1994) found indicative and dominant species of phytoplankton for rivers in South America. She found multiple genera and several species that are similar to this study, including *Crucigenia quadrata, Monoraphidium contortum, M. arcuatum, Pediastrum simplex, P. duplex, Eudorina elegans,* and *Synedra ulna*. In

her study, O'Farrell (1994) found *C. quadrata* to represent waters with low conductivity and neutral pH, and *E elegans* represented moderate conductivity and pH; in this study *C. quadrata* was not dominant or indicative and occurred across the range of conductivity and pH, and *E. elegans* was also not dominant, but was mainly found in waters with moderate conductivity and pH. In O'Farrell's (1994) study *M. contortum* and *P. simplex* were indicative of low conductivities and neutral pH and *M. arcuatum*, *P. duplex* and *S. ulna* were indicative of high conductivities and alkaline pH. This study supported the findings of *M. arcuatum* and *P. duplex* representing high conductivities and alkaline pHs, but this study also found *M. contortum* and *P. simplex* as indicators of high conductivities and alkaline pHs, contrasting O'Farrell's findings. O'Farrell found *Synedra ulna* in high conductivity and alkaline pH whereas I found it in moderate conductiviy and pH. Clearly there is room for much more research to allow us to understand how particular species in different locations differ in their responses to environmental factors: the 'species' may in fact be cryptic siblings, they may show local adaptations, or they may be affected by interactions with other species, or interactions between environmental variables. In the absence of this sort of information, it will prove difficult to use these species as indicators of water quality or ecosyustem health.

Phytoplankton assemblages not only differed substantially between river systems, but also between seasons within in the river systems, as discussed below.

Keelbottom Creek

There were strong seasonal and diel variations in physico-chemical parameters. The most significant variables were the same as in the general survey (Chapter 3), but with the addition of nitrogen and total phosphorus as important variables. Levels of total phosphorus and Chlorophyll *a* increased from the late-dry to the post-wet season (Figures 4.4b and 4.5), indicating a potential phosphorus limitation of phytoplankton species in this system. Phosphorus is usually thought to limit `primary productivity in fresh water (Schindler 1977). Phytoplankton assemblages did not vary within the study site, but did vary with season (Table 4.12). Phytoplankton species in this river were highly correlated with turbidity and phosphorus (Figure 4.15 and Table 4.13). The late-dry season was primarily dominated by chlorophytes, and secondarily by ochrophytes but only one diatom species. The post-wet season was also dominated by chlorophytes, but only 2 species, *Staurastrum chaetoceras* and *Closterium kurtzingii*, persisted in both seasons (Table 4.14). As phytoplankton in this system clearly closely relates to season and seasonal changes in physico-chemical parameters, they are good potential indicators of water quality.

Basalt River

The Basalt River showed strong differences in physico-chemical parameters between season and time of day. Again, the main parameters identified were the same as in the general survey with the addition of phosphorus and nitrogen also being important and showing significant differences between seasons and times of day. The Basalt River, unlike the other rivers, showed differences in physico-parameters and phytoplankton species between strata (Figure 4.18 and Table 4.17). The phytoplankton was most highly correlated with conductivity in this system (Table 4.18), which itself is related to the geology of the catchment and sediment of the river. Similarly, DeNicola et al. (2004) found that stream and wetland periphyton communities in Ireland were closely related to conductivity, reflecting catchment geology and land use. A large suite of species were significant indicators in the post-wet season samples, but the only significant indicator species in the late-dry season were *Cosmarium binum*, *Scenedesmus ellipticus* (chlorophytes) and *Aphanizomenon gracile* (cyanobacteria), indicating that these species may specialize and may be adapted to the extremely high conductivity in the late-dry season (over 1000 $\mu S*cm^{-1}$) and very alkaline pH (9-10) (Table 4.19). It is suprising to see *C. binum* as an indicator of this river, as it is known commonly in the tropics as an acidophile (John et al. 2002). *Scenedesmus ellipticus* is a cosmopolitan species and occurs in a variety of aquatic habitats (John et al. 2002). Lüderitz and Scholz (1989) found evidence that *A. gracile* has increased activity at higher pHs, it had a significant uptake of copper at pH 8.4, but there was no significant uptake at acidic pHs (e.g. 5.4). These species should be scrutinized more in the laboratory to determine if they are indicating high conductivity and/or alkaline pH.

Suttor River

Physico-chemical parameters and especially nutrients differed significantly between season and time of day (Figure 4.22). Total nitrogen and phosphorus levels were much higher for this river than in the other rivers (Figure 4.5). As there were no aquatic macrophytes at the sampling site, which was extremely turbid, it is probable that the high levels of nutrients reflect the lack of uptake by plants (including phytoplankton), in contrast to the other rivers. The indicator species for this system were dominated by euglenoids, and only one species (*Strombomonas tambowika*) was a significant indicator for the late-dry season (Table 4.24). The dominance of euglenoids in this system may be a result of the high nutrient status of this river, as some euglenoids tend to be associated with eutrophic waters. Clearly there is scope here for further experimental investigation.

4.4.3 Comparable studies

There have been several studies investigating the influence of physical and chemical parameters on phytoplankton assemblages in rivers. Talling and Rzoska (1967) found that phytoplankton species were closely associated with water flow and discharge in the Blue Nile. A study by Leland (2003) found that major taxa within the San Joaquin River indicated differing responses to the longitudinal variation in water depth and flow regime. A change in phytoplankton species composition was attributed to water depth and flow regime in two rivers in Brazil (Bovo-Scomparin and Train 2008). In a study of the Fitzroy River, Queensland, Fabbro and Duivenvoorden (2000) found that algal assemblages separated according to clarity of the water column, temperature and water flow between seasons. In the Paraguay River, phytoplankton species richness and composition varied significantly between seasons (Zalocar de Domitrovic 2002). The phytoplankton assemblages of the Nakdong River in Korea varied by season and river placement; nutrients appeared everywhere to be in excess of algal requirement, and did not influence markedly the downstream and seasonal phytoplankton compositional differences in this river (Ha et al. 2002). A comparable study of several rivers in the River Plate Basin found that variation in phytoplankton assemblages were due to differences in temperature, pH, turbidity and conductivity which varied with geographic location (O'Farrell 1994). In the Reconquista River in Argentina, phytoplankton assemblages correlated with nutrients, temperature, turbidity, water flow and season (Olguin et al. 2004).

While these studies provide some opportunity for comparison with the Burdekin system, the intermittent nature of this river sets it apart from those studies. However, dryland rivers that become a series of water holes in the dry season occur commonly across northern Australia, and often provide the only freshwater habitat over vast areas. Some efforts in understanding these systems have been made recently. In a study of the Fitzroy River, Queensland, Fabbro and Duivenvoorden (2000) found that algal assemblages separated according to clarity of the water column, temperature and water flow between seasons. Many of the genera found were the same as in this study, but only a few species were found to be the same: they were *Pediastrum duplex, P. simplex Trachelomonas oblonga, Synedra ulna, Aphanizomemnon gracile* and *Anabaena spiroides*. The model created by Fabbro and Duivenvoorden (2000) predicts the dominance of phytoplankton genera under various physico-chemical conditions. They found *Trachelomonas* to be dominant with high turbidity and high conductivity, whereas this study found *Trachelomonas* to be an indicator in the Suttor River with high turbidity and lower conductivity. However, this study's findings support the model for the cyanobacteria genera, *Aphanizomenon* and *Anabaena*, which are indicative of high pH, high conductivity and low turbidity. Townsend (2006) found that phytoplankton assemblages correlated with the river's hydraulics between the seasons in the Mary River in the Northern Territory, but no clear evidence of domination by any single phytoplankton

group was found. The majority of the phytoplankton were identified only to genus, with a few exceptions, *Chroomonas acuta, Peridinium inconspicuum, Synedra ulna, Navicula cryptocephala,* and *Nitzschia agnita.* This study found that while the river was flowing the abundant species belonged to *Anabaena, Chroomonas acuta, Cryptomonas, Scenedesmus* and *Euglena* (Townsend 2006), which is similar to the findings of this study, where during the wet season there was greater abundance of *Anabaena spiroides, Scenedesums quadricauda, S. ellipticus* and *Euglena granulate*. The dry-season water holes in that study were large – e.g.,14 km long and 6-9 m deep – unlike the systems in this study. During the dry season (lacustrine state) the abundant species belonged to *Peridinium inconspicuum, Chroomonas acuta, Thodomonas, Cryptomonas, Acanthoceras, Nitzschia agnita, Synedra ulna, Trachelomonas,* and *Sphaerocystis* (Townsend 2006). The investigation of this thesis also found *Peridinium* spp. during the dry season, but also in the wet, whereas the *Trachelomonas* spp. and *Synedra ulna* were found to be indicative of the riverine state (wet season). Two studies conducted in the river systems of the Lake Eyre Basin had similar water-hole morphology to the present study. Costelloe et al. (2005) found that phytoplankton assemblages differed between rivers as well as seasons, as a result of differences in conductivity, flow connectivity (the length of the river upstream of sampling site) and water flow (discharge from previous year). Costelloe et al.'s study identified phytoplankton to genus level. *Anabeana*, *Anabaenopsis* and *Gyrosigma* were found at sites with high conductivities, which the present study also found, except that *Gyrosigma* was found in moderate conductivities. The highest diversity was in sites with low-moderate conductity and coincided with the end of the flood season, and representative genera were *Euglena, Phacus, Strombomonas, Trachelomonas, Pediastrum, Eudorina, Aulocoseira, Synedra, Isthmochloron, Botryococcus* and *Oocystis* (Costelloe et al. 2005). These observations are broadly in agreement witht the present study, where the greatest diversty of phytoplankton was found in the Keelbottom Creek, which is of moderate conductivity and neutral pH, during the post-wet season. McGregor et al. (2006) studied phytoplankton assemblages in another river in the Lake Eyre Basin and in the Murray-Darling Basin, finding that assemblages differed significantly between catchments, although the magnitude of the difference was not large, with 50% of the dissimilarity between the catchments attributed to eight species, *Campylomonas reflexa, Chlamydomonas biocca, Trachelomonas oblonga* var. *australica, Chlamydomonas sp., Anabaena spiroides, Trachelomonas hispida, Anabaena circinalis, Aphanocapsa holsatica* (in order of % difference from least to most). The most common species across the sites were *Campulomonas reflexa, Chroomonas acuta, Trachelomonas hispida, T. oblonga* var. *australica, Euglena* sp. 1 and *Chlamydomonas biocca; Monoraphidium* spp. and *Scenedesmus* spp. increased significantly in the post-wet season and *Anabaena spiroides* increased significantly in the dry season (McGregor et al. 2006). I also found an increase in *Scenedesmus* spp. and *Monraphidium* spp. in the post-wet season; however *Anabaena spiroides* increased in abundance in the post-wet season in the Burdekin catchment. In

contrast to the study presented here, they found that phytoplankton assemblages were poorly correlated with environmental variables. There was also no clear trend in variability between landscape scales (waterhole and catchment), suggesting no measurable difference in phytoplankton community composition as a result of spatial patterns; communities in close proximity within the landscape were no more similar to each other than communities from regions further away (McGregor et al. 2006). Bunn et al. (2003) found that phytoplankton production of dryland systems in the upper Murray catchment were regulated by hydrologic processes which determined their transport along the system and within each waterhole.

These studies provide a similar picture to the current one of the Burdekin system: phytoplankton assemblages are largely governed by a rather obvious suite of physical and chemical variables, especially relating to seasonal change in flow and temperature, and factors such as turbidity, pH and nutrient concentrations, which relate to lithology, soils and land use. Causal links between these variables are readily made and the results of this brief review (and supported by the results of this study) are not surprising. In the present study, two of the rivers were intermittent in flow, with phytoplankton assemblages surviving the dry season in refugial water holes limiting impact of the landscape to areas in immediate vicinity. That is, phytoplankton of these systems are probably governed by the local landscape (geology, vegetation, land use) rather than by the upstream catchment, as is the case for most rivers. The Basalt River did sustain flow throughout the dry season and maintained conditions of water quality which reflected the basaltic nature of the catchment. This system stood out as being distinct from the others.

The water holes of dryland rivers in northern Australia have diverse phytoplankton assemblages that develop following reduction or cessation of wet-season flow, which may be very short lived. Water holes develop their own characteristic assemblages, according to local factors, but communities within rivers are more similar than those between rivers, as a result of underlying differences in catchment characteristics. Water quality and other variables' influences therefore operate at river catchment scales for part of the annual cycle, but at a local scale (= water hole catchment scale) for the majority of it during the dry season. Understanding the dynamics of biophysical processes in relation to these different scales is vital for good management of these water bodies, which may be the only aquatic environment in the landscape, and for designing appropriate monitoring programs. This theme is taken further in Chapter 6.

Chapter 5 . Growth responses of three species of *Scenedesmus* isolated from the Burdekin catchment study sites to changes in conductivity and temperature

5.1 Introduction

A large number of inter-linked variables collectively describe or affect 'water quality'. They include physical variables such as flow, temperature, light environment, conductivity, suspended solids and dissolved oxygen; chemical variables such as dissolved nutrients and pH; and biological variables such as productivity, respiration and chlorophyll levels. In the field studies (Chapters 3 and 4) the importance of several of these variables to the phytoplankton assemblages was elucidated, suggesting strong links between the phytoplankton and water quality indicators of ecosystem health. However, despite strong correlative evidence for the links between the biota and water quality, causal links are best demonstrated by controlled experiments. While such links are routinely elucidated in single-species monitoring of water quality in industrial situations (Voltolina et al. 2005, Ma et al. 2007), they are rarely pursued for whole-assemblage or field indicators of ecosystem health (Connolly and Pearson 2007). In this chapter, I describe experiments that link three common phytoplankton species with two of the most obvious drivers of the observed algal assemblages, namely temperature and conductivity, as case studies of the utility of developing explicit cause-effect relationships. Clearly further work on other variables and species would be necessary to fully develop explicit water-quality–algal-assemblage profiles to be used in a monitoring program.

