The relationship between periphyton, flow and nutrients in foothill rivers of the south-western Cape, South Africa

By

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DECLARATION

This thesis reports original research carried out in the Freshwater Research Unit, Department of Zoology, University of Cape Town. It has not been submitted in whole or in part for a degree at any other university. Data presented here are original. A subset of the data used in Chapter 8 were collected jointly for this project and a related study undertaken in partial fulfilment of a BSc honours degree, Department of Botany, University of Cape Town by D. Basic in 2011. Any assistance received is fully acknowledged.

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ABSTRACT

This thesis examines spatial and temporal patterns in periphyton community composition and biomass and the environmental factors responsible for shaping these communities in south-western Cape rivers. The study focused on two perennial foothill rivers in the south-western Cape: the Berg River, which is oligotrophic but has a large dam (The Berg Dam) situated in its upper reaches; and the Molenaars River, which has a natural flow regime but is moderately enriched by trout farm effluent. Two sites on the Berg River (one upstream and one downstream of the Berg Dam) and two on the Molenaars River (one upstream and one downstream of the Du Toit's Kloof trout farm) were used to study temporal dynamics in periphyton communities over a 21-month period between September 2007 and May 2009.

Quantitative monthly samples of periphyton biomass (Chlorophyll *a* and Ashfree Dry Mass) and community composition were collected from all four sites over the 21-month sampling period, which encompassed two dry seasons, separated by a wet season. Data representing six environmental factors, namely solar radiation, temperature, flow, physico-chemical, nutrients and invertebrate grazing were also collected on each sampling occasion or from continuous loggers in the case of solar radiation, temperature and flow. Clear seasonal cycles in environmental conditions in the Berg and Molenaars Rivers that are typical of rivers in Mediterranean climates were demonstrated under natural flow conditions. Principle Component Analysis (PCA) of all environmental variables provided an understanding of the key variables responsible for differences between sites.

Under natural flow conditions, periphyton communities follow a cycle of peak biomass towards the end of the growing season in late summer/early autumn, and minimum biomass during mid-winter with a small peak during late winter/early spring. Winter and early spring communities were dominated by diatoms, particularly *Eunotia rhomboidae*, whereas the peak in late summer/early autumn 2008 was dominated by green filamentous taxa such as *Mougeotia* spp. and *Spirogyra* spp. and by cyanobacteria, particularly *Chamaesiphon* spp. and *Aphanocapsa* spp. in summer/early autumn 2009. Peak biomass differed between years and between sites with different trophic status reflecting differences in the availability of nutrients between systems. Periphyton communities were highly dynamic both within and between seasons, particularly over the winter and early spring suggesting that these communities shift at a temporal scale similar to the occurrence of flood events within seasons, rather than at the broader scale defining seasonal changes in environmental conditions.

The frequency of flood disturbance over the wet season, was the single most important driver of periphyton communities and accounted for almost 75% of the total variation in periphyton biomass under natural flow and nutrient-poor conditions, and was therefore identified as the primary driver of periphyton communities. Temperature and light, which define seasonal changes in environmental conditions described a much smaller portion of the observed temporal variation in periphyton communities and were therefore identified as secondary drivers of periphyton dynamics. The role of flood frequency as a driver of periphyton was smaller under moderately enriched conditions but was still the primary driver of these communities and accounted for 45% of the total variation. These findings emphasize the decreasing role of flow and increasing role of nutrients in shaping periphyton communities over time, as nutrient availability increases.

Of the within-year floods DRIFT Class 2 floods or larger correlated significantly with Chl *a* biomass suggesting that Class 2 floods defines the disturbance threshold for periphyton communities in the south-western Cape under natural flow conditions. However, pre- and post-flood sampling showed that the effects of floods differ across a range of flood sizes depending on antecedent base flow conditions and different levels of enrichment. Defining the disturbance threshold of different flood events is therefore complicated by the resistance of the community to disturbance. An assessment of temporal patterns in periphyton communities and invertebrate grazer densities suggested that the spring peak in periphyton biomass may be a result of reduced grazing pressure at a time when recovery of invertebrate grazers lags behind algal growth and provides a window of opportunity for rapid biomass accrual. Simple linear regression analyses provided further evidence for the role of grazers as potential top-down controllers on periphyton

The effects of grazing on periphyton communities were quantified by an *in situ* grazer exclusion experiment in the Berg River during late spring (November/December 2010). Within the 13-day experimental period, conspicuous filaments of the green alga, *Mougeotia* spp. developed under low grazing pressure (< 1000 individuals m^{-2}) and Chl *a* biomass was 6-fold higher compared with enclosures that included ambient densities of grazing invertebrates during this time. These results confirmed the role of invertebrate grazers, particularly baetid mayflies as a top-down control on periphyton biomass during spring. By contrast, few grazer effects on periphyton communities were evident from the results of a similar experiment in autumn 2011.

Unlike sites with natural flow regimes, temporal patterns of periphyton were erratic downstream of the Berg Dam with no clear winter biomass minimums and summer maximums typical of those under natural flow conditions. A shift to a community dominated by heterotrophs was evident downstream of the dam, particularly during the summer months when base flows were unnaturally elevated. Differences in base flows and associated water quality conditions, rather than differences in the disturbance regime over the wet season were the primary drivers of differences in periphyton communities between sites with and without flow alteration.

An assessment of differences in periphyton communities between hydraulic habitats showed that periphyton communities respond differently to differences in near-bed velocity depending on the level of nutrient enrichment and the age of the periphyton mat. These findings support the subsidy-stress response of periphyton described in the literature, although there is evidence to suggest that differential invertebrate grazing activity and disturbance history may contribute to the heterogeneity of periphyton communities at the reach scale.

Through both field assessments and controlled experiments, this thesis contributes to our understanding of the factors which drive periphyton communities in Mediterranean rivers. Key findings and their implications for river management are discussed and a conceptual model of the proposed changes in periphyton community structure associated with altered flow and nutrient conditions in south-western Cape rivers is proposed. Finally, this thesis constitutes the first detailed assessment of periphyton community structure in South African rivers and further research over a wider geographic range is therefore recommended.

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CHAPTER 1 GENERAL INTRODUCTION

1.1 INTRODUCTION

South African rivers are threatened by flow and nutrient-related changes due to increasing human demand and factors such as the drier conditions predicted by global climate change. Besides threats to biodiversity, unmanaged exploitation of rivers reduces their resource value and consequently both social and economic development.

In recent years, resource managers and scientists in South Africa have made concerted efforts to manage rivers in a more sustainable way. One of the key aspects of these management efforts is the *Ecological Reserve* which defines the water needed to sustain aquatic ecosystems (Kleynhans *et al.* 2005). Defining flows necessary to meet the Ecological Reserve is heavily dependent on our ability to understand and quantify the relationship between any one ecosystem component and the flow regime. In South Africa fish, riparian vegetation, and macro-invertebrates are the main biotic components of river ecosystems currently considered in both the setting of Ecological Reserves and monitoring the effects of altered flow on ecosystem integrity.

In the United States, Australia and New Zealand, periphyton[1], or benthic algae found on riverbeds, is increasingly included in the suite of organisms used for predicting and assessing ecosystem integrity (Chessman *et al.* 1999; Hill *et al.* 2000; Growns and Growns 2001; Downes *et al.* 2003; Chester and Norris 2006). In open-canopied temperate rivers, periphyton plays a key role by converting dissolved nutrients into food that provides the main source of energy for higher trophic levels (Biggs 1996). Periphyton communities thus occupy a pivotal position in aquatic ecosystems at the interface of the chemical-physical and biotic components of the food web (Lowe and Pan 1996). As a critical link in the functioning of aquatic ecosystems, changes in the biomass or structure of periphyton can profoundly influence the rest of the aquatic community. Periphyton is particularly responsive to flow and nutrient alterations (e.g. Biggs and Close 1989; Grimm and Fisher 1989; Biggs 1995; Biggs and Smith 2002; Uehlinger *et al.* 2003; Bergey and Resh 2006). Periphyton communities are therefore ideally suited for monitoring the cause and effects of an altered flow regime, which would significantly improve our ability to manage water resources more efficiently. The ability to identify the relative importance of factors that control periphyton dynamics under natural conditions is one of the most critical aspects of assessing the impact of enrichment and flow alteration on the structure of periphyton communities in streams (Villeneuve *et al.* 2011).

Very little research on the periphyton of South African Rivers has been undertaken in the past. Diatoms, a component of the periphyton, have long been researched in South Africa as indicators of pollution and in this regard have been the subject of recent research in South African rivers (De la Rey *et al.* 2004, Taylor *et al.* 2007a, Taylor *et al.* 2007b). The primary goal of that research is confined

[1] Periphyton is the complex assemblage that comprises algae, protozoa, bacteria and fungi found on substrata in lotic ecosystems. In terms of biomass, benthic algae generally make up the largest proportion of the periphyton and as such, the terms periphyton and benthic algae are often used synonymously.

to the development of a biotic index of water quality (Taylor *et al.* 2007a; Taylor *et al.* 2007b), rather than being rooted within the discipline of community ecology, which seeks to understand patterns and mechanisms of change in communities based on the interaction of water quality with energy sources, habitat structures and the flow regime (e.g. Chessman *et al.* 1999).

The primary objective of this thesis was to develop an understanding of patterns in periphyton community structure and biomass in cobbled, foothill rivers of the south-western Cape. This formed the basis for identifying those factors that drive these patterns and the relative importance of key factors for the maintenance of intact periphyton communities in these ecosystems.

Given the ecological importance of periphyton and its significance in ecological monitoring and river management, it is important to understand all the factors that potentially shape periphyton communities in rivers. The following sections therefore review what is currently known about periphyton community structure and functioning and the primary drivers of these patterns in temperate rivers elsewhere.

1.2 WHAT IS PERIPHYTON AND WHY IS IT IMPORTANT IN STREAM ECOSYSTEMS?

1.2.1 Defining Periphyton

Periphyton refers to assemblages of freshwater algae, cyanobacteria and prokaryotes such as bacteria, fungi and protozoa that are found on substrata in inland aquatic ecosystems. Strictly speaking, the term periphyton includes micro-invertebrates but the inclusion of fauna in the definitions of periphyton reported in the literature is rare. In most riverine ecosystems with open canopies, the assemblage consists predominantly of algae and cyanobacteria, collectively termed benthic algae in some cases (Stevenson *et al.* 1996). Other terms used to describe periphyton in the literature include "Aufwuchs" which means 'to grow upon' and is commonly used in Europe (Stevenson *et al.* 1996). The term "biofilm" is commonly used in Australia to define these communities (e.g. Burns and Ryder 2001) and sometimes the term "phytobenthos" is included in the literature (Law 2011). Periphyton has become the most widely used term among stream ecologists to describe this community (Biggs 2000b; Larned 2010) and therefore this term has been adopted for use in this study.

Within this group of organisms however, the term periphyton is further refined to distinguish between the different substratum types upon which the community grows. For example, "Epilithon" refers to those communities found on hard substrata such as cobbles and boulders that are many times larger than the size of individual algal cells, while "epiphyton" are those communities that grow on the filaments of aquatic plants or large filamentous algae (Biggs 2000b). "Epipsammon" describes communities growing on sand grains that are generally smaller than the individual cells of most algal taxa, whereas "epipelon" is the periphyton growing on mud and silt grains that are organic or inorganic and smaller than the cells of most unicellular taxa (Biggs 2000b). This study is concerned specifically with the epilithon growing on cobbles in riverine ecosystems and with epiphyton that often grow on large filamentous algae.

1.2.2 The role of periphyton in sustaining ecosystem functioning

Algal components of periphyton are autotrophic and thus obtain their energy from sunlight and abiotic resources. Together with other autotrophs such as aquatic macrophytes, and microbial heterotrophs, periphyton constitutes the primary source of energy in riverine food webs (Allan and Castillo 2009). Particularly in shallow, fast flowing, open-canopied rivers periphyton is an important primary producer and therefore a significant source of energy for maintenance of the rest of the ecosystem (Stevenson 1996). However, the actual energy provided by the periphyton can vary both spatially and temporally. According the river continuum concept (RCC) of Vennote *et al.* (1980), the balance between heterotrophic and autotrophic energy input shifts along the length of a river. In particular, the RCC postulates that autotrophic energy input, mainly from benthic algae, is the primary source of energy for aquatic food webs in the middle reaches of a river. However, the nutritional quality of periphyton can shift over time with changes in periphyton taxa between seasons (Lamberti 1996; Ledger and Hildrew 1998). Besides the provision of energy for sustaining aquatic food webs, benthic algae are able to absorb metal ions and assist with the breakdown of organic matter contamination (Law 2011). Therefore, periphyton can help to purify water (Biggs and Kilroy 2000). Also, periphyton mats provide habitat for aquatic invertebrates and can protect fauna from predation as well as provide a haven from high flows near the river bed (Law 2011).

1.2.3 Instream values affected by periphyton proliferations

Altered flow regimes and nutrient enrichment often result in proliferations of periphyton that affect a number of instream values. Cyanobacterial blooms, for example, can cause gastric illnesses in cattle, skin irritations in humans and affect the odour and taste of water. Algal proliferations can result in the outbreak of pest organisms such as blackflies. Excess production of filamentous algae can reduce the recreational value of rivers, clog water intakes and irrigation nozzles, and reduce biodiversity by reducing the streambed habitat unsuitable for many sensitive fish and invertebrate species (Biggs 2000b). These impacts can be costly and difficult to rectify and are therefore cause for concern. Thus, the ability to understand the flow and nutrient characteristics necessary to prevent such undesirable consequences will considerably improve our ability to use these ecosystems in a sustainable way. In South Africa little is known about the structure and functioning of periphyton in riverine ecosystems and thus the consequences of anthropogenic change to these communities are poorly understood and difficult to predict.

1.3 FACTORS MOST COMMONLY REGULATING ACCRUAL AND LOSS PROCESSES OF PERIPHYTON IN STREAMS

1.3.1 Conceptual framework for understanding patterns in benthic algae in streams

Geology, climate and human activities affect landscape features such as topography, slope, vegetation and land-use, which in turn control the fundamental physical variables of streams, *viz* hydrology, water quality and physical habitat. A complex series of interactions between these

physical factors controls biological responses and interactions which determine the growth form, species composition and biomass of periphyton (Biggs 1996).

In his conceptual overview of the factors that influence periphyton dynamics, Biggs (1996) describes factors that directly control biomass accrual and those regulating the counteracting process of biomass loss (Figure 1.1). Biomass accrual is directly affected by the supply of nutrients (Horner *et al.* 1990; Mulholland *et al.* 1991; Stevenson *et al.* 1996; Anderson *et al.* 1999; Dodds 2003), and light intensity (DeNicola *et al.* 1992; Hill 1996; Hill and Dimick 2002). When both nutrient and light requirements are fully met then temperature, which affects the metabolic rate of algal cells, controls the growth of algae and thus controls biomass accrual (DeNicola 1996).

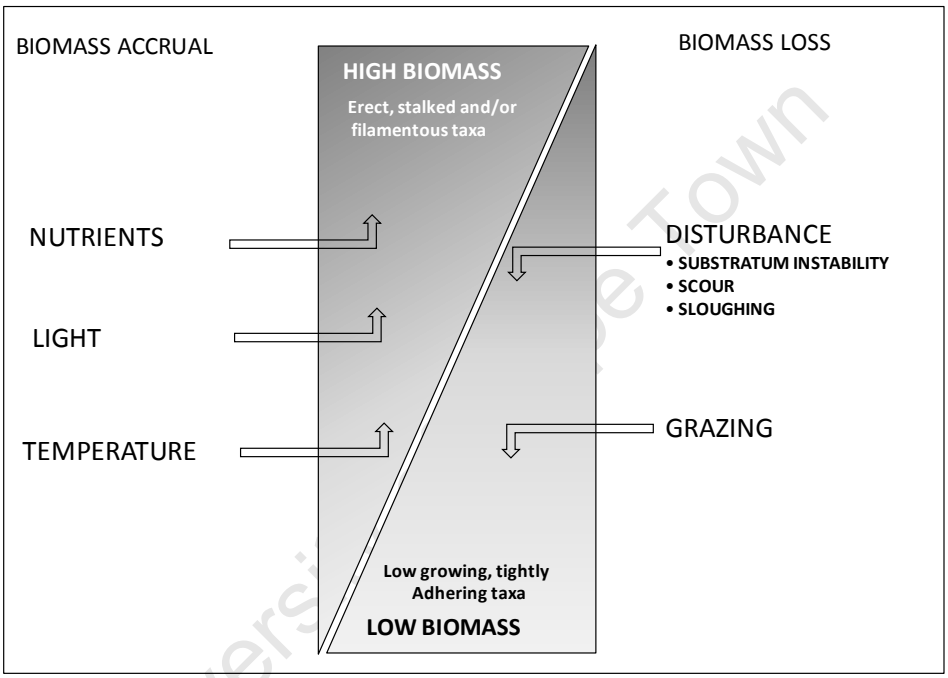


Figure 1.1 **Summary of the conceptual framework for understanding the factors that control periphyton development in streams (after Biggs 1996).**

Disturbance[2] is the main factor controlling algal loss. Disturbance can include both physical disturbances such as flood events and desiccation, which operate over large spatio-temporal scales, as well as small-scale disturbances such as grazing (Grimm 1994). In lotic ecosystems, disturbance is usually caused by floods through substratum instability, abrasion by suspended sediments (Grimm and Fisher 1989; Biggs *et al.* 1999; Francoeur and Biggs 2006) and shear stress (Biggs and Thomsen 1995; Biggs 1996, Biggs *et al.* 1998; Biggs 2000b). Grazing by invertebrates (Steinman *et al.* 1991; Steinman 1996; Wellnitz and Ward 2000) and fish (Power and Matthews 1983; Power *et al.* 1988) is a biotic process that leads to biomass loss in many aquatic ecosystems.

While Biggs' (1996) conceptual framework (Figure 1.1) draws on nutrients, light and temperature as the resources necessary for algal growth, with disturbance by floods and grazing as key factors leading to algal loss, the interaction between these variables is complex and not always intuitive.

[2] For the purpose of this study, disturbance is defined as a relatively discrete event that removes organisms at a rate faster than the rate of accrual or recruitment (Larned 2010; Stanley *et al.* 2010).

For example, floods as a means of physical disturbance might directly lead to a loss of algal biomass but they also mobilise nutrients, thereby potentially promoting algal growth (Larned *et al.* 2004). Furthermore, floods can reduce grazer densities, and affect predator-grazer dynamics that in turn influence periphyton growth and biomass. Besides the direct role of flood flows, other aspects of the hydrological regime can indirectly control algal dynamics over time and space. Low flows, for example, indirectly affect periphyton by influencing the supply rate of nutrients to algae, water depth and turbidity, which in turn affect light penetration, and hydraulic biotope characteristics. Also, hydrodynamic drag forces can affect the colonization of propagules, growth, survival and morphology of periphyton (Nikora *et al.* 1998).

In a recent review of research focused on periphyton ecology, Larned (2010) discussed the role of additional factors controlling the presence, abundance, composition and growth of periphyton. These include exposure to environmental stressors such as ultraviolet radiation (Kelly *et al.* 2001; Kelly *et al.* 2003; Frost *et al.* 2007), thermal stress (Wilde and Tilly 1981; DeNicola 1996) and pH stress or acidification (DeNicola 2000; Sabater *et al.* 2003). Biological mechanisms such as competitive interactions that lead to the dominance by certain taxa also affect periphyton assemblage structure (Smith and Doan 1999; Leflaive and Ten-Hage 2009). These controlling factors and the interactions between them are discussed in detail in Sections 1.3.2 to 1.3.5.

The relative importance of any one of these factors, or the nature of the interactions between them, determines whether the periphyton community is characteristically low in biomass with low-growing tightly adhering taxa, or high in biomass with a community of erect, stalked and or filamentous taxa (Biggs 1996). These patterns in taxonomic structure, biomass and morphology, which vary over different temporal and spatial scales, are discussed in Section 1.4.

1.3.2 Resource supply

1.3.2.1 Nutrients

Nitrogen (N) as nitrate and ammonia, dissolved organic phosphorus (P) and to a lesser extent silica, are generally considered the most critical nutrients for algal production (Dodds and Welch 2000; Francoeur 2001; Dodds 2006; Mulholland and Webster 2010). From the vast literature concerning the relationship between nutrients and algal growth, most has dealt with phytoplankton, with relatively few studies focused on periphyton. Nevertheless, within the field of periphyton ecology, nutrient limitation, particularly nitrogen (N) and phosphorus (P) limitation, is one of the best studied topics (Larned 2010). Other elements that have received some attention in the periphyton literature are Silicon (Si), which is important to the growth of diatoms, and iron (Fe), but these are not well studied (Borchardt 1996; Mulholland and Webster 2010).

By definition, periphytic algae are attached to substrata and thus spatial organisation of periphyton assemblages is very different from that of phytoplankton. Whereas phytoplankton is generally suspended in the water column with nutrients available across the entire cell surface, periphyton tends to form mats that can be many cells thick. Thus the major pathway for external nutrient supply is either from overlying water or from underlying substrata in the case of upwelling zones (Borchardt

1996, Henry and Fisher 2003, Wyatt *et al.* 2008, Larned 2010). This can lead to a vertical nutrient gradient within the algal mat with high nutrient availability at the surface-water interface and low nutrient availability at the base. Also, the movement of dissolved nutrients and gasses from the water column to the mat surface can be impeded in flowing waters because of a zone of non-mixing, a "dead zone", created by laminar flow within the boundary layer at the surface of the mat (Borchardt 1996). Consequently, algal cells within the mat can be separated from the nutrient source within the water column.

Steinman *et al.* (1995) found that nutrient recycling within dense algal mats provided a significant source of nutrients for P-limited periphyton streams. Similarly, Mulholland *et al.* (1995) showed that internal recycling of nutrients within periphyton mats contributed 60-70% of the P uptake during an eight-week study of longitudinal patterns in nutrient cycling and periphyton in laboratory streams. In a study of a N-limited desert stream ecosystem, Peterson and Grimm (1992) showed that the development of a thick periphyton mat during late successional stages of development reduced availability of both water-column and substratum-derived nutrients and increased reliance for growth on internal nutrient recycling. Thus nutrient recycling within an algal mat can be important for sustained growth (Mulholland *et al.* 1991; Stevenson *et al.* 1991; Peterson and Grimm 1992) but it cannot sustain periphyton indefinitely (Larned 2010). It is likely that periphyton assemblages take their nutrients from multiple sources simultaneously, with algal cells within the matrix and at the base of the matrix reliant on nutrients from the sediment and internal nutrient recycling whereas surface cells are more reliant on nutrients derived from the water column (Mulholland 1996).

Although several chemical constituents can become limiting under certain conditions, growth of benthic algae in streams is usually limited by the availability of N or P (Borchardt 1996, Mulholland and Webster 2010) because demand is often high relative to availability (Larned 2010). A plethora of nutrient enrichment studies in natural and artificial streams provide clear evidence that either P or N, or both, limit periphyton growth in streams (see Borchardt 1996 for a review). Through manipulation of nutrients, many of these studies have identified the single nutrient that limits periphyton growth because, relative to other nutrients, its availability is in short supply relative to demand. For example, in a study of a coastal rainforest stream in Canada, Stockner and Shortreed (1978) showed that enrichment of natural streams with ammonia produced little change in periphyton biomass but periphyton grew rapidly following the addition of inorganic P. By contrast, (Flecker *et al.* 2002) showed that enrichment of a tropical stream in South America with ammonia resulted in significant increases in periphyton biomass, whereas additions of P had no effect on the biomass of periphyton. Dodds and Welch (2000) reviewed nutrient enrichment studies across the USA and found that 13% showed an increase in algal biomass with N addition, 18% with P addition and 44% with both N and P. 26% of cases showed no difference in algal biomass to either N or P suggesting that co-limitation by N and P is fairly common.

Nitrogen to phosphorus (N:P) ratios are often used as a benchmark for establishing which of these two macronutrients is potentially limiting in freshwater benthic habitats (Borchardt 1996; Allan and Castillo 2009), based on the Redfield ratio of 16 (Redfield 1958), derived from marine algal studies.

In general, ambient N:P ratios greater than 20:1 are considered P-limited, less than 10:1 are N-limited. Between 10:1 and 20:1 the distinction as to which nutrient is limiting is uncertain (Schanz and Juon 1983 in Borchardt 1996). The single limiting nutrient paradigm (or Liebig's Law of the Minimum), which states that only one nutrient can be in shortest supply relative to demand at any given time (De Baar 1994 in Larned 2010), has been confirmed in experiments using cultures of single algal species (see Borchardt 1996). However, both Liebig's Law of the Minimum and the applicability of the N:P ratio as an indicator of nutrient limitation in multispecies benthic algal assemblages has been questioned by some researches in recent years (Francoeur *et al.* 1999; Dodds and Welch 2000; Francoeur 2001; Dodds 2002). Their concern is largely because nutrient requirements can differ considerably between different species in an algal mat and thus different species may be limited by different nutrients (Francoeur 2001). The N:P ratio varies considerably between different benthic algal taxa because of the significant variability in nutrient requirements between algal species (Borchardt 1996). For example, filamentous chlorophytes require more P than do diatoms to maximise growth (Welch *et al.* 1988). In his meta-analysis of 237 streams with nutrient addition experiments spanning the USA, Canada, New Zealand, Australia and India, Francoeur (2001) challenged the application of the single nutrient paradigm for multispecies algal assemblages. He found that algal assemblages comprising different species are unlikely to be limited by a single nutrient. Rather, simultaneous stimulation of benthic algal community biomass by more than one nutrient was the rule, not the exception as previously considered. He concluded that while field studies of individual species suggest that algal biomass can be increased by adding a single nutrient, meta-analyses of these data provide the statistical power to detect subtle increases in biomass by independent addition of a different nutrient, thus supporting the notion that nutrient co-limitation is common in periphyton communities, as suggested by Dodds and Welch (2000) and Tank and Dodds (2003). Although attempts have been made to generalise about geographical and temporal patterns of nutrient limitation, particularly in temperate regions of North America (Borchardt 1996), the validity of these generalisations is not widely accepted.

In addition to nutrient limitation studies, nutrient enrichment studies have also addressed the relationship between nutrients and periphyton community structure. Using substrata with different levels of enrichment, Peterson and Grimm (1992) showed that nitrogen-fixing cyanobacteria dominated unenriched substrata, while substrata enriched with N were colonised by diatoms and filamentous chlorophytes but were later dominated by cyanobacteria. Others (e.g. Peterson *et al.* 1993) showed that nutrient enrichment does not always lead to a change in algal species composition. While P enrichment leads to dominance by cyanobacteria in phytoplankton communities (Borchardt 1996), the effect of P enrichment on periphyton community structure is less predictable because sometimes, but not always (Hill and Knight 1988; Lohman *et al.* 1991), it leads to dominance by cyanobacteria (Fairchild *et al.* 1985; Peterson and Grimm 1992). Pringle (1990) found that taxon richness and diversity were greatest when ambient nutrient levels were low but the nutrient-diffusing substratum was enriched. She attributed these findings to spatial heterogeneity of nutrient resources from the substratum suggesting that high diversity is maintained in nutrient-poor systems by the natural heterogeneity of nutrients from the substratum relative to

those in nutrient-rich environments, which offer a homogenous supply of nutrients from the water column. Thus, a generalisation of the response of periphyton communities to nutrient enrichment remains elusive (Larned 2010).

In summary, despite clear evidence in the literature that periphyton biomass and community structure are affected by either N, P or both nutrients, very few generalisations can be drawn from this research. Perhaps it is the complexity of the interactions between nutrients and other variables acting on periphyton communities that prevent the development of a general understanding of how periphyton communities respond to nutrient changes.

1.3.2.2 Light

Light is fundamentally important to periphyton because most periphytic organisms are autotrophic, relying on photosynthetically active radiation (PAR) to convert inorganic compounds into living matter. Light can thus strongly influence periphyton dynamics and community structure in stream ecosystems (Steinman *et al.* 1987; Hill and Knight 1988; Steinman *et al.* 1989; Ledger and Hildrew 1998). Investigations on the role of light as a limiting resource for periphyton growth have focused mainly on comparisons of periphyton community structure and biomass between areas with dense riparian canopies and those with open canopies (e.g. Shortreed and Stockner 1983; Hill and Knight 1988; DeNicola *et al.* 1992). These studies observed higher periphyton growth rates in unshaded areas than in those with dense canopies. Their findings have been supported by experimental studies such as that carried out by Quinn *et al.* (1997), who used shade cloth to reduce PAR by 60%, 90% and 98% in artificial channels in New Zealand. By contrast, Stockner and Shortreed (1978) found no significant difference in periphyton production between shaded and open sites in British Colombia, but under oligotrophic conditions. Similarly, Mosish *et al.* (1999) found no difference in chlorophyll *a* concentrations with differing shade treatments. The addition of N, however, resulted in an increase in chlorophyll *a* in the unshaded treatments with a steady decline as shade increased. The lack of a response in benthic algal production to shading in the oligotrophic stream of Stockner and Shortreed (1978) and that of Mosish *et al.* (1999) prior to the addition of N, thus suggests that under conditions of nutrient limitation, light limitation may be insignificant. In other studies, biomass responses to differences in light intensity were observed only after the elimination of grazers (Steinman 1992; Hill *et al.* 1995), suggesting that high grazing intensities in unshaded reaches can reduce biomass relative to that in shaded reaches, thus counteracting the effects of canopy cover.

Despite differences in periphyton biomass between shaded and unshaded river reaches, riparian canopy cover is rarely uniform across the channel, even in densely forested reaches (Hill 1996) and there can be considerable heterogeneity in the availability of light across channels and between channels with different riparian characteristics. Differences in periphyton biomass can therefore be attributed in some cases to differences in the availability of light at different spatial and temporal scales (Rosemond *et al.* 2000; Hill and Dimick 2002).

As light reaches the water column, it is attenuated exponentially because dissolved organic matter, suspended sediments and water itself scatter and absorb light (Hill 1996). Such attenuation is generally not significant and rarely limits benthic algal growth, although significant reductions in light attenuation have been reported in rivers carrying very high silt and clay loads (Davies-Colley *et al.* 1992).

Once light reaches the periphyton mats attached to substrata in rivers, the vertical matrix of algal cells and inorganic particles causes variation in light transmission to cells at the base of the mat (Hill 1996, Larned 2010) and attenuation increases with an increase in thickness of the periphyton mat (Dodds 1992; Johnson *et al.* 1997; Biggs *et al.* 1999, Higgins *et al.* 2008).

Some algal taxa can cope better than others with suboptimal light conditions. Mechanisms that allow periphyton communities to persist under severe light limitation include facultative heterotrophy, motility within the algal matrix, and acclimation to low light by certain shade-tolerant taxa (Larned 2010). Tuchman *et al.* (2006) showed that facultative heterotrophy by diatoms allowed periphyton communities to persist under conditions of severe light limitation when concentrations of dissolved organic carbon (DOC) are high. Larned (2010) suggested that facultative heterotrophy may thus be restricted to dark conditions rich in DOC which, in the case of rivers, would apply to algal cells at the base of benthic algal mats. Some taxa (such as raphe-bearing motile diatoms) can avoid extreme conditions by moving along the light gradient whereas others such as prostrate benthic algae (e.g. *Achnanthes*) are at a distinct disadvantage in vertically developing algal mats and may therefore decline in abundance when light levels decline (Hill 1996). Photo acclimation of benthic algal cells to maximise photosynthetic efficiency under limited light conditions has been reported by a number of researchers. Hill *et al.* (1995), for example, found that at sites with low light intensities due to shading by canopy cover, primary productivity by periphyton was twice that of periphyton from open sites and that photosynthesis reached saturation at lower light intensities at the shaded site. At the scale of the benthic algal mat, evidence for photo acclimation by algal cells is given by Tuchman (1996) who found an increase in photopigment concentrations in cells at the base of an algal mat. Dodds (1992) also showed that photosynthetic efficiency increases with an increase in depth from the surface of a periphyton mat.

In terms of broad taxonomic groups, it is evident that chlorophytes require high light conditions to thrive, whereas diatoms and cyanobacteria often dominate in areas with reduced irradiance (Steinman *et al.* 1987; Steinman *et al.* 1989; Hill 1996). Cyanobacteria are excellent competitors for light, unlike chlorophytes, because they have phycobilins or pigments that absorb light in the green region (Dodds 2002). Based on their experimental study using laboratory streams, Steinman *et al.* (1989) predicted that under conditions of moderate grazing activity, diatoms would dominate at irradiances < 50 µmol $m^{-2}s^{-1}$, diatoms and some cyanobacteria and chlorophytes would constitute the community at 50-100 µmol $m^{-2}s^{-1}$ and chlorophytes would dominate at irradiances > 100 µmol $m^{-2}s^{-1}$. Despite their ability to survive at low light levels, Hill (1996) suggests that most taxa probably grow best under moderately high irradiances (between 200-400 µmol $m^{-2}s^{-1}$). The dominance of filamentous chlorophytes at these light intensities might be because their growth form allows them

to overgrow prostrate taxa such as diatoms and cyanobacteria and not because these latter groups grow sub-optimally at these light intensities (Hill 1996).

Recent research in New Zealand (Lange *et al.* 2011) showed significant differences between diatom functional groups exposed to different light intensities (as well as nutrient conditions and grazing pressure), rather than between taxonomic groups. Their study categorized diatoms as either 'low profile' (i.e. adnate, prostate), 'high profile' (i.e. erect, filamentous, stalked, colonial) or 'motile' (i.e. fast moving).

Photoinhibition is the phenomenon of a reduction in photosynthesis (and thus biomass) exhibited by primary producers when they are exposed to high light intensities (Han *et al.* 2000). Most knowledge on this subject is taken from studies on phytoplankton, although a few studies have measured changes in periphyton growth rates under high Ultra Violet Radiation (UVR). A reduction in periphyton growth rates has been measured in both laboratory and field experiments (Bothwell *et al.* 1993; Kiffney *et al.* 1997; Frost *et al.* 2007), although other field-based studies showed no adverse effects of high UVR on the growth rates of periphyton (DeNicola and Hoagland 1996; Weidman *et al.* 2005). The role of photoinhibition in structuring periphyton community structure therefore remains unclear and is generally regarded in the literature as understudied (Larned 2010).

1.3.3 Temperature

Temperature directly affects the production and biomass of benthic algae in streams because it influences growth rates through its control of enzyme reaction rates that in turn control photosynthetic metabolism (DeNicola 1996; Larned 2010). Most research concerned with understanding the role of temperature in regulating periphyton communities has examined correlations between temperature and seasonal changes in community structure, biomass and primary production. Water temperature is often correlated with other environmental factors that change seasonally such as light or nutrient concentrations (Larned 2010; Law 2011). Temperature therefore interacts with other abiotic factors in a complex manner to affect algal growth rates, and it is often difficult to separate the response by algae to different factors. Nevertheless, the effects of temperature should be most apparent when all other abiotic factors are optimal for maximising periphyton growth (DeNicola 1996). Several studies have measured the effects of temperature on algal production in streams. In artificial channels, Lamberti and Resh (1983) showed a 40-fold increase in chlorophyll a by artificially raising the water temperature from 20 ^{0}C to 30 ^{0}C. Based on a series of *in situ* experiments where water temperature was manipulated, Patrick (1971) found that an increase in temperature at the lower end of the range of tolerance lead to an increase in the number of species in the community as well as an increase in biomass, but that when temperatures were near optimal range, changes in diatom community characteristics were less predictable. Moving away from the limits of tolerance at either end of the range resulted in significant decreases in biomass and a severe degradation in community structure (Patrick 1971).

Although each algal species has its own optimum temperature for growth and range for survival, Several studies have shown that temperature changes generally cause shifts in the representation of

the major algal divisions (DeNicola 1996). Diatoms tend to dominate between 5 and 20 ^{0}C, chlorophytes and yellow-green algae between 15 and 30^{0}C and cyanobacteria above 30^{0}C (Wilde and Tilly 1981; Lamberti and Resh 1985, in DeNicola 1996). Also, species diversity tends to increase from approximately 0 to 25^{0}C and decrease at temperatures greater than 30^{0}C, when only a few cyanobacteria taxa dominate. Some changes in community structure with temperature at lower taxonomic levels than algal division have been observed. For example, Vinson and Rushforth (1989) found that *Achnanthes* spp. and *Navicula* spp. were most abundant at temperatures below 14^{0}C with *Cocconeis* spp. dominant above 25^{0}C and *Nitzschia* spp. dominant in temperatures as high as 39^{0}C. Much variation in the dominance of algal species at certain temperatures has been reported in the literature and therefore these generalisations are not well supported.

Regardless of the shifts in dominance in algal taxa with changes in temperature, temperatures between 10^{0}C and 30^{0}C are optimal the growth of most benthic algae in freshwater ecosystems, other than those occurring in hot water springs (Larned 2010). This suggests that temperature may be a limiting factor for algal growth under extreme conditions. The effects of temperature limitation may be mitigated to some extent by temperature acclimation. Relative to non-acclimated taxa, algae acclimated to low temperatures have higher maximum photosynthetic rates, lower temperature optima for photosynthesis and higher concentrations of enzymes for fixing carbon (Davison 1991). Nevertheless, temperature is rarely the sole limiting factor in nature (DeNicola and Hoagland 1996, Larned 2010) although it does set an upper limit for production when other abiotic factors such as light and nutrients are optimal (DeNicola and Hoagland 1996).

1.3.4 pH

The pH of a stream can influence the growth and taxonomic composition of the benthic algal assemblage (Ledger and Hildrew 1998; Sabater *et al*. 2003). Although acidified rivers are often associated with anthropogenic impacts such as mining, naturally acidic rivers, such as those in the south-western Cape of South Africa, can occur naturally as a result of drainage from acidic, fynbos-covered slopes (Davies and Day 1998). Acidification can mobilise P, providing greater availability for growth and thus promoting algal biomass (Law 2011) but humic substances often adsorb P, so availability is often lower in "humically" acid systems such as those in the south-western Cape (Prof. J. Day, Freshwater Research Unit, University of Cape Town, pers. comm.). The diatom, *Eunotia* spp. is often dominant in acidic rivers but algal species diversity is lower compared with more neutral streams (Winterbourn *et al.* 1992; Ledger and Hildrew 1998; Passy 2006). In a study of temporal changes in the benthic algae of an acidic river in the UK, Ledger and Hildrew (1998) found that, in addition to the acidobiontic diatom, *Eunotia* spp., small coccoid green algae and filamentous green algae of the group Zygnematales, were also common.

1.3.5 Stream flow

Stream flow has opposing effects on the accrual of benthic algae. These effects relate specifically to different components of the flow regime through time and space. Under low-flow conditions (i.e. the inter-flood period), differences in current speed can contribute to both accrual and loss

processes, as described by the 'subsidy-stress response' (Odum *et al.* 1979; Biggs *et al.* 1998). High flows (i.e. floods) can affect algal communities differently depending on the magnitude, frequency and timing of these events and the resistance and resilience of benthic algal communities. The response of periphyton to both high and low flows is discussed in this section.

1.3.5.1 Subsidy-stress response

The relationship between current speed and periphyton has been well documented. Both field studies and experimental research show that the highest algal biomasses occur at intermediate current speeds in most habitats (Borchardt 1996; Steinman 1996). The unimodal relationship between current speed and periphyton biomass (i.e. the subsidy-stress response) implies that two direct but opposing forces related to current affect periphyton biomass (Biggs and Hickey 1994; Biggs and Thomsen 1995). Firstly, increases in current speed from near zero flow conditions stimulate algal growth and reproduction by promoting mass transfer of nutrients to algal cells (Lock and John 1979; Dodds 1989; Poff *et al.* 1990; Biggs and Stokseth 1996; Biggs *et al.* 1998; Larned *et al.* 2004). Secondly, further increases in current velocity lead to increased shear stress, decrease in immigration rates and an increase in export rates. Beyond a certain optimal current speed when periphyton growth and loss processes reach an equilibrium, further increases in current speed result in a decrease in periphyton biomass (Biggs and Thomsen 1995; Borchardt 1996; Biggs *et al.* 1998 and others). The effects of current speed are, however, not always that simple. Horner *et al.* (1990) showed that maximum biomass occurred at a current speed of 0.6 ms^{-1}. By contrast, Poff *et al.* (1990) reported velocity optima of <0.17 ms^{-1}, while in other cases a negative linear relationship between algal biomass and current speed with peak biomass at near zero velocity has been observed (Biggs *et al.* 1998). This suggests that the effects of current speed on periphyton biomass are mediated by other factors.

Stevenson and Glover (1993) studied nutrient fluxes through low- and high-density algal communities (measured as the number of algal cells per m^2) and found that the negative effects of the increased density of periphyton mats on the transport of nutrients through the mats can be ameliorated by increases in current speed. Thus the optimal speed (or saturating velocity, above which current no longer has a positive effect on periphyton biomass) is probably higher for high-density communities than for low-density communities (Stevenson 1996). Horner and Welch (1981) suggested that periphyton accrual in response to different current speeds changes according to changes in ambient nutrient concentrations. They found that where ortho-P was in sufficient supply (above 45 µg l^{-1}); periphyton biomass increased as current speed increased with an optimal velocity at 0.5 m s^{-1}. At low P concentrations, periphyton biomass decreased steadily as current speed increased because under conditions of low nutrients, the positive advantages of current on nutrient mass transfer was outweighed by the frictional shear as speed increased. Horner and Welch (1981) showed that current speed positively affected growth rates and maximum biomass accrual mostly under high nutrient conditions. Biggs *et al.* (1998) found that the biomass of one mucilaginous diatom-cyanobacteria assemblage increased with increasing current speed as expected, presumably because of the advantages of increased nutrient mass transfer. A second assemblage with the same

growth form characteristics, however, did not respond to an increase in velocity because it was not nutrient limited. Therefore, increases in nutrient concentrations seem to enhance the positive relationship between current speed and periphyton growth but where nutrients are not limiting, increases in current speed do not provide the added advantage of increases in nutrient mass transfer to algal cells.

Besides the influence of different nutrient regimes on the relationship between periphyton biomass and current speed, Biggs *et al.* (1998) found that community growth form is also a key determinant in the response of periphyton biomass to current speed. Firstly, loosely-woven growth forms characteristic of filamentous green algal communities enable high rates of nutrient mass transfer, regardless of current speed. For communities with these growth forms, the highest biomass was found at low velocities, decreasing monotonically as a function of increasing velocity due to an increase in drag on the filaments (Figure 1.2). In a second community dominated by moderately loosely woven stalked diatoms and short filamentous taxa, biomass increased with current speed due to increased nutrient mass transfer, reaching a unimodal peak at near-bed velocities of 0.23 m s^{-1} and then declined sharply at current speeds of 0.41 to 0.45 m s^{-1} (Figure 1.2). In a third community characterised by mucilaginous diatoms mats, lowest biomass was recorded at near-zero near-bed velocities but increased monotonically as near bed current speed increased to 0.6 m s^{-1} (Figure 1.2). This supports the notion that prostrate taxa are less vulnerable to shear stress, and thus sloughing, than filamentous forms. Therefore, prostrate taxa may be more reliant on current speed to promote nutrient mass transfer than upright or erect taxa (Biggs *et al.* 1998).

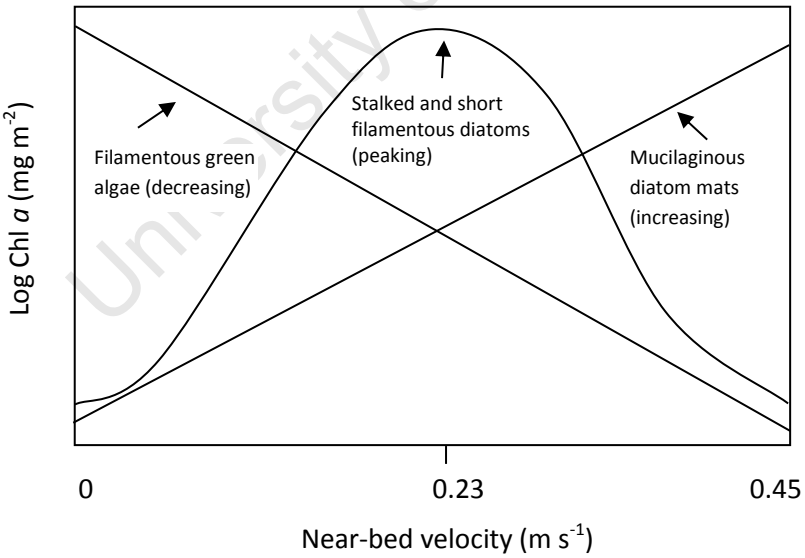


Figure 1.2 **Biomass responses to spatial variations in current speed in streams by three different periphyton communties (adapted from Biggs 2000b)**

Periphyton growth form, mat density and nutrient status influence the role that current speed plays on the growth and reproduction of algae, and thus the accrual and loss of biomass *per se.* Current speed also influences benthic algal immigration and export rates, however, thereby directly affecting biomass (Stevenson 1996). Peterson and Stevenson (1989) found that immigration rates were 10

times higher in current speeds of 0.12 m s^{-1} than those in 0.28 m s^{-1} as algal cells are less able to establish under higher current speeds. Also, small algal cells seem to be most effective at colonizing areas of fast flow whereas both large and small taxa colonise areas of slow flow (Stevenson 1996). Similarly, export rates of benthic algae (or emigration) increase with an increase in current speed (Horner *et al.* 1990). Stevenson (1996), however, stated that the relationship between benthic algal export and current is probably not as simple as that between benthic algal immigration and current speed. This is because export is probably also affected by the physiological condition of benthic algae, which deteriorates as the mat thickens and as algal cells within the mat become light- and nutrient-limited.

Spatial differences in patterns of periphyton community structure and biomass at the biotope level, as well as colonisation processes following disturbance are probably affected primarily by differences in current speeds (Stevenson 1996). These patterns are discussed further in section 1.4.

1.3.5.2 Flood events (Hydrological disturbance)

Disturbance theory has been debated extensively in the freshwater ecological literature, largely because natural events such as flooding do not fall neatly into the classical definitions of ecological disturbance. A classical definition that has been widely used but subject to debate by river ecologists is that given by White and Pickett (1985). They define disturbance as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resource, substrate availability or the physical environments". This definition emphasises (a) the requirement for biotic consequence and (b) the lack of reference to scale and the need to specify the spatial or temporal extent of disturbance (Stanley *et al.* 2010). Resh *et al.* (1988) addressed this issue by highlighting the need to quantify disturbance in terms of the physical nature of the event such as the magnitude of a flood. However, they also stipulate that disturbance includes only unpredictable events (Resh *et al.* 1988). In the case of benthic algal communities in south-western Cape rivers, this would preclude floods from the definition of disturbance. However, Larned (2010) pointed out that the predictability of events is irrelevant for immobile periphyton because no clear thresholds separate predictable and unpredictable disturbance events. In a recent review of disturbance in stream ecology, Stanley *et al.* (2010) emphasised that disturbance should be quantified simultaneously by physical measures of the event itself (e.g. flood intensity and duration) as well as the biotic response. Furthermore, the relevant spatial and temporal scales at which the physical force and biotic response are measured should be specified (Poff 1992). Hydrological disturbance defined by different levels of physical force may therefore result in a variety of biological responses including resistance (i.e. the ability to withstand displacement by disturbance), resilience (the ability to return to the pre-disturbance state) and/or reductions in populations (Peterson 1996; Ractliffe 2009). As stated by Larned (2010), ecologists generally define physical disturbances as "episodic events that remove organisms at rates faster than rates of accrual or recruitment". This definition is considered the most applicable to the effects of hydrological disturbance on periphyton communities in this study.

Periphyton in rivers can be affected by a number of disturbance factors, including desiccation, anoxia, freezing, acute contaminant exposure, bed movement, rapid increases in temperature and

light, as well as hydraulic forces (Peterson 1996; Larned 2010). Substratum movement and rapid increases in hydraulic forces induced by flood events are the most relevant to this study and are therefore discussed further.

Flood disturbance can greatly reduce periphyton biomass and alter community composition (Grimm and Fisher 1989; Uehlinger 1991; Peterson 1996; Francoeur and Biggs 2006; Holomuzki and Biggs 2006; Webb *et al.* 2006). In some rivers, floods are considered the dominant organising factor affecting spatial and temporal patterns (see Section 1.5) in periphyton community structure and biomass (Resh *et al.* 1988; Poff and Ward 1989; Biggs 1995).

Flood events affect periphyton community structure through a number of different physical mechanisms associated with increases in hydraulic forces. Firstly, increased stream velocity during flood events increases shear stresses and drag, which remove algal cells directly (Biggs and Thomsen 1995; Biggs *et al.* 1999). Secondly, flood events can mobilise the sediment which in turn leads to abrasion of periphyton mats by suspended sediment (Biggs and Close 1989; Biggs *et al.* 1999; Francoeur *et al.* 1999; Bond and Downes 2000; Francoeur and Biggs 2006; Webb *et al.* 2006). Thirdly, flood events can result in bed movement where periphyton are directly scoured by abrasion as substrate particles tumble, or indirectly affected by light deprivation as stones are overturned and algal mats are buried (Power and Stewart 1987; Power 1990; Biggs *et al.* 1999; Holomuzki and Biggs 2006).

Depending on the size of the flood event, sediment load and propensity for substratum movement, shear stress acts either alone or in concert with abrasion by sediments and bed movement to remove periphyton. Based on laboratory experiments, Horner *et al.* (1990) reported that small increases in sediment load caused some loss of benthic algal biomass but did not change the community composition. Biggs and Smith (2002) found that, in rivers with high bed mobility, periphyton species diversity declined following a flood event but in rivers with highly armoured beds, even the most intense floods had no effect on periphyton communities. In a laboratory experiment, Francoeur and Biggs (2006) found that although increased velocities alone removed benthic algae, high suspended sediment concentrations together with increased velocities were more effective at algal removal. In contrast to these findings, however, simulated flood experiments undertaken by Heinlein (2000), cited in Francoeur and Biggs (2006) reported that suspended sediment scour was less influential than water velocity in removal of benthic algae. Although the relative effects of increased shear stress and sediment abrasion on periphyton have received some attention in recent years (Biggs and Thomsen 1995; Heinlein 2000; Francoeur and Biggs 2006; Webb *et al.* 2006), only a few studies have quantified the effects of disturbance-induced light deprivation as a result of overturned stones and burial of algal mats. Of these, Power (1990) found that the degree to which productivity is compromised after burial in fine sediments immediately after a flood is directly related to the amount of sediment deposited. Nevertheless, it seems that sediment abrasion and tumbling dominate the negative effects of floods on periphyton (Larned 2010). Therefore, flood events of equal velocity and hydraulic forces at the bed level affect periphyton

differently because sediment supply and bed mobility can be considerably variable across river systems (Biggs *et al.* 1999; Biggs *et al.* 2001).

For any given periphyton community, it appears that a hydraulic threshold exists, beyond which most of the biomass is lost within a short duration following the point at which the hydraulic threshold is reached. In their laboratory experiments, Biggs and Thomsen (1995) found that as long as flows were above the hydrological threshold, almost all biomass loss occurred within the first 10 minutes of their experimental trials. Similarly, Francoeur and Biggs (2006) found that 83 to100 % of biomass was lost within the first five minutes of disturbance, reaching an asymptote at around 30 minutes with no further loss beyond that point. These studies suggest that flood intensity is more influential in periphyton removal than flood duration.

The biotic response of periphyton to flood events is not only dependent on the physical mechanisms that potentially remove periphyton during floods, but also on the physiognomic, taxonomic and physiological properties of the periphyton community itself (Peterson 1996). Indeed, Biggs and Thomsen (1995) showed that resistance to flooding varied significantly between communities with different species composition and physiognomy i.e. communities with filamentous green algae and diatoms were less resistant to physical forces of flooding compared with communities dominated by adnate diatoms. Also, a community composed of a thin layer of diatoms may be resistant to increased velocities but vulnerable to removal by sediment abrasion (Blenkinsopp and Lock 1994; Biggs and Thomsen 1995; Peterson 1996). Thus, the relative effect of sediment scour versus increased velocities in removing algae may be greater in adnate communities.

Francoeur and Biggs (2006) reported that the efficacy of biomass removal by flood events with and without sediment abrasion was community-specific, even when the initial biomass and dominant taxa of the communities was similar. The communities in their study differed however in their attachment strength, such that removal of loosely attached communities increased strongly with increasing suspended sediment concentrations but removal of tightly adherent communities was low and unrelated to sediment concentrations. They suggest that the physiological state of the same species between these communities may have differed such that algal taxa at the base of the periphyton mat in the loosely adherent taxa were “less healthy” (possibly due to light and/or nutrient limitation) and therefore more vulnerable to flood events (Peterson 1996; Francoeur and Biggs 2006). Thick mats with loose attachment (because of basal cell senescence) usually develop when disturbance events are infrequent. Therefore susceptibility of periphyton communities to flooding is greater where flooding is less frequent (Larned 2010).

Besides resistance to shear stress and sediment abrasion, some taxa are resistant to burial by sediments. Although the ability of benthic algae to resist such disturbance is not well studied, Peterson (1996) suggests that taxa tolerant of low light at the base of well developed mats are likely to exhibit resistance to disturbance-induced light deprivation. Resistance to sediment burial, however, may be more dependent on anoxia, rather than light deprivation (Peterson 1996). In his review of algal response to floods, Peterson (1996) reported that the most resistant periphyton communities are those dominated by an interwoven matrix of filamentous cyanobacteria, those

covered by thick cohesive cyanobacteria surface films (Peterson *et al.* 1990) or those with relatively uniform surfaces (Blenkinsopp and Lock 1994). These include adnate diatoms and the basal cells of chlorophytes (Larned 2010). Extreme flood events remove the majority of periphyton biomass, regardless of the physical mechanisms of removal and community characteristics. These community characteristics therefore become increasingly influential in dictating the immediate effects of scour as flood intensities decline (Peterson 1996).

Whereas resistance to flooding is a response to an actual disturbance event, resilience is a response to the cessation of flooding. Resilience is a function of recolonisation or regrowth from persistent cells and subsequent community succession. Recolonisation and succession are discussed in section 1.4.1.1, which deals with short term temporal patterns of periphyton in rivers.

1.3.6 Grazing

Research using both field and laboratory experiments provides strong evidence that grazing can have a significant impact on periphyton biomass and community structure. In particular, an extensive meta-analysis of herbivory on algal resources (Feminella and Hawkins 1995) showed that herbivores strongly regulate algal resources in streams. Their study contradicted a popular belief that stream communities are regulated primarily by abiotic factors (Steinman 1996). In his extensive review on the effects of grazers on freshwater benthic algae, Steinman (1996) found that algal biomass almost always declines in the presence of herbivores. Grazers responsible for a decline in algal biomass include snails (e.g. Steinman *et al.* 1987; Lowe and Hunter 1988; Underwood and Thomas 1990; Rosemond *et al.* 1993), aquatic insects (e.g. Colletti *et al.* 1987; Jacoby 1987; Hill and Knight 1988; Lamberti *et al.* 1992; Poff and Ward 1995; Holomuzki and Biggs 2006), crustaceans (Flint and Goldman 1975; Pringle *et al.* 1993), fish (e.g. Stewart 1987; Power *et al.* 1988) and tadpoles (e.g. Lamberti *et al.* 1992; Peterson and Boulton 1999).