5.1.1. Phytoplankton as indicators of water quality

Water quality assessment using bioindicators has been studied for over one hundred years (Cohn 1853, Chandler 1970, Chutter 1972, De Pauw and Vanhooren 1983, Davis and Simon 1995) and multiple bioindicator organisms have been used, e.g. water fowl (Peris et al. 1992, Bowerman et al. 1998, Rattner et al. 2005, Wayland et al. 2006), fish (Kruger 1985, Adams et al. 1993, Ham et al. 1997, Adams et al. 1999, Bouchereau et al. 2006), crustaceans (Sanchez-Moyano and Garcia-Gomez 1998, Lignot et al. 2000, Sanchez-Moyano et al. 2000), insects (Extence and Ferguson 1989, Stark 1993, Lang and Reymond 1995, Lemly 1998), zooplankton (Marneffe et al. 1998, Gesteira and Dauvin 2000, Raposa et al. 2003), benthic algae (Wu 1999, Lai et al. 2003, Verb and Vis 2005) and phytoplankton (Stoermer 1978, Laal et al. 1994, Korneva and Mineeva 1996, McNair and Chow-Fraser 2003, Lei et al. 2005). Research has favoured the use of heterotrophic over autotrophic bioindicators (Adamus et al. 2001). Autotrophic indicators include vascular plants, used to assess wetlands and lakes (Adamus et al. 2001) and benthic algae and phytoplankton for water quality assessment of streams and rivers (Lowe and Pan 1996). Phytoplankton respond readily to changes in water column nutrients and other chemical and

physical changes (Wetzel and Likens 2000). As single-celled and small groups of single-celled organisms, they exhibit high productivity and rapid turnover (Reynolds 1984, Lamberti 1996), which allows them to reflect the changes in water quality faster than vascular plants. In summary, the direct response of phytoplankton to physico-chemical variables, and their rapid turnover rate and high primary productivity renders them potentially ideal organisms for monitoring water quality changes resulting from different or changing land uses, providing a direct measure for ecological changes in rivers (Reynolds 1984, Noppe et al. 1999).

The immediate response of phytoplankton to changes in water quality could thus be exploited for monitoring ephemeral or seasonal systems, where the water chemistry and physical factors change quickly. Numerous studies have been conducted to determine the effects of temperature (e.g. Andersson et al. 1994, Weyhenmeyer 2001, Bouterfas et al. 2002), pH (e.g.Gonzalez 1997), light (e.g. Litchman 1998, 2000, Litchman et al. 2004), nutrients (e.g. Berman et al. 1991, Beardall et al. 2001a, Beardall et al. 2001b, Ahlgren and Hyenstrand 2003), carbon dioxide (Beardall 1981, 1991, Aksmann and Tukaj 2004), metals (e.g. Karez et al. 1994, Nalewajko and Olaveson 1995, Awasthi and Rai 2005) and herbicides (Badr and Abou-waly 1997, Aksmann and Tukaj 2004) on phytoplankton Chlorophyll *a,* growth, metabolism and community composition, while studies investigating the direct effect of conductivity have been few (e.g. Rott et al. 1998, DeNicola et al. 2004).

Field studies

Temperature, pH, and light are known to play a role in structuring phytoplankton communities in freshwater ecosystems. Phytoplankton species show different tolerance and physiological responses to temperature change (Reynolds 1989). Suzuki and Takahashi (1995) showed that maximum growth rates of various diatoms occurred at temperatures similar to the environment they were isolated from, suggesting that temperature can affect species distribution in the field. Similarly, a study conducted in lakes in Sweden demonstrated that overall phytoplankton biomass did not respond to global warming, but summer biomass of chlorophytes and cyanobacteria did increase at the expense of the other phytoplankton groups (Weyhenmeyer 2001). In a Spanish study, temperature and stratification tended to drive the algal composition in a wastewater regeneration pond (Arauzo et al. 2000). Stratification occurred during the higher temperature summer months and was dominated by Chlorococcales and Volvocales, whereas Euglenophyceae dominated during the autumn and winter months of destratification. Beardall and Raven (2004) suggested that elevated temperatures act upon different species in different ways, causing increased metabolic activity and growth of some species and pushing others beyond their temperature optima, resulting in changed phytoplankton community compositions.

There have been very few studies in which conductivity is addressed as a main environmental variable affecting phytoplankton. Many field studies note conductivity, but do not focus on it as a main factor. One study conducted in the lakes of Ireland found that conductivity (62-424 μS cm^{-1}) was strongly correlated with periphyton distribution from the various lakes, which reflected the geology and land use in the watersheds (DeNicola et al. 2004). Conductivity was identified as one of the main drivers of phytoplankton community composition in the field during this study.

Laboratory studies

Temperature can affect biochemical pathways in algae. Gao et al. (2000) investigated nitrate reductase activity under controlled laboratory conditions in chromophytic and chlorophytic algae. They found that NR activity peaked between 10 and 20°C in chromophytic phytoplankton, while the temperature optimum for chlorophytic phytoplankton NR was above 30°C. These results demonstrate the potential of temperature to change phytoplankton community composition through species-specific physiological processes. Coles and Jones (2000) isolated three species of cyanobacteria, *Microcystis aeruginosa*, *Merismopedia tenuissima* and *Oscillatoria* sp. and one diatom, *Aulocoseira granulata*, from the Potomac River and cultured them at 15, 20, 25, and 30°C. Photosynthetic rates were positively correlated with temperature over the entire range for the cyanobacteria and between 15 and 25°C for the diatom, suggesting an advantage for cyanobacterial growth at higher temperatures. Bouterfas et al. (2002) isolated three species of freshwater chlorophytic phytoplankton, *Coelastrum microporum*, *Selenastrum minutum* and *Cosmarium subportumidum*, from a Moroccan lake. They showed that temperature influenced growth rates: at 35°C, *C. subportumidum* showed the lowest growth rate, 1.00 d^{-1}, followed by *C. microporum* and *S. minutum* with 1.64 d^{-1} and,1.73 d^{-1} respectively. In contrast, (Dauta et al. 1990) found that optimal temperature ranged from 27 to 35°C for cyanobacteria and below 20°C for diatoms.

As temperature and conductivity were strong correlates with algal assemblages, suggesting that they might be major determinants of algal assemblages, and as both are readily manipulated in the laboratory, they were selected as the two variables to test in this preliminary study of cause-effect relationships between water quality and selected algal species.

5.1.2 *Scenedesmus* spp. as indicators of water quality

Field studies

Studies conducted in the field on algal species and community composition have identified *Scenedesmus* spp. as common components of the chlorophytes (Talling and Rzoska 1967, Ganf 1974a, Lung'Ayia et al. 2000). Other studies established that *Scenedesmus* spp. show seasonal

variation in abundance between the dry and wet seasons (Talling 1986a, Talling 1986b, Zafar 1986, Zalocar de Domitrovic 2002). By taking phytoplankton samples at three different times during the day, Ganf (1974b) showed, that *Scenedesmus* spp. were evenly distributed throughout the water column throughout the day despite different thermal stratification. This study also found that during intense thermal stratification there was no significant difference between surface accumulations of *Scenedesmus* spp. and accumulations below 10 cm (Ganf 1974b). Another study in tropical Australia showed that artificial destratification (mechanical aeration) of the water column correlated with a shift in phytoplankton composition from cyanobacteria and diatoms to chlorophytes, mainly *Scenedesmus denticulatus*, *S. acuminatus, Dictyosphaerium* sp., and *Ulothrix* sp. (Hawkins and Griffiths 1993).

A field study in Argentina established that *Scenedesmus opoliensis* was one of the most abundant species in small rivers with high conductivities (1310 – 5830 µS cm^{-1}) (O'Farrell 1994), while a study of an Argentinean lake found that the number of phytoplankton genera decreased as conductivity increased during water input (Fazio and O'Farrell 2005). Before water input, the conductivity ranged from 1980-3570 µS cm^{-1}; during water input from the local aquifer, and after water input, it ranged from 2650 to 23000 µS cm^{-1} and 1550 to 10010 µS cm^{-1}, respectively. *Scenedesmus* spp. were most abundant in the after-input period.

Laboratory studies

Increasingly from the 1990s, species of *Scenedesmus* isolated from tropical to polar regions have been used for laboratory experiments (Avendano and Orus 1990, Dauta et al. 1990, Egan and Trainor 1991, Miernik et al. 1993, Martinez et al. 1999, Voltolina et al. 1999, Fargasova et al. 2000, Martinez et al. 2000, Fargasova 2001, Fathi 2002, Voltolina et al. 2004, 2005). Many studies have focused on anthropogenic effects on freshwater, such as effects of herbicides and insecticides on growth (Badr and Abou-waly 1997, Ma et al. 2003, Ferraz et al. 2004, Ma et al. 2004a, Ma et al. 2004b); effects of heavy metals on growth (Greger et al. 1992, Fargasova et al. 2000, Mallick and Mohn 2003, Greger and Johansson 2004); use of *Scenedesmus* in bioremediation of wastewater (Kaya and Picard 1996, Nunez et al. 2001, Voltolina et al. 2005); and use in aquaculture (Wiltshire and Lampert 1999, Chen 2001, Lurling 2003).

The effects of temperature on growth of *Scenedesmus* and nutrient uptake have been studied previously. Martinez et al. (1999) suggested that growth and phosphorus uptake in *S. obliquus* is temperature-dependent. The cells were studied in phosphorous concentrations of between 0 and 372 µM and temperatures of 20, 25, 30 and 35 °C. The affinity of *Scenedesmus obliquus* for phosphorus was positively correlated with an increase in temperature up to the optimal temperature of 30 °C; exceeding the temperature optimum resulted in reduced growth rates and

lower phosphorus affinities (Martinez et al. 1999). Not only is growth rate affected, but temperature can also affect the cell cycle. Cepak et al. (2007) showed that the length of the cell cycle was prolonged with decreasing temperature (from 12 h at 33 °C to 70 h at 15 °C). The size of mother cells and consequently the size of daughter cells increased with decreasing temperature (Cepak et al. 2007).

There appear to be no studies concentrating on the effects of conductivity on *Scendesmus* cultures.

Although the effects of water quality on phytoplankton growth and composition have been investigated in detail, the majority of these studies relate to temperate regions or were conducted with temperate species. In addition, these studies focused either on laboratory experiments or field studies. The study presented here aimed to integrate field data and laboratory results to determine whether the drivers of phytoplankton community structure identified in the field would have a similar effect on growth of selected phytoplankton species when investigated as isolated variables under controlled environmental conditions. The three *Scenedesmus* species responded differently to conductivity and temperature in the field (Chapter 5 discussion), therefore these species were chosen for these laboratory studies as these species were represented at the field sites and were relatively easy to culture and grow in the laboratory.

5.2 Methods

5.2.1 Sample collection

Phytoplankton samples were obtained by vertical haul through the water column (bottom to surface) with 25 µm and 50 µm phytoplankton nets fitted with 1 L sample jars at the bottom. The samples were immediately placed on ice in the dark. They were returned to the laboratory, divided into 150 mL samples in 250 mL Duran Schott Erlenmeyer flasks and placed in a culture room at 25°C, with a light intensity of 45 µmol photons m^{-2} s^{-1} and a 12:12 h light/dark cycle. Samples were allowed to acclimate to culture conditions for two weeks before single species were isolated for controlled temperature and conductivity experiments.

5.2.2 Establishment of unialgal cultures

Unialgal cultures were established via serial dilutions in 24-well tissue culture plates on samples taken from each of the nine sites (Chapter 2 section 2.2). Three serial dilutions were carried out for both culture media using Bold's Basal medium (Sigma catalog # B5282) after Nichols (1973) and Cyanobacteria medium (Sigma catalog # C3061) after Rippka *et al*. (1979) (recipes are given in Appendix 3)) and for each study site (=54 tissue culture plates). Homogeneous samples were

diluted 1:1 with the respective medium in well 1 and samples in well 1 were diluted 1:2 in well 2. The 1:2 dilutions were carried out for subsequent wells up to well 23. Samples from well 23 were diluted 1:1 with the respective medium in well 24. The tissue culture plates were sealed with parafilm to minimize evaporation. All serial dilutions used sterile equipment and autoclaved media and were carried out in a Laminar flow (AES Environmental Pty LTD fitted with HEPA filter, Australian Standard 4260, NATA certified) under sterile conditions. Plates were incubated at 24°C in a Contherm cross-flow controlled environment phytoplankton growth chamber (Contherm, New Zealand) at a light intensity of 45 µmol photons m^{-2} s^{-1} and a 12:12 h light/dark cycle. Samples were examined for growth daily for 7 days using an inverted high resolution microscope (Olympus CKX41).

Monoclonal cultures of *Scenedesmus quadricauda*, *S. dimorphus*, and *S. ellipticus* (Figure 5.1) were established from serial dilution using the capillary-capturing technique (Anderson and Kawachi 2005) in 20 mL Petri dishes filled with either 10 mL Bold or Cyanobacteria medium. Monoclonal cultures were maintained under the same conditions described for serial dilution cultures and were re-cultured weekly or fortnightly depending on densities.

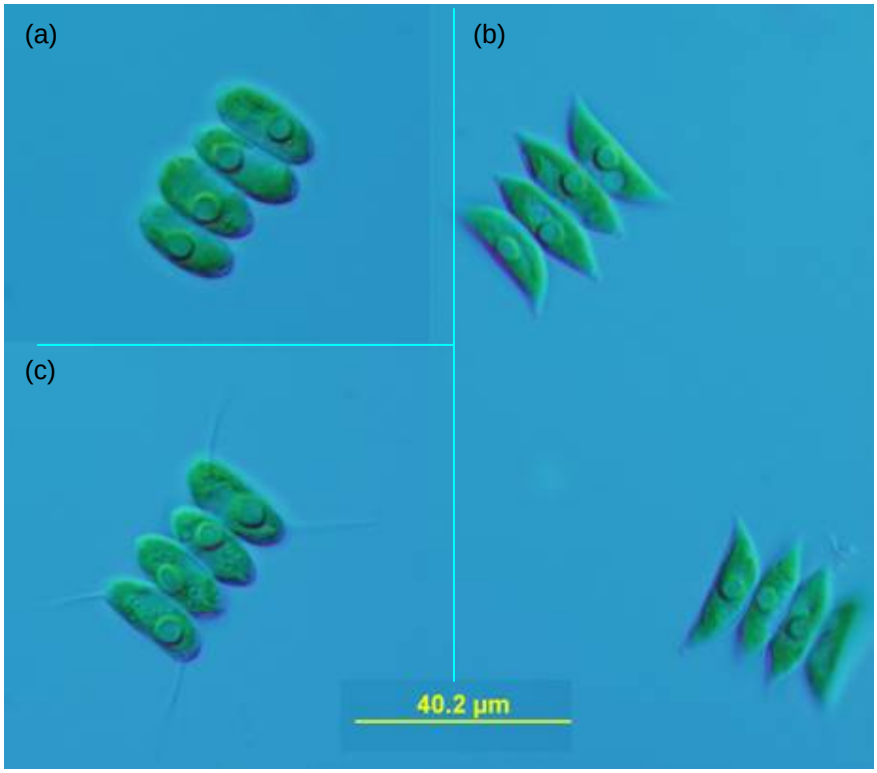


Figure 5.1 - Micrographs of Scenedesmus spp. used in laboratory experiments: (a) S. ellipticus, (b) S. dimorphus, (c) S. quadricauda; courtesy of S. Hudson

5.2.3 Culture maintenance

Cultures of *Scenedesmus* spp. were maintained in triplicate in 250 mL Duran Schott Erlenmeyer flasks containing 150mL of Bold or Cyanobacteria medium under sterile conditions. Growth was determined daily for two weeks directly via cell counts on Lugol preserved culture samples using a Neubauer improved haemocytometer and indirectly on live material using turbidity (% Transmission) at λ 750 nm (Varian DMS 90 UV Visible Spectrophotometer). These data were used to create growth calibration curves using linear regression between turbidity (% transmission) and counted cells mL^{-1} (Figure 5.5).