Despite the large volume of literature showing a decline in periphyton biomass in the presence of grazers, there are exceptions to this general pattern, where algal biomass either remains unchanged (Jacoby 1987; Feminella *et al.* 1989; Karouna and Fuller 1992) or increases (Lamberti and Resh 1983; McCormick and Stevenson 1991) in response to grazers. Steinman (1996) indicated that the outcome of a periphyton-grazer interaction (i.e. the extent to which periphyton biomass is regulated by grazers) depends on algal traits and the herbivore involved. Algae with spiny armament or a low-lying prostrate growth form, for example, are difficult to consume (Luring and Beekman 1999), while some algae produce chemicals that deter herbivores. Cyanobacteria in particular, produce microcystins or saxitoxins that render them unpalatable to certain herbivores (Holomuzki *et al.* 2010). Certain algal taxa form colonies that increase their size and thus exclude certain herbivores. *Scenedesmus*, for example, forms an eight-celled colony (or coenobium) thereby providing size refugia from certain grazers as a defence against herbivory (Luring and Beekman 1999). Some algal taxa produce mucilage that can be a defence against herbivory (Malej and Harris 1993). In some instances grazers can limit producer biomass to extremely low levels such that periphyton reaches a spatial refuge from grazing (McIntire *et al.* 1996) and no further reduction in biomass is measurable. The risk of consumption is therefore dependent on the palatability and availability of benthic algae.

Herbivores too exhibit specific traits that determine the extent to which periphyton is regulated by grazing. In particular, the mode of feeding influences what algal species and growth forms are edible (Holomuzki *et al.* 2010). Some trichopteran larvae, for example, have scraping mouthparts best suited to feeding on low-profile, tightly attached algae whereas heptageniids and leptophebiids generally have brushing and gathering feeding structures and therefore tend to feed on loosely attached portions of the periphyton mat. The type of grazer therefore not only affects periphyton biomass but also profoundly influences the resulting periphyton community structure and growth form. Of the different feeding modes, it appears that rasping and scraping grazers most affect algal biomass and community structure (Holomuzki *et al.* 2006) because in the process of rasping and scraping, much of the overstory forms are dislodged from the substratum and exported downstream (Rosemond *et al.* 2000). In instances where grazers tend to harvest upright, overstory or loosely attached algal taxa, significant shifts in benthic communities are common, moving towards one that is characterised by prostrate, understory forms (Holomuzki and Biggs 2006). Communities dominated by understory forms can actually flourish under these conditions because of the competitive release from overstory forms (Power *et al.* 1988).

Foraging behaviour and mobility of grazers can also have a profound effect on periphyton biomass and community structure. Lamberti *et al.* (1987) found that while baetid nymphs, trichopteran larvae and molluscs all reduced periphyton biomass in their study, trichopteran larvae had the greatest impact on periphyton biomass, despite low densities of this grazer. They attributed this result to the greater mobility (relative to molluscs) and consumption rates (relative to baetid nymphs) of foraging trichopteran larvae.

Increases in algal biomass in response to grazing are rare and restricted to situations with low grazer densities (Steinman 1996). Herbivory can result in nutrient regeneration from within the periphyton matrix, and so in cases of nutrient limitation, the positive response from more available nutrients may outweigh the negative response of herbivory.

1.4 PATTERNS OF BENTHIC ALGAE IN RIVER ECOSYSTEMS

Rivers are characteristically heterogeneous systems that are variable in pattern and process over time and space (Poff and Ward 1990, Palmer and Poff 1997). The complex interplay of abiotic and biotic factors discussed in the previous section operate over a wide range of spatial and temporal scales, resulting in heterogeneous patterns of periphyton community composition and biomass both at fine scales measured within microhabitats and over days, and at broad scales defined over years and between catchments (Stevenson 1997).

1.4.1 Temporal patterns

1.4.1.1 Short-term: recovery following disturbance

Experiments on artificial substrata or in artificial channels (e.g. Poff *et al.* 1990) and changes in periphyton community dynamics following a disturbance in natural streams (e.g. Stevenson 1990) both indicate that periphyton characteristically follows an initial phase of biomass accrual after a

flood either through immigration/colonisation of propagules from upstream or growth/reproduction of residual cells remaining or a combination of both (Poff *et al.* 1990; McCormick and Stevenson 1991; Biggs 1996; Larned 2010). Following a point at which the algal assemblage reaches peak biomass, loss processes dominate the assemblage, through death, emigration, sloughing and grazing later in the sequence (Poff *et al.* 1990; Biggs 1996) and the carrying capacity is reached when rates of accrual and loss are in balance (Biggs 1996).

Short-term patterns in periphyton development are influenced by a number of abiotic and biotic factors. Firstly, the rate of colonisation is governed by the size and type of the propagule pool from upstream refugia; the near-bed velocity, which might impede settlement of new cells; and substratum texture. Also the relative contribution of either colonisation or growth/reproduction to initial recovery depends on the severity of the disturbance event and, if this was a flood, on whether cells were left on rocks afterwards. Despite these variations, which could lead to very different initial algal assemblages, Korte and Blinn (1983) proposed that colonisation and succession of lotic algal assemblages follow a predicable sequence during the accrual phase. Their model states that an organic film of detrital mucilage, bacteria and fungi develops and conditions the substratum within a period of hours following disturbance. Within days, adnate diatoms become established in a 2-dimensional matrix, followed by a 3-dimensional matrix of algae, bacteria, fungi and polysaccharides. While successional sequences predicted by model have been observed (Steinman and McIntire 1987; Peterson *et al.* 1990; Peterson 1996), such a sequence of events is not inevitable. Poff *et al.* (1990), for example, demonstrated the absence of a diatom-to-filamentous-algae taxonomic sequence in their study using artificial substrata at four different current regimes in the upper Colorado River. *Ulothrix zonata*, a green filamentous species dominated both fast- and slow-flowing treatments for the first two weeks of their experiment. Thereafter diatoms became dominant and remained dominant in the fast flowing treatments, while cyanobacteria became dominant only in the slow-flowing experimental channels at the end of the 42-day study period.

Patterns of recovery and succession may simply be a result of differences in the starting point of the succession trajectory as determined by recolonisation only or recolonisation and regrowth/reproduction processes. In the case of studies using artificial substrates or following a catastrophic disturbance event that removes all living periphyton from the substratum, colonisation from upstream refugia dominates recovery. Following less severe disturbance events, however, resilient taxa remain after disturbance has largely cleared the substratum of more vulnerable taxa. Regrowth from residual cells often leads to early dominance by these taxa and thus the trajectory of recovery will differ between patches where the initial phase of biomass accrual is reliant on colonisation of propagules from upstream or regrowth and reproduction of residual cells. Also, the time taken to reach peak biomass is largely governed by whether or not initial recovery is driven by new colonists or by regrowth and reproduction of residual cells or a combination of both. For example, Stevenson (1990) showed that benthic algae in Wilson Creek, a third- order cobble-bed river in Kentucky, USA, recovered to maximum standing crops within 10 days after a storm caused bank-full discharge. He attributed rapid recovery to reproduction and regrowth of residual cells following the storm event. However, under more severe flood conditions, time to peak biomass can

take as long as 100 days (Biggs and Stokseth 1996). Therefore, the severity of disturbance is an important factor to consider when assessing the pattern of recovery in benthic algal assemblages.

Differences in the resistance of certain taxa to disturbance can be an important factor influencing short term patterns of recovery. Taxa with a low profile, those with strong adhesion and those with a tightly interwoven structure are the most resistant to flood disturbance (Peterson 1996). Low-profile taxa include prostrate algae such as the diatoms *Achnanthes* and small *Navicula* spp. while those that are tightly adherent include the basal cells of heterotrichous green algae such as *Stigeoclonium*. Interwoven mats of filamentous cyanobacteria are also resistant to flood disturbance (Biggs and Hickey 1994). By contrast, filamentous green algae such as *Spirogyra* spp. attach loosely to substrata and are therefore susceptible to removal by flood disturbance (Power and Stewart 1987).

Besides morphological differences between taxa, certain life history traits are also influential to the pattern of recovery in algal assemblages, McCormick and Stevenson (1991) suggest that early successional species exhibit adaptations for maximising early growth that are differentially favoured in different current regimes. For example, their research showed that *Surirella ovate* was a fast immigrating and early successional dominant in fast flows because it has a high probability of invading from the plankton (high immigration coefficient). By contrast, *Gomphonema angustatum* was an early successional dominant in slow-flow habitats because of its dominance in the drift, despite its low immigration coefficient. Also, higher net growth rates tend to characterise late-successional taxa rather than those that occur early in succession.

A number of studies have shown that grazing affects the trajectory of recovery following hydrological disturbance (Poff and Ward 1995; Hart and Finelli 1999; Rutherford *et al.* 2000; Opsahl *et al.* 2003; Bertrand *et al.* 2009). Steinman *et al.* (1987) for example found that caddisflies and snails were able to remove overstory algal forms which led to a community of prostrate algae. Grazing activity was therefore able to inhibit succession. In an *in situ* experiment on an open-canopied mountain stream, Poff and Ward (1995) also found that grazers essentially maintained the periphyton community in an early successional state. The effects of grazers on succession may however depend on the characteristics of both grazers and periphyton (Steinman 1996; Rosemond *et al.* 2000). DeNicola *et al.* (1990) showed that baetids were able to change algal succession when introduced at the start of the successional process but had no effect when introduced 16 days after the start of succession.

Besides the role of differences in the processes that govern biomass accrual early in the accrual cycle, growth and reproduction are dependent on the availability of resources, mainly light and nutrients, together with temperature and on modes of reproduction (Bothwell 1989; Biggs 1996). Although one would expect the time to peak biomass to be shorter in enriched than unenriched conditions, this is not always the case because, in nutrient poor conditions, diffusion of nutrients through the algal mat to basal cells becomes limiting, resulting in death of these cells and thus sloughing of the algal mat early in the accrual cycle (Lohman *et al.* 1991; Lohman *et al.* 1992; Biggs 1996). Nevertheless, the maximum standing crop at the time of peak biomass is usually greater in

enriched environments under similar velocity, light and temperature conditions (Biggs and Close 1989; Biggs 1995). Also, values for peak biomass vary significantly depending on the periphyton assemblage structure at this time. Values between 300 to 400 mg m^{-1} chlorophyll *a* have been reported for peak biomass assemblages dominated by diatoms and cyanobacteria (Bothwell 1989 and Horner *et al.* 1990) whereas values in excess of 1200 mg m^{-1} chlorophyll *a* have been recorded for filamentous green and chrysophyte algae (Biggs 1995). Eventually loss processes through death due to age, parasitism and disease dominate. Autogenic sloughing as a function of resource stress of the underlying layers often occurs, resulting in very rapid loss in biomass.

1.4.1.2 Medium- to long- term (2-15 months)

In his review of medium- to long term-term temporal patterns in periphyton dynamics in rivers, Biggs (1996) argued that these patterns tend to reflect the outcome of flood frequency and its interaction with nutrient and light resources (Biggs 1996). These temporal patterns follow one of three main generalisations as follows: a) relatively constant low biomass throughout the year; b) cycles of accrual and sloughing in streams with a moderate frequency of flood events and moderate supplies of nutrients and light, c) seasonal growth with intervening period of moderate to low biomass (Biggs 1996; Biggs 2000b).

A) Relatively constant, low biomass throughout the year

Relatively constant, low biomass can occur under conditions of frequent flooding (i.e. about every 10 days) such that biomass never has the opportunity to grow and accrue. The disturbance created by frequent flooding therefore overrides the positive effects of nutrients and light on the growth and accrual of periphyton biomass (Biggs 1995). Periphyton communities in these spate-prone streams are usually dominated by prostrate (mainly diatom) taxa that are less vulnerable to abrasion and turbulent flow than more complex, upright taxa (Bergey and Resh 2006; Francoeur and Biggs 2006). In some situations, relatively constant low biomass is the result of particularly low supply of nutrients and light (Biggs 1995) which limit the growth and accrual of periphyton biomass, regardless of the flood regime characteristic of the system. Rivers with a high flood frequency are often oligotrophic (Biggs and Close 1989) and it is often difficult to establish whether biomass accrual is constrained by disturbance events or resource supply in these systems (Biggs 1996). There are also situations where flooding is so infrequent that high densities of invertebrate grazers develop (Rosemond 1994), such that grazing is the dominant regulator of biomass accumulation. Intense grazing activity can therefore prevent periphyton accrual and then the periphyton mat is usually dominated by grazing-resistant diatoms such as *Achnanthidium, Cocconeis, Cymbella, Synedra,* the basal structures of *Stigeoclonium* and tightly adhering cyanobacterial crusts (Biggs and Gerbeaux 1993; Rosemond 1994). Rosemond *et al.* (2000), however, suggest that the importance of any given factor in maintaining periphyton biomass at these low levels can shift seasonally. They found that that grazing, nutrients and light availability all limited the development of periphyton mats. Although grazing by snails seemed to depress periphyton biomass year round, light availability was limiting in autumn and summer but not in spring when nutrients were limiting.

B) Cycles of accrual and sloughing

In streams with a moderate frequency or seasonal flood events that allow for extended periods of flow stability (i.e. > 1month) and at least moderate supplies of nutrients and light, periphyton mats can develop with a succession in taxonomic structure (Fisher and Grimm 1988; Biggs and Close 1989, Uehlinger 1991; Yang *et al.* 2009). Floods do not always result in complete removal of biomass and removal is dependent on the flood intensity, resistance to scour, and pre-flood biomass (refer to Section 1.4.4.2). The rate of recovery can therefore vary quite considerably. Stable periods between disturbance events result in the accrual of biomass. In some systems, periphyton biomass accrues rapidly during the window of time immediately following a major flood when invertebrate densities are considerably reduced. Algae can grow and accumulate unconstrained by grazing pressure during this window, because invertebrate recolonisation and reproduction is slower than that of algae, but eventually biomass losses are observed either through increases in grazing pressure as invertebrates recover (Power 1992), or through autogenic sloughing (Biggs and Close 1989). Eventually, grazing may become the dominant regulator of algal biomass during the interflood period. This suggests that factors regulating algal biomass shift from "abiotic" to "biotic" with time since the last flood disturbance. However, Biggs (1996) adds that the shift from abiotic to biotic control of algal biomass is yet to be demonstrated in many streams.

C) Seasonal patterns in the growth with intervening periods of moderate to low biomass

Biggs (1996) suggests that seasonal differences in the growth and biomass of periphyton is mediated by seasonality in the disturbance regime, grazer densities, or light availability. A number of studies have found that hydrological disturbance correlates with seasonal patterns in periphyton biomass (e.g. Duncan and Blinn 1989; Junior *et al.* 1991; Sabater and Sabater 1992; Tornes and Sabater 2010), whereas others have shown that the resource availability is the dominant factor determining seasonal patterns in stream periphyton community structure and biomass (e.g. Hill and Dimick 2002; Bernhardt and Likens 2004). For example, Sabater and Sabater (1992) found that periphyton biomass in a temperate stream in Spain peaked in mid-summer, following high discharge events during spring. They concluded that the hydrological regime governs seasonal changes in periphyton biomass in these systems. By contrast, Hill and Dimick (2002) found that primary production was greatest in the spring, with a smaller, secondary peak in autumn, separated by a mid-summer minimum in algal production. In these Tennessee streams, this pattern was primarily attributed to changes in light availability due to riparian leaf dynamics. During the spring prior to leaf-fall, light is unlimiting and conditions favour the growth and accrual of periphyton biomass. After leaf emergence, periphyton biomass decreased during the summer when light becomes limiting and then increases again in the autumn after leaf fall (Hill and Dimick 2002).

Flood disturbance frequently interacts with resource availability or grazers to exhibit seasonal patterns in periphyton communities. This scenario is typical of temperate streams in Mediterranean climates, which are characterised by long periods of hydrological stability during the summer when rainfall is low and temperatures are high. These periods are interrupted by floods during the cold winter months. In these systems, periphyton communities undergo an annual cycle where abiotic

forces dominate during the winter, whilst biotic forces are more important controllers of periphyton communities in summer (Tornes and Sabater 2010). In particular Power (1992) found that the control of periphyton in certain Californian streams shifts from hydrological disturbance during the winter season to grazer control during the dry, hydrologically stable summer season. Considering the general shift in abiotic variables between seasons in temperate rivers, it is likely that hydrological disturbance, resource availability and grazers are all important in determining seasonal patterns in stream periphyton in temperate streams such as cobble-bed foothill rivers in the Mediterranean climate of the south-western Cape.

1.4.2 Spatial patterns

Spatial patterns in periphyton community structure have been studied at the scale of habitat type (or hydraulic biotope[3]) within a reach, between river reaches and across catchments. Because spatial patterns in periphyton are generally governed by the spatial distribution in environmental factors that vary across habitats, river reaches and catchments, periphyton communities tend to be structured as a mosaic of patches that together create patterns at various scales (Matthaei *et al.* 2003).

1.4.2.1 Small scale: biotopes

At the smallest scale, differences in periphyton biomass and community structure can be observed between areas with different substratum and flow conditions characterising different hydraulic biotopes. Particle size is often correlated with stability of the substratum. Thus, biomass is generally lower on smaller, less stable sandy substrata while the highest biomass is often recorded on boulders or bedrock (Biggs 2000a, b). There are situations where thick algal mats can develop on fine sand and silts but this only occurs at very low flows when silts and sand become stable. Diatoms are usually the first colonisers of these substrates but cyanobacterial mats may eventually develop. These mats often bind the sand or silt particles and can form a significant biomass. For example, thick mats of *Phormidium*, a filamentous cyanobacterial taxon, can become established in areas with very low velocity during the summer in New Zealand (Biggs 2000a, b). The importance of substratum type under different flow conditions was recently highlighted by Tornes and Sabater (2010). They found that the differences in algal communities between different substrata were greatest during the high flow period (winter), possibly because the differences in stability between different substrata are greatest at high flows.

Differences in periphyton communities between biotopes such as riffles, runs, pools and slack-waters with similar substrata generally reflect spatial differences in near-bed velocities (hence shear stress) and nutrient mass transfer (see Section 1.3.5.1). Although several studies have addressed differences in community structure between habitats with different flow characteristics using experimental channels (Poff *et al.* 1990; Horner *et al.* 1990; Humphrey and Stevenson 1992; Stevenson 1996; and others), few have published data on differences between biotopes *in situ*.

[3] Hydraulic biotope is defined as a spatially distinct instream flow environment determined by the hydraulic and substratum characteristics of the river channel (Rowntree and Wadeson 1999).

Biggs (1996) suggested that patterns of biomass accrual between biotopes differ depending on whether a river is nutrient enriched or not. Biggs (1996) elaborated further by proposing that in enriched rivers, higher periphyton biomass will develop in slower flowing runs, slack-waters and pools, compared with faster flowing riffles where higher shear stress restricts periphyton mat development. By contrast, he proposes that highest biomass in unenriched rivers will develop in riffles compared with runs, slack-waters and pools because increased mass transfer of metabolites will promote mat development in faster flowing conditions where ambient nutrient concentrations are low (Biggs 1996).

1.4.2.2 Medium scale: patterns between different river reaches

The River Continuum Concept (RCC)(Vannote *et al.* 1980) predicts an increase in the biomass of primary producers down the length of a stream, reaching a maximum in mid reaches and then decreasing again in the lower reaches. While data from undisturbed river systems support the RCC (Cushing *et al.* 1983), naturally disturbed systems such as those characteristic of Mediterranean climates show erratic longitudinal patterns in benthic algal biomass (measured as chlorophyll concentrations) that do not support the RCC (Sabater and Sabater 1992). Sabater and Sabater (1992) argued that rivers with a highly variable hydrological regime are more likely to exhibit a patchy distribution in both biological and environmental variables at a local scale, which govern spatial patterns in benthic algae. Also, the RCC applies largely to streams with forested headwaters, where light availability in the upper catchment limits algal growth. Wiley *et al.* (1990) showed that rivers traversing prairies and grassland ecotypes do not support the RCC. Similarly, the validity of the RCC to open canopied fynbos streams in the south western Cape of South Africa has been questioned (King *et al.* 1988; Davies and Day 1998).

1.4.2.3 Broad-scale: inter-catchment patterns

Assessment of broad-scale regional patterns in periphyton biomass and community composition emphasises the importance of recognising the hierarchical structure of factors that regulate periphyton in lotic ecosystems (Stevenson 1997). In a regional study of New Zealand rivers, Biggs (1990) found that composition and biomass of periphyton communities were linked primarily to catchment geology, with land use of secondary importance. Leland and Porter (2000) found that catchment geology was a significant factor contributing to variation in benthic algal community structure but that most of the variation could be attributed to broad-scale differences in land use across the catchment.

Biggs (1995) showed that the effects of nutrients, light and stream size on periphyton are only apparent after accounting for large scale "ultimate variables" such as geology and climate. He showed that broad-scale patterns in mean monthly periphyton biomass are defined primarily by the frequency of flood disturbances, the proportion of the catchment in high intensity agricultural land-use and the proportion covered by alkaline rocks. That study also demonstrated a strong interaction between disturbance and enrichment in determining broad scale spatial patterns. For example, where flood disturbances are very frequent, and/or levels of enrichment are very low, then mean

monthly biomass is very low. Under the same disturbance regime but with greater enrichment, mean monthly biomass is greater because the regeneration of algae between floods is greater as enrichment increases. He concluded that that flood disturbance and catchment enrichment regimes could therefore be used to explain and predict broad-scale difference in periphyton development among other temperate stream ecosystems.

1.5 BENTHIC ALGAE IN BIOLOGICAL MONITORING

McCormick and Cairns (1994) commented that although algae are an ecologically important group in freshwater ecosystems, they are often ignored as indicators of ecosystem change. In recent years however, the usefulness of benthic algal assemblages for monitoring of ecosystem change has been recognised and incorporated into many management initiatives internationally, particularly in New Zealand (Biggs 2000a, b; Biggs and Kilroy 2000; Bierwagen *et al.* 2007), Australia (Burns and Ryder 2001; Ryder and Mascarenhas 2007) and the United States (Hill *et al.* 2000).

Biotic indices derived from the presence/absence of diatoms species at a site have received considerable attention in the literature (e.g. Round 1991; Kelly *et al.* 1998; Rott *et al*. 2003) and are widely used for water quality assessments (e.g. Round 1991; Eloranta and Soininen 2002; Kwandrans *et al.* 1998). Nevertheless, Chessman *et al.* (1999) argue that "the conceptual basis for biological assessment of rivers has gradually shifted from the notion of biological indicators of water quality to that of indicators of 'biological integrity[4]' or 'ecosystem health[5]'". Biological integrity and ecosystem health are determined not simply by water quality conditions, but by energy sources, habitat heterogeneity, flow characteristics and biological interactions which interact with water quality to define the condition of a river (Chessman *et al.* 1999).

Biological assessments of ecosystem integrity or health in South Africa are based largely on indices developed for macro-invertebrates, riparian vegetation, fish and habitat characteristics. Diatom indices are currently being developed and tested throughout South Africa (De la Rey *et al.* 2004; Taylor *et al.* 2007a,b) but their purpose is mostly confined to detecting water quality changes, rather than ecosystem integrity or health.

Effective indicators need to be applicable across a wide range of freshwater habitats, to have a wide range of quantitative attributes, to respond to change in disturbance regimes at spatial and temporal scales relevant to river management, to have a scientific basis, and to be cost-effective (Burns and Ryder 2001). The short generation time and sessile nature of periphyton make it particularly suitable as a monitoring tool for detecting nutrient- and flow-related changes (Burns and Ryder 2001; Ryder and Mascarenhas 2007). Many algal taxa have specific environmental tolerances and preferences such that the entire assemblage represents an information-rich system for environmental monitoring (Lowe and Pan 1996). Also, changes in periphyton taxonomic composition and biomass have been shown to follow even slight nutrient enrichment and/or

[4] Biological integrity is defined as 'the capacity to support and maintain a balanced, integrated, adaptive biologic system having the full range of elements and processes expected in the natural habitat of a region' (Karr 1995).

[5] Ecosystem health is defined by a set of societal goals for ecosystem condition (Chessman *et al.* 1999).

changes in the flow regime (Hildrew and Giller 1994; Biggs 1995; Biggs and Kilroy 2000), and therefore, are usually the first organisms to respond to and recover from stress (Lowe and Pan 1996; Reavie *et al.* 2010).

Although diatoms have been recognised traditionally for their usefulness as water quality indicators, diatoms as indicators of ecological integrity of rivers have been tested and applied, mainly in Australia (Chessman *et al.* 1999; Growns and Growns 2001) and the United States (Blinn and Herbst 2003; Wang and Stevenson 2005). Growns and Growns (2001), for example, used indicators of both macro-invertebrates and diatoms to demonstrate that altered hydrology, rather than altered water quality, was the primary cause for degradation of ecosystem integrity of the Hawkesbury-Nepean River system in Australia. In a study of Californian rivers, Blinn and Herbst (2003) assessed the use of both diatoms and soft algae as indicators of environmental conditions. They found that diatoms were better indicators of stream conditions than soft algal communities, although some green filamentous taxa were useful indicators of impaired ecological integrity.

Besides indicator taxa among diatoms or the periphyton community as a whole, taxonomic descriptors such as diversity indices and taxon richness have been described and used in assessments by several authors (e.g. Weitzel 1979 cited in Hill *et al.* 2000) but their usefulness as indicators of anthropogenic change has been questioned. For example, Patrick (1977) demonstrated that assemblages with similar diversity scores could represent rivers with significantly different chemical conditions. Also, some unpolluted streams exhibit naturally low species diversity (Hill *et al.* 2000). Herbst and Blinn (2007) found that diversity was greater at disturbed sites that at undisturbed sites. Indeed, many studies have shown that the chemical enrichment associated with mild or moderate human influence stimulates diatom species richness (e.g. Marcus 1980; Chessman 1986; Lobo *et al*. 1995; Juttner *et al.* 1996). Based on extensive temporal and spatial surveys across New Zealand, thresholds of change in periphyton biomass are used to determine trophic status of lotic ecosystems and thereby detect shifts in ecosystem condition (Biggs and Kilroy 2000). Also, the autotrophic index (AI) (which is a measure of the proportion of photosynthetically active matter *vs.* heterotrophic matter within a periphyton mat) has been used to detect shifts in river condition in the United States, New Zealand and Australia (Lowe and Pan 1996; Biggs and Kilroy 2000; Burns and Ryder 2001). Another commonly cited parameter is the phaeophyton: Chl *a* ratio which can be used as a measure of algal senescence within the periphyton mat as an indicator of stress (Peterson and Stevenson 1992; Burns and Ryder 2001). In Australia, monitoring programmes combine both structural (i.e. taxonomic and biomass parameters) and functional attributes such as metabolism and food web interactions, allowing the best assessment of impacts in riverine systems (Burns and Ryder 2001; Ryder and Mascarenhas 2007). Despite the use of functional attributes, these are often costly and difficult to measure correctly (Burns and Ryder 2001).

Hill *et al.* (2000) argue that indices of biotic integrity based on periphyton should be designed to use a suite of attributes based on species richness, trophic structure (i.e. biomass) and organismal abundance, thus incorporating both taxonomic and non-taxonomic parameters. The overall sum of these metrics should create an index that is responsive to both specific sources of stress as well as

general cumulative perturbations (Karr 1993). A periphyton index of biotic integrity (PIBI) was developed and tested in the united States, based on 10 metrics of periphyton communities including relative taxon richness, relative abundance of indicator taxa (i.e. total diatoms, total cyanobacteria, dominant diatoms, acidophilic diatoms, eutraphentic diatoms and mobile diatoms as separate metrics), chlorophyll biomass, total biomass and phosphatase activity (Hill *et al.* 2000). The PIBI accurately detected anthropogenic impacts such as stream acidity, stream substratum composition, and stream and riparian habitat integrity in the United States.

One of the first steps towards the development of an index of environmental change is the development of a good understanding of the causal relationships between biotic and abiotic regulators of ecosystems and the biotic response to changes in these factors. Thus, developing an understanding of natural heterogeneity in river ecosystems and the associated temporal and spatial patterns in periphyton dynamics, as well as factors that regulate changes over time and space is fundamental to the development of a multi-metric index of change based on periphyton. Parameters that drive one ecosystem may act differently in another; however, most reliable statements of the ecological consequences of anthropogenic change require considerable understanding of the biotic and abiotic relationships that govern each river under consideration. If natural patterns of periphyton biomass accrual and removal are not known, then it is difficult to predict how periphyton will respond to anthropogenic alterations. Also, such predictions require that the spatial and temporal *scale* of observation be defined and that the pattern of hierarchical dominance of factors controlling periphyton be identified (Stevenson 1997).

Before periphyton can be included in ecological monitoring programmes in South Africa, it is therefore imperative that we first develop an understanding of the spatial and temporal dynamics of periphyton communities across the region. Once a suite of metrics has been evaluated, only then can a composite index be tested and applied (Karr 1993; Hill *et al.* 2000).

1.6 OBJECTIVES AND STUCTURE OF THIS THESIS

This thesis is the culmination of a study funded by the Water Research Commission (WRC) to investigate periphyton dynamics in foothill rivers of the south-western Cape (Ewart-Smith and King 2012). The project was motivated by the recognition that periphyton could contribute to the management of South African rivers and therefore the study focused on exploring the potential of including periphyton in the suite of tools used for ecological monitoring of rivers in South Africa. Data collected and analysed for the WRC project formed the basis of this thesis, although additional components, particularly the two grazer exclusion experiments, included in this thesis were not part of the WRC project.

Central to this thesis is the development of an understanding of the temporal changes in periphyton community structure and biomass over an annual cycle and the key factors that drive these changes. The study focused on two foothill rivers in the south-western Cape, namely the upper Berg River and the Molenaars River, which differ in trophic status. The upper Berg River is also the location of a large dam, resulting in an altered flow regime in the downstream reaches. A comparison of

periphyton community structure between these rivers, with sites representing different flow and nutrient conditions, was used to develop an understanding of changes in periphyton community structure in response to anthropogenic changes in flow and nutrient enrichment in south-western Cape foothill rivers. A primary focus on understanding *temporal* changes in periphyton communities meant that this study was spatially limited to only four sites and thus extrapolation to a broader spatial scale was limited. Nevertheless, this research constitutes a first step towards considering periphyton among the suite of biological components used in the management of flow regimes for the maintenance of biological integrity of open-canopied rivers of South Africa.

Based on the collection of monthly periphyton data and environmental variables over 21-months and the outcome of *in situ* flood studies, and grazer experiments, the main objectives of this research were to:

1. describe and quantify temporal patterns of periphyton community structure and biomass within foothill reaches with different trophic status and thus nutrient availability;
2. identify and develop an understanding of the key factors (abiotic and biotic) that control periphyton community composition and biomass in foothill reaches;
3. describe and quantify reach-scale (hydraulic biotope) spatial patterns of periphyton community structure and biomass within foothill rivers differing in nutrient status and under different seasonal conditions;
4. quantify the effects of floods of different sizes on periphyton biomass and community composition within rivers with different nutrient status in different seasons; and
5. quantify the effects of grazing on periphyton biomass and community composition under different seasonal conditions.

This thesis comprises nine chapters which address these objectives. Briefly, Chapter 1 provides a review of periphyton dynamics in temperate rivers. A conceptual framework for understanding the abiotic and biotic factors that affect periphyton communities is the key focus of the review. With the exception of the general methods section (Chapter 2), each chapter revisits specific aspects discussed in the review, with specific emphasis on open-canopied rivers in Mediterranean climates.

Chapter 2 provides a description of the study area and sampling sites and the methods employed in field sampling and processing of sampling material in the laboratory. This section highlights the basic design of the project.

Chapter 3 provides a quantitative assessment of the temporal patterns in environmental conditions monitored monthly over 21 months (October 2007 to May 2009) and highlights potentially important abiotic and biotic determinants of periphyton dynamics. This chapter therefore provides a foundation for describing and quantifying temporal patterns in periphyton community structure under different flow and nutrient conditions.

Chapter 4 describes and quantifies temporal patterns in periphyton community structure and biomass under different flow and nutrient conditions over the 21-month sampling period.

Chapter 5 links the environmental information in Chapter 3 with the temporal patterns in periphyton community structure in Chapter 4 and therefore identifies the relative importance of the abiotic and biotic determinants of periphyton communities over an annual cycle. Chapters 3, 4 and 5 together address Objectives 1 and 2 of the thesis.

Chapter 6 describes spatial patterns of periphyton community structure at a reach scale based on collection of samples in three main hydraulic biotopes (i.e. slack-waters, runs and riffles) during February 2009 and October 2009. This chapter specifically addresses Objective 3.

Chapter 7 analyses the role of floods in structuring periphyton communities through a series of sampling events immediately prior to and immediately following selected flood events at various sites in both the Molenaars and Berg Rivers and during different seasons. Chapter 7 therefore addresses Objective 4.

Chapter 8 investigates the effects that aquatic invertebrate grazers have on periphyton biomass and community structure during late spring and early autumn using controlled *in situ* experiments. This chapter addresses Objective 5.

Chapter 9 provides a general discussion on spatial and temporal patterns of periphyton communities in relation to key biotic and abiotic variables. The understanding of what drives periphyton communities through this research and within the context of the international literature is used as a basis for making recommendations for the use of periphyton as a tool for assessing ecological integrity, particularly under conditions of altered flow regimes. This chapter therefore relates specifically to the management of rivers in South Africa.

CHAPTER 2 STUDY AREA, GENERAL SAMPLING STRATEGY AND PROCEDURES

2.1 STUDY AREA

All sampling was undertaken within the south-western Cape of South Africa. This region of the country has a Mediterranean climate characterised by a distinct winter rainfall period with heavy rain in the mountain catchments (over 1000 mm a^{-1}). The winter therefore marks a period of frequent, intense flood disturbance, mostly between April and September in response to the north-westerly frontal systems. The high winter rainfall period is followed by an extended period of flow stability over the warm summer months.

Sampling focused specifically on the upper foothill reaches of two perennial rivers within this region, namely the Berg and Molenaars Rivers (Figure 2.1). Both rivers are naturally acidic, with low nutrient concentrations and dissolved solids, partly because of the humic compounds that leach from the fynbos and partly because of the geology of the region which is characterised by quartzitic sandstones of the Table Mountain Group (Davies and Day 1998). Both the Berg and Molenaars Rivers have their source in the Cape Fold Mountains typical of the south-western Cape, although they drain separate catchments. The Berg River is the second largest river in the south-western Cape, while the Molenaars River is one of the main tributaries of the Breede River, which is the largest river in the south-western Cape. The Molenaars and Berg Rivers therefore provide a good spatial representation of those typical of the south-western Cape of South Africa.

From its source in the Drakenstein and Franschhoek Mountains south of Franschhoek, the Berg River flows northwards and forms the main stem of the Berg River catchment which drains into the Atlantic Ocean at Velddrif on the west coast of South Africa, some 285 km away. In 2007, the Berg Dam was constructed on the main stem of the Berg River in its foothill reach. The Berg Dam drains an area of about 70 km^2 which is less than 0.8% of the entire Berg Catchment. The reservoir has a surface area of approximately 4.8 km^2 and a storage capacity of 126.4 x 10^6 m^3. Historically, the catchment of the Berg River above the dam was dominated by commercial pine plantations but these were clear-felled by the Working for Water programme in 1998. The natural vegetation of this region, mostly sandstone Fynbos, has recovered over the past 12 years and therefore the catchment upstream of the dam has a natural flow regime and is largely unimpacted by human activities. Prior to the construction of the Berg Dam, the foothill reach of the Berg River downstream of the dam was impacted by an Interbasin transfer (IBT) from the Theewaterskloof Dam, which resulted in summer time flow reversals (i.e. higher base flows in the summer than in the winter) and therefore this section of the river has a history of flow-related impacts.

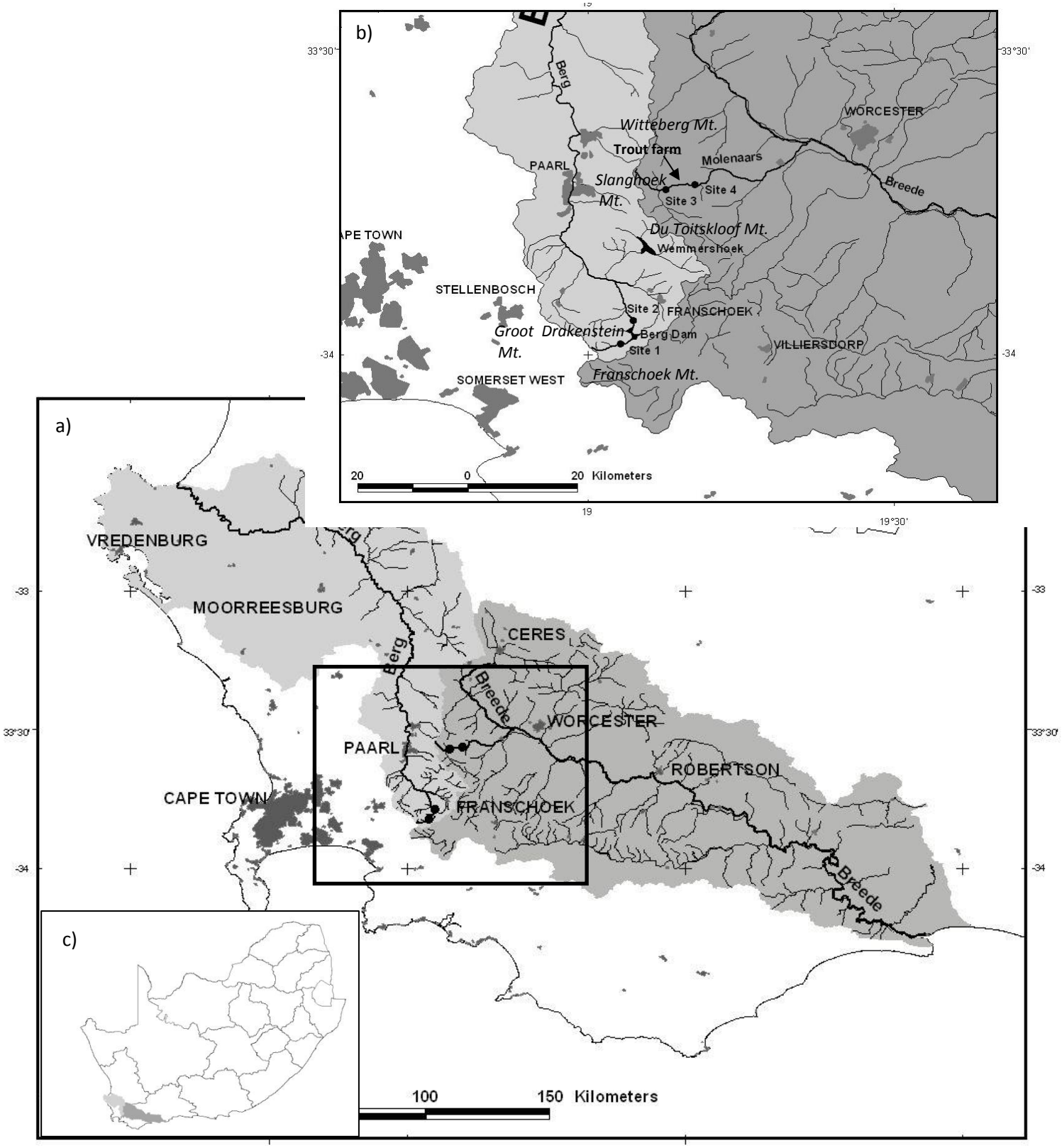


Figure 2.1 **The study area (a) showing the Berg and Breede catchments, (b) Sites 1 and 2 on the Berg River and Sites 3 and 4 on the Molenaars River and (c) the location of the study area within the south western Cape region of South Africa.**

The Molenaars River has its source in the Klein Drakenstein range and drains eastwards, receiving flow along its length from a number of streams on the southern slopes of the Witteberg, the northern Dutoitsberge and the south-eastern slopes of the Slanghoekberge. The Huguenot Tunnel runs under the Slanghoekberge and immediately downstream of its eastern portal, the Krom and Elands Rivers combine and continue through the Du Toits Kloof Valley as the Molenaars River. This river eventually flows into the Breede River at Worcester, some 33 km from its source. The Molenaars River is enriched within its upper reaches by fish-farm effluent entering from the Krom and Elands Rivers, as well as in the main stem of the Molenaars River at the Du Toits Kloof Resort. Prior to recent efforts to eradicate alien vegetation, the riparian fringe of the Molenaars River in the Du Toits Kloof Valley was heavily invaded by alien acacias. However, the natural vegetation, mostly granite and sandstone fynbos, has recovered in recent years. Despite impacts to water quality, the flow regime of the Molenaars River is natural. Although in different catchments, all four study sites are within 20 km of each other and share the same geology, vegetation and climatic conditions.

2.2 STUDY SITES

In order to establish the relative importance of flow and nutrients as key drivers of periphyton dynamics (Chapter 1), the general design of the project involved the selection of sites upstream and downstream of points at which a) the natural flow regime or b) the nutrient status is altered. Consequently, two sites within the Upper Foothill reach of the Berg River and two sites within the Upper Foothill reach of the Molenaars River were selected as sampling sites for this study (Figure 2.1). All four sites have open canopies and relatively steep gradients. With the exception of the site below the Berg Dam, all sites are characterised by short, intense floods. The substratum at these sites is dominated by boulders and cobbles derived predominantly from the Table Mountain group sandstones, although gravel and sand do occur in some habitats.

Within the reaches representative of these sites, the present-day Mean Annual Runoff (MAR) of the Molenaars River is 164 Mm^3 while that of the Berg River upstream of the dam is 136 Mm^3, indicating that the Molenaars River is a slightly bigger system. The general characteristics of each site are described below:

Site 1 (Figure 2.2) on the Berg River is situated upstream of the Berg Dam, about 100 m from the new gauging weir located at the inflow to the dam (G1H076). In this reach, the river has a natural flow regime and is nutrient poor (oligotrophic). This site has a steep mountainous bank on its left and a lateral bar of deposited cobbles on the right bank. The substratum is dominated by boulders and cobbles with some pebbles and gravel-sized particles. The river flows as a single channel, approximately 40 m wide and the site is characterised by a series of riffles, separated by runs with slack-waters along the margins during low flow conditions. This site therefore represents natural flow conditions and low nutrient availability.

Site 2 (Figure 2.3) is situated about 300 m downstream of the Berg River Dam on the Berg River. Its flows are highly regulated by the existence of the Berg River Dam which has been operational since late July 2007, two months prior to the collection of the first samples for this study. Nutrient levels

are slightly higher than Site 1 due to decomposition of vegetation drowned by the dam. The Berg River valley opens into a wide, unconfined section downstream of the dam and therefore the site is completely unconfined and thus characterised by lateral cobble and sand bars on both banks. The substratum is dominated by cobbles and boulders with bedrock outcrops, although sand rather than gravel and pebbles occupies the space between larger bed particles. The single active channel width is approximately 40 m and the site extends from a cobble-riffle at its upstream extent, to a long, deep, uniform run and ends immediately upstream of a bedrock cascade. This site therefore represents managed flow conditions and low nutrient availability.

Site 3 (Figure 2.4) is situated immediately downstream of the confluence of the Krom and Elandspad rivers which together become the Molenaars River. It is upstream of the Du Toit's Kloof Trout Farm and Hotel, and is slightly enriched by effluent from a number of trout farms upstream on the Elandspad River. Nevertheless, the flow regime is natural. The active channel is approximately 40 m and the site has relatively well defined banks on both the right and left of the channel. It consists of a riffle/rapid section with a fairly uniform run section, bordered by slack-waters along the margins. It ends in a stepped riffle further downstream. This site therefore represents natural flow conditions and nutrient enrichment.

Site 4 (Figure 2.5) on the Molenaars River is situated about 100 m downstream of the effluent outlet from the du Toit's Kloof Trout Farm and Hotel. Enrichment is greater than at Site 3 but the flow regime is natural. The active channel is approximately 45 m wide at the site and the left bank is confined by a steep mountainous bank while the right bank extends as a narrow cobble bar that extends as a low gradient seep away from the site towards the N1 highway. The substratum is characterised by boulders and cobbles and the site consists of a mosaic of cobble-riffles, shallow runs and rapids. This site therefore represents natural flow conditions and greater nutrient enrichment, relative to Site 3.

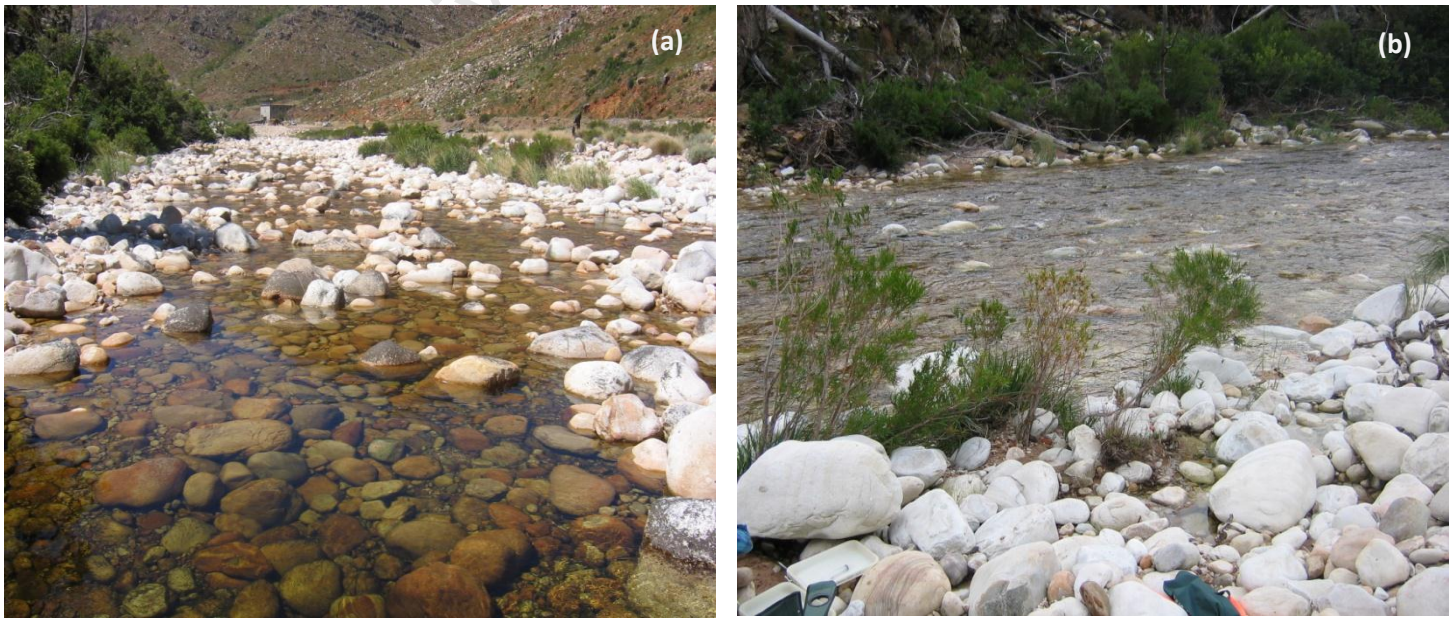


Figure 2.2 **Site 1 on the Berg River photographed during the summer (a) in January 2008 and (b) winter in June 2008.**

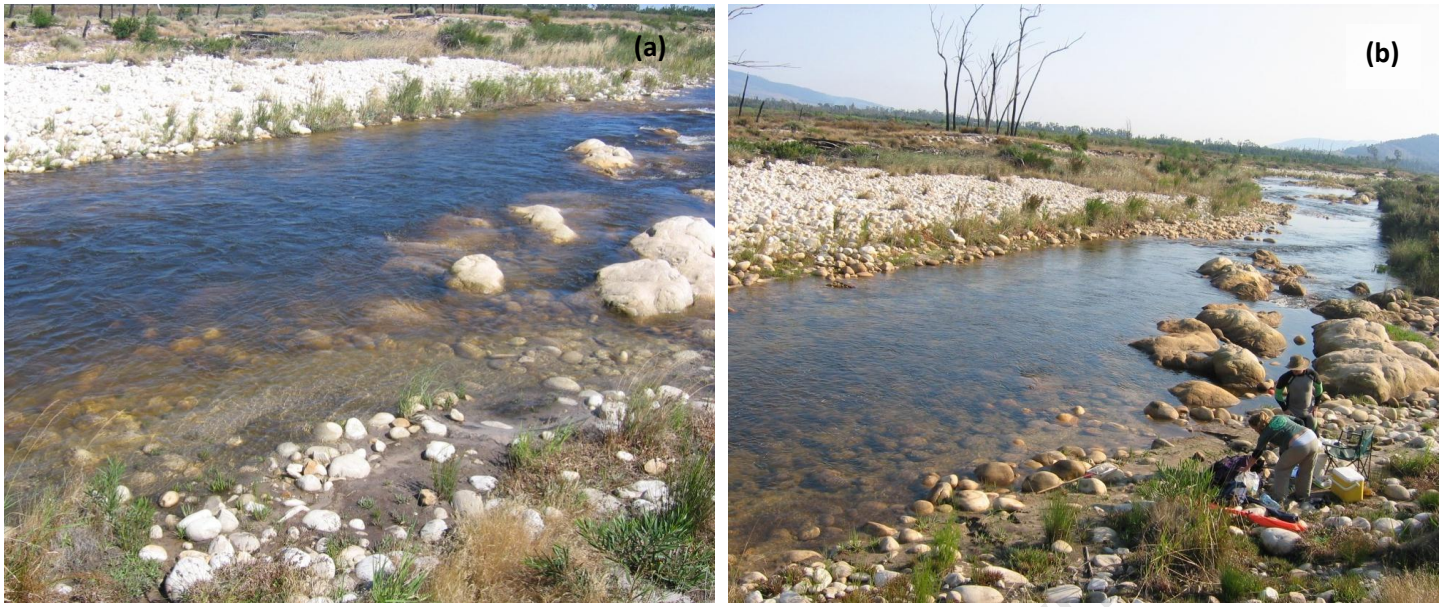


Figure 2.3 **Site 2 on the Berg River photographed during the summer (a) in February 2008 and (b) winter in June 2008.**

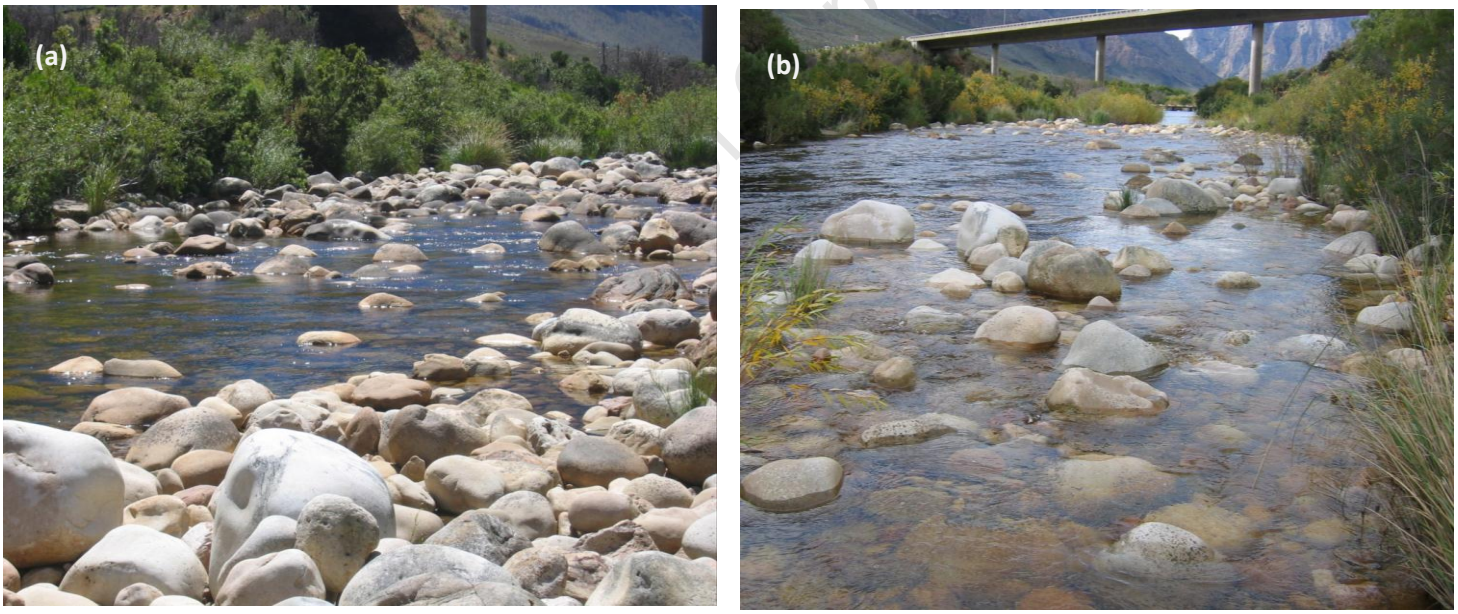


Figure 2.4 **Site 3 on the Molenaars River photographed during the summer (a) in February 2008 and (b) winter in June 2008.**

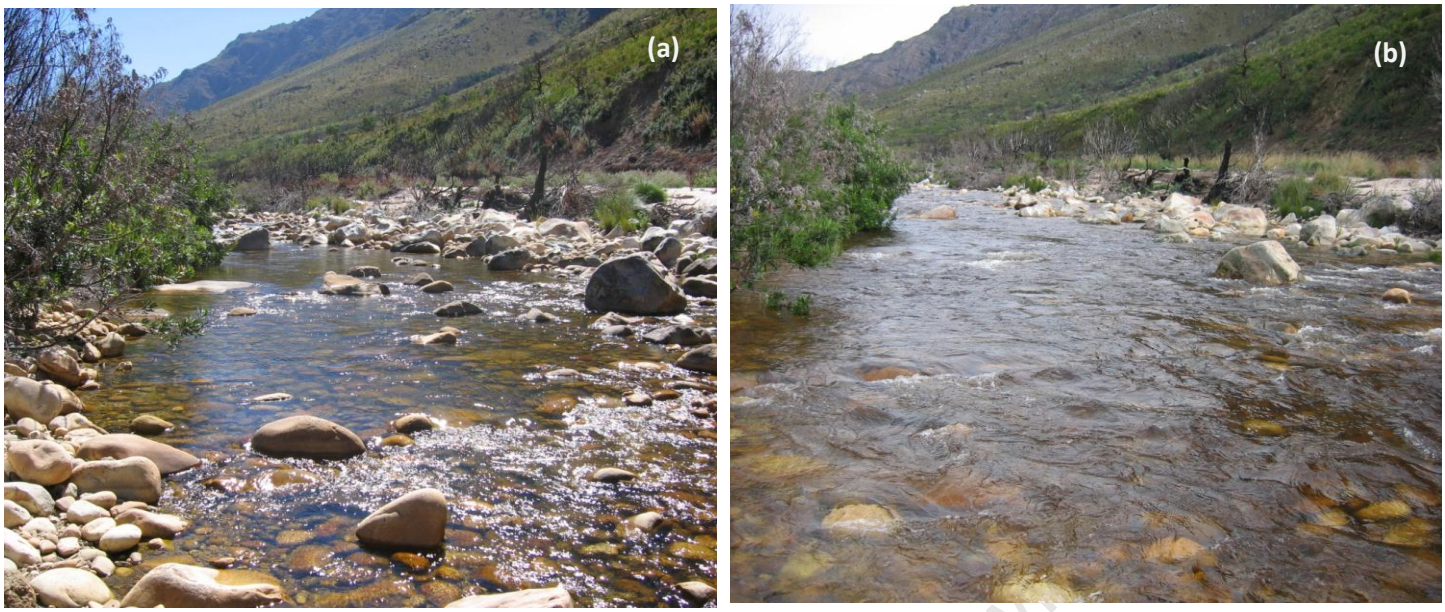


Figure 2.5 **Site 4 on the Molenaars River photographed during the summer (a) in February 2008 and (b) winter in June 2008.**

2.3 GENERAL SAMPLING STRATEGY AND FIELD PROCEDURES

Methods for the collection of periphyton and invertebrate samples and physic-chemical data, as well as the processing of sample material in the laboratory, were consistent throughout the project and are described in this section.