5.2.4 Conductivity experiments

Based on conductivity values encountered at the field sites, conductivity experiments were conducted over a range from 760-960 $\mu S\ cm^{-1}$ in increments of 50 $\mu S\ cm^{-1}$ for the three *Scenedesmus* species. Mother culture and experimental culture set-up and maintenance are summarized in Figure 5.2. Nine mother cultures (three independent cultures per *Scenedesmus* species) were inoculated with an initial inoculation density of 10^5 cells mL^{-1} in 150 mL Bold medium (final volume) in 250 mL Erlenmeyer flasks. Mother cultures were allowed to acclimate to the new conductivity levels for two weeks before inoculating experimental flasks for that particular conductivity level to avoid measuring effects on growth that could be due to conductivity shock rather than changed conductivity itself. Experimental flasks were inoculated at the same initial inoculation density, volumes, medium, and conductivity from acclimated mother cultures with a replication of three independent cultures per species (between culture replicates) and three within culture replicates (total of 27 flasks; 9 replicates per species). A completely randomized design was applied with 9 experimental flasks and 3 mother culture flasks per light shelf in a three light shelf Contherm cross-flow controlled environmental phytoplankton growth chamber at 24ºC, a light intensity of 45 μmol photons $m^{-2}\ s^{-1}$ and a 12:12 h light/dark cycle. Experimental cultures were allowed to acclimate to the new conductivity levels for two days before samples for growth evaluations were collected.

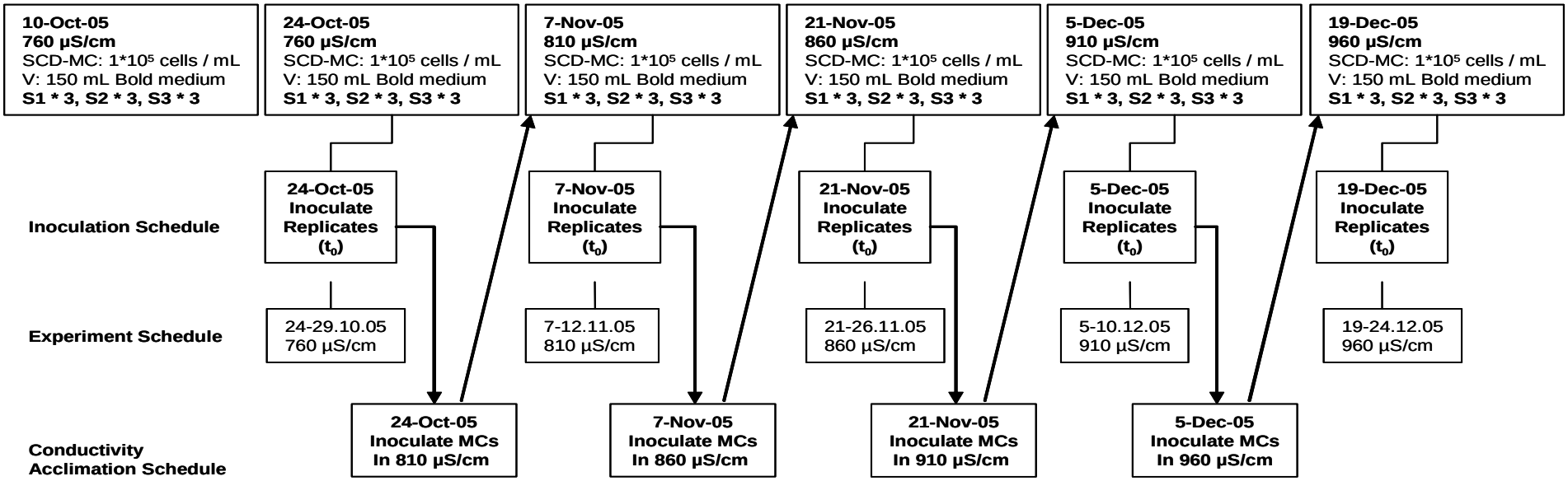


Figure 5.2 - Flow chart for conductivity acclimation experiments between 760-960 µS/cm in 250 mL Erlenmeyer flasks for *Scenedesmus* spp. SCD=starting cell density; MC=mother culture; V=culture volume; S1=*S. quadricauda*; S2=*S. dimorphus*; S3=*S. ellipticus*

For growth evaluation via turbidity measurements (Section 5.3.1), a 5 mL sample of each experimental flask was taken once a day for 5 days (time 0, 24, 48, 72, 96, 120 h). Random cell counts were conducted on three random samples at every measurement time to ensure that turbidity measurements were in line with the previously established calibration curves (Figure 5.5). pH measurements were taken concurrently for each sample with a Eutech Instruments Cyberscan pH/Ion 510 probe.

Controlled conductivity changes in Bold's medium

To increase the conductivity of Bold medium, potassium chloride (KCl) was chosen as an electrolyte. Potassium chloride was used because potassium is a major ingredient in plant fertilizers, which are more likely to be washed into rivers during the wet season, therefore potassium is more likely than sodium to contribute to conductivity changes in the dry season. 50x Bold medium concentrate was added to 600mL of Millipore deionised water (DI; Millipore, Elix 5 with a Progard 2 filter) and mixed for 10 minutes. Conductivity was adjusted via titration using a 0.01 M KCl prepared in DI water and mixed for 10 minutes (Table 5.1). The conductivity-adjusted Bold media were made to almost 1L with DI water. Due to its temperature-dependence, conductivity was measured at 25^{o}C with an YSI 556 Multiprobe and conductivity was fine-tuned by adding either DI water or 0.01 M KCl until the desired conductivity level was reached, before the final volume adjustment to 1L medium.

Table 5.1 - Titration table for conductivity adjustments of Bold medium

Conductivity of experimental Bold's Medium [µS/cm]	Conductivity of Bold's Medium [µS/cm]	Difference in conductivity [µS/cm]	Addition of 0.01 M KCl in DI [mL]
810	765.75	44.25	31.40
860	765.75	94.25	66.88
910	765.75	144.25	102.35
960	765.75	194.25	137.83

5.2.5 Temperature experiments

Temperature experiments were conducted using a 2ºC step-wise acclimation protocol over the range of 16-34°C (Figure 5.3), reflecting the temperature regime observed in the field. Replication, randomization, culture conditions, acclimation conditions, sample collection for growth evaluation, and measurement periods followed the experimental design described for conductivity experiments. Temperature experiments used the same monoclonal *Scenedesmus* species as for the conductivity experiments. Experiments were conducted in Bold's medium.

5.2.6 Statistics

Data were analyzed using Statistica 7. Statistical analyses were based on three-factor ANCOVAs (proc. GLM) following a complete factorial design to analyse the effects of the independent variables conductivity, temperature, species, and culture (independent replicate) on growth rates of the three monoclonal *Scenedesmus* species. ΔpH was used as a co-variate. Tukey's post-hoc tests were used for comparison of means. Homogeneity of variance was confirmed using Cochran's C test. To compare the correlation and significance of conductivity and temperature laboratory data with physico-chemical field data, main effects ANOVAs (proc GLM) were conducted for effects of minimum and maximum conductivity, minimum and maximum pH, and minimum and maximum temperature on the abundances of *S. quadricauda*, *S. ellipticus*, *S. dimorphus*, *S. bernardii*, *S. denticulatus*, and *S. arcuatus* collected in the general field study (Chapter 3). Main effect ANOVAs were also conducted for the effect of conductivity, pH, temperature, PAR, turbidity, ammonium, nitrate, and total phosphorous for the significance of these parameters on abundances of *S. quadricauda*, *S. ellipticus*, and *S. dimorphus* at the field sites in the intensive field study (Chapter 4).

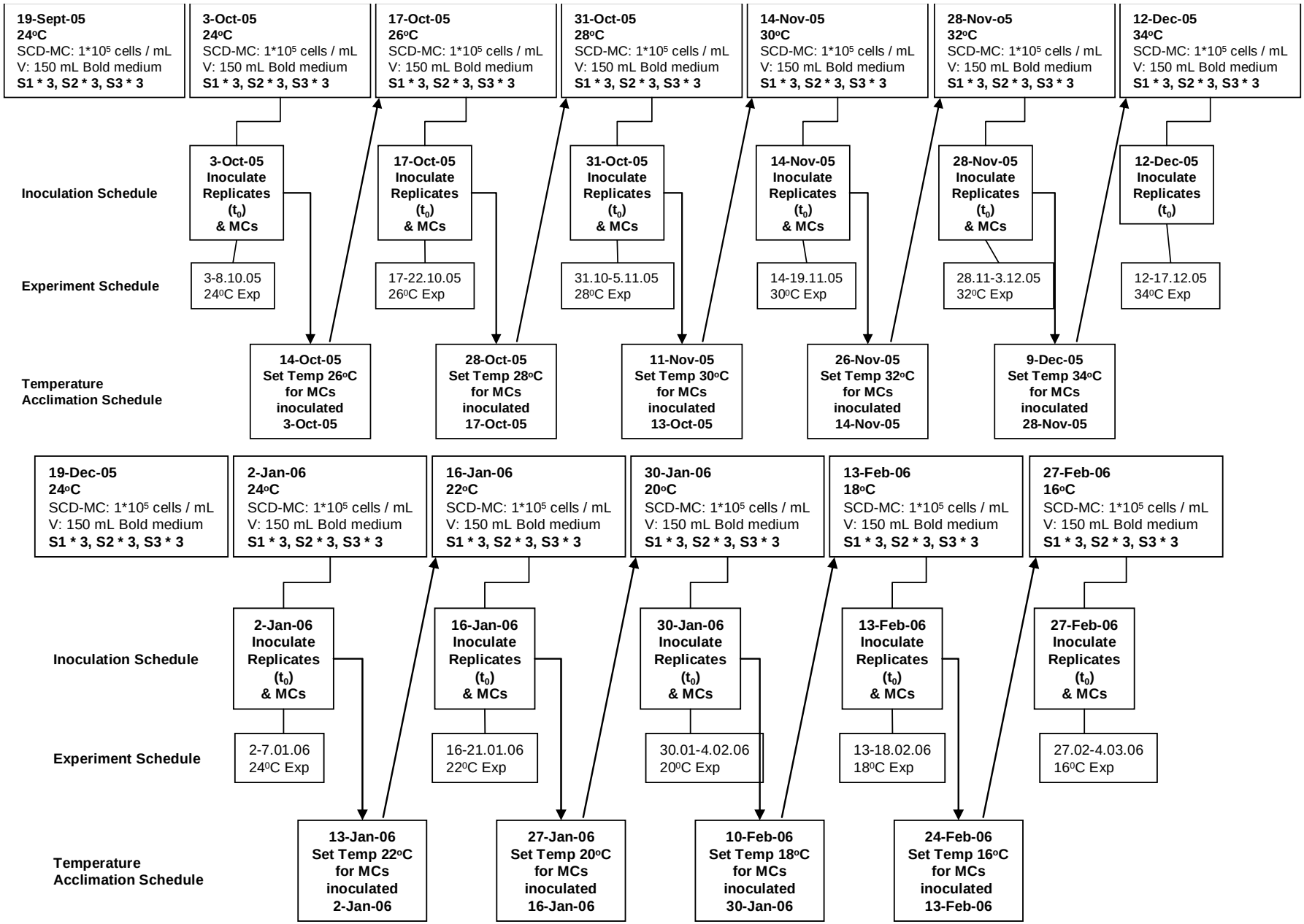


Figure 5.3 - Flow chart for temperature acclimation experiments between 24-34° and 24-16°C in 250 mL Erlenmeyer flasks for *Scenedesmus* spp. SCD=starting cell density; MC=mother culture; V=culture volume; S1=*S. quadricauda*; S2=*S. dimorphus;* S3=*S. ellipticus*

5.2.7 Representative growth curve examples

Three basic growth curves were encountered using the three *Scenedesmus* spp. Specific growth rates were calculated using the linear portion of the growth curves for each experimental variable measured and for each *Scenedesmus* spp. *Scenedesmus dimorphus* showed all three types of growth behaviours (Figure 5.4).

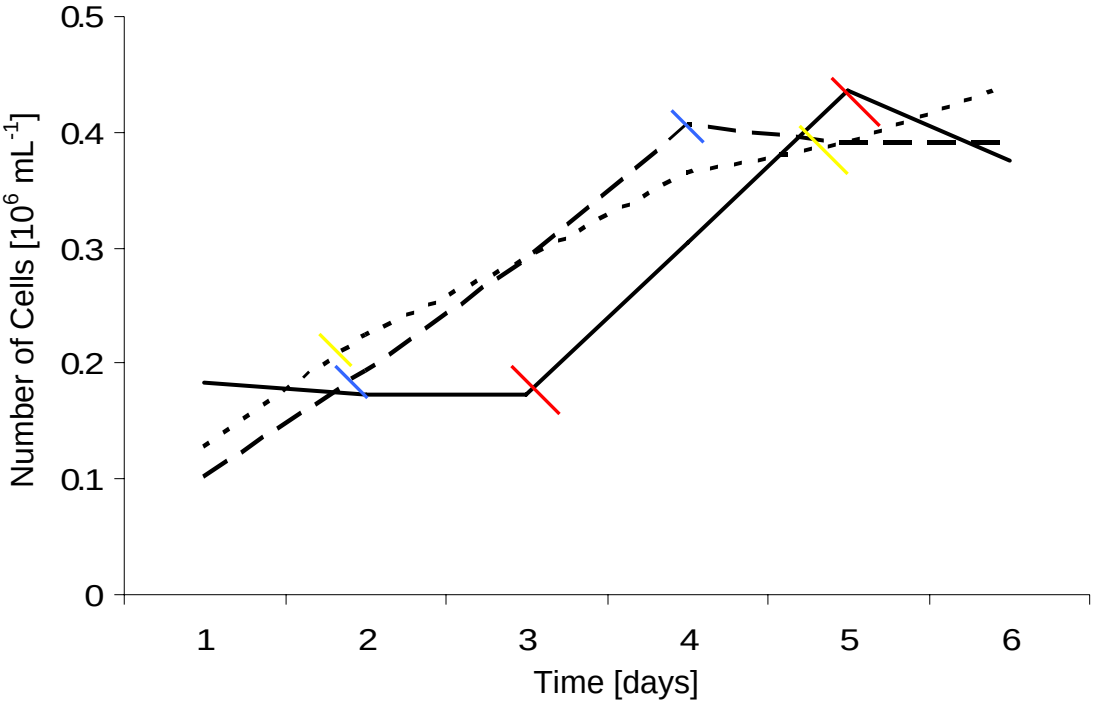


Figure 5.4 - Representative growth curves for *Scenedesmus dimorphus*. Perpendicular lines indicate the portion of the growth curve used to calculate specific growth rates.