On each sampling occasion, individual stones were selected as replicate samples at each of the four sampling sites for the collection of physico-chemical data and biota, including periphyton and invertebrates. Replicate samples were randomly selected within specific hydraulic biotopes that varied depending on the objectives of different components of the study. The number of replicates collected also varied between different components of this study and are described further in the methods section of relevant chapters.

2.3.1 Physico-chemical data collection

Prior to the removal of each stone from the river bed, near-bed velocity and depth were measured *in situ* for each of the eight replicate stones collected on each sampling occasion. Velocity was measured with a Marsh-MacBirney FLOW-MATE Model 2000 portable electromagnetic flow meter attached to a top-setting wading rod. At site level, three replicate measurements of pH, conductivity, turbidity and dissolved oxygen were taken randomly from the run biotopes where periphyton and invertebrate samples were collected with hand-held portable instruments. pH was measured with a Crison Portable 506 field pH meter, accurate to 0.01 pH units. Conductivity measurements were taken with a Crison Conductimeter Portable 523 field meter which is accurate to 0.01 mS m^{-1} and compensates for temperature at 25^0C. Turbidity (NTU) was measured with a Hach 2100P turbidimeter. Dissolved oxygen (as % and in mg l^{-1}) was recorded using a Crison OXI45 oxygen meter.

Water samples were collected and stored on ice in the field and later frozen for analysis of phosphate (PO_4^{3+}-P), nitrate (NO_3^--N), nitrite (NO_2^--N), ammonium (NH_4^+-N), silicate (SiO_4) and total phosphorus (TP). Samples were analysed by flow injection using a Lachat QuikChem® 8500 Automated Analyser. NH_4^+-N was measured using Lachat's QuikChem® Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated (detection limit: 5µg.L^{-1}); NO_3^--N and NO_2^--N were estimated using Lachat's QuikChem® Method 31-107-04-1-E, in which NO_3^--N is converted to NO_2^--N and diazotized with sulfanilamide to form an azo dye (detection limit: 2.5µg.L^{-1}); SRP was measured by forming anantimony-phospho-molybdate complex using QuikChem® Method 31-115-01-1. QuikChem® (detection limit: 15µg.L^{-1}). QuikChem® Method 31-114-27-1-D was used for the determination of silicate concentrations.

Continuous temperature loggers were deployed during the project so that seasonal changes in the physical environment could be linked to the biological data. Hobo U22 Water Temp Pro v2 temperature loggers were fixed to the riverbed at Site 1 (upstream of the dam on the Berg River), Site 2 (downstream of the dam on the Berg River) and Site 4 (below the du Toitskloof Hotel on the Molenaars River) in October 2007 and at Site 3 (upstream of the du Toitskloof Hotel on the Molenaars River) in November 2008. These loggers are capable of storing approximately 42 000 measurements ranging between 0°C and 50°C. Data were generally downloaded during each monthly sampling occasion between September 2007 and May 2009.

2.3.2 Periphyton and Invertebrate sampling

Considering the potential influence of stone size on periphyton biomass, replicate stones of a similar size (with the longest axis ranging between 100 mm and 250 mm) were selected to reduce any potential influence of stone size. Stones were collected randomly, rather than along transects, either within each hydraulic biotope or only in the runs (in the case of monthly sampling). Invertebrates present on each stone were collected using an 80 µm mesh net, with an aperture area of 300 x 300 mm. This was achieved by lifting each stone and placing it within the net and then gently brushing the stone surface, taking care not to remove periphyton. Collection involved placing an 80 µm mesh net downstream of the stone being sampled and then lifting the stone and placing it in the net making sure that all invertebrates were washed into the net. Invertebrates that did not dislodge from this procedure were gently picked from the stone surface with forceps to ensure that algal loss was minimal. Invertebrate samples were fixed in 10% Formaldehyde in the field and transported back to the laboratory where the samples were strained through an 80 µm mesh and preserved in 70% ethanol until further analysis.

Once all invertebrates were removed, each stone was placed in a sampling tray and the periphyton removed by scrubbing until no change in the rinsing water was evident. A sub-sample of 50 ml was removed from the sample (which was later quantified in the laboratory) and preserved in Lugol's iodine solution for identification of algal taxa. The remainder of the sample slurry was immediately placed in a dark container on ice for transport back to the laboratory where the samples were frozen within 10 hours of collection for biomass analysis.

The dimensions of each stone were measured as the longest axis (x), the longest horizontal axis perpendicular to x, (y) and the longest vertical axis of the stone (z). The surface area of each stone was then calculated using the regression equation for stone area:

$$\text{surface area in } cm^2 = 0.014x + 33.819\ (xy + xz + yz)$$

This equation was derived from stones collected during the Pilot study (Ewart-Smith 2007) for which the x, y and z axes were measured and the surface areas were calculated. Surface area was measured for these stones (n=39) by covering them with metal foil that was then weighed. Area was calculated from the known weight per unit area. The combination of stone dimensions that best predicted area was xy + xz + yz ($r^2 = 0.93$).

Stone surface area was corrected by estimating the percentage of each stone that was embedded in finer sediments and therefore unavailable as habitat for benthic algae.

2.4 GENERAL LABORATORY PROCEDURES

2.4.1 Periphyton biomass determination

Frozen periphyton samples were defrosted over night in the dark. When defrosted, each sample was mixed and divided into two portions for the measurement of three periphyton biomass indicators (normalized to mg m^2) i.e. total dry mass, ash free dry mass (AFDM), and Chlorophyll *a* (Chl *a)*. Total dry weight was measured by filtering the sample portion through Whatmann GFF 4 glass fibre filter papers which were then dried at 60 ^{0}C overnight. The samples were then ashed in an oven at 400 ^{0}C for 4 hours. The difference between the dry weight and the weight of the ash is the organic component (i.e. AFDM) of the periphyton.

The other portion of the sample was used for Chl *a* determination. Chl *a* is abundant in most periphytic algae, including cyanobacteria (Biggs and Kilroy 2000) and therefore the quantity of this pigment was used as a measure of live algal biomass in each sample. Chl *a* was extracted with methanol AR, boiled at 70 ^{0}C for 3 minutes to increase extraction efficiency and to fix the chlorophyll by destroying the enzymes. Absorbance was measured at a wavelength of 665 nm with a spectrophotometer (Spectroquant Pharo 100). Background absorbance was measured at 750 nm. Chlorophyll degrades naturally as communities age and die, resulting in degradation products called phaeopigments which interfere with the measurement of live Chl *a* using spectrophotometry (Biggs and Kilroy 2000). The sample was therefore acidified with 0.1.M Hydrochloric Acid (HCL) to correct for phaeopigments in the sample measurements and then neutralised with 0.1 M NaOH. Absorbance was re-read at both 665 nm and 750 nm following the acidification and neutralisation step and the measurements were corrected for the presence of turbidity and phaeopigments. These procedures are based on those given by Nusch (1980) and Sartory and Grobbelaar (1984). These values were then multiplied by 36.9, which is the absorption coefficient for methanol, to determine Chl *a* concentrations for each rock sampled (Sartory and Grobbelaar 1984).

To ensure statistical rigour in the sampling protocol, the number of samples required to account for spatial variability in periphyton communities was based on a pilot study undertaken in September

2005 (Ewart-Smith 2007). Chl *a* concentrations for 30 replicates collected in three different biotopes during September 2005 from Site 1 on the Berg River were used for this analysis. The analysis requires an iterative process of using a different number of replicates each time to satisfy a level of confidence for detecting differences that are not a result of natural variability in communities within a site. The following formula was therefore applied (Zar 1996, p.133):

$$n \geq 2sp2 \ / \delta 2 \ (t\propto(2), v + t\beta(1),v)2$$

where:

n is the size of the sample from each biotope,

δ is the minimum difference in populations that we wish to detect. This was set at 30% (Biggs and Kilroy 2000) i.e. any change of less than 30% would be considered ecologically unimportant because of natural spatial variability),

sp is the pooled within-population standard deviation determined from the samples collected,

t∝(2), v is the critical value for the t-distribution for v = 2(n-1) degrees of freedom and with ∝, the nominated significance level i.e. 0.05,

β is the probability of committing a type II error (i.e. erroneously concluding that there is no difference between populations),

tβ(1),v is related to the probability that the desired exceedence will be detected (see Table B3, Appendix B of Zar 1996 for the t values).

Based on three iterations, it was established that 10 stones should be collected in the backwater/slackwater biotope, six in the runs and seven in the riffles to detect a 30% change in Chl *a* concentrations between biotopes with a power of 90%. This analysis indicated that runs were there least variable of the three dominant biotopes characteristic of the study sites and therefore was used to guide a more efficient collection of samples during this study.

2.4.2 Taxonomic analysis of algae

The sub-samples used for taxonomic analysis were stored in a dark refrigerator. During sample processing, each 50 ml sub-sample was first homogenised for 30 seconds using a simple hand blender. This procedure ensures that clumps of filamentous algae are broken up so that a non-aggregated, even cell distribution in the sample is achieved. This process is essential for ensuring any further sub-sampling is representative of the overall sample such that variability is reduced and precision increased.

A number of different approaches to preparation and analysis of samples for taxonomic purposes were tested at the onset of sample analysis. Firstly, a few sub-samples collected during September and October 2007 were divided into two halves following homogenisation, one for the identification of green (chlorophytes) and blue-green algae (cyanobacteria) and the other for the identification of diatoms. The latter sample was cleaned according to the bleaching techniques described in (Taylor

et al. 2007), to remove organic coatings and reveal the morphology of the silica frustules used for identification. A droplet of each cleaned sample was mounted on a glass slide with a cover slip using entallan and then analysed at 1000 x magnification with an oil immersion lens associated with a compound microscope for diatom identification. From the other half of the sample, a droplet was placed on a glass slide with a cover slip and analysed at a range of magnifications from 400 x to 1000 x, depending on the size of individual cells. When available, a Nikon Eclipse 50i Compound Microscope, fitted with a Nikon DS Camera Control Unit and Digital 3D Imaging was used to assist with identifications. Although this approach proved useful for the *identification* of algal taxa, it did not provide a means by which to *enumerate* different taxa for quantification of the sample. Thus, the possibility of using a haemocytometer was employed for enumeration of algal cells. The haemocytometer provides a grid of known area with a set depth of 0.1 mm and thus it is possible to count the number of cells visible in each unit with a known area and thereby quantify the sample. However, because of the depth of the haemocytometer chamber, it is not possible to focus the compound microscope at a magnification of either 650 x or 1000 x. Thus, algal identification using a compound microscope with a haemocytometer for enumeration of cells is limited to what is visible at a magnification of 400 x. A review of the international literature indicated that, where possible, an inverted microscope with a sedimentation chamber is generally employed for enumeration of algal taxa to overcome this problem. This method (known as the Utermohl method), is not limited to lower magnifications because the objectives are below the sample. Nevertheless, the current project did not have access to an inverted microscope and thus algal enumeration was limited to identification at 400 x magnification using a haemocytometer. Stream periphyton research in New Zealand (Biggs and Kilroy 2000) suggests that most enumerations are possible at this magnification, however, it is necessary to scan the sample at 1000 x magnification to determine whether any taxa present in significant numbers are being missed at the lower magnification. A comparison between a sample analysed at 1000 x with that at 400 x showed that many of the smaller cyanobacteria taxa, as well as some of the smaller diatoms are not identifiable at 400 x and therefore the approach may be biased towards larger diatoms and green algae. Nevertheless, an initial scan of the samples was undertaken at a magnification of 1000 x so that if present in significant numbers, these smaller taxa were detected and identified for enumeration at 400 x magnification.

A haemocytometer with a total volume of 0.9 mm^3 was therefore used for algal taxon identification at 400x following an initial scan at a magnification of 1000x. After the samples were homogenised, a 5 ml subsample was extracted and centrifuged for 10 minutes at 3000 rpm to concentrate the algal cells. The supernatant (4.5 ml) was discarded and the cells remaining in the pellet (0.5 ml) were resuspended. Once the sample was adequately concentrated to ensure approximately 30 to 50 cells per field of view at 400 x magnification, a volume of 0.9 mm^3 was placed in the haemocytometer for enumeration of cells. All cells were identified and enumerated within an area ranging between 1 mm^{-2} and 9 mm^{-2}, regardless of whether they occurred as single cells, within colonies or within filaments. The volume sampled was then calculated by multiplying the area sampled by the depth of the haemocytometer chamber (0.1 mm). The Identification guides used in the identification of taxa

included Morris (1967), Bellinger (1980), Cox (1996), Janse Van Vuuren *et al.* (2006) and Taylor *et al.* (2007)

Following identification and enumeration, algal density per sample was then quantified as the number per m^2 of stone surface area. The longest axis of at least 10 cells per taxon was measured from a random collection of samples and averaged. These data were used to weight the densities of individual taxa and thereby reduce the over-representation of very small cells in the sub-samples as follows:

Length weighted density (number m^{-2}) = density (number m^{-2}) x ((length of long axis in µm)/ 170µm).

170 µm is the length of the largest cell recorded in the data set and therefore data were weighted as a fraction of this length. Although this technique does not measure algal biovolume directly, it includes an estimation of relative algal cell size and thus provided a means of comparing taxa over a wide range of cell sizes.

2.4.3 Identification and enumeration of invertebrate grazer biomass

All invertebrates were identified to family-level using a dissecting microscope and taxonomic keys (Day *et al.* 1999) when necessary. Those invertebrates recognized as potential grazers (Table 2.1) were counted and classified into between 3 and 5 size categories. A number of individuals per size category for each taxonomic group were dried over night to a constant weight at 60 ^{0}C for the determination of biomass. A Mettler AE 100 laboratory balance with an accuracy of ± 0.001 mg was used to weigh a number of individuals within each size category per taxa. Length to weight relationships for each size category and each taxonomic group were then calculated. These data were used for converting the number of individuals to grazer biomass per taxonomic group.

Potential grazers were separated into functional feeding groups (FFG) according to their mode of feeding and their primary food source, based on the functional feeding groups defined by Schael (2005) and modified according the feeding modes defined by Arens (1989) (Table 2.1).

Table 2.1 **Functional feeding group definitions used for determining potential grazers, modified from Schael (2005) using the definitions for different feeding modes in Arens (1989) .**

FFG	Feeding Mode	Dominant Food Type	Invertebrate taxa
Deposit feeder	Excavate algal mats with shovel-like appendages	Most large algae and detritus in comparative amounts dependent on availability.	baetids, plecopterans (early instars); teloganodids; leptophlebiids
Scraper	scrape thin films of micro-organisms off substrata	Algae and some detritus	chironomids, elmids (adults and larvae), glossosomatids, hydraenids, blephacerids, hydroptilids;
Brusher	Brush algal mats with large scraping brush on the terminal segment of their labial palps	Mostly large algae	heptageniids; helodids

In the case of Chironomidae, which include predators and grazers, monthly invertebrate data collected from the Molenaars River by Ractliffe (2009) over a three year period and identified to species level were used to calculate the relative proportion of chironomids that feed on periphyton in each monthly sample collected during this study (Figure 2.6). These proportions were used to correct the total numbers of Chironomidae collected during this study. Aquatic invertebrate genera and species within families other than the chironomids also fall into different functional feeding groups. Individuals were therefore assigned to functional feeding groups based on prior knowledge of the dominant species representative of a given family (Table 2.1).

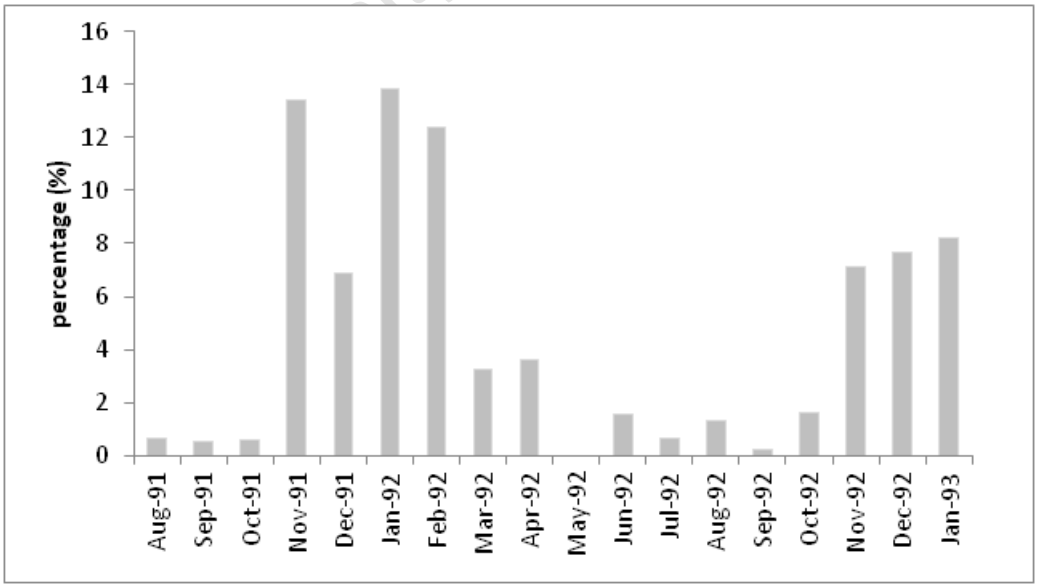


Figure 2.6 **The % contribution of Tanypodinae (which are predators) to the total number of chironomids collected monthly at 6 sites on the Molenaars River between August 1991 and January 1993 (Ractliffe 2009). These data were used to adjust the total number of chironomids counted in each sample to compensate for possible inclusion of predators in the estimation of algal feeder density and biomass because invertebrates were only identified to family level.**

CHAPTER 3 TEMPORAL PATTERNS IN ENVIRONMENTAL CONDITIONS

3.1 INTRODUCTION

The Berg and Molenaars Rivers fall within a Mediterranean type climatic area characterised by high seasonality in rainfall and temperature, with distinct wet and dry periods. Under natural conditions, both the abiotic and biotic components of these systems show distinct seasonal patterns but these can vary between years depending to a large extent on the frequency and intensity of flooding over the wet winter season (Gasith and Resh 1999). Although seasonal cycles of fauna in Mediterranean climates have received attention over the last two decades, very few studies have focused on periphyton community structure, particularly those of the south-western Cape. Extensive field-based surveys of the temporal patterns in periphyton biomass and/or taxon composition have been undertaken in mesic (e.g. Biggs and Close 1989; Uehlinger 1991) or xeric (e.g. Grimm and Fisher 1989) regions, which have described the general pattern of periphyton biomass and taxon composition over time and have determined the relative importance of the factors that drive these temporal patterns. Although some principles of the structure and function of periphyton in these ecosystems may apply to those in Mediterranean regions, the strong seasonality in rainfall (during the cool wet season) and the associated seasonal flooding followed by a hot dry summer clearly distinguish the Mediterranean climate from most mesic or xeric climates (Gasith and Resh 1999). An investigation of periphyton dynamics and the factors that drive these patterns in rivers of the south-western Cape, a typically Mediterranean region is therefore warranted.

This chapter describes patterns in environmental variables that potentially control periphyton dynamics in foothill rivers of the south-western Cape. The aims are specifically to 1) describe temporal changes in abiotic and biotic factors under natural flow and nutrient conditions; 2) compare these patterns with those under altered flow conditions and nutrient enrichment. This chapter is therefore a precursor to Chapter 4, which describes temporal patterns in periphyton biomass and community structure under different flow and nutrient conditions. A number of predicted patterns in these variables are addressed in this study and summarised in Table 3.1.

Both the Berg River above the dam and both study reaches on the Molenaars River experience similar rainfall conditions and natural flow regimes. It is therefore predicted that these reaches will show a strongly seasonal pattern of high flows during the winter with a similar frequency and duration of flood events and low flow conditions with no large flood events during the hot dry season (Prediction 1, Table 3.1). Seasonality in the flow regime should reflect seasonal differences in habitat characteristics at these sites with deeper, faster flowing conditions during the winter and shallow, slower flow during the summer.

Water temperature too should reflect a seasonal cycle of higher temperatures in the summer months and lower temperatures during the winter, with distinct shifts in conditions coincident with seasons (Prediction 2, Table 3.1). Considering shorter day length and denser cloud cover over the

cold wet winter relative to the summer, a cycle of lowest solar radiation in the winter and highest radiation in the summer is expected (Prediction 2, Table 3.1).

Table 3.1 A summary of the expected temporal patterns of abiotic and biotic variables potentially driving periphyton communities in the south-western Cape.

Prediction	Relevant sites
1) The flow regime will be strongly seasonal under a natural flow regime with an absence of flood events over the summer and a similar frequency and duration of flood events between rivers, confined to the winter.	Sites 1, 3 and 4
2) Water temperature and solar radiation will reflect seasonal cycles with higher temperatures in the summer and lower temperatures during the winter with abrupt seasonal shifts.	Sites 1, 3 and 4
3) No seasonal pattern in flow, temperature or habitat characteristics such as depth and velocity will be evident downstream of the Berg Dam and the distinction between sites with natural flow regimes and that below the Berg Dam will be driven by differences in the size, frequency and duration of flood events	Sites 1 and 2
4) Sites with and without enrichment under a natural flow regime will differ only during the stable, low flow period.	Sites 1, 3 and 4

The two study reaches on the Berg River are separated by the Berg Dam. It is therefore anticipated that the size, frequency and duration of flood events over the study period will differ between these two reaches. Alterations in the flow regime downstream of the dam may also result in differences in low flow conditions between these two reaches with no clear seasonality in discharge, water temperature and habitat variables such as depth and velocity downstream of the dam (Prediction 3, Table 3.1).

The upper-most study reach of Molenaars River receives trout farm effluent from one of its tributaries, while the downstream reach receives additional effluent from a second trout farm immediately upstream of the site. It is therefore expected that the upper Molenaars reach will be nutrient enriched relative to the upper Berg reach, while the downstream reach will have the highest nutrient concentrations of all four study reaches because of the cumulative effect of trout farm effluent along its course. Because of the dilution effects during the winter period, differences in environmental conditions between the oligotrophic upper Berg River (Site 1) and the Molenaars River (Sites 3 and 4) will only be evident during the summer months when flows are stable.

Seasonal differences in invertebrates that potentially feed on algae (including scrapers, grazers and deposit feeders and collectively termed grazers for this study) will also be explored in all four study reaches. Considering the higher nutrient availability in the Molenaars River, relative to the Berg River, both biomass and densities of grazers are expected to be higher in the Molenaars River over the dry summer months with no difference between the Berg and Molenaars Rivers in the winter period (Table 3.1).

Finally, analyses of these hydrological, chemical and biological parameters over time should assist with identifying the key determinants for predicting temporal dynamics of periphyton communities addressed in Chapter 4.

3.2 METHODS

3.2.1 Experimental design

This study compared sites where flow is unregulated and regulated and which are enriched and unenriched over time. Therefore Site 1 on the Berg River upstream of the dam was compared with Site 2 downstream of the dam to address the effects of flow regulation. Site 1 on the Berg River was also compared with Sites 3 and 4 on the Molenaars River to address the effects of enrichment. Samples were collected monthly at all four sites over a 21-month period between September 2007 and May 2009. Although replication for some variables was at the level of individual rocks, some variables were only measured for the site on each sampling occasion. Replicate data for individual rocks were therefore averaged for comparison with site level data for this component of the study.

3.2.2 Sampling strategy for *in situ* measurements

3.2.2.1 Physico-chemical measurements

Physico-chemical parameters characterizing benthic habitat and water chemistry conditions were measured *in situ* in conjunction with the collection of invertebrate and periphyton samples during each sampling occasion. In particular, near-bed velocity and depth were measured for each of the eight replicate stones collected randomly within runs from all four sites over the 21-month sampling period. At least three replicates each of turbidity, dissolved oxygen, pH and conductivity were measured *in situ* on each sampling occasion, while water samples were collected for the analyses of nutrient concentrations. Details of the collection and processing of data for describing the chemical and physical parameters measured on each sampling occasion are described in Section 2.3.1.

3.2.2.2 Invertebrate samples

Benthic invertebrates found on each replicate stone selected for periphyton sampling were collected separately for the estimation of grazer density and biomass. Field sampling procedures for the collection and processing of invertebrate samples are detailed in Section 2.3.2. The approach used for selecting potential grazers and the methods used for biomass determination and enumeration are described fully in Section 2.4.3.

3.2.3 Time series data

3.2.3.1 Daily solar radiation

Total daily radiation values calculated from hourly data in megajoules per square meter (M m^{-2}) were obtained from the Institute for Soil, Climate and Water at the Agricultural Research Council (ARC) in Stellenbosch. Daily data were not available for the weather station situated in the Franschhoek Valley near the Berg River sites (La Motte, Lat: -33.8813; Long: 19.07148) for the

sampling period and therefore daily irradiance data measured at a weather station in the Du Toitskloof Valley near the Molenaars River sites (Meulplaas: Klipdrift, Lat: -33.6666; Long:19.30245) were used at all four sites for the analysis of temporal patterns.

3.2.3.2 Water temperature

In situ temperature measurements

Temperature loggers (Hobo U22 Water Temp Pro v2) were fixed to the riverbed at Site 1, Site 2 and Site 4 in October 2007. Due to concerns around possible differences in water temperature between Sites 3 and 4, an additional logger was installed at Site 3 on the Molenaars River in November 2008. Data from Site 4 were used to estimate water temperature for the missing period (October 2007 to November 2008) based on the relationship between the two sites when both were being monitored.

In March 2009 however, just four months after installation, the logger at Site 3 was stolen. Nevertheless, data for this site were collected independently by a separate research project (Dallas *et al.* 2011) that had installed loggers over the same period. Data were logged hourly and thus provided detailed information on mean daily water temperature and the range of these temperatures on a daily basis.

Simulated water temperature

In situ measurements of water temperature began in late October 2007 and therefore time series water temperature data were not available for the period prior to and during the first two months of sampling. Daily minimum and maximum air temperature data were available, however, from two weather stations situated in the Franschhoek and Du Toit's Kloof Valleys respectively. These data were provided by the Institute for Soil, Climate and Water at the Agricultural Research Council (ARC) in Stellenbosch. A multiple linear regression model, which uses mean daily air temperature data and daily discharge data to derive maximum daily water temperature (Rivers-Moore *et al.* 2005), was used to simulate water temperature data for the period 2007 to 26 October 2007.

The multiple linear regression model (Rivers-Moore *et al.* 2005) employed for the simulation is given by the following equation:

$$WTx = \beta + (a \times Tn) - (b \times \text{daily discharge}^{-1})$$

Where: WTx = Maximum daily water temperature;

$\beta = 4.004$, $a = 0.8995$ and $b = 0.4827$ (coefficients of the model);

Tn = mean daily air temperature; and

Daily discharge^{-1} = the inverse of the mean daily discharge

Mean daily air temperature records are generally not available from the ARC or any of the weather stations that record air temperature. Therefore, mean daily temperature data were estimated by calculating the average of the daily minimum and maximum temperature data provided by the Institute for Soil, climate and Water at the ARC. The average maximum daily temperatures from the modeled and measured water temperature data at Site 4 were highly correlated ($R^2 = 0.833$, $p < 0.0001$) with an average difference of 0.68 ^{0}C. However, differences of 7^{0}C were calculated during

the colder months between June and August 2008 suggesting that the model is more accurate during warmer water conditions. Similarly, the measured and simulated average maximum daily water temperature at Site 1 were correlated (R^2=0.705, p<0.001). Differences of 11 or 12^{0}C were calculated during some months suggesting that these data are less reliable than that for Site 4.