5.3 Results

5.3.1 Correlation between % transmission and cells counts

Figure 5.5 shows at least 95% linear correlation between turbidity (measured as % transmission at λ 750nm) and counted cells mL^{-1} for the three *Scenedesmus* species. Growth for conductivity (Section 5.3.1) and temperature (Section 5.3.2) experiments was determined using turbidity as an indirect proxy. Turbidity measured as % transmission at λ 750nm was converted to number of cells mL^{-1} using the linear equations shown in Figure 5.5 for the three *Scenedesmus* species.

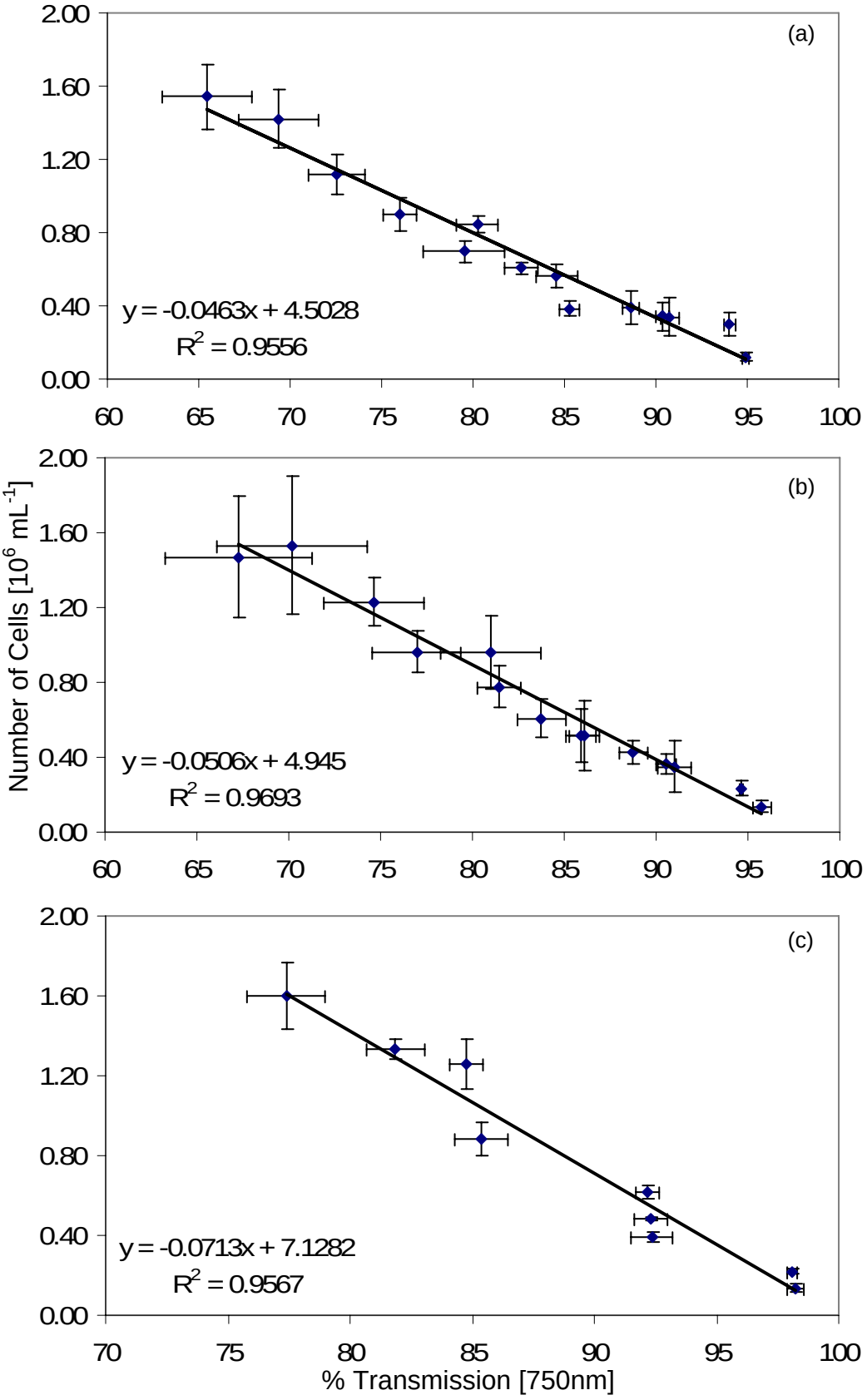


Figure 5.5 - Linear regression between counted cells mL-1 and turbidity (% transmission) for established monoclonal *Scenedesmus* spp cultures. (a) *Scenedesmus quadricauda*; (b) *Scenedesmus dimorphus*; (c) *Scenedesmus ellipticus*. n = 3; standard error is shown.

5.3.2 Conductivity experiments

Average growth rates were highest for *S. ellipticus*, intermediate for *S. dimorphus* and lowest, bordering on the detection limit, for *S. quadricauda*. Growth rate for *S. ellipticus* was positively correlated with increasing conductivity (Figure 5.6a). The growth rate of *Scenedesmus ellipticus* ranged from 0.17 at 760 μS cm^{-1} to 1.0 at 960 μS cm^{-1}. *Scenedesmus dimorphus* showed a decrease in growth rate, from 0.43 to 0.25 at lower conductivities (760 to 860 μS cm^{-1}, respectively) and then an increase in growth rate to 0.33 and 0.53 at higher conductivities (910 and 960 μS cm^{-1}), respectively (Figure 5.6a). Growth rates for *S. quadricauda* remained constant, 0.085 at 760 μS cm^{-1} to 0.10 at 960 μS cm^{-1}, with a slight decrease at 810 μS cm^{-1} to 0.06, showing no effect of conductivity changes on growth (Figure 5.6a).

Cochran's C test confirmed homogeneity of variances for growth rate (0.392) and ΔpH (0.303) (Appendix 4). An ANCOVA (proc GLM) determined that there was no significant effect of ΔpH for each species ($F_{(1, 88)} = 0.850$; $p = 0.359$) (Figure 5.7b) and the effect of culture (replicate) was also not significant ($F_{(2,88)} = 0.682$; $p = 0.508$). There was, however, a significant effect of conductivity on growth rate ($F_{(4, 88)} = 19.56$; $p < 0.001$) and the effect of species was also significant ($F_{(2,88)} = 67.67$; $p < 0.001$). There was a significant interaction for species×conductivity ($F_{(8,88)} = 20.593$; $p < 0.001$), culture×conductivity ($F_{(8,88)} = 2.405$; $p = 0.021$), and species×culture×conductivity ($F_{(16,88)} = 3.035$; $p < 0.001$), while species×culture showed no significant interaction ($F_{(4,88)} = 1.085$; $p = 0.369$) (Appendix 4.2). Tukey's post-hoc tests determined that the effect of conductivity on growth rate was significant at higher conductivity levels, but not between 760-860 μS cm^{-1} and all species of *Scenedesmus* contributed to the significant effect of species on growth rates (Appendix 5). The post-hoc test showed that the significance of species×conductivity interaction was driven predominantly by *S. ellipticus* (S3) and to a lesser extent by *S. dimorphus* (S2) and *S. quadricauda* (S1), with *S. ellipticus* exhibiting significant effects on growth rate across the conductivity spectrum, while growth rates of *S. dimorphus* and *S. quadricauda* individually were not significantly affected by conductivity changes (Appendix 5). Similarly, Tukey's post-hoc test confirmed that the significant interaction of culture×conductivity was driven by the effect of high conductivity (960 μS cm^{-1}) for all cultures, while the significance of species×culture×conductivity interaction was mainly due to *S. ellipticus* (S3) (Appendix 5).

In contrast, a main effect ANOVA showed no significant effect of either minimal or maximal conductivity on abundance of *Scenedesmus* spp. in the general field survey (Appendix 4.3). Analysis of abundances of *S. quadricauda*, *S. ellipticus*, and *S. dimorphus* in the intensive field study by main effect ANOVA showed that conductivity had a significant effect on the abundance

of *S. ellipticus* ($F_{6,24} = 4.79$; $p < 0.002$), while abundances of *S. quadricauda* and *S. dimorphus* were not significantly affected by conductivity at the field sites (Appendix 4.4). While pH had no significant effect on growth of *Scenedesmus* spp. in the laboratory conductivity study, both minimum and maximum pH significantly affected abundance of *S. dimorphus* at the field sites in the general study ($F_{(6,79)} = 3.84$; $p < 0.002$ and $F_{(6,79)} = 3.719$; $p < 0.002$, respectively), while abundances of the other *Scenedesmus* species were not significantly affected by pH (Appendix 4.3). In contrast to the significant effect of pH on *S. dimorphus* in the general field study, a main effect ANOVA on the effect of pH on abundance of *Scenedesmus* species in the intensive field study showed that abundance of *S. dimorphus* was not significantly affected by pH, but abundance of *S. ellipticus* was at those sites and times ($F_{(6, 24)} = 4.194$; $p < 0.005$, Appendix 4.4).

5.3.3 Temperature experiments

Average growth rates were highest for *S. ellipticus*, intermediate for *S. dimorphus* and were lowest, bordering the detection limit, for *S. quadricauda.* As for the conductivity experiments, growth rates for *S. quadricauda* were not affected by temperature change. Growth rates were low and oscillated between 0.12 to 0.06 (Figure 5.7a). Growth rates for *S. dimorphus* showed no discernable pattern with temperature, fluctuating around 0.25 (Figure 5.7a), and were highest (0.40 and 0.38) at 26 and 34°C. Growth of *Scenedesmus ellipticus* also showed no clear pattern with temperature, oscillating between 0.50 – 0.60 for most temperatures. At extreme temperatures of 16, 32, and 34°C, however, growth rate was significantly lower (0.18, 0.40, 0.42, respectively) than at median temperatures (Figure 5.7a).

Cochran's C test confirmed homogeneity of variances for growth rate (0.193) and ΔpH (0.308). An ANCOVA (proc. GLM) established that there was no significant effect of ΔpH or culture (replicate) ($F_{(1, 176)} = 0.021$; $p = 0.197$) on growth rate ($F_{(1,176)} = 0.753$; $p = 0.387$), while there was a significant effect of temperature ($F_{(8,176)} = 5.295$; $p < 0.001$) and species ($F_{(1,176)} = 136.615$; $p < 0.001$) (Appendix 4.1). A Tukey's post-hoc test found that the significant effect of temperature was mainly due to the effect of 16ºC on growth rates, which was significantly different from all other temperature regimes except for growth response at 32ºC and all species contributed equally to the significant effect of species (Appendix 5). No significant interaction of species×culture ($F_{(3,176)} = 0.463$; $p = 0.709$), culture×temperature ($F_{(17,176)} = 1.587$, $p = 0.072$), or species×culture×temperature ($F_{(35,176)} = 0.659$; $p = 0.928$) were observed. There was, however, a significant interaction between species×temperature ($F_{(17,176)} = 5.059$, $p < 0.001$). Tukey's post-hoc analysis showed that the significance of species×temperature interaction was driven predominantly by *S. ellipticus* (S3) and to a lesser extent by *S. dimorphus* (S2) and *S. quadricauda* (S1), with *S. ellipticus* exhibiting significant effects on growth rate across the temperature

spectrum, while growth rates of *S. dimorphus* and *S. quadricauda* individually were not significantly affected by temperature changes (Appendix 5).

A significant effect of minimum temperature ($F_{(10,79)} = 2.113$; $p < 0.03$), but not maximum temperature ($F_{(10,79)} = 1.566$; $p < 0.132$), was also observed for abundance of *S. ellipticus*, but not for the other *Scenedesmus* species in the general field study (Appendix 4.3). Temperature also had a significant effect on the abundance of *S. ellipticus* ($F_{(6,24)} = 3.311$, $p < 0.016$) in the intensive study, while abundances of *S. quadricauda* and *S. dimorphus* were not significantly affected (Appendix 4.4).

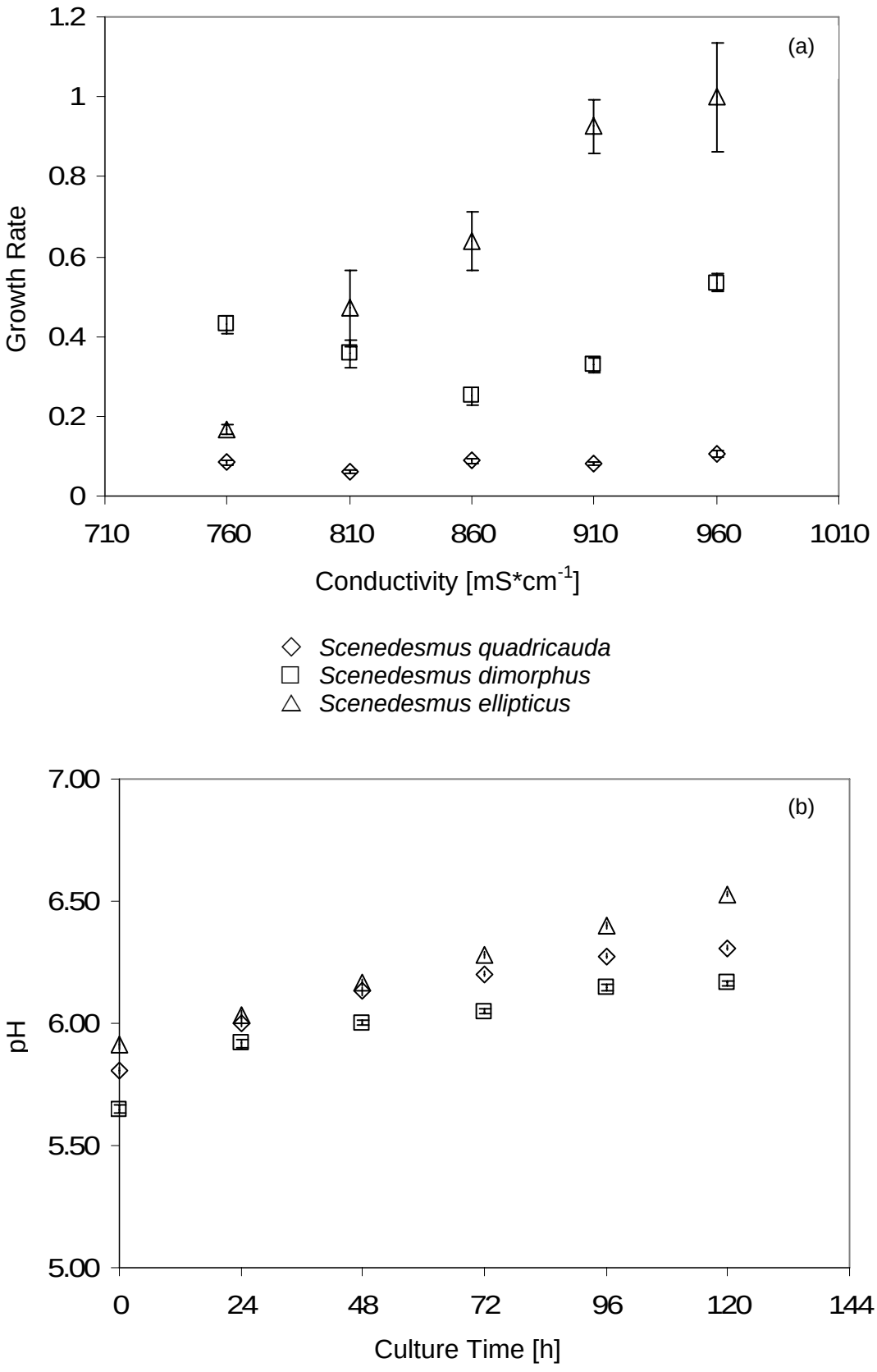


Figure 5.6 – Effect of conductivity on growth of three species of *Scenedesmus*. (a) Growth rate of *S. quadricauda, S. dimorphus, S. ellipticus* at conductivities between 760-960µS cm^{-1}. (b) Mean pH of cultures of *Scenedesmus* species over a range of conductivity (760-960µS cm^{-1}) over time. n = 3 independent culture replicates with a replication of 3 for each culture. Standard error is shown.

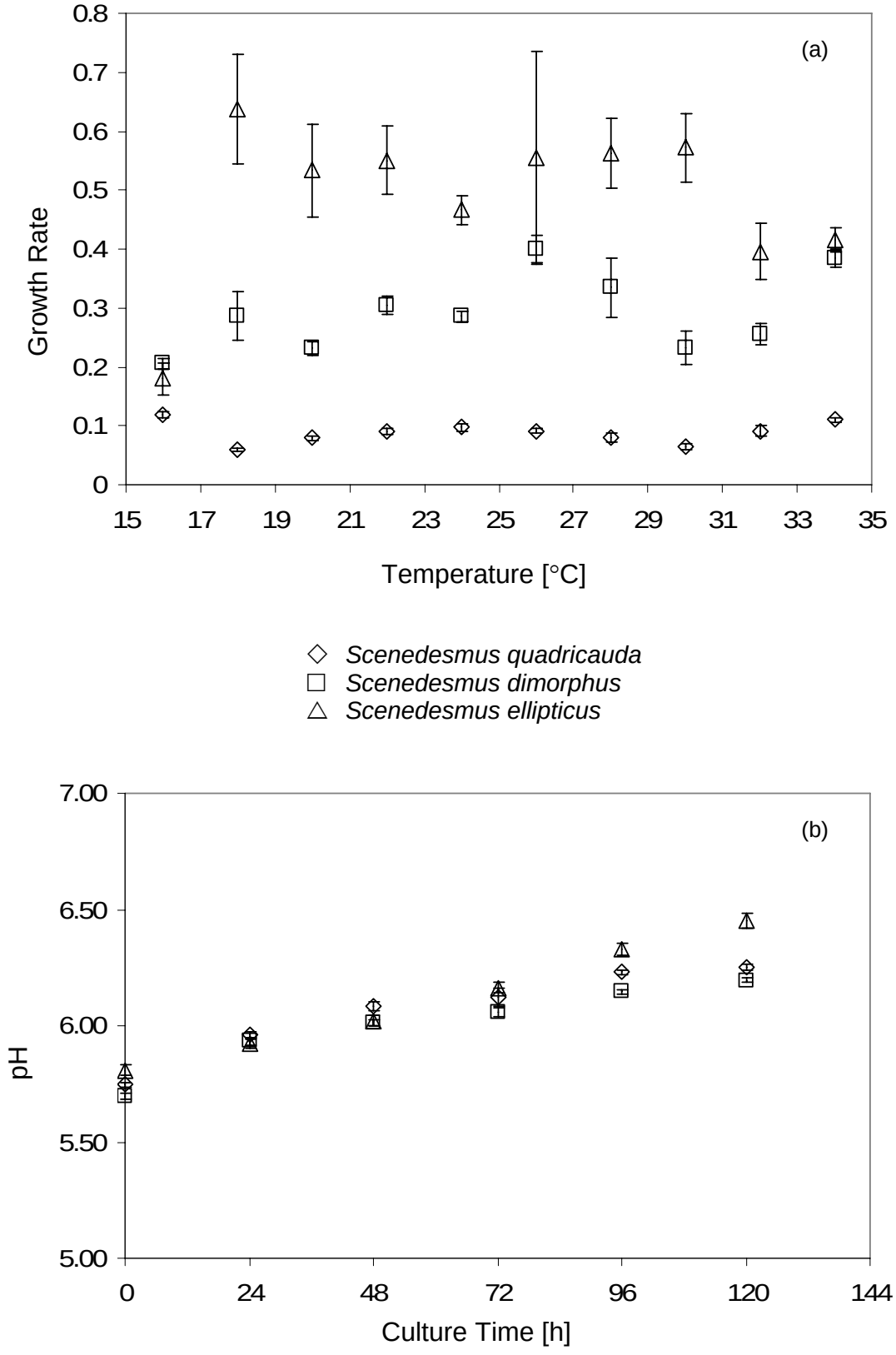


Figure 5.7 - Effects of controlled temperature changes on 3 species of *Scenedesmus* spp. (a) Growth rate of *S. quadricauda, S. dimorphus, S. ellipticus* from 16 - 34°C. (b) Mean pH for cultures of three *Scenedesmus* species over the temperature range of 16 - 34°C over time. n = 3 independent culture replicates with a replication of 3 for each culture, Standard error is shown.

5.4 Discussion

The genus *Scenedesmus* has been used in laboratory experiments for a long time. Species of this genus have high uptake kinetics for nutrients (Giddings 1977, Kunikane et al. 1984, Gonzalez 1997), heavy metals (Baos et al. 2002, Awasthi and Rai 2005) and pesticides (Behra et al. 1999, Aksmann and Tukaj 2004) allowing these species to show direct and immediate responses to their surrounding environment. *Scenedesmus* species (e.g., *S. quadricauda* (Ahlgren and Hyenstrand 2003), *S. acuminatus* (Ovie and Egborge 2002), *S. obliquus* (Xi et al. 2002), *S. abundans* (Tahiri et al. 2000)) have also been found to have a relatively high nutrient content for use as a quality food for aquaculture. Within the Chlorococcales, the genus *Scenedesmus* is the most common and taxonomically diverse (John et al. 2002), making species of this genus ideal study organisms for ecological experiments, since they are representative of many ecosystems. *Scenedesmus* species that occurred in the field were chosen for the laboratory experiments. *Scenedesmus quadricauda*, *S. ellipticus* and *S. dimorphus* were found in all rivers studied.

5.4.1 Conductivity

Growth rates for *S. ellipticus* were positively correlated with conductivity; growth rates *S. dimorphus* decreased at lower and increased at higher conductivities; while growth rates for *S. quadricauda* were not affected by changes in conductivity. There was a significant effect of conductivity on growth rate of *S. ellipticus*, but no significant effect for *S. dimorphus* and *S. quadricauda*. As observed in the laboratory study, conductivity significantly affected the abundance of *S. ellipticus* in the intensive field study, while it had no significant effect on the abundances of *S. quadricauda* or *S. dimorphus*. In contrast, no significant effect of conductivity on field abundances was observed for any of the *Scenedesmus* species including *S. ellipticus* in the general study, while pH significantly affected the abundance of *S. dimorphus* in the general study and *S. ellipticus* in the intensive study.

The field component of this study found that the sites with the lowest conductivity (Suttor/Belyando Rivers) tended to be dominated by euglenoids. As conductivity increased (Keelbottom Creek and Basalt River), chlorophytes and cyanobacteria became more dominant. Multivariate statistics from Chapter 3 showed that *S. dimorphus* and *S. quadricauda* correlated with Axis 2 which mainly represented conductivity. In contrast, multivariate analysis showed that *Scenedesmus ellipticus* did not correlate with conductivity in the field; however, it did correlate to Axis 3, which related mainly to a dissolved oxygen gradient. As shown in these laboratory experiments, increasing conductivity has a direct positive effect on growth of *S. ellipticus*, which is also supported by a main effects ANOVA of data obtained in the intensive study but not the general study. However, multivariate analysis of the effect of conductivity did not correlate

significantly with *S. ellipticus* in the field, but was shown to be correlated significantly with *S. dimorphus* and *S. quadricauda*. Analysis of the general field data by main effect ANOVA showed that abundance and thus growth of *S. dimorphus* is more likely significantly influenced by pH, a parameter influenced by changes in conductivity. In contrast, the main effect ANOVA supports the laboratory findings that growth (abundance) of *S. quadricauda* is not significantly affected by conductivity or pH. Therefore, the use of multivariate statistics to analyse field data may reveal significant indirect effects of available light, nutrients and/or temperature rather then the direct impact of conductivity on abundances of *Scenedesmus* species. However, using a main effect ANOVA, no significant effect of light (PAR, turbidity) or nutrients (ammonium, nitrate, total phosphorous) on abundance of the three *Scenedesmus* species was observed on data obtained in the intensive field study (Chapter 4, Appendix 4), while temperature did significantly affect the abundance of *S. ellipticus* in both the general and intensive field study, but not *S. dimorphus* or *S. quadricauda*.

No published field studies have specifically investigated the effect of conductivity on *Scenedesmus* species' abundance and species composition, but some studies have investigated the effect of conductivity changes on overall phytoplankton composition. O'Farrel (1994) found an increase in species richness and chlorophyte abundance with increased conductivity in Argentina, and Fazio and O'Farrell (2005), also in Argentina, showed that conductivity changed species' abundance patterns to dominance by chlorophytes and cyanobacteria. A study by Zalocar de Domitrovic (2002) also found a correlation between conductivity in the field and phytoplankton composition: chlorophytes, including *Scenedesmus*, dominated in the lower part of the Paraguay River, which had a higher conductivity than the upper part of the river. The laboratory conductivity results in the present study and the main effect ANOVA (intensive field study) support these findings for *Scenedesmus ellipticus*, which showed a significant increase in growth rate with increased conductivity. However, *S. dimorphus* did not show a significant increase in growth rate and the main effect ANOVA suggest that its growth may be more susceptible to changes in pH rather than conductivity alone, effects that are difficult to separate from one another as changes in one affect the other. *S. quadricauda* exhibited poor growth under the selected culture conditions and it is therefore not surprising that no growth responses due to conductivity changes were detected; however, the main effect ANOVA suggests that growth of *S. quadricauda* is unlikely to be influenced by changes in either pH or conductivity.

The poor growth performance by *S. quadricauda* under selected culture conditions is surprising, as the organism is widely used in laboratory experiments: for example, it has been used in experiments investigating bioremediation potential (Patil 1991, Della Greca et al. 2008, Faramarzi et al. 2008), heavy metals (Awasithi and Rai 2006, Mathad et al. 2006, Perales-Vela et al. 2007), pesticides/herbicides (Lei et al. 2007, Ma et al. 2007), nutrient limitation (Beardall et al. 2001a),

grazing (Loiterton et al. 2004, Acuna et al. 2008), medium composition (Kim et al. 2007), temperature (Zargar et al. 2006, Wu et al. 2008), UV radiation (Beardall and Raven 2004, Holzinger and Lutz 2006), and light exposure (Furusato et al. 2004) on growth rates. The low observed growth rate could be due to genetic differences between the isolated strain and other commonly used ones. A search of the Integrated Taxonomic Information System (ITIS - on-line database, http://www.itis.gov, retrieved 28.07.2008) revealed that eight varieties of *S. quadricauda* are accepted, which suggests morphological and, therefore, genetic diversity of this species. Based on temperature effects and culture age of *S. communis* – the suggested neotype for *S. quadricauda* as no type material exists – Trainor (1991b) points out that the quadricaudate appearance is an unstable character typically expressed in mid to late log-phase and under low temperature selection in many spiny species, which has most likely led to many misidentifications. In contrast, under the selected culture conditions here, the character appears to be stable but the growth rate is uncharacteristically low for any *S. quadricauda* described in the literature (Ahlgren 1987, Oh and Rhee 1991). The species identity would need testing by sequence comparison of the isolated strain with published sequences of *S. quadricauda* in Genbank. A search of Genbank revealed only six entries for *S. quadricauda*: one for the UTEX strain 614 for partial sequences of the 28S rRNA, 5.8S rRNA, and ITS2 (internally transcribed spacer), three for the strain NIES-96 for the chloroplast psaB gene, the chloroplast rbcL gene, and the chloroplast atpB gene, and two entries lacking strain identification for the mitochondrial cox1 and the partial 18S rRNA, 5.8S rRNA and ITS1 genes. Given the lack of genetic sequences and certainty regarding the true identity of those published sequences, the lack of an existing type material, and the temperature/growth phase dependent phenotypic plasticity of this genus, it is beyond the scope of this thesis to verify the species identity.

5.4.2. Temperature

There was a significant effect of temperature on growth rate of *Scenedesmus* spp. mainly due to the effect of 16ºC on growth rates and was driven predominantly by *S. ellipticus,* exhibiting significant positive growth responses to increases in temperature, while growth rates of *S. dimorphus* and *S. quadricauda* individually were not significantly affected. A main effect ANOVA supports these laboratory findings for *S. ellipticus* showing a significant effect of low temperature but not maximum temperature on the abundance of this species, but no significant effect for *S. dimorphus* and *S. quadricauda* in the general and intensive field studies. As for the conductivity experiments, *S. quadricauda* showed extremely slow growth under selected culture conditions, potentially for reasons explained above. It is possible that the majority of cells under the selected media and culture conditions exist in a dormant state. *Scenedesmus* spp. were found to stay in a dormant state instead of recruiting into the water column in the presence of the predator *Daphnia magna* (Hansson 1996). Kajan et al. (1992) found that dormancy (encystment)

of *Scenedesmus obliquus* could be induced by temperatures of 85°C or higher. The high temperature treatment was conducted to rid the culture of bacteria to enable large-scale culture set up for aquaculture. A study conducted on *Scenedesmus quadricauda* indicated that only the highest temperature, 39°C, caused changes in growth rate, biomass and cholorphyll *a*, and the heat shock protein, lisp, was expressed (Zargar et al. 2006). Staehr and Birkeland (2006) found that with increasing acclimation temperature (5, 15, and 25°C) *Scenedesmus acutus* (culture from Scandinavian Culture centre for Algae and Protozoa, University of Copenhagen) increased growth rate, as well as cellular pigment content and showed decreased cell size. This species also had increased optimal temperature for photosynthesis with increased acclimation temperature. The latter two studies did show a significant effect of temperature on growth rate, but did not mention dormancy in the species. Other studies conducted on *Scenedesmus* spp. highlight growth-inhibiting conditions such as salinity (Mohapatra et al. 1998) and light quality (Oh and Rhee 1991), but do not mention dormancy.