3.2.3.3 Daily discharge data

Daily average discharge data for the 21-month sampling period were obtained primarily from the Department of Water Affairs' hydrology department. These data were taken from three gauging stations located on the Berg River and Molenaars River, namely G1H076 located upstream of the Berg Dam near Site 1, GhH077 located immediately downstream of the Berg Dam near Site 2 and H1H018 located immediately downstream of Site 4 on the Molenaars River. G1H076 and G1H077 were only constructed after commencement of sample collection for this project and therefore it was necessary to simulate daily flow data for the period September 2007, when the first sample was collected, and March 2008, when G1H076 and G1H077 became operational and began recording daily flow data. Daily flow between July 2007 and March 2008 was provided by the Berg River Dam Management team. It was based on a dam balance model that uses the daily change in water level as a surrogate for daily flow data. While daily flow data are more accurate than those generated by the model, a comparison of data generated from the model and that recorded at G1H076 for a period of overlap between March 2008 and August 2008 (Figure 3.1) suggests that the simulated data provides a relatively good surrogate for daily flow data. This was supported by an R^2 value of 0.80 (p <0.001) for the correlation between simulated and measured daily flow data for this period. It is noteworthy however that high flows are less well represented by the dam balance model than low flow conditions (Figure 3.1).

Hydrology for the period between September 2007 and March 2008 at Site 2, when gauging station G1h077 below the dam became operational, were based on daily records of dam releases provided by the Berg River Dam Management team.

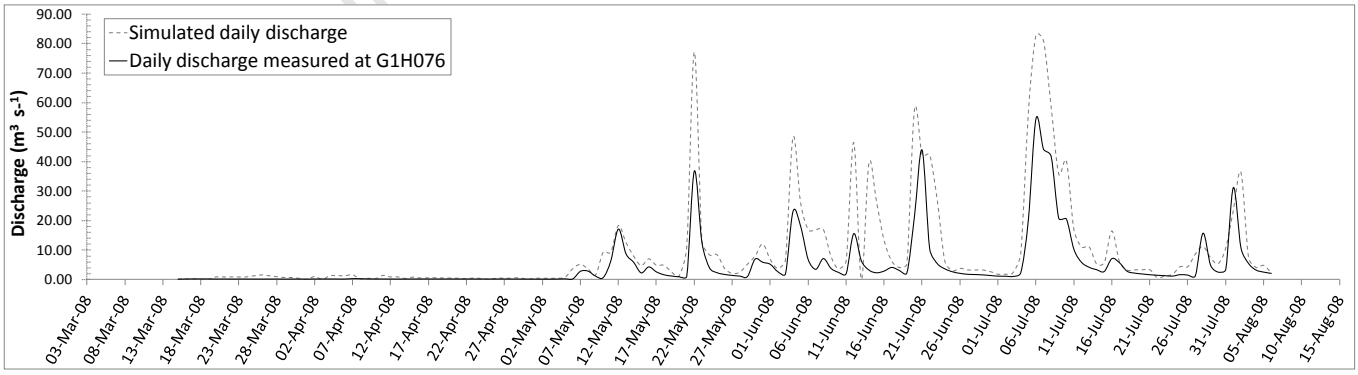


Figure 3.1 A comparison of daily discharge data recorded at G1H076 upstream of the Berg Dam and daily discharge data generated by the Dam balance model between March and August 2008

Flood classes defined according to the DRIFT method (Section 3.2.4.3) were based on long term data records (1980 – 2007) from G1H004, a gauging station that has since been flooded by the Berg Dam, and H1H018 between 1970 and 2000 on the Molenaars River.

3.2.4 Analytical methods

3.2.4.1 Analysis of time-series temperature data

Time series water temperature data for the sampling period were used for two purposes. Firstly, summary statistics that describe the temporal pattern of water temperature on a monthly basis were calculated as potential predictors of temporal change in periphyton biomass. These parameters included the average daily inter-sampling water temperature (WT_{ave}), the coefficient of variation (CV) of average daily water temperature over the inter-sampling period (WT_{CV}), and the average daily range of water temperature over the inter-sampling period (WT_{range}). These are summarised together with the flow parameters in Table 3.2.

Secondly, these data were used to define seasons over the 21-month sampling period.

3.2.4.2 Temperature and the identification of seasons

Categorisation of monthly data into seasons was based on a sequential regime-shift detection method of continuous water temperature data measured in the field or simulated from air temperature. The regime shift detector (RSD) is written in Visual Basic for Application (Excel), version 3.2. The method is based on a sequential t-test analysis of regime shifts (STARS) that can signal a possibility of a regime shift in real time (Rodionov *et al.* 2004). The method was developed for the detection of regime shifts in biological and climatic variables in oceans based on time-series data collected at various levels over several years or decades. In this study, STARS was used to detect shifts in both daily mean temperature between October 2007 and May 2009 and daily maximum temperature between April 2007 and May 2009. STARS can be tuned to detect the regimes of specific time intervals and magnitudes. For the assessment of shifts in both daily mean and maximum water temperature, running averages were based on a period of 15 days. The probability level was set at p=0.01 for the daily mean temperature, while that for daily maximum temperature was set at p=0.035 because the daily maximum temperatures were more variable and a higher p value reduced the detection of stochastic events unrelated to seasons.

Time-series water temperature data collected at Site 4 and simulated from air temperature provided the most complete set of time-series water temperature data over the 21-month sampling because problems with faulty loggers or theft of equipment resulted in incomplete time-series data actually measured *in situ* at the other three sites. A comparison of simulated and measured data between Sites 1 and 4 (Figure 3.2 and 3.3) indicated a better relationship between measured and simulated data at Site 4. Thus detection of seasonal shifts was based on data for Site 4 only. Each of the 21 months sampled was then categorised into seasons for further analyses at all four sites.

3.2.4.3 Analysis of the flow regime

The hydrological regimes characterising the Berg and Molenaars Rivers based on daily flow data was condensed into a number of descriptors that are potentially important for understanding periphyton dynamics in these rivers.

DRIFT (Downstream Response to Instream Flow Transformations) is a method of classifying flood flows and low flows using long-term average daily flow data (King *et al.* 2003). According to the method, eight flow classes represent dry and wet season low flows and inter- and intra-annual flood events. The larger, inter-annual floods are classified as those with a return period of 1-2 years (Class 5), 2-5 years (Class 6), 5-10 years (Class 7) and 10-20 years (Class 8).

Classes 1 to 4 floods are the intra-annual flood events and the flood size for each class is defined by the class 5 floods (i.e. those with a return period of 1-2 years). The magnitude of this event is halved to define the upper threshold of the Class 4 flood. This flood size is then halved to define the upper threshold of the Class 3 flood and so on until the upper threshold of the Class 1 flood is defined.

Flood events for both the Berg (Howard 2004) and Molenaars Rivers (Brown and Louw 2003) have been assigned to the classes defined by DRIFT using long-term daily flow records at G1H004 and H1H018 respectively. G1H004, which is now inundated by the Berg dam, includes flows from the Wolwekloof tributary which is downstream of Site 1. It was therefore anticipated that the size of flood events on the Berg River recorded at G1H076 would be smaller than those recorded historically downstream at G1H004, and thus the thresholds for flood classes defined in previous work would be overestimated for the Berg River alone. On the contrary, however, the thresholds defined previously (Howard 2004) seemed smaller than those defined from the new weir's data. The data record for G1H004 between 1980 and 2000 was therefore re-analysed to determine the flood size with a return period of 2 years and thus the upper thresholds of the Class 1 to 4 floods were adjusted (Table 3.2). The DRIFT classes defined by Brown and Louw (2003) for the Molenaars River were used directly for the classification of flood events recorded over the 21-month sampling period (Table 3.2).

Table 3.2 DRIFT classification and magnitude of floods based on average daily flows ($m^3 s^{-1}$) for G1H004 on the Berg River taken from previous work (Howard 2004) and recalculated for this study and H1H018 on the Molenaars River taken from Brown and Louw (2003). No floods greater than a class 5 flood was experienced during this study and therefore flood classes 6 to 8 were not recalculated.

Flood Class	G1H004 (Berg River) from Howard (2004)	G1H004 (Berg River) adjusted, this study	H1H018 (Molenaars River) from Brown and Louw (2003)
1 (intra-annual)	3.6	4.5	5.0
2 (intra-annual)	7.2	9.0	16.0
3 (intra-annual)	14.5	17.8	31.0
4 (intra-annual)	29.0	35.6	61.0
5 (1:2 years)	58.7	71.2	93.7
6 (1:5 years)	75.3	Not assessed	146.0
7 (1:10 years)	78.8	Not assessed	181.0
8 (1:20 years)	85.6	Not assessed	187.0

Monthly low flows for the Berg and Molenaars Rivers were also defined using the DRIFT method by Howard (2004) and Brown and Louw (2003) respectively. Low flows were analysed using flow duration/exceedance curves for each month. These low flow duration curves exclude flood events but capture the increased flow "underneath" each flood. Because the interbasin transfer (IBT) on the Berg River was operational between 1980 and 2007, the low flows for this period are unnaturally elevated for the Berg River and therefore the low flow record for G1H004 is taken from the flow record pre-1980. The 50th percentile (or median low flow data) at G1H004 and H1H018 are provided in Table 3.3 and were used to define base flow conditions for further analysis of the flow regime during the 21-month sampling period.

Table 3.3 50th percentiles for low flow data at G1H004 and H1H018 based on long-term low flow averages with floods removed from the flow record Flow values are in m^3s^{-1}.

Station	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
G1H004 (pre-1980)	0.32	0.21	0.20	0.66	1.52	2.75	3.22	4.17	2.64	1.92	1.05	0.49
H1H018 (full record)	3.23	2.69	2.25	1.17	1.28	2.68	3.98	2.99	2.91	1.43	1.81	2.75

Aquapak Version 1.05 2007 (Gordon *et al.* 2004), was used to summarise daily flow data into a number of flow parameters that potentially explain temporal patterns of periphyton. Using the thresholds defined by DRIFT for flood events in the Berg and Molenaars Rivers, it was possible, using Aquapak, to determine the frequency and duration of the intra-annual flood events (i.e. Class 1-4) and the 1 in 2 year flood (Class 5) over the 21-month sampling period. If the time between two consecutive flow peaks was less than 3 days, or the minimum flow between two consecutive peaks exceeded the winter base flow discharge (an average of the base flows over the winter period from the long term record Table 3.3), the peaks were combined as a single event. The number of days between sampling and the preceding Class 1, 2, 3, 4 and 1:2 floods were also calculated for each sampling occasion, as parameters that might describe recovery since a particular flood event.

The number of days since the onset of both the wet and the dry season and each sampling occasion provided a means of describing the timing of the start or cessation of the flood season. The onset and cessation of the wet season (and hence the start of the dry season) were described as the first and last crossing of the mean annual flow calculated from the long term flow record for both the Molenaars and Berg Rivers using the method described in King and Brown (2010). In addition to these parameters, average daily discharge (Q_{ave}) , the Coefficient of Variation (CV) of daily discharge (Q_{CV}), and the peak discharge (Q_{max}) in the inter-sampling period (i.e. the period between two consecutive sampling occasions which was usually about 30 days) were calculated as hydrological factors that could explain temporal variance in periphyton biomass (Table 3.4).

3.2.5 Statistical analyses

3.2.5.1 Univariate statistical analyses

Analysis of Variance (ANOVA) in STATISTICA v10, 2011 was used to detect changes in specific environmental variables between sites and seasons. Data were log (x+1) transformed where necessary to fulfil the assumptions of normality and to reduce variance prior to analysis. Where significant differences were found ($p < 0.05$), Tukey multiple *post-hoc* comparisons of differences between sites and seasons were used.

3.2.5.2 Characterisation of sites based on environmental variables

Once the general pattern of temporal change in the variables under different flow and enrichment conditions was established, relations among variables were determined using Principle Components Analysis (PCA) in PRIMER v6 (Clarke 1993; Clarke and Warwick 1994). Firstly, the values of 42 parameters characterizing the five environmental variables (Table 3.4) were calculated. Draftsman plots of each set of parameters were used to check for normality and where obvious skewness was detected, data were either log (x+1) or square root transformed. In many cases, several parameters measure similar entities and therefore there was the potential for including redundant information. The output correlation matrices for each set of environmental variables were therefore used to identify highly correlated and effectively redundant information. Parameters values with an $r > 0.9$ were considered strongly correlated and thus only one of a number of highly correlated predictor variables that contain biologically and statistically redundant information was retained for inclusion in the PCA analysis as indicated in Table 3.4. All data in the reduced set of parameters were then normalized prior to PCA by subtracting the mean and dividing by the standard deviation for each variable so that all parameters are of potentially equal importance in determining the principle components. Principle components with eigenvalues (i.e. explained variances) near zero do not contribute significantly to explaining variability in the dataset and can therefore be excluded. The set of environmental parameters were therefore reduced further by excluding those with eigenvalues between -0.2 and 0.2 in the first three axes of the PCA.

Table 3.4 **A list of the variables characterising the six key environmental factors measured during this study. Parameters in bold text were included in the initial PCA analyses describing temporal and spatial differences in Section 3.3.3 after correlation analysis to exclude redundant variables.**

Environmental factor	Variable
Irradiance	Mean daily solar radiation during the inter-sampling period (RS_{ave}) **Minimum daily solar radiation during the inter-sampling period (RS_{min})** Maximum daily solar radiation during the inter-sampling period (RS_{max}) **Cumulative solar radiation during the inter-sampling period (RS_{cum})**
Temperature regime	Average inter-sampling temperature (WT_{ave}) **CV of inter-sampling water temperature (WT_{CV})** **Minimum daily water temperature during the inter-sampling period (WT_{min})** Maximum daily water temperature during the inter-sampling period (WT_{max}) **Average daily range in temperature of the inter-sampling period (WT_{range})** **Cumulative degree days during the inter-sampling period (WT_{cum})**
Flow regime	Average inter-sampling discharge (Q_{ave}) **Median of inter-sampling discharge (Q_{med})** **CV of inter-sampling discharge (Q_{cv})** **Peak discharge over the inter-sampling period (Q_{max})** Number of floods ≥ Class 1 **Number of floods ≥ Class 2** Number of floods ≥ Class 3 Number of floods ≥ Class 4 Number of days in flood ≥ Class 1 flood (FLW duration-Class 1_{dur}) **Number of days in flood ≥ Class 2 flood (FLW duration-Class 2_{dur})** Number of days in flood ≥ Class 3 flood (FLW duration-Class 3_{dur}) Number of days in flood ≥ Class 4 flood (FLW duration-Class 4_{dur}) days since flood ≥ CLASS 1 **days since flood ≥ CLASS 2** days since flood ≥ CLASS 3 days since flood ≥ CLASS 4
Physico-chemical	**Water depth** **Near-bed velocity** **Conductivity** **Turbidity** **pH** **Oxygen (mg l^{-1})** Oxygen (%)
Nutrients	**PO_4^{3+}-P** **TP** **SiO_4** TIN **NH_4^+-N** NO_3^--N + NO_2^--N **NO_2^--N** **NO_3^-N** N:P ratio
Grazing	Total biomass of grazers Total biomass of scrapers **Total biomass of brushers** **Total biomass of deposit feeders** Total density of grazers **Total density of scrapers** **Total density of brushers** **Total density of deposit feeders**

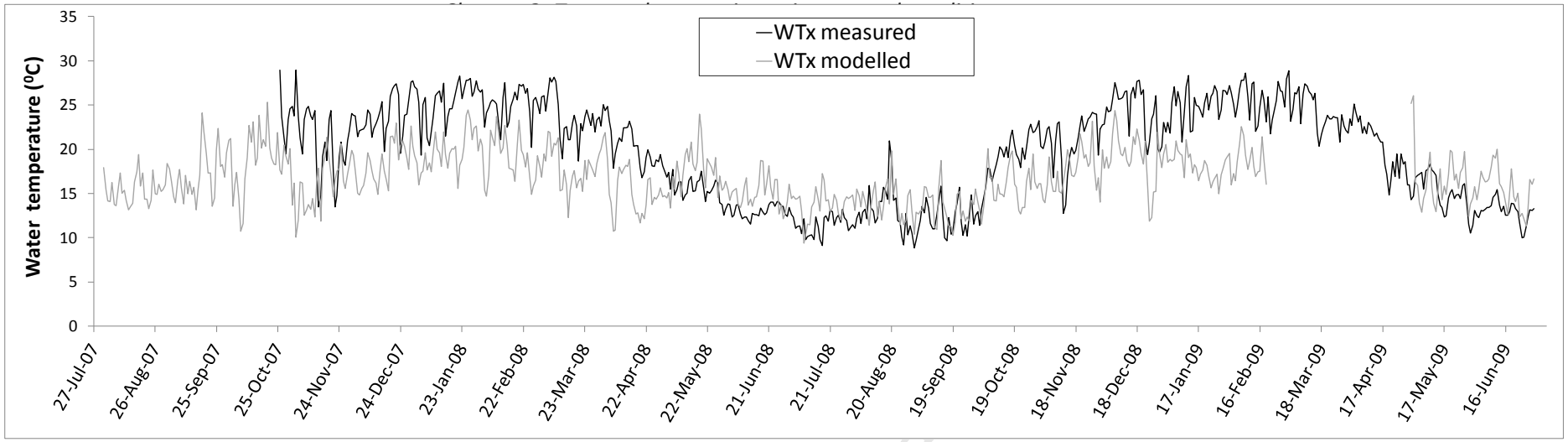


Figure 3.2 **Daily maximum water temperatures measured in the Berg River at Site 1 between late October 2007 and June 2009 compared with simulated daily maximum water temperature derived from daily air temperature and flow data between August 2007 and June 2009**

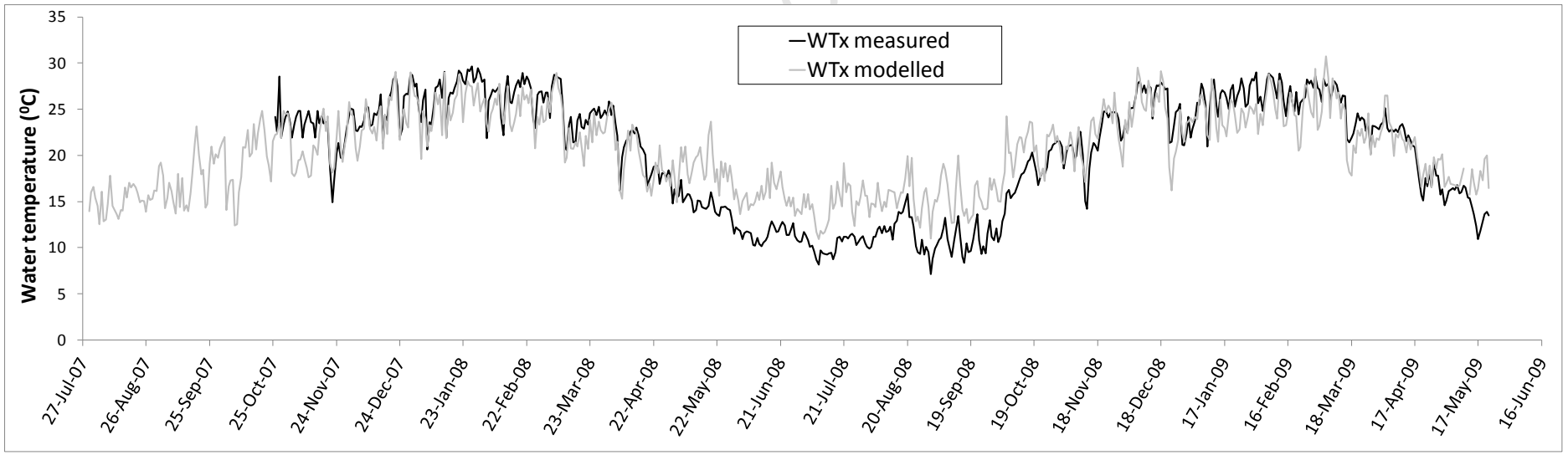


Figure 3.3 **Daily maximum water temperatures measured in the Berg River at Site 2 between late October 2007 and May 2009 compared with simulated daily maximum water temperatures derived from daily air temperature and flow data between August 2007 and May 2009**

3.3 RESULTS

3.3.1 Temporal changes in abiotic conditions

3.3.1.1 Daily radiation over the study period

Total daily radiation measured at La Motte in Franschhoek showed a distinct cycle with lowest daily radiation measured between May and July each year and highest daily radiation recorded between October and January, peaking around mid-December (Figure 3.4). These temporal changes in the maximum total radiation reflect temporal changes in day-length over an annual cycle and appear fairly consistent between years based on data over a three year period (Figures 3.4). The range of total radiation from day to day, however, varied considerably as observed during January 2008 when minimum values of 6.9 MJ m^{-2} and maximum values of 35.77 MJ m^{-2} were measured within the same week. One-way ANOVA between months shows distinct monthly differences in the total daily radiation (MS=2051, df=19, F=60.03, p<0.0001). Based on the Tukey post-hoc analysis of differences between months, September 2007 and September 2008 were significantly different from August and October of both years showing a distinct increase in total radiation over this three-month period (Figure 3.5). Similarly, significant changes in total daily radiation were found between February 2008 and May 2008 signally a marked decline in total radiation. The same pattern could not be verified the following year because of a lack of available data.

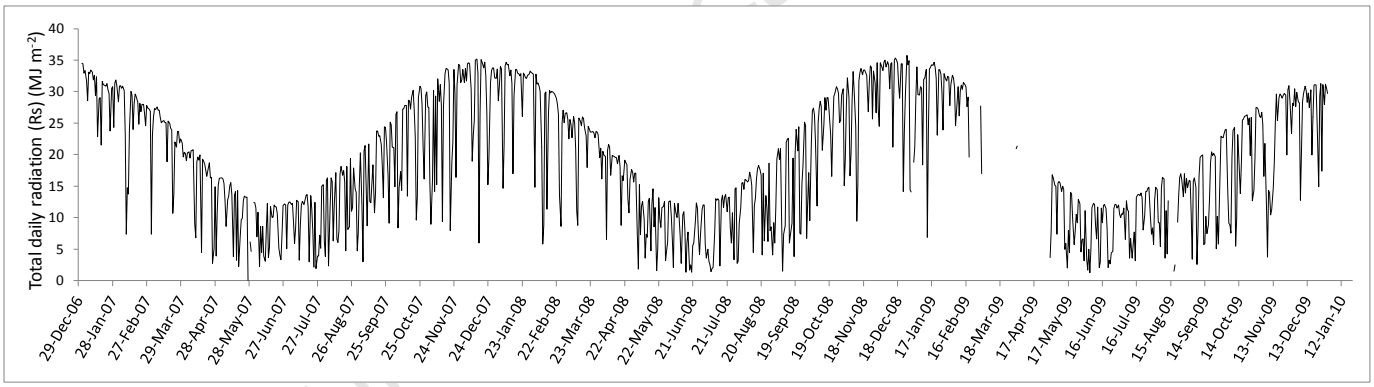


Figure 3.5 Total daily radiation (MJ m^{-2}) measured at La Motte in the Franschhoek Valley between January 2007 and January 2010.

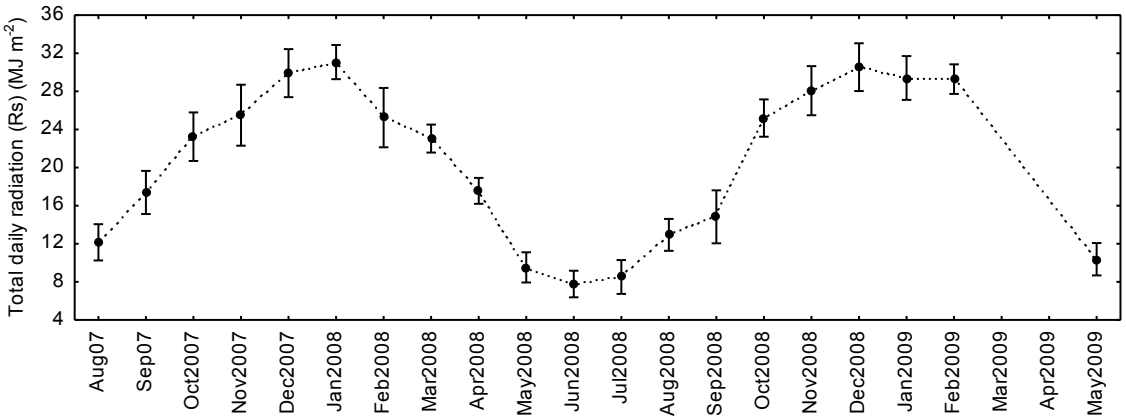


Figure 3.4 Monthly averages of total daily radiation measured at La Motte in the Franschhoek Valley between August 2007 and May 2009. The vertical bars denote the 0.95 confidence intervals.

3.3.1.2 Characteristics of water temperature

Mean monthly temperature (WT_{ave}) at each of the four sites, like daily radiation, showed a cyclical trend of lower WT_{ave} between May and September and higher WT_{ave} between late December and early March (Figure 3.6). A two-way ANOVA showed significant differences in WT_{ave} between sites (Table 3.5). A post-hoc analysis of differences (Tukey test), indicated that there were no significant differences in WT_{ave} between Sites 3 and 4 on the Molenaars River in all months. On the Berg River however, Site 2 downstream of the dam was significantly warmer than Site 1 during April, May and June 2008.

There were no significant differences in WT_{ave} between Sites 1, 3 and 4 in all months sampled, with the exception of October2008 when Site 3 (upper Molenaars site) was significantly warmer than Site 1 (Upper Berg site). By contrast, Site 2 (lower Berg site) was significantly warmer than both Sites 3 and 4 (lower Berg site) during the colder months (i.e. between April 2008 and October 2008).

While Site 1 on the Berg River had the lowest amplitude of change in WT_{ave}, Site 4 on the Molenaars River had the highest amplitude of change over the study period. Site 3 was generally the coolest site, whereas Site 2 below the Berg Dam was the warmest.

Table 3.5 Two-way ANOVA of daily mean water temperature (WT_{ave}) between sites over 21 months

	SS	Df	MS	F	p
Site	217	2	108	46	< 0.001
Month	30891	16	1931	813	< 0.001
Site x Month	638	54	12	5	< 0.001
Error	4975	2094	2		

At Sites 1, 3 and 4, the colder months were associated with a much narrower range in daily temperatures, varying generally within 3 or 4^{o}C of the daily mean, than the warmer months, which are associated with a much broader range in daily temperatures (Figure 3.7). A two-way ANOVA showed that the water temperature range (WT_{range}) differed between sites and months (Table 3.6). Site differences were evident during the warmer months when WT_{range} at Site 3 was significantly lower than at Site 1 and Site 4 (Figure 3.7), possibly due to cooler water from the Krom River gorge and significant shading from the narrow valley characterizing Site3. Fewer site differences in WT_{range} were evident during the colder months (Figure 3.7).

While a seasonal trend of lower WT_{ave} during the colder months and higher WT_{ave} mean water temperatures during the warmer months was evident at Site 2 (Figure 3.6), no clear seasonal pattern in WT_{range} was apparent downstream of the dam (Figure 3.7). Nevertheless, WT_{range} was not significantly different from the other three sites during the colder months between April 2008 and August 2008. The temperature differences between Site 2 and the other three sites during the warmer months were probably a result of altered conditions associated with the Berg Dam and the daily release characteristics since the dam has been operational.