Temperature is an important factor driving phytoplankton growth and reproduction (Smith 1950). A laboratory study conducted by Lurling and Van Donk (1999) found that *Scenedesmus acutus* (culture from Max-Planck-Institute for Limnology) grown at 9.5, 16.5, 24 and 29 °C showed a significantly reduced growth rate (0.453) at 9.5°C and had an optimal growth rate (1.839) at 24°C. (Coles and Jones 2000) found that growth rates of species of cyanobacteria and diatoms, isolated from a temperate river in the United States, were affected by temperature. *Oscillatoria* (cyanobacteria) had the highest growth rate (0.557 – 1.098) across all temperatures investigated (15 – 30 °C), whereas the cyanobacterium *Microcystis* showed a high growth rate (1.096) at the higher temperature of 30 °C and the diatom *Aulocoseira* showed high growth rates (0.418) at lower temperatures (15 and 20 °C). It seems that some *Scenedesmus* spp. grow better at higher temperatures (Ahlgren 1987, Lurling and Van Donk 1999), while high temperatures appear to enhance growth rates of species of cyanobacteria and low temperatures are suitable for diatoms (Coles and Jones 2000, Staehr and Birkeland 2006).

Multivariate analyses in Chapter 4 showed that in the field phytoplankton composition was significantly correlated with season, temperature, conductivity, nutrients and pH. Specifically, *S. ellipticus* was a significant indicator species in Keelbottom Creek during the post-wet season, which had a temperature range of 24-25.5°C, and was correlated with season, pH (6.7-7.3), turbidity (11-16.3 NTU) and total phosphorus (52-64 mg L^{-1}). In the Basalt River, *S. ellipticus* was an indicator species during the late dry season, which had a temperature range of 28.1-30.2°C and *S. quadricauda* was an indicator species during the post-wet season, which had a temperature range of 27.3-29.6°C. Both species were highly correlated with season, conductivity, pH, total nitrogen and ammonia. Conductivity ranged from 1049-1055 µS cm^{-1} during the late-dry season to 675-680 µS cm^{-1} during the post-wet season. pH ranged from 8.8-9.35 to 7.1-8.3 from the late-

dry to the post-wet season. Total nitrogen and ammonia ranged from 508-1622 to 350-758 mg L^{-1} and 7-804 to 3-54 mg L^{-1} respectively during the late-dry and post-wet seasons. In contrast, main effect ANOVA analyses tie in better with the laboratory results obtained in that abundance of *S. ellipticus* is significantly influenced by temperature and to a certain extent conductivity, while abundance of *S. dimorphus* is significantly influenced by pH, but not conductivity or temperature, and abundance of *S. quadricauda* is influenced by none of the parameters. The main effects ANOVAs also show that none of PAR, turbidity, ammonium, nitrate or total phosphorous significantly affected the abundance of any of the three *Scenedesmus* species taken into culture. Therefore the laboratory study in combination with main effect ANOVAs of field data confirm conductivity and temperature as the main drivers of phytoplankton abundance, as suggested by multivariate analyses of community structure of the field sites.

It is most likely that, in the field, temperature in conjunction with other variables substantially influences *Scenedesmus* spp. The literature generally supports the notion that temperature affects phytoplankton growth in combination with light (Litchman 2000, Bouterfas et al. 2002), nutrients (Kunikane et al. 1984, Beardall et al. 2001a, Beardall et al. 2001b, Ahlgren and Hyenstrand 2003) and carbon dioxide (Hanagata et al. 1992). Main effects ANOVAs of field data in this study only showed a significant effect on *S. ellipticus* (Appendix 4.3 and 4.4), with regards to minimum temperature (general field study) and temperature in general (intensive field study). The other *Scenedesmus* species showed no effect of temperature in both field studies. In the field component of this study, phytoplankton assemblage composition showed a strong correlation with seasonality, mainly based on light availability, temperature and water movement (i.e. flooding). During summer seasons a successional shift to chlorophytes has been observed in tropical regions, which correlates with increased temperature and increased water flow (Talling 1986b, Zafar 1986, O'Farrell et al. 2002, Zalocar de Domitrovic 2002, Costelloe et al. 2005), a pattern also seen in the field investigations for this study.

The laboratory experiments in this study were designed to validate the identified drivers of phytoplankton community composition in the field. Unfortunately, growth responses of *Scenedesmus* spp. to either temperature or conductivity changes alone under laboratory conditions did not show the same strong correlations as seen in the field, but in combination with main effect ANOVAs of field data the main drivers identified by multivariate statistics were confirmed. It is likely that main drivers of field phytoplankton compositional changes (e.g., temperature or conductivity) cannot be properly simulated with regards to their individual impact under otherwise constant laboratory conditions because of the loss of associated physical and/or chemical field-based interactions.

Chapter 6 . General discussion

6.1 Physical environment

Like most other large catchments, the Burdekin River system comprises a complex series of sub-catchments whose characteristics are governed by climate, hydrology, geology and lithology. Although the sites for this study were selected geographically, they clearly paralleled the major physico-chemical differences between the sub-catchments – the Upper Burdekin, Lower Burdekin and Coastal Plains, Bowen-Broken and Belyando-Suttor systems.

The climate in the catchment ranges between wet tropical on the coastal ranges to semi-arid in the west. Temperature can range from below zero to 45 °C across the catchment, usually with the extremes being inland. Rainfall varies notably throughout the catchment from over 2500 mm per annum in the coastal ranges to less than 600 mm in the central southern regions (NRM 2002).

More than 80% of stream flow occurs between December and April. It is common for rivers within the Burdekin catchment to cease flowing between May and November (Roth et al. 2002). The Upper Burdekin sub-catchment, which includes the Basalt River and the Keelbottom Creek from this study, covers less than 30% of the total catchment, but contributes more than 50% of the annual stream flow. The Belyando Suttor sub-catchment comprises 60% of the catchment, but only contributes about 30% of the annual stream flow (NRM 2002). However, the rainfall received within a sub-catchment, and therefore flow, can vary substantially between river systems, and there may be high variability within and between years. Cyclones are an important contributing factor to high flows across the Burdekin system (Mabin and Lowry 1996).

6.2 Water quality

Water quality varies considerably across the Burdekin catchment. Water temperature was similar across the sub-catchments and was directly linked to season and depth. pH was slightly acidic to alkaline (6-10), with the Basalt River having the highest values. Conductivity varied significantly across the catchment. The Basalt River had the highest values (>1000 $\mu S.cm^{-1}$) and the Belyando River had the lowest readings (92 $\mu S.cm^{-1}$). Geology varies significantly between the sub-catchments and conductivity and pH reflect this (DeNicola et al. 2004). Turbidity was highest in the Belyando-Suttor sub-catchment and lowest in the Basalt River (due to its groundwater source (NRM 2002), although high turbidity was associated with all the sub-catchments during high-rainfall-high-flow events. Historic records note the Burdekin catchment region as highly turbid and support the Burdekin River's classification as a naturally turbid watercourse (DNR 2000). Nutrients, too, varied across the catchment as well as by season, with nitrogen concentrations of

346-1622 µg N L^{-1} and total phosphorus concentrations of 35-389 µg P L^{-1} across the catchment. A National Land and Water Audit found that 75-100% of stream lengths in the Burdekin catchment showed increased phosphorus loads, and 50-75% increased nitrogen loads, linked to grazing in most of the catchment and sugarcane growing in the floodplain. In general the water quality of the water holes was tightly linked to local conditions, because of their isolation, rather than being governed by upstream events as in most river systems.

Water quality can vary within water bodies across small distances. In this study, there was distinct thermal stratification of deeper water holes, but not of shallow systems. Replicated samples across individual water holes showed surprisingly small variation, and assessing most water quality parameters required much less effort than was applied in this study. This is a useful outcome as it suggests that good results from water quality monitoring can be achieved without excessive replication within sites.

6.3 Algal diversity

Diversity of phytoplankton was moderate compared with systems elsewhere (Table 6.1). The physico-chemical differences between sub-catchments clearly contributed to increased diversity across the catchment. The latitudinal pattern of species diversity, with highest diversity in the tropics, is typical of most groups of organisms, with particular exceptions of cool-adapted species (e.g., the Plecoptera, or stoneflies). Similarly, algal diversity is usually greatest in the tropics, as Table 6.1 indicates. However, Table 6.1 includes a variety of types of systems from which it is difficult to draw unequivocal conclusions.

None of the species from this study were endemic, and most had cosmopolitan distributions. However there have been reports of endemic phytoplankton species found for tropical north Australia, among them are: *Xanthidium multicorne, Cosmarium securiforme, Triploceras gracile, T. verticillatum, Pleurotaenuim australianum, Staurastrum elegans, S.tyleri, Micrasterias alata*, and *Peridinium lingii* (Thomasson 1986).

6.4 Relationships between algal assemblages and water quality

Unlike the study by McGregor et al. (2006) which found that algal assemblages were poorly correlated with the measured environmental variables (temperature, conductivity, pH, turbidity, PAR, dissolved oxygen), phytoplankton species in this study correlated highly with temperature, conductivity, pH, turbidity and nutrients. McGregor et al. (2006) found that a non-metric multidimensional scaling ordination of phytoplankton assemblages showed a separation of water holes based on catchment and season, which was also observed in the presented study. However,

phytoplankton assemblages in this study were also driven by physico-chemical parameters, which suggest a potential for using phytoplankton assemblages as indicators of water quality in these rivers. The phytoplankton assemblages in the Basalt River were dominated by cyanobacteria and chlorophytes. The Keelbottom Creek was indicated by a variety of phytoplankton groups, but was mainly dominated by chlorophytes and euglenoids. The Belyando/Suttor River was dominated by euglenoids. Some euglenoids are known for representing eutrophic waters and the Belyando/Suttor River had values of total nitrogen and total phosphorus that were higher than for the other rivers.

Field studies strongly suggested that several water quality parameters affect phytoplankton assemblages, strongly suggesting a link between water quality, phytoplankton and ecosystem health. Laboratory experiments, designed to investigate the utility of developing explicit cause-effect relationships, linked three common *Scenedesmus* species with two of the most obvious drivers of these algal assemblages, namely temperature and conductivity.

This study is not unique in its investigation of phytoplankton assemblages in relation to water quality and hydrology of a river system, but it is unusual in its attempt to confirm identified drivers of algal assemblages in the field through controlled laboratory experiments and, to use specific physico-chemical water quality parameters and whole phytoplankton assemblages as indicators of ecosystem health. The Burdekin system itself would appear to stand out from other systems that have been studied, in its variability across the catchment in flow and water quality characteristics, related to geology and topography, its temporal variability and in its algal assemblages, which reflect these environmental characteristics.

Table 6.1 - Species richness of phytoplankton in sites across the latitudinal gradient

Site	Latitude*	# of spp.	Reference
Kasari River Estonia	58°N	150	(Piirsoo et al. 2007)
Lakes of County Clare Ireland	52°N	84	(DeNicola et al. 2004)
Lake Salsbury Canada	49°N	26	(Longmuir et al. 2007)
Lake Doïrani Greece	41°N	19	(Temponeras et al. 2000)
Wetlands USA, N. America	40°N	58	(Casamatta et al. 1999)
Lake Contreras Spain	39°N	60	(Dasi et al. 1998)
Lake Aroncio Sicily	38°N	69	(Barone and Naselli-Flores 1994)
Nakdong River Korea	36°N	206	(Ha et al. 2002)
Rainbow River USA, N. America	29°N	79	(Cowell and Dawes 2008)
Tehuantepec River Mexico	17°N	273	(Moreno-Ruiz et al. 2008)
Man made lakes, India	11°N	120	(Zafar 1986)
Lake Naivasha Kenya, Africa	0.8°S	141	(Kalff and Watson 1986)
Lake Victoria, Kenya, Africa	1°S	103	(Lung'Ayia et al. 2000)
Burdekin Catchment, Australia	20°S	138	This study
Paraguay River, Argentina, S. America	20°S	332	(Zalocar de Domitrovic 2002)
Barra Bonita Reservoir, Brazil, S. America	22°S	131	(Calijuri et al. 2002)
Lake Erye Basin, Australia	28°S	237	(Costelloe et al. 2005)
Parana River, Argentina, S. America	33°S	290	(Izaguirre et al. 2001)
Darling River Australia	33°S	102	(Hotzel and Croome 1994)

6.5 Ecological dynamics in the Burdekin system

A summary model of the ecology of the Burdekin water holes would appear to be as follows:

1. Wet season: strong or flooding flows; connectivity between locations and sub-catchments; resetting of system by flushing and homogenizing of biophysical variables;
2. brief flood characteristics diminish (current velocity, depth, width, turbidity);
3. dry season conditions emerge: contraction of river, reduction or loss of surface flow, and loss of connectivity, increase clarity of water, establishment of macrophyte and algal assemblages characteristic of water holes;

4. further drying and increasing influence of local conditions rather than upstream influences or whole catchment influences; macrophyte and algal assemblages well established, as are invertebrate assemblages (Betts 2003);
5. towards the end of the dry season, conditions may deteriorate in smaller water holes, but remain stable in large water holes, with little change in the phytoplankton assemblages;
6. minor flushes due to local storms may wash organic material into water holes and/or cause mixing of hypoxic and surface strata, causing deterioration of dissolved oxygen levels;
7. wet season flushing resets the system.

This pattern of events is not dissimilar to that described for inland Queensland systems (e.g. Sheldon 2005) except for the more prolonged flow in the coastal-mountain-sourced streams (e.g., Keelbottom Creek), and those arising in basalt (e.g., Basalt River). Anthropological disturbance might be expected to exacerbate the pressures on health – for example, cattle accessing small water holes may severely pollute them, pushing the above model down the deterioration sequence. But natural 'deterioration' – due to increasing hypoxia or persistent turbidity – must be regarded as an essential feature of these ecosystems as they now function (historically, when the continent was wetter, they were probably very different). However, human impacts, such as abstraction of water, or input of nutrients by cattle, -may be sufficient to push particular water holes over the 'edge' of system integrity. This effect was not observed directly in this study and can only be inferred currently because of the lack of published material; however, this scenario parallels findings in the Cooper system (Sheldon 2005).

It is expected that the gradual seasonal change in water quality will act as a filter for tolerant species in the short term (in 'ecological time'), but importantly may act as a driver for adaptation and evolution in the long term. So even the least aesthetically pleasing of water holes, such as the permanently turbid ones in the Belyando/Suttor catchment, may be important in sustaining current biodiversity and in driving biodiversity development.

6.6 River health

Over the last 20 years, emphasis has been directed towards the use of biological patterns as indicators of ecological health rather than focusing on water quality alone (Hart et al. 1999, Smith et al. 1999). The terms 'ecosystem health' and 'river health' have been coined to capture the intent of contemporary ecosystem assessments – terms which are analogous with the concept of human health, giving a general sense of understanding (Rapport 1989). However, it is not clear which aspects of 'river health' are identified by ecosystem-level indicators, nor how physical, chemical and biological characteristics may be integrated into measures rather than just

observations of possible cause and effect (Norris and Thoms 1999). According to Karr et al. (1986) 'a biological system can be considered healthy when its inherent potential is realized, its condition is stable, its capacity for self-repair when perturbed is preserved, and minimal external support for management is needed.' The primary needs for a healthy ecosystem are biotic integrity and sustainability (Karr 1999). Norris and Thoms (1999) suggest that acceptable reference conditions for 'river health' should be based on ecological understanding (e.g. biotic integrity); features should be set *a priori* and sites to represent the reference condition selected and classified to provide site-specific comparisons of indicators of 'river health'.

Phytoplankton assemblages are typically regarded as highly sensitive indicators of 'ecosystem health' that are nutrient-controlled and physically forced (by vertical mixing, flushing, residence time), which makes them relevant, readily detectable indicators of biogeochemical and ecological change (Paerl et al. 2007). Each of the rivers studied had distinct phytoplankton assemblages which changed with season. The less turbid waters of the Basalt River were characterized by chlorophytes and cyanobacteria during the stratified dry season, which shifted to dominance by multiple taxa during the wet season. The extremely turbid Belyando/Suttor Rivers were characterized by euglenophyte species during both seasons, whereas Keelbottom Creek was characterized by chlorophytes and dinoflagellates in the dry season, and chlorophytes and euglenoids in the wet season. The successional shift of these phytoplankton assemblages was due to physico-chemical properties within the river systems, identified by multivariate analyses as temperature, conductivity, pH, turbidity and nutrients.

Therefore, it might appear possible to use the phytoplankton assemblages to indicate the ecological and physico-chemical changes in the individual rivers. However, as the Burdekin water holes are influenced predominantly by the immediate local conditions, reflected in patchy distributions of phytoplankton species within an individual river, consistency of indicators across the system would be hard to achieve. The Burdekin tributaries cannot be used as references for each other: although they are in the same major catchment, they are significantly different from each other, such that each river requires a different set of reference conditions, as suggested by Norris and Thoms (1999). After each wet season, the flush of water through each system resets the water quality, as well as amalgamating the phytoplankton species giving an overall view of the whole river's water quality and phytoplankton assemblages. But as the dry season progresses, different reference conditions are required as the water ceases to flow and the systems become more closely linked to the local geology and landscape, rather than the upstream catchment. McGregor et al. (2006) drew similar conclusions for water holes in Cooper Creek of the Lake Eyre Basin. During the dry season, these water holes were influenced by localized successional changes, occurring independently at the water-hole scale as a result of a diminished influence of

flow and connectivity. However, after flushing flow, the water holes had far more homogeneous phytoplankton assemblages (McGregor et al. 2006). The alternation of states between longitudinally homogeneous river phytoplankton communities during periods of flow, and heterogeneous disconnected water-hole communities resembling shallow lake phytoplankton following extended periods of no flow (Padisak 1993) has been observed elsewhere (Angeler et al. 2000, Costelloe et al. 2005) and appears to characterize phytoplankton communities in arid to semi-arid systems (McGregor et al. 2006).

In the opinion of Fairweather (1999), many of the ecological indicator programs under development around the world focus on static or structural aspects of ecosystems. He suggests that a further step be taken to develop indicators from a process or dynamic perspective, entailing more direct measurers of ecological processes and of the changes that are occurring, followed by assessing these processes in the laboratory. This study attempted to do just that. I elucidated the phytoplankton assemblage composition of dry inland rivers in Australia and the water quality parameters that affected these assemblages. Testing of the identified drivers of field phytoplankton community assemblages (temperature and conductivity) under controlled laboratory conditions of three common and ubiquitously distributed *Scenedesmus* species supported the field results. However, there were differences in the responses of the tested species in the field and the laboratory, presumably because of the interactions of the water quality variables in the field. The strong turbidity signal in the Belyando/Suttor River appeared to be reflected in the algal composition, but it remains uncertain as to whether the association was coincidental. Unfortunately, much 'ecosystem health' monitoring suffers this problem, with few attempts to elucidate cause-effect relationships. The preliminary attempt to elucidate cause and effect relationships by means of the laboratory experiments demonstrate that this approach has its own problems, especially in over-simplifying the real situation by focusing on a small subset of species and physico-chemical variables. To use phytoplankton as robust indicators of 'river health', reference sites are required, and complex water quality interactions need to be recreated in the laboratory.

Typically, in the absence of supporting laboratory testing, cause and effect are deduced from strong associations in the field, coupled with understanding of the functioning of the ecosystem, and with exclusion of alternative explanations. Thus, these relationships are deduced from strong inference rather than experimental proof. In some cases this approach appears to work well, especially where the biota is well known (e.g., the British RIVPACS methodology). It is too early to erect such a system using algae in tropical rivers despite the strong gradients demonstrated in this study, because of the sheer variability of the assemblages. Expansion of the current data set with additional intensive studies over more seasonal cycles might, however, allow for the above

approach to work equally well in the dry tropics. However, such an approach appears to be in the more distant future for this region, as the identification of the diverse flora in multiple samples is tedious and costly. This may be the greatest obstruction to further development of the method. Currently, the use of algal assemblages for regular or routine assessment of tropical river health is not practical because of their patchy and variable distribution and because more tractable taxa are available (e.g., fish, invertebrates). This situation may change as DNA screening of samples becomes a viable method of assessing biodiversity on large ecosystem scales.

6.7 Conclusion

This study has described the diversity and dynamics of phytoplankton across a large tropical catchment. It has demonstrated relationships between catchment characteristics and algal assemblages across three contrasting sub-catchments, and across the seasons. Evidence from water quality and phytoplankton samples indicates that permanent water holes undergo rapid and large-scale change as a result of wet-season flooding, but then settle down to a relatively stable existence through most of the dry season.

Several aspects of the ecology of these systems are unknown. Their permanence is maintained by the water table, but the dynamics of this are not known. The contribution of the phytoplankton to the food web is unknown as there have been no studies of zooplankton or other aspects of food webs in the system. It is unknown what the importance of these systems is to frog, reptile, bird or mammal species in the region. The dynamics of benthic invertebrate assemblages have been described (Betts 2003) and the composition of the fish community is well known at a catchment, if not a water-hole, scale (Pusey et al. 1998). But the links between these elements of the ecosystem, and how they change with time, remain to be elucidated.

While the biota of the water holes appears to comprise cosmopolitan, or at least widespread, species, they would appear to be of some conservation significance because they are the only significant water bodies across a vast and dry landscape, and are therefore worthy of careful management. There was no indication that the water holes in this study were under major threat from current land use, probably because their size buffers any effects of cattle. In this regard they are therefore important, because it is known that there are severe impacts of cattle in other smaller water holes in the catchment (Burrows 2008). A useful outcome of this and complementary studies would be a partnership between scientists, land-owners and the Natural Resource Management Board to develop a management plan for these systems.

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Appendix 1 - Raw data

The raw data for this study are recorded on the accompanying CD which contains the following files:

1. Chapter 3 Raw Data General Study.xls

This file includes the water quality measurements and phytoplankton identification for three seasons, three sites on three rivers, as well as the data set up for analysis in the program PC Ord version 4.

2. Chapter 4 Raw Data Intensive Study.xls

This file includes the water quality measurements, nutrients and phytoplankton identification for two seasons, 6 replicate sites on three rivers, as well as the data set up for analysis in the program PC Ord version 4.

Codes used for measurements and samples in the data sheets are explained below.

General study raw data key:

Sample Names

Examples - 1B1OPD; 2K2MPM; 3S3OPA

- The first number denotes the season – 1=Mid-dry season (August); 2=Late dry season (November); 3=Post wet season (April)
- The second letter denotes the river system – B=Basalt River; K=Keelbottom Creek; S=Suttor/Belyando Rivers
- The third number denotes which site (1 out of 3) on the river
- The fourth and fifth letters denote habitat – OP=open water; MP=macrophyte
- The sixth letter denotes time of day – D=dawn; M=mid-day; A=afternoon

The first sheet of the file contains all of the water quality data from the 3 sites on the 3 rivers during the mid-dry season. The second sheet is during the late dry season and the third sheet is the post wet season. Each of the other sheets is labelled with the data they contain. All data is keyed to sample as explained above. The sheets with the label PC ORD are the data that is set up to import into PC Ord program for analysis.

Intensive study raw data key:

Sample Names

Examples – 1B1aD; 1K3bM; 2S5cA

- The first number denotes the season – 1=Late dry season (November); 2=Post wet season (April)
- The second letter denotes the river – B=Basalt River; K=Keelbottom Creek; S=Suttor River
- The third number denotes the replicate sample (1 out of 6) at the river site
- The fourth lower case letter denotes the stratum within the water column – a=1st stratum; b=2nd stratum; c=3rd stratum; d=4th stratum
- The fifth letter denotes time of day – D=dawn; M=mid-day; A=afternoon

The first sheet of the file contains all of the water quality data from the 6 replicates over 3 days at each river. Turbidity, chlorophylls, and phytoplankton samples were taken from day 3 dawn and mid-day only at all replicate sites (1-6) and strata. Nutrients were taken the same times and all strata, but at replicate sites 2, 4, and 6 only. All data is keyed to sample as explained above. The sheets with the label PC ORD are the data that is set up to import into PC Ord program for analysis.

Appendix 2 - Algal species identified from the field study sites. Taxonomy after Guiry and Guiry (2007) and Graham and Wilcox (2000)

Species Classification	Basalt River			Keelbottom Creek			Suttor River		
	Emu Valley	Bluff Downs	Hill-grove	Dots-wood	Miram-beena	Bridge	Mt. Douglas	Disney	Mt. Hope
Ochrophyta									
Achnanthales									
Achnanthes sp. 1				X	X	X			X
Achnanthes sp. 2					X	X			
Bacillariales									
Nitzschia sp. 1	X	X				X			
Nitzschia sp. 2				X		X			
Nitzschia reversa						X			
Cymbellales									
Cymbella sp. 1	X	X	X	X	X	X			
Cymbella sp. 2		X			X				
Gomphonema sp. 1	X	X	X	X	X	X			
Gomphonema sp. 2		X			X	X			
Gomphonema sp. 3				X	X	X			
Gomphonema sp. 4		X				X			
Eunotiales									
Eunotia sp. 1				X		X			
Eunotia sp. 2		X							
Fragilariales									
Fragilaria sp. 1	X	X		X	X	X	X	X	X
Fragilaria sp. 2		X	X						
Fragilaria sp. 3						X			X
Synedra ulna		X	X	X	X	X			
Mastogloiales									
Mastogloia sp. 1	X	X	X			X			
Mastogloia sp. 2		X			X				
Meloseirales									
Melosira sp. 1					X	X			
Melosira sp. 2				X	X	X	X		
Naviculales									
Gyrosigma sp. 1	X	X		X	X	X	X	X	X
Gyrosigma sp. 2		X		X	X				
Gyrosigma sp. 3	X	X	X	X	X	X			
Navicula sp. 1	X	X	X	X	X	X	X		X
Navicula sp. 2			X						
Navicula sp. 3					X	X			
Pinnularia sp. 1	X	X	X	X	X	X	X	X	X
Pinnularia sp. 2		X				X			
Pinnularia sp. 3		X				X			
Stauroneis sp. 1		X			X				
Thalassiophysales									
Amphora sp.1					X		X	X	X
Amphora sp. 2	X					X			
Unknown Diatom 1		X	X						
Unknown Diatom 2	X	X	X						

Chlorophyta									
Chlorococcales									
Coelastrum sp. 1					X		X		
Crucigenia quadrata var. *secta* Playfair		X	X	X	X	X		X	X
Crucigeniella crucifera (Wolle) Komárek			X	X	X	X			
Dictyosphaerium sp. 1				X		X			
Kirchneriella lunaris (Kirchner) K. Möbius		X		X	X	X			
Lagerheimia longiseta (Lemmermann) Wille						X			
Monoraphidium arcuatum (Korshikov) Hindák		X			X	X			X
Monoraphidium contortum (Thuret) Komàrková-Legnerová	X	X	X	X	X	X	X		
Pediastrum boryanum (Turpin) Meneghini	X	X	X	X	X	X			
Pediastrum duplex Meyen	X		X	X	X	X			
Pediastrum simplex Meyen	X	X	X	X		X			
Scenedesmus arcuatus Lemmermann			X	X	X	X			
Scenedesmus bernardii G.M. Smith			X	X	X				
Scenedesmus denticulatus Lagerheim								X	
Scenedesmus dimorphus (Turpin) Kützing			X	X	X	X		X	X
Scenedesmus ellipticus (Corda)	X	X	X	X	X	X		X	X
Scenedesmus quadricauda (Turpin) Brébisson	X	X	X	X	X	X	X	X	X
Tetraedron gracile (Reinsch) Hansgrig					X			X	X
Tetraedron incus (Teiling) G.M. Smith							X		
Tetraedron regulare Kützing		X	X	X	X			X	X
Tetrastrum elegans Playfair				X					
Treubaria triappendiculata C. Bernard				X	X	X		X	X
Cladophorales									
Cladophora fracta (O.F. Müller ex Vahl) Kützing	X	X	X						
Rhizoclonium hieroglyphicum (C. Agardh) Kützing	X								
Klebsormidiales									
Koliella spiculiformis (Vischer) Hindák					X				
Microsporales									
Microspora sp. 1									X
Oedogoniales									
Oedogonium sp. 1					X	X			
Tetrasporales									
Gloeocystis sp. 1			X	X	X	X			X
Volvocales									
Actinastrum hantzschi Lagerheim			X	X		X			
Eudorina elegans Ehrenberg					X	X			
Gonium sp. 1					X				

Zygnematales									
Closterium acerosum (Schrank) Ehrenberg ex Ralfs	X	X	X	X	X	X			
Closterium dianae Ehrenberg ex Ralfs		X				X			
Closterium kuetzingii Brébisson						X			
Closterium moniliferum Ehrenberg ex Ralfs	X		X	X	X	X	X		X
Closterium parvulum Nägeli									X
Closterium praelongum Brébisson						X			X
Closterium sp. 1		X							X
Cosmarium binum Nordstedt	X	X	X	X	X	X	X		
Cosmarium contractum O. Kirchner						X			
Cosmarium pseudopyramidatum P. Lundell	X	X	X	X	X	X	X		X
Cosmarium retusiforme (Wille) Gutivinski	X		X	X					
Euastrum bidentatum Nägeli						X			
Euastrum didelta Ralfs ex Ralfs						X			
Euastrum elegans Kützing ex Ralfs					X				
Euastrum sp. 1				X					
Euastrum sp. 2						X			
Micrasterias decemdentata (Nägeli) W. Archer					X	X			
Micrasterias mahabuleshwarensis J. Hobson					X				
Mougeotia sp. 1	X	X	X	X	X	X			
Sphaerozosma pulchrum Bailey					X				
Spinoclosterium cuspidatum (Bailey) Hirano			X						
Spirogyra sp. 1		X	X	X	X	X			
Staurastrum chaetoceras (Schröder) G.M. Smith		X	X	X	X	X		X	X
Staurastrum gracile Ralfs ex Ralfs	X	X	X	X	X	X			
Staurastrum muticum Brébisson ex Ralfs									X
Staurastrum tetracerum Ralfs	X			X	X	X	X		X
Staurastrum wildemanii var *unispiniferum* A.M. Scott & G.W. Prescott						X			
Staurastrum sp. 1		X				X			
Staurodesmus gibberulus (Joshua) Teiling					X	X	X		
Staurodesmus triangularis (Lagerheim) Teiling							X		X
Staurodesmus sp. 1					X	X			X
Zygnema sp. 1	X								
Cyanobacteria									
Chroococcales									
Microcystis sp. 1				X	X				
Spirulina sp. 1	X	X	X						
Nostocales									
Anabaena oscillarioides Bory de Saint-Vincent	X	X	X		X	X			
Anabaena spiroides Klebahn	X	X	X						
Anabenopsis elenkinii V.V. Miller		X							X