Table 3.6 **Two-way ANOVA of daily water temperature range (WT_{range}) between sites over 21 months**

	SS	Df	MS	F	p
Site	1262	2	631	292	< 0.001
Month	4910	16	307	142	< 0.001
Site x Month	1581	54	29	14	< 0.001
Error	4527	2094	2		

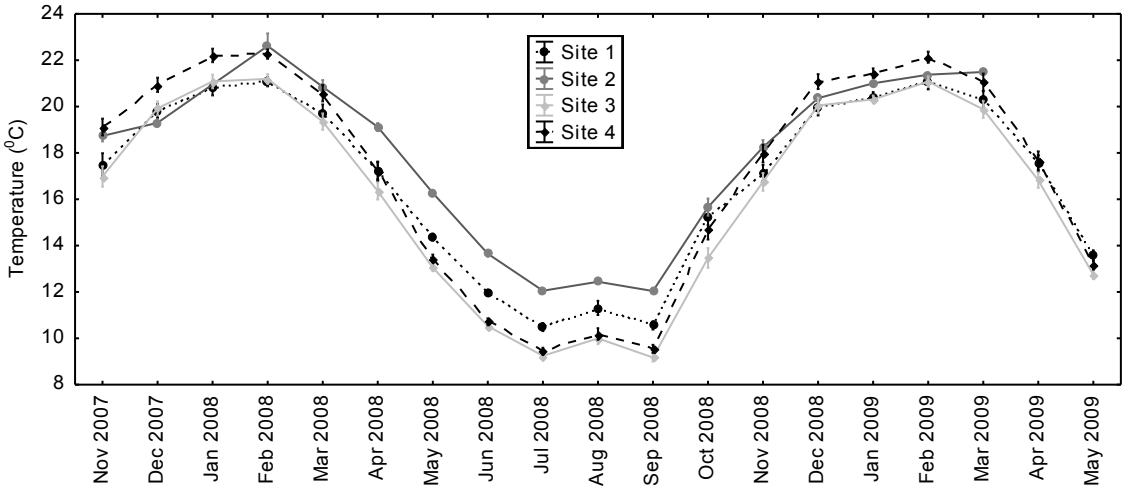


Figure 3.6 **Monthly mean water temperature (WT_{ave}) between October 2007 and May 2009 at Sites 1 and 2 on the Berg River and Sites 3 and 4 on the Molenaars River. The vertical bars denote the 0.95 confidence intervals.**

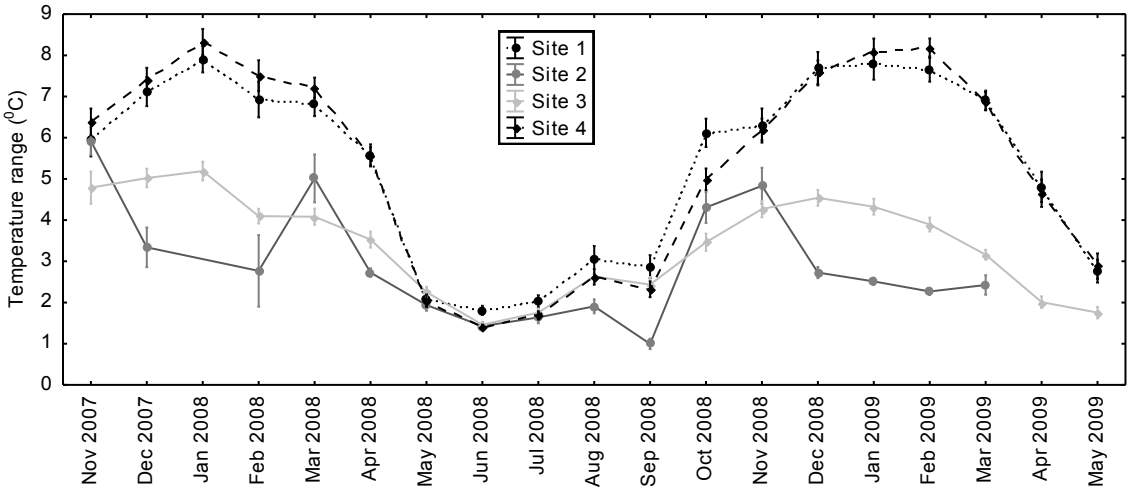


Figure 3.7 **Daily range in water temperature (WT_{range}) averaged over each month between October 2007 and May 2009 at Sites 1 and 2 on the Berg River and Sites 3 and 4 on the Molenaars River. The vertical bars denote the 0.95 confidence intervals**

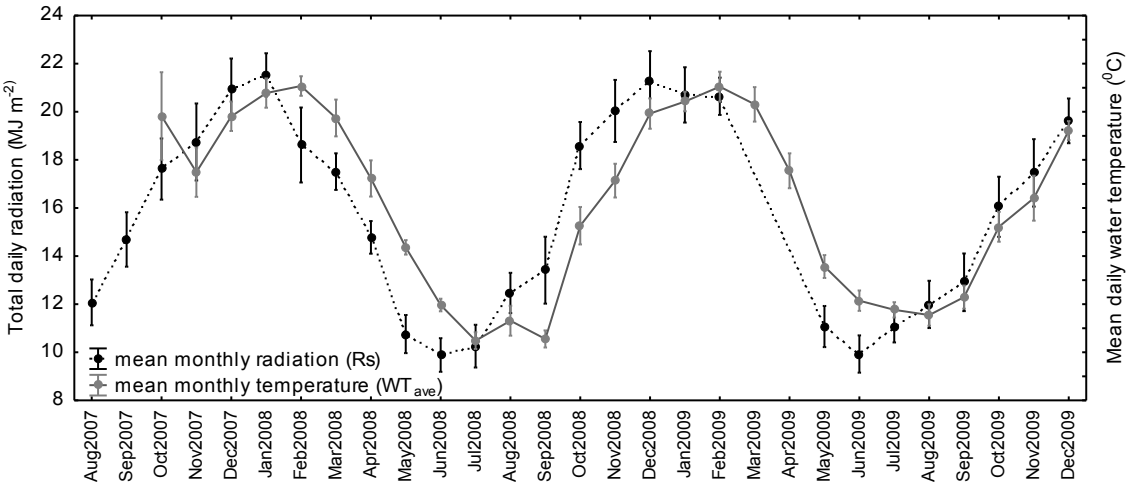


Figure 3.8 **A comparison between mean monthly radiation (Rs) and mean monthly water temperature (WT_{ave}) at Site 1 showing the offset in the annual cycle of these two variables. Both the increase and decrease in WT_{ave} is delayed by approximately one month, relative to changes in Rs.**

3.3.1.3 Determination of seasons based on Maximum daily temperature

STARS was applied to maximum daily temperature measured at Site 4 for the sampling period and to the simulated data for the period prior to the onset of sampling (see section 3.2.4.2), to determine shifts in temperature that could be used to define the boundaries of different 'seasons'. Between November 2007 and May 2009, the measured temperature data returned ten shifts in the average maximum daily temperature reflecting ten different seasonal periods (Figure 3.9). While the measured data detected a seasonal shift in temperature between October 2008 and December 2008, no such distinction was evident for the same period based on simulated data (Figure 3.10). Apart from this anomaly, the shifts detected for both the measured and simulated data were similar and therefore the seasonal shifts defined using simulated data for the period prior to sampling (May 2007 to November 2007), were accepted as a sound basis for detecting seasons for this period. Thus a total of 11 seasons over the 21-month sampling period were identified, representing early spring, late spring, summer, early autumn, late autumn and winter over two seasonal cycles (Figures 3.9 and 3.10). The same seasonal boundaries were applied to all study reaches because a comparison of the seasonal shifts derived at Site 4 were similar to those determined for Site 1 on the Berg River (not presented) suggesting that the seasonal signature as depicted by water temperatures are not catchment specific. Indeed, these data showed similar cut-off dates to water temperature data collected over the same period in the Palmiet River in a neighbouring catchment (G. Ractliffe, unpublished data), and it is probable that foothill rivers in the winter rainfall region of the south-western Cape show similar seasonal changes. Evidently, these seasonal changes do not coincide with the generalized seasonal categorisation of spring, summer, autumn and winter which are based on the conventional allocation of three months of the year to each season. Essentially categorisation of monthly data into six seasons (i.e. early spring, late spring, summer, early autumn, late autumn and winter) should assist with the interpretation of temporal changes in periphyton biomass and community structure within any given annual cycle, which is presented in Chapter 4.

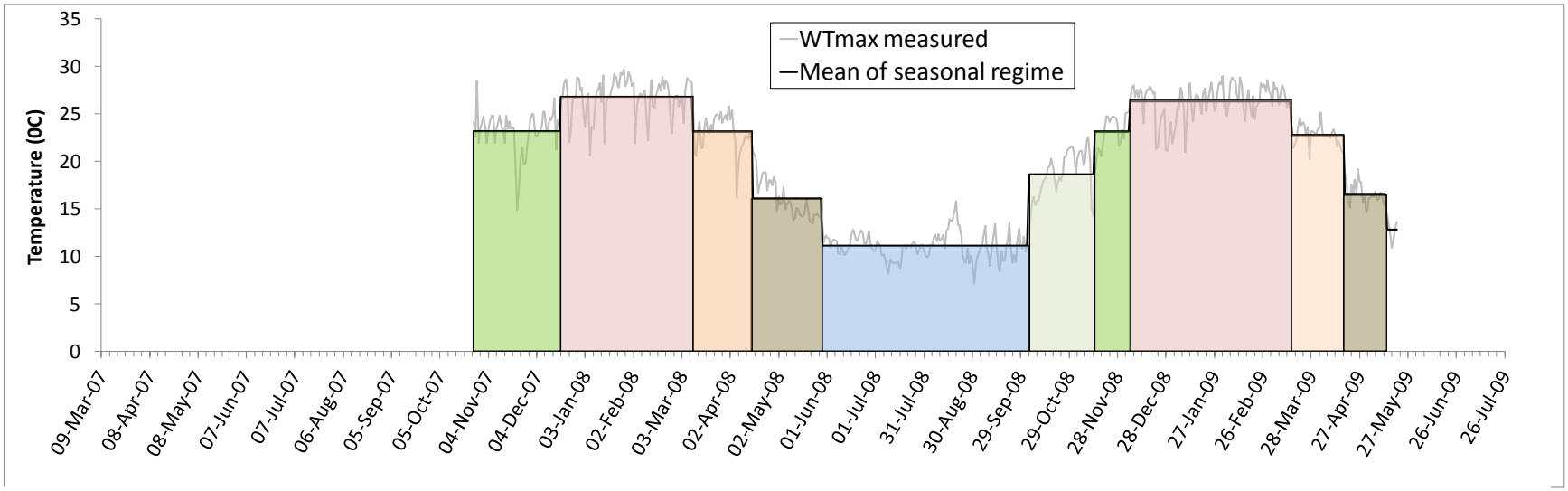


Figure 3.9 **Maximum daily water temperature (WT_{max}) measured at Site 4 on the Molenaars River between October 2007 and May 2009 plotted with the 11 regimes representing seasons detected using the STARS procedure p=0.04, length = 15, subsample = 9, IP4. CV = 0.311. Yellow = early spring; green = late spring; pink = summer; orange = early autumn; brown = late autumn, blue = winter**

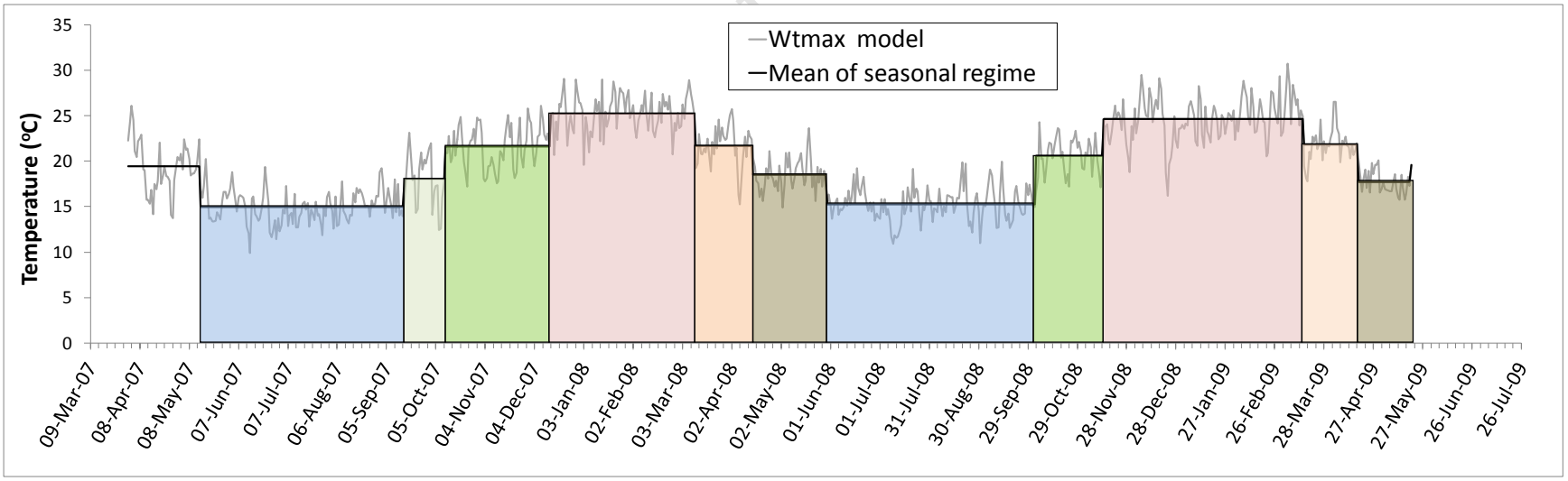


Figure 3.10 **Maximum daily water temperature (WT_{max}) at Site 4 on the Molenaars River between October 2007 and May 2009 plotted with the 11 regimes representing seasons detected using the STARS procedure p=0.05, length =15, subsample=15, IP4 cv=0.223. Yellow = early spring; green = late spring; pink = summer; orange = early autumn; brown = late autumn, blue = winter**

3.3.1.4 Flow characteristics of the Berg and Molenaars Rivers

Under natural conditions, the Berg River at G1H004 has a Mean annual Runoff (MAR) of 136 Mm^3 and a mean annual discharge of 4.7 m^3s^{-1}. The Molenaars River in its foothill reaches at H1H018 immediately downstream of Site 4 is somewhat larger with a natural MAR of 164 Mm^3 and a mean annual discharge of 5.2 m^3s^{-1}. During the sampling period, annual discharge in both the Molenaars and Berg Rivers did not differ significantly from the long term average, indicating that natural flow conditions over the sampling period were representative of these systems over the long term. Under natural conditions both rivers show a strong cyclical pattern of low flows during the warm summer months followed by a distinct wet season characterised by frequent floods when water temperatures are low as was evident during the period of sampling between September 2007 and May 2009 (Figures 3.11 to 3.14).

Interestingly, mean daily discharge was lower than expected for the winter at Site 1 and differed considerably from that in the Molenaars River (Sites 3 and 4[6]) (Figure 3.11). The unexpected drop in mean daily discharge at Site 1, relative to that at Sites 3 and 4 during this period might be due to the difference in size between these two rivers. August 2008 was a warm month with little or no rainfall which is atypical of conditions in the south-western Cape during the winter. Thus, the Berg River, being a smaller system than the Molenaars River, may have been less buffered to change in response to these conditions with a significant change in base flows at Site 1 but not at Sites 3 and 4.

During the sampling period for this study (Figures 3.11 to 3.14), the onset of both the wet and dry seasons was fairly consistent under natural conditions between years and sites (Table 3.7) with the dry season extending from December to May during both the 2007/08 and 2008/09 period and May 2008 and 2009 marking the onset of the wet season in both systems (Figures 3.11 to 3.14).

By contrast, Site 2 (Figures 3.11 and Figure 3.15) experienced a completely different pattern of wet and dry conditions over the study period. Although floods occurred during the winter of 2008, unnaturally extended periods of low flow separated these events during the winter (Figure 3.15). Elevated base flows characteristic of small spates under natural conditions were evident during the summer months, particularly between November 2008 and April 2009 (Figure 3.12).

Table 3.7 Julian day and date of the onset of the wet and dry season between 2007 and 2009 under natural flow conditions on the Berg and Molenaars Rivers. The onset of the wet and dry season each year is defined as the first and last crossing respectively of the hydrograph over long term mean daily discharge.

	2007		2008		2009
	Onset of the wet season	**Onset of the dry season**	**Onset of the wet season**	**Onset of the dry season**	**Onset of the wet season**
Berg River @ G1H077	116 (25 Apr 07)	310 (6 Nov 07)	131 (10 May 08)	320 (15 Nov 08)	120 (30 Apr 09)
Molenaars River @ H1H018	117 (26 Apr 07)	313 (9 Nov 07)	132 (11 May 08)	321 (16 Nov 08)	135 (15 May 09)

[6] Note: flow data from H1H018 on the Molenaars River was used to describe the flow regime for both Sites 3 and 4. The flow data were not adjusted to account for a greater catchment area at Site 4, relative to Site 3 because there are no major tributaries that confluence with the Molenaars River between these two sites.

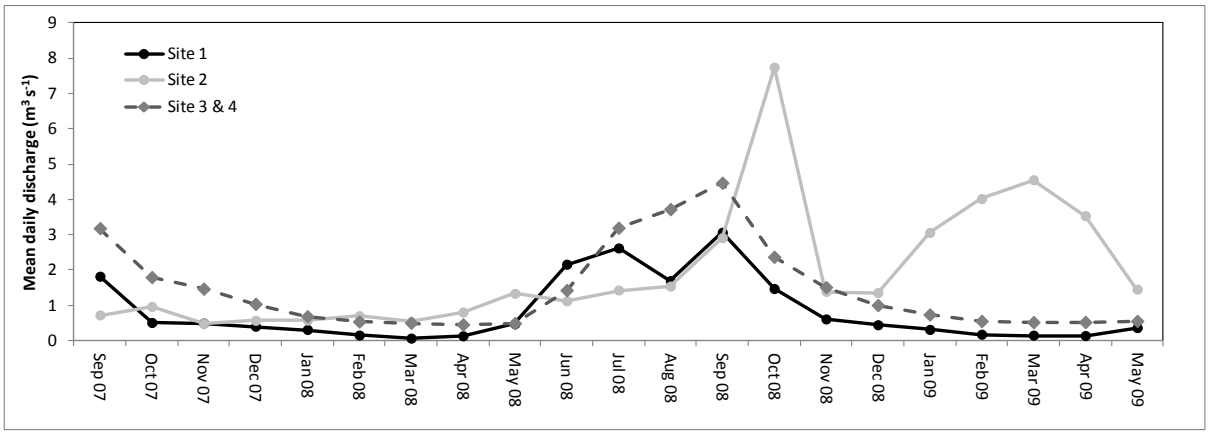


Figure 3.11 **Mean daily discharge over the 21-month sampling period for Sites 1 to 4 on the Berg and Molenaars Rivers showing a distinct difference in flows over the summer months between regulated (Site 2) and unregulated Sites (Sites 1, 3 and 4).**

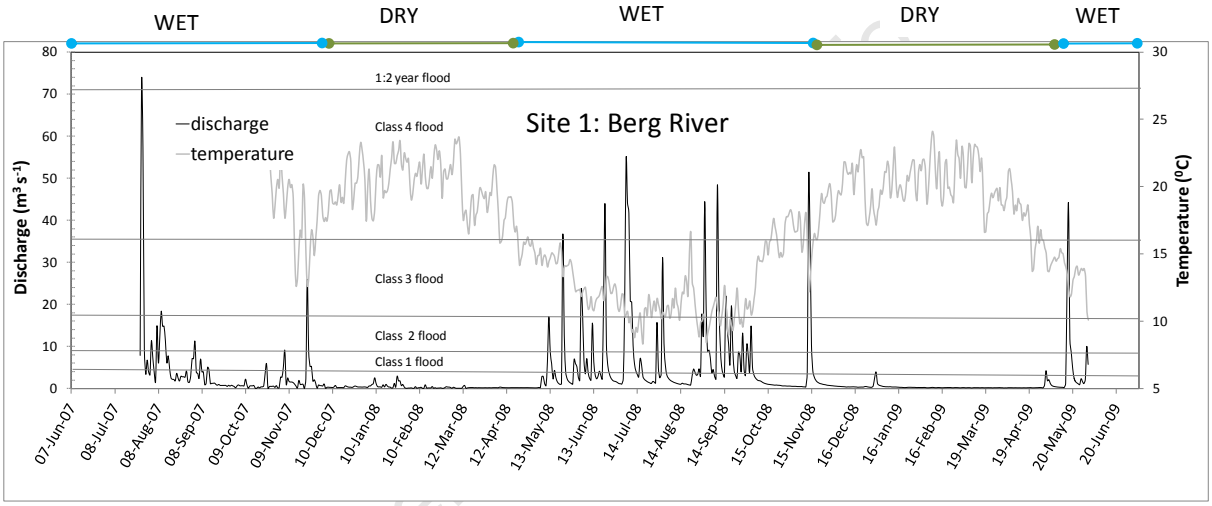


Figure 3.12 **Site 1: Mean daily discharge with mean daily water temperature over the sampling period.**

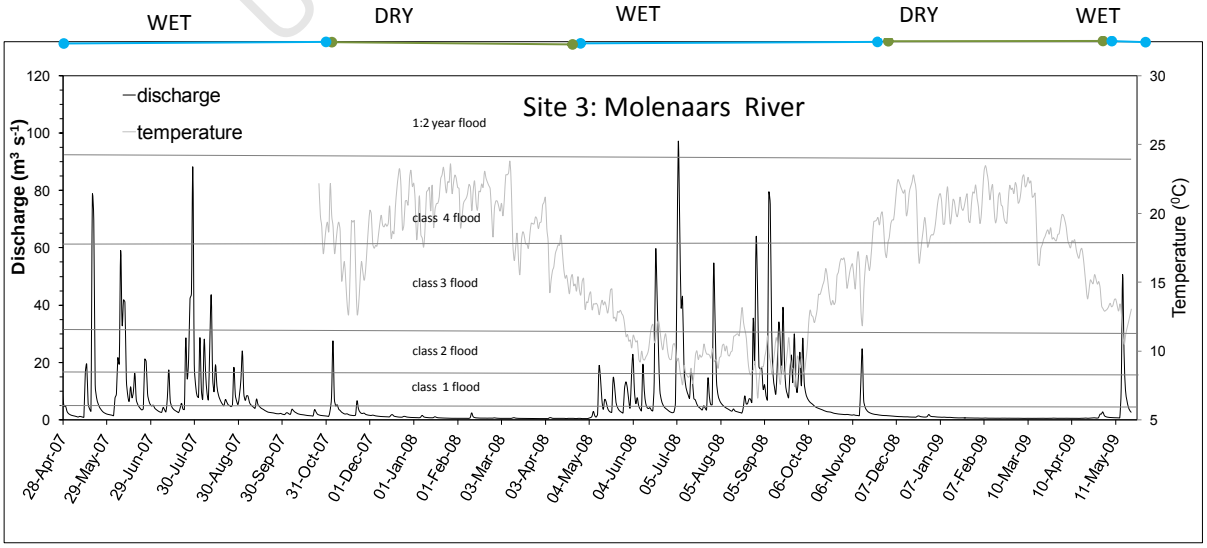


Figure 3.13 **Site 3: Mean daily discharge with mean daily water temperature over the sampling period. Note: Sites 3 and 4 have the same discharge taken from H1H018.**

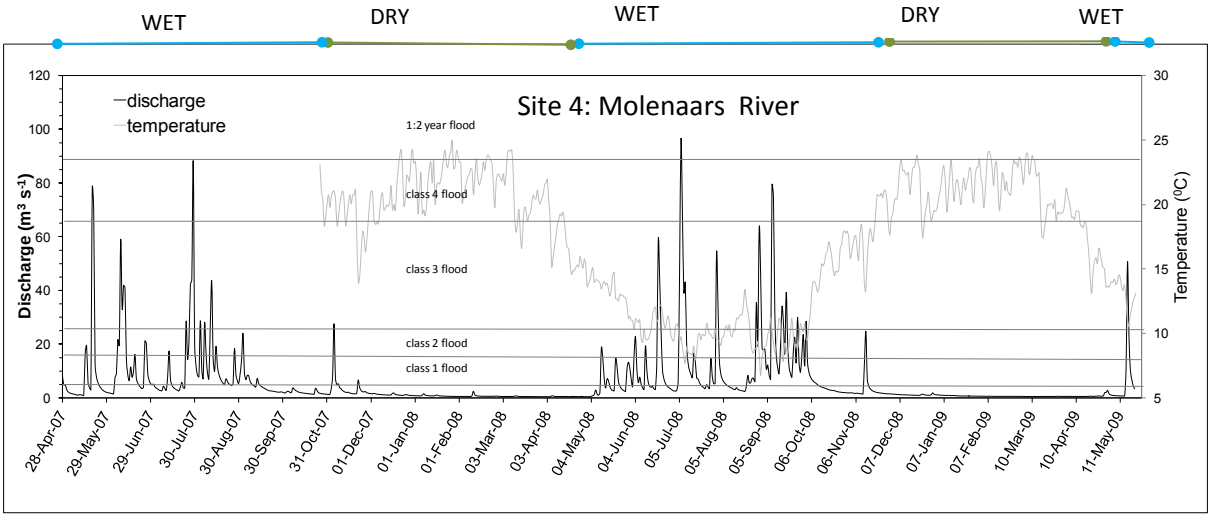


Figure 3.14 Site 4: Mean daily discharge with mean daily water temperature over the sampling period. Note: Sites 3 and 4 have the same discharge taken from H1H018.

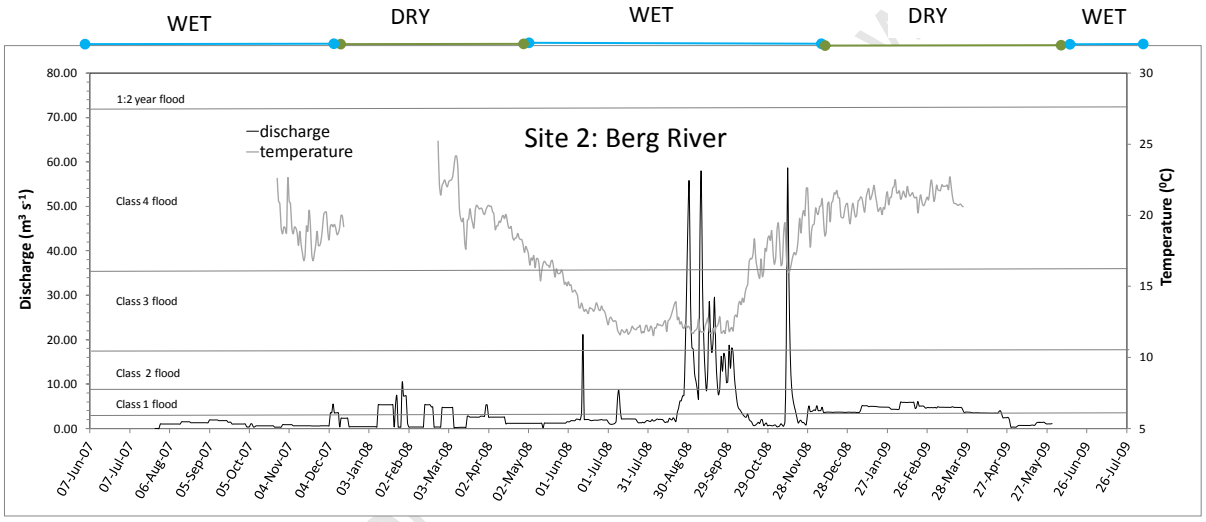


Figure 3.15 Site 2: Mean daily discharge with mean daily water temperature over the sampling period

Flood characteristics

The size, frequency and duration of flood events categorized by the DRIFT methodology are summarized in Figure 3.16. At Site 1 on the Berg River upstream of the Berg Dam, it is evident that the size, frequency and duration of flood events at the end of the wet season in 2007 (September 2007 to November 2007) differed substantially from that during the same period in 2008. Between September 2007 and November 2007, two class 1 and two class 2 floods were experienced during this period with a class 3 flood towards the end of November 2007. Also, the duration of these floods ranged between one and four days. By contrast, two class 4 floods and one 1:2 year flood were experienced in September 2008 alone, with a combined duration of 13 days. A class 3 flood occurred during October 2008 lasting for six days and another Class 4 flood with a duration of five days occurred during November 2008.