Aphanizomenon gracile Lemmermann	X	X	X		X			X	X
Cylindrospermum sp. 1		X							
Oscillatoriales									
Oscillatoria tenuis C. Agardh	X	X	X						X
Oscillatoria sp. 1	X								X
Phormidium sp. 1		X							X
Pseudanabaenales									
Limnothrix redekei (van Goor) M.E. Meffert	X	X	X		X	X	X		X
Synechococcales									
Merismopedia glauca (Ehrenberg) Kützing		X	X		X	X			X
Euglenoids									
Euglenales									
Euglena acus Ehrenberg	X	X	X	X	X	X	X	X	X
Euglena granulata (G.A. Klebs) Schmitz	X	X	X	X	X	X	X	X	X
Euglena limnophila Lemmermann	X	X	X	X	X	X		X	X
Phacus acuminatus Stokes		X							X
Phacus alatus G.A. Klebs						X			
Phacus caudatus Hübner	X		X	X	X	X	X	X	X
Phacus curvicauda Svirenko						X			
Phacus longicauda (Ehrenberg) Dujardin				X	X	X			X
Strombomonas acuminata (Schmarda) Deflandre	X			X		X	X	X	X
Strombomonas tambowika (Svirenko) Deflandre	X	X	X	X			X	X	X
Strombomonas sp. 1				X			X	X	X
Trachelomonas armata (Ehrenberg) F. Stein				X	X	X			
Trachelomonas curta A.M. Cunha		X				X			X
Trachelomonas granulata Svirenko				X		X	X	X	X
Trachelomonas hispida (Perty) F. Stein	X	X	X	X	X	X	X	X	X
Trachelomonas oblonga Lemmermann	X	X	X	X	X	X	X	X	X
Trachelomonas planctonica Svirenko				X	X	X	X		X
Trachelomonas volvocina Ehrenberg	X	X		X	X	X	X	X	X
Dinophyta									
Peridiniales									
Glenodinium sp. 1					X	X			
Peridinium bipes F. Stein	X	X	X	X	X	X	X	X	X
Peridinium inconspicuum Lemmermann		X	X	X	X	X	X		
Peridinium lomnickii Woloszynska	X	X	X	X	X	X	X	X	
Peridinium volzii Lemmermann		X							
Other Ocrophyta									
Eustigmatales									
Pseudostaurastrum enorme (Ralfs) R. Chodat						X			

Mischococcales									
Centritractus belanophorus Lemmermann	X	X	X	X	X		X	X	X
Goniochloris smithii (Bourelly) Fott						X			
Ophiocytium capitatum Wolle									X
Tribonematales									
Tribonema affine (Kützing) G.S. West			X						

Appendix 3 – Recipes for medium used in laboratory experiments

Bold's Basal Medium (BBM)
Into 936 mL of dH_2O, add 10 mL of the first six stock solutions. Add 1 mL each of the alkaline EDTA, acidified iron, boron and trace metals solutions.

Component	Stock Solution ($g\ L^{-1}\ dH_2O$)	Quantity Used	Concentration in Final Medium (M)
Macronutrients			
$NaNO_3$	25.00	10 mL	2.94×10^{-3}
$CaCl_2 \cdot 2H_2O$	2.50	10 mL	1.70×10^{-4}
$MgSO_4 \cdot 7H_2O$	7.50	10 mL	3.04×10^{-4}
K_2HPO_4	7.50	10 mL	4.31×10^{-4}
KH_2PO_4	17.50	10 mL	1.29×10^{-3}
NaCl	2.50	10 mL	4.28×10^{-4}
Alkaline EDTA Solution		1 mL	
EDTA	50.00		1.71×10^{-4}
KOH	31.00		5.53×10^{-4}
Acidified Iron Solution		1 mL	
$FeSO_4 \cdot 7H_2O$	4.98		1.79×10^{-5}
H_2SO_4			
Boron Solution		1 mL	
H_3BO_3	11.42		1.85×10^{-4}
Trace Metals Solution		1 mL	
$ZnSO_4 \cdot 7H_2O$	8.82		3.07×10^{-5}
$MnCl_2 \cdot 4H_2O$	1.44		7.28×10^{-6}
MoO_3	0.71		4.93×10^{-6}
$CuSO_4 \cdot 5H_2O$	1.57		6.29×10^{-6}
$Co(NO_3)_2 \cdot 6H_2O$	0.49		1.68×10^{-6}

Cyanobacteria BG-11 Medium
Into 900 mL of dH2O, add 1 mL of the Fe citrate solution, and then add the remaining components.

Component	Stock Solution ($g\ L^{-1}\ dH_2O$)	Quantity Used	Concentration in Final Medium (M)
Fe Citrate Solution		1 mL	
Citric acid	6	1 mL	3.12×10^{-5}
Ferric ammonium citrate	6	1 mL	$\sim 3 \times 10^{-5}$
Macronutrients			
$NaNO_3$	--	1.5 g	1.76×10^{-2}
$CaCl_2 \cdot 2H_2O$	36	1 mL	2.45×10^{-4}
$MgSO_4 \cdot 7H_2O$	75	1 mL	3.04×10^{-4}
$K_2HPO_4 \cdot 3H_2O$	40	1 mL	1.75×10^{-4}
$MgNa_2EDTA \cdot H_2O$	1.0	1 mL	2.79×10^{-6}
Na_2CO_3	20	1 mL	1.89×10^{-4}
Trace Metals Solution		1 mL	

Trace Metals Solution			
H_3BO_3	--	2.860 g	4.63×10^{-5}
$ZnSO_4 \cdot 7H_2O$	--	0.220 g	7.65×10^{-7}
$MnCl_2 \cdot 4H_2O$	--	1.810 g	9.15×10^{-6}
$Na2MoO_4 \cdot 2H_2O$	--	0.391 g	1.61×10^{-6}
$CuSO_4 \cdot 5H_2O$	79.0	1 mL	3.16×10^{-7}
$Co(NO_3)_2 \cdot 6H_2O$	49.4	1 mL	1.70×10^{-7}

Appendix 4 – Statistics tables for temperature and conductivity experiments. Statistics generated using Statistica 7.

4.1 Laboratory experiments: temperature

Analysis of homogeneity of variance. Effect: Temperature		
	Hartley F-max	Cochran C
Growth rate	24.29250	0.193306
Δ pH	18.57210	0.307694

Univariate Test of Significance (ANOVA-proc. Glm) to determine if temperature has a significant effect on growth rate. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
Δ pH	0.009634	1	0.009634	0.7528	0.386758
(1) Species	**1.748295**	**1**	**1.748295**	**136.6149**	**0.000000**
(2) Culture	0.021425	1	0.021425	1.6742	0.197397
(3) Temperature	**0.542111**	**8**	**0.067764**	**5.2952**	**0.000006**
1 * 2	0.017769	3	0.005923	0.4628	0.708590
1 * 3	**1.100504**	**17**	**0.064736**	**5.0586**	**0.000000**
2 * 3	0.345238	17	0.020308	1.5869	0.071820
1 * 2 * 3	0.294955	35	0.008427	0.6585	0.927753
Error	2.252315	176	0.012797		

4.2 Laboratory experiments: conductivity

Analysis of homogeneity of variance. Effect: Conductivity		
	Hartley F-max	Cochran C
Growth rate	7.623820	0.391748
Δ pH	4.827993	0.303310

Univariate Test of Significance (ANOVA-Proc Glm) to determine whether conductivity has a significant effect on growth rate. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept	**0.071102**	**1**	**0.071102**	**4.54721**	**0.035758**
Δ pH	0.013294	1	0.013294	0.85018	0.359023
(1) Species	**2.116330**	**2**	**1.058165**	**67.67361**	**0.000000**
(2) Culture	0.021332	2	0.010666	0.68212	0.508199
(3) Conductivity	**1.222975**	**4**	**0.305744**	**19.55346**	**0.000000**
1 * 2	0.067856	4	0.016964	1.08491	0.368973
1 * 3	**2.575922**	**8**	**0.321990**	**20.59248**	**0.000000**
2 * 3	**0.300797**	**8**	**0.037600**	**2.40463**	**0.021407**
1 * 2 * 3	**0.759266**	**16**	**0.047454**	**3.03487**	**0.000455**
Error	1.375995	88	0.015636		

4.3 Field experiments: general study

Univariate Test of Significance for Conductivity minimum. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	1410386	15	94025.7	1.016625	0.447496
S. ellipticus	814643	10	81464.3	0.880809	0.554565
S. dimorphus	483281	6	80546.8	0.870889	0.520171
S. bernardii	242414	7	34630.6	0.374433	0.914647
S. denticulatus	129820	1	129819.6	1.403636	0.239668
S. arcuatus	43	1	42.7	0.000462	0.982912
Error	7306558	79	92488.1		

Univariate Test of Significance for Conductivity maximum. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	1394243	15	92949.6	0.996482	0.467308
S. ellipticus	823976	10	82397.6	0.883358	0.552257
S. dimorphus	491731	6	81955.2	0.878615	0.514584
S. bernardii	237817	7	33973.9	0.364223	0.920346
S. denticulatus	132671	1	132671.0	1.422322	0.236591
S. arcuatus	47	1	47.0	0.000503	0.982156
Error	7368941	79	93277.7		

Univariate Test of Significance for pH minimum. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	14.19474	15	0.946316	1.226989	0.270111
S. ellipticus	7.28349	10	0.728349	0.944373	0.498076
S. dimorphus	**17.78725**	**6**	**2.964541**	**3.843809**	**0.002049**
S. bernardii	8.15708	7	1.165297	1.510917	0.175705
S. denticulatus	0.53575	1	0.535749	0.694649	0.407100
S. arcuatus	2.43265	1	2.432652	3.154164	0.079584
Error	60.92883	79	0.771251		

Univariate Test of Significance for pH maximum. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	12.64629	15	0.843086	1.075196	0.392425
S. ellipticus	5.94521	10	0.594521	0.758199	0.667919
S. dimorphus	**17.49534**	**6**	**2.915890**	**3.718663**	**0.002620**
S. bernardii	7.42149	7	1.060213	1.352100	0.237455
S. denticulatus	0.80484	1	0.804842	1.026424	0.314093
S. arcuatus	2.15112	1	2.151116	2.743340	0.101627
Error	61.94572	79	0.784123		

Univariate Test of Significance for Temperature minimum. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	221.6734	15	14.77823	1.546575	0.109120
S. ellipticus	**201.9130**	**10**	**20.19130**	**2.113065**	**0.032814**
S. dimorphus	10.3300	6	1.72167	0.180177	0.981507
S. bernardii	99.2118	7	14.17312	1.483249	0.185334
S. denticulatus	0.0181	1	0.01812	0.001896	0.965380
S. arcuatus	0.0434	1	0.04336	0.004537	0.946465
Error	754.8810	79	9.55546		

Univariate Test of Significance for Temperature maximum. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	160.6969	15	10.71312	1.315438	0.213198
S. ellipticus	127.5141	10	12.75141	1.565713	0.132504
S. dimorphus	23.3515	6	3.89191	0.477878	0.822894
S. bernardii	51.0770	7	7.29671	0.895945	0.513952
S. denticulatus	2.3115	1	2.31150	0.283832	0.595702
S. arcuatus	0.0007	1	0.00072	0.000089	0.992505
Error	643.3881	79	8.14415		

4.4 Field experiments: intensive study

Univariate Test of Significance for Conductivity. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	2754453	13	211881.0	2.018689	0.065878
S. ellipticus	**3017357**	**6**	**502892.9**	**4.791296**	**0.002417**
S. dimorphus	327512	5	65502.4	0.624072	0.682905
Error	2519033	24	104959.7		

Univariate Test of Significance for pH. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	8.85480	13	0.681138	0.988317	0.490081
S. ellipticus	**17.34388**	**6**	**2.890647**	**4.194266**	**0.005048**
S. dimorphus	2.07825	5	0.415650	0.603099	0.698092
Error	16.5457	24	0.689190		

Univariate Test of Significance for Temperature. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	42.94541	13	3.303493	1.155099	0.365971
S. ellipticus	**56.82367**	**6**	**9.470611**	**3.311492**	**0.016128**
S. dimorphus	2.99004	5	0.598009	0.209100	0.955367
Error	68.63815	24	2.859923		

Univariate Test of Significance for PAR. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	3847174	13	295936.4	1.524669	0.179396
S. ellipticus	1121981	6	186996.9	0.963411	0.470416
S. dimorphus	2300798	5	460159.6	2.370749	0.069718
Error	4658371	24	194098.8		

Univariate Test of Significance for Turbidity. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	503825	13	38755.77	0.550936	0.867974
S. ellipticus	395474	6	65912.28	0.936982	0.487062
S. dimorphus	4562	5	912.49	0.012972	0.999936
Error	1688286	24	70345.27		

Univariate Test of Significance for Ammonium. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	133593	13	10276.38	0.221946	0.996531
S. ellipticus	143225	6	23870.86	0.515556	0.790645
S. dimorphus	13761	5	2752.18	0.059441	0.997383
Error	1111230	24	46301.24		

Univariate Test of Significance for Nitrate. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	34974.0	13	2690.305	0.412186	0.950448
S. ellipticus	25507.0	6	4251.174	0.651329	0.688748
S. dimorphus	244.0	5	48.800	0.007477	0.999984
Error	156646.1	24	6526.920		

Univariate Test of Significance for Total Phosphorus. Sigma-restricted parameterization. Effective hypothesis decomposition. SS = Sum of Squares, DF = Degrees of Freedom, MS = , F = F value, p = p value (Significant values are bold).

Effect	***SS***	***DF***	***MS***	***F***	***p***
Intercept		0			
S. quadricauda	96737.6	13	7441.35	0.457352	0.928033
S. ellipticus	57755.7	6	9625.96	0.591620	0.733813
S. dimorphus	1308.9	5	261.78	0.016089	0.999891
Error	390492.3	24	16270.51		

Appendix 5 – Post hoc statistics tables for temperature / conductivity experiments. Statistics generated using Statistica 7.

The Tukey's post hoc statistics tables for this study are recorded on the accompanying CD which contains the following file:

1. Chapter 5 Statistics Tables.xls
   This file includes the entire Tukey's Post Hoc statics tables for the temperature and conductivity laboratory experiments for this study.