A similar inter-annual difference in the characteristics of flood events during this period was observed at H1H018 on the Molenaars River (Sites 3 and 4). A comparison of the frequency and

duration of floods during the spring in 2007 (September to November) with spring 2008 shows that only a few Class 1 and 2 floods with short durations occurred in 2007, while a number of Class 4 floods (or larger) and a Class 3 flood with far greater durations occurred over the spring of 2008 (Figure 3.16). These inter annual comparisons for natural flow conditions on both the Berg and Molenaars Rivers suggest that 2008 experienced more frequent floods with a greater magnitude and longer duration compared with 2007 for the latter part of the wet season.

Besides the inter annual differences in flood characteristics, a comparison between floods at Site 1 with that at Sites 3 and 4 (based on H1H018) on the Molenaars suggests some differences between these two rivers under natural conditions (Figure 3.16) . During the winter wet season of 2008, Site 1 on the Berg River experienced six class 4 floods and one event classified as a 1:2 year flood. Besides these larger flood events, five class 3 floods were experienced over this period in 2008 upstream of the Berg Dam. Whereas the Berg River experienced class 3 and 4 floods at the onset of the wet season (i.e. May and June 2008), these larger intra-annual floods only arrived during mid-winter (i.e. July 2008) on the Molenaars River, with the early part of the wet season characterised by smaller spates (Class 1 and 2 floods). Nevertheless, a series of larger floods was experienced between July and September 2008 in the Molenaars River and the duration of these events ranged between six days in September and a total of 23 days during July 2008 (Figure 3.16).

A comparison between natural flow on the Berg and Molenaars with altered conditions downstream of the Berg Dam at Site 2 shows a lack of the distinction between a period of flooding over winter followed by a period of no flooding during the dry summer months. In particular, flow conditions characteristic of class 1 and 2 floods were experienced throughout the summer of 2007/8 and again during the summer of 2008/09 lasting for extended periods (Figure 3.15). During the winter and early spring of 2007, when flooding occurred at Sites 1, 3 and 4 under a natural flow regime, no flood events were experienced at Site 2 downstream of the dam (Figures 3.15 and 3.16). Also, May 2008 marked the onset of the wet season during natural conditions at Site 1 but no flooding took place until June 2008 at Site 2 when a single Class 3 flood with duration of one day was released from the dam. Besides this flood and a single Class 1 flood in July 2008 (which is usually indistinguishable from wet season base flows under natural conditions), wet season conditions commenced at the end of August 2008 at Site 2 when the dam over topped resulting in a Class 4 flood at the end of August and another in early September 2008. A third Class 4 flood occurred in November 2008 downstream of the dam, which, under natural conditions would have marked the end of the wet season, although true low flow conditions typical of this river under natural conditions were not apparent during the warmer summer season that followed in 2009.

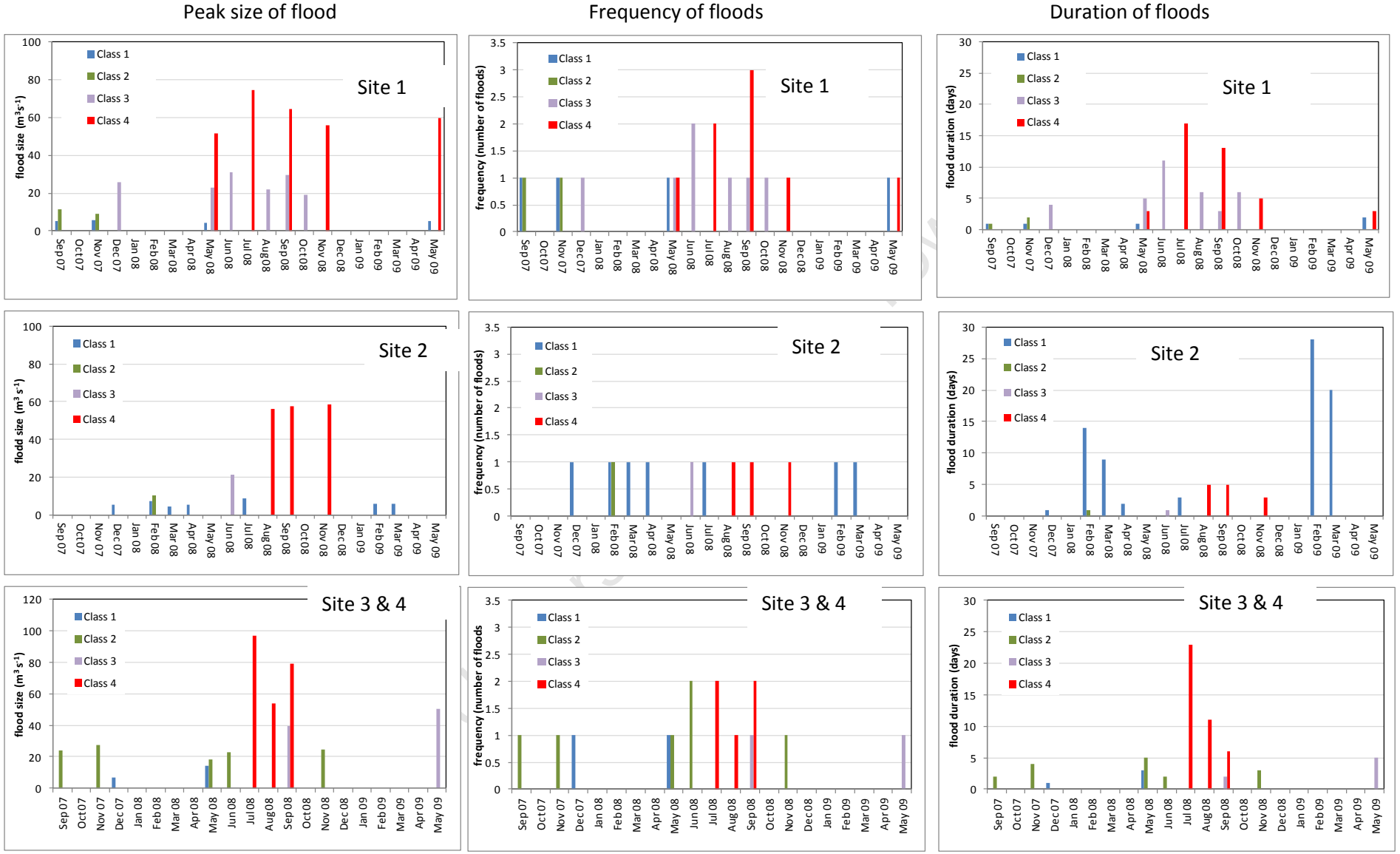


Figure 3.16 Summary of the different flood size characteristics determined using DRIFT

Base flows

During the study period, base flows in the Molenaars River were consistently higher than in the Berg by about 0.3-0.6 m^3 s^{-1} under natural conditions during the dry summer months (i.e. December to March) each year. This difference in base flows reflects the slight difference in size between these two systems. During the late spring and summer of 2007/08, base flows downstream of the Berg Dam were consistently higher than those upstream of the dam due to elevated irrigation releases from the dam between December and April (Figures 3.12 and 3.15). By contrast, base flow conditions downstream of the dam the following summer (i.e. 2008/09) were significantly higher than natural conditions with mean monthly base flows similar to those typical of winter under natural conditions (Figure 3.15). Essentially, base flows at the onset of the dry season were elevated relative to natural conditions and remained above the threshold for a class 1 flood for an extended period over the summer months. Whereas base flows are typically higher during the winter period under natural conditions the winter between June 2008 and August 2008 at Site 2 was marked by a period with lower than expected base flows, followed by exceptionally high base flows during October 2008 (Figure 3.15).

These flood and base flow comparisons suggest that the annual flow regime downstream of the Berg Dam was considerably altered from natural conditions during the study period. Also, comparisons between flood characteristics during winter/early spring 2007 and 2008 suggest inter- annual differences in the flow regime over the study period.

3.3.1.5 Temporal patterns in nutrient concentrations

Phosphorus and nitrogen are the key nutrients responsible for algal growth and therefore differences in monthly concentrations between sites may be responsible for changes in periphyton characteristics under different nutrient loads and in different seasons. Monthly concentrations of ortho-phosphates (PO_4^{3+}-P) and total phosphorus (TP) are presented in Figures 3.17 and 3.18. Monthly concentrations of nitrite nitrogen (NO_2^--N), ammonium nitrogen (NH_4^+-N) and total inorganic nitrogen (TIN), which is the sum of NH_4^+-N, NO_3^--N and NO_2^--N, were presented separately in Figures 3.19 to 3.21. The TIN concentrations were driven largely by NO_3^--N concentrations and reflect similar patterns. NO_3^--N concentrations are therefore not presented. Although silica (SiO_4) does not influence algal growth *per se,* diatoms require silica for the construction of their frustules and thus concentrations of silica potentially influence patterns of diatom biomass and composition. Monthly silica concentrations are therefore presented in Figure 3.22.

Concentrations of phosphorus and nitrogen were generally lower in the Berg River, relative to the Molenaars River (Figures 3.17 to 3.21). Unexpectedly, both PO_4^{3+}-P and TP were higher at Site 3 upstream of the Du Toit's Kloof trout farm compared with concentrations downstream of the trout farm at Site 4 (Figures 3.17 and 3.18). Phosphorus concentrations in the Berg River did not show any seasonal pattern over the study period (Figures 3.17 and 3.18). By contrast, both PO_4^{3+}-P and TP concentrations in the Molenaars River were generally elevated at Site 3 during the warm summer

months, with a peak in March 2008 and again the following summer in February 2009 (Figures 3.17 and 3.18).

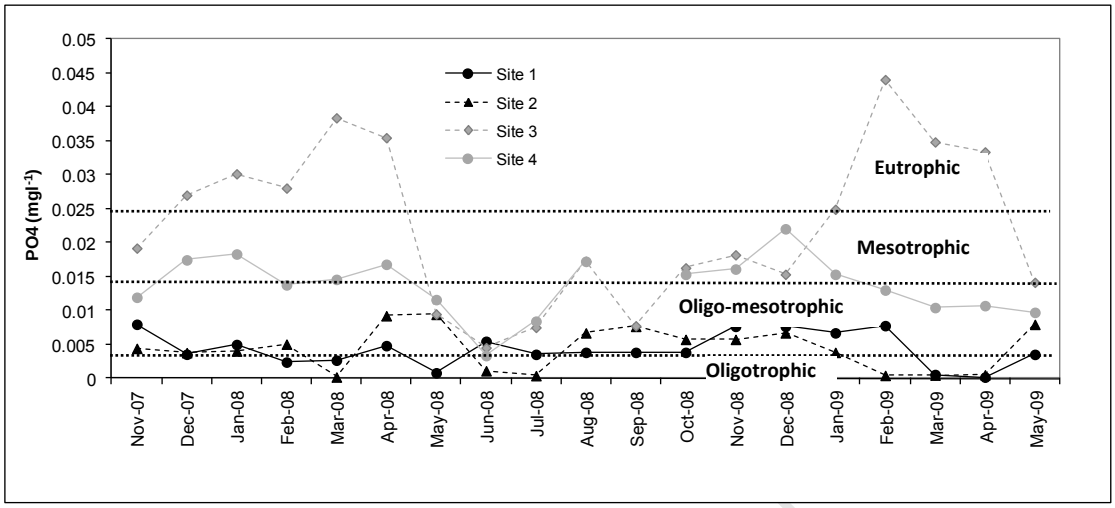


Figure 3.17 **Orthophosphate (PO_4^{3+}-P) in mgl^{-1} measured monthly from all four sampling sites on the Berg and Molenaars Rivers between November 2007 and May 2009. The dashed lines indicate the threshold between various trophic conditions as reported by DWAF 2008.**

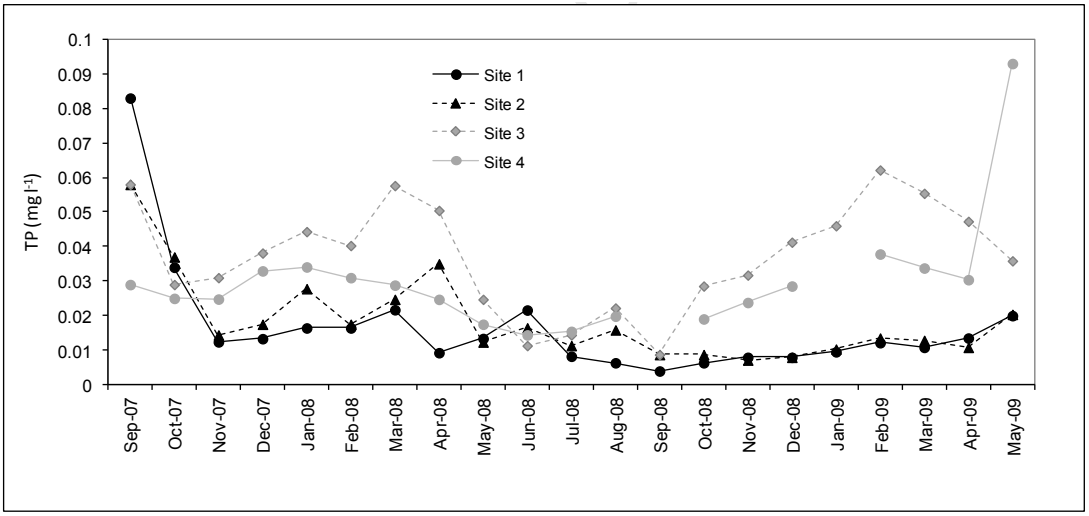


Figure 3.18 **Total phosphorus (TP) in mgl^{-1} measured monthly from all four sampling Sites on the Berg and Molenaars Rivers between September 2007 and May 2009.**

Similarly, higher concentrations of TIN were recorded at Site 3 on the Molenaars during the dry summer period from February 2008 to April 2008 and again between December 2008 and April 2009 (Figure 3.19). TIN concentrations in the Molenaars River were generally low during the winter (dropping with the onset of the wet season in May 2008), and reaching an annual minimum in June

2008. By contrast, TIN concentrations in the Berg River upstream of the dam peaked during May 2008, although no clear seasonal pattern in TIN was evident at this site (Figure 3.19).

Although no seasonal pattern in NO_2^--N concentrations were evident at all sites, the first annual cycle between November 2007 and July 2008 showed greater monthly fluctuations relative to the second annual cycle between August 2008 and May 2009 at all four sites (Figure 3.20). Also, NO_2^--N concentrations are generally lower during the first cycle (2007/2008) relative to the second (2008/2009). No seasonal pattern in NH_4^+-N was evident either over the sampling period (Figure 3.21). NH_4^+-N concentrations at Site 2 downstream of the Berg Dam, however, were considerably higher than at the other three sites during the first annual cycle. NH_4^+-N concentrations dropped off during the second cycle, particularly between November 2008 and April 2009 when concentration were only slightly higher than those recorded at the other three Sites. A sharp increase in May 2009 to concentrations similar to those recorded the previous year (May 2008) was once again recorded (Figure 3.21).

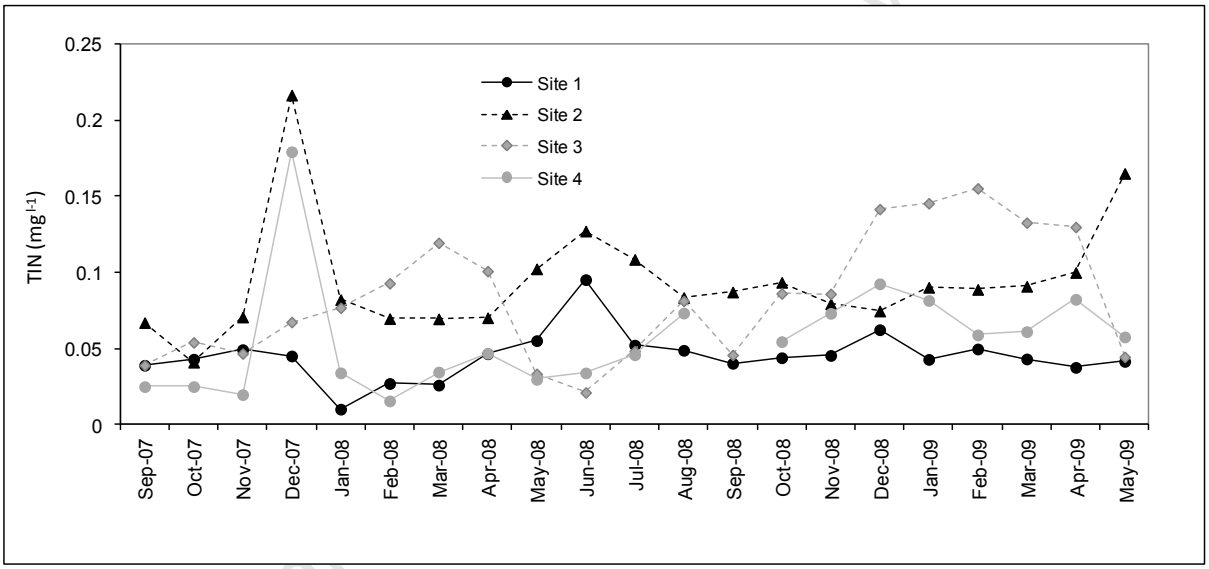


Figure 3.19 **Total inorganic nitrogen (TIN) in mgl^{-1} measured monthly from all four sampling Sites on the Berg and Molenaars Rivers between September 2007 and April 2009. All concentrations are within the range of "oligotrophic" according to DWAF 2008.**

Essentially, these data show that the Molenaars River at both sites was more enriched than the Berg River under natural flow conditions. Also, phosphorus and TIN concentrations changed seasonally in the Molenaars River, whereas no seasonality in these nutrients was evident in the Berg River. Although no seasonal patterns in NO_2^--N concentrations were evident at all four sites, NO_2^--N was elevated during the first summer relative to the second suggesting inter-annual differences in NO_2^--N. Nutrient concentrations upstream and downstream of the dam were similar with the exception of NH_4^+-N concentrations, which was far higher downstream of the dam relative to Site 1 upstream.

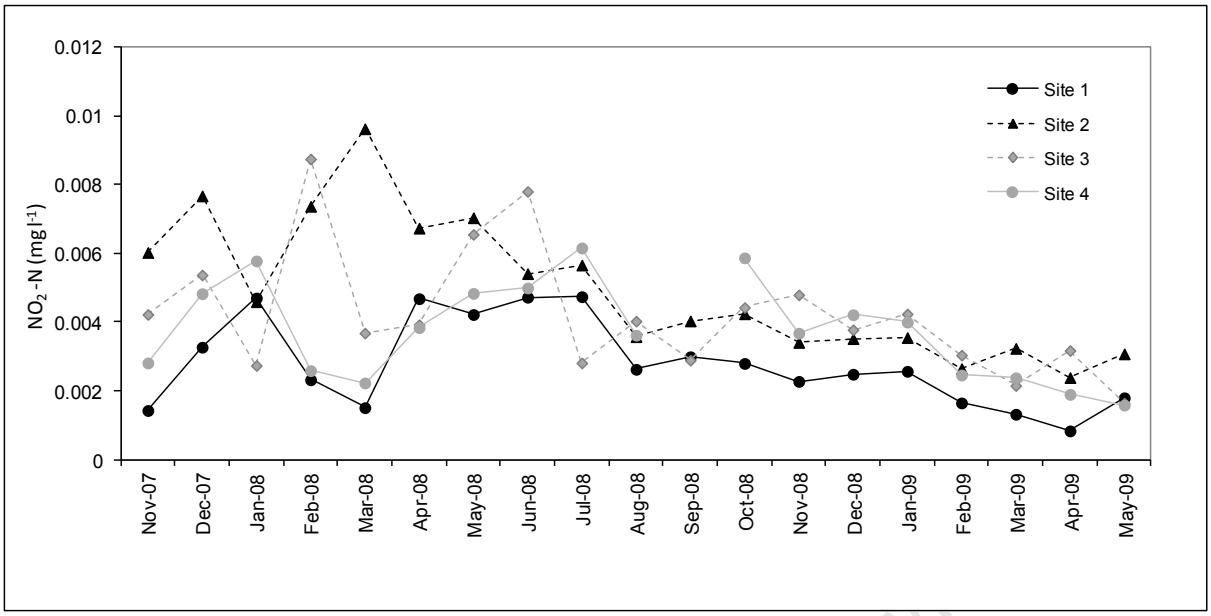


Figure 3.20 **Nitrites (NO_2^--N) in mgl^{-1} measured monthly from all four sampling Sites on the Berg and Molenaars Rivers between November 2007 and May 2009.**

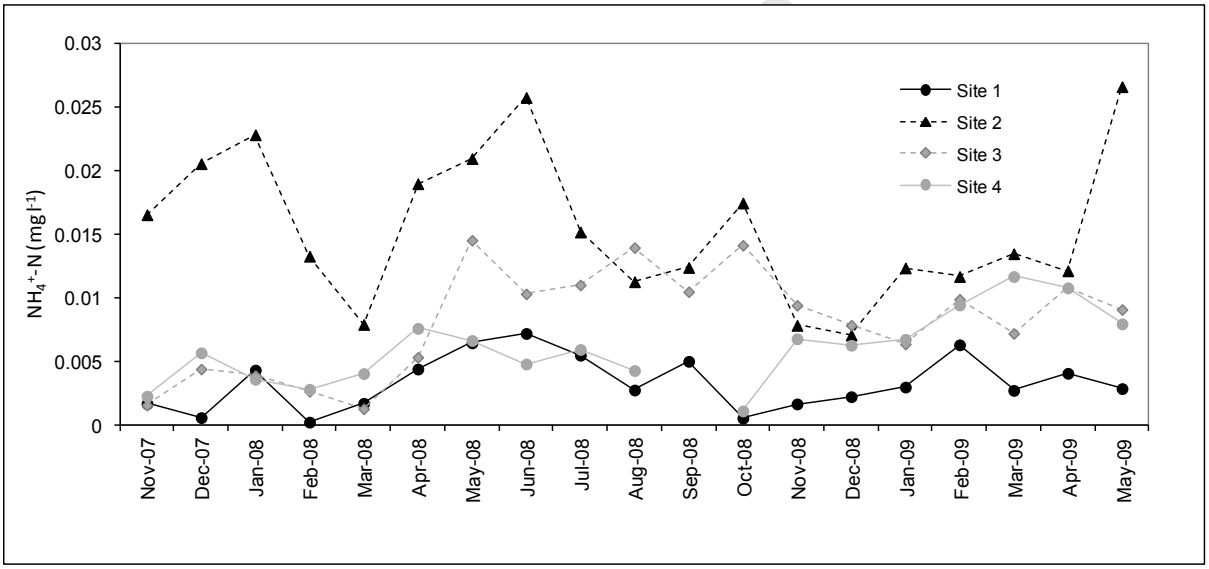


Figure 3.21 **Ammonia (NH_4^+-N) in mgl^{-1} measured monthly from all four sampling sites on the Berg and Molenaars Rivers between November 2007 and May 2009.**

Using the guidelines provided by DWA (DWAF 2008) for thresholds of different trophic status for both nitrogen and phosphorus, it would appear that all sites in all months were well within the oligotrophic range for nitrogen over the 21-month sampling period. Similarly, Sites 1 and 2 on the Berg River were generally within the range for oligotrophic conditions in terms of phosphates, although conditions in some months, particularly during the dry summer period, extended into the oligo-mesotrophic range. Elevated PO_4^{3+}-P concentrations during the summer at Site 3 placed the Molenaars River well within the eutrophic category (according to the guidelines) for the dry summer season. A drop in phosphate concentrations during the colder wet season placed this site in either a mesotrophic or even oligotrophic state during this period. Site 4 on the Molenaars River reached

mesotrophic conditions at the onset of the dry summer period but was generally within the range of oligo-mesotrophic for the rest of the sampling period.

Silica also showed a clear seasonal pattern with high concentrations over the dry summer months in the Molenaars River, particularly at Site 4 (Figure 3.22). Sites 1 and 2 on the Berg River did not reflect the elevated silica concentrations recorded on the Molenaars River and remained low throughout the sampling period, with no clear seasonal pattern. During the winter period, particularly between May 2008 and July 2008, silica concentrations on the Molenaars River dropped sharply to near zero levels. During this period there was no discernible difference between silica concentrations at all four sites (Figure 3.22).

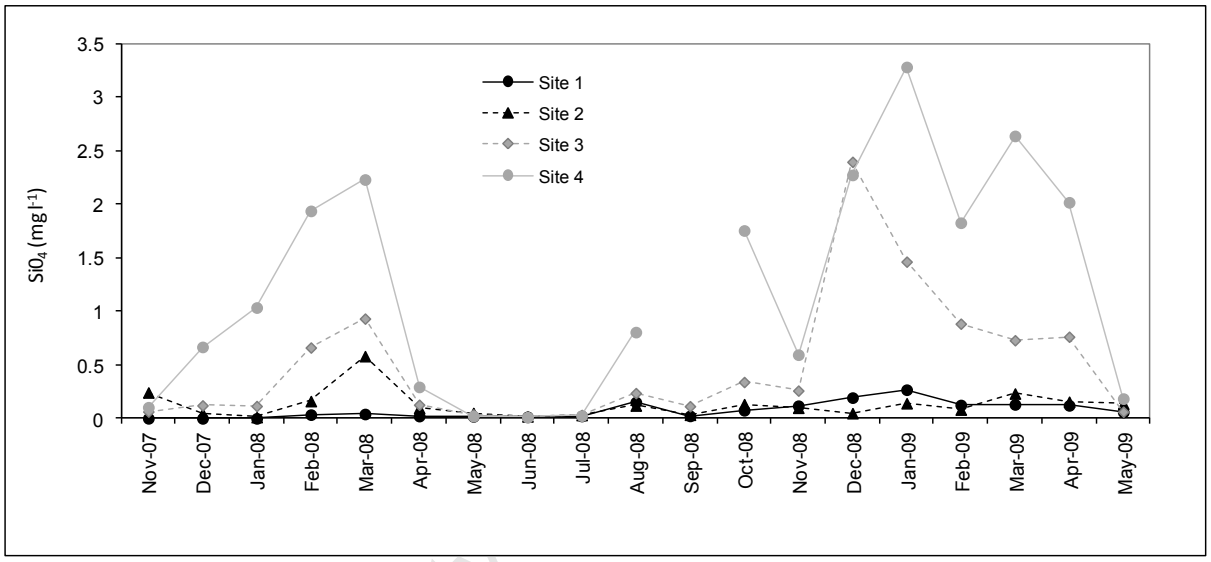


Figure 3.22 Silica (SiO_4) in mgl^{-1} measured monthly from all four sampling sites on the Berg and Molenaars Rivers between November 2007 and May 2009.

3.3.1.6 Temporal patterns in water chemistry

Turbidity

No seasonal pattern in turbidity over the study period was evident at any of the four sampling sites (Figure 3.23). Turbidity at Site 2 was considerably higher than that at the other three sites during most months. Turbidity was particularly high during the first annual cycle at Site 2 when values were generally above 4 NTU with peaks of 10 NTU during November 2007 and March 2008 (Figure 3.23). During the March 2008 sampling visit, turbidity increased within a few hours from 8.5 NTU to 13.44 NTU, hence the large variability at this time indicated by the error bars in Figure 3.23 at this time. This sudden increase in turbidity was associated with irrigation releases from the Berg Dam, which resulted in significant increases in discharge over a very short period.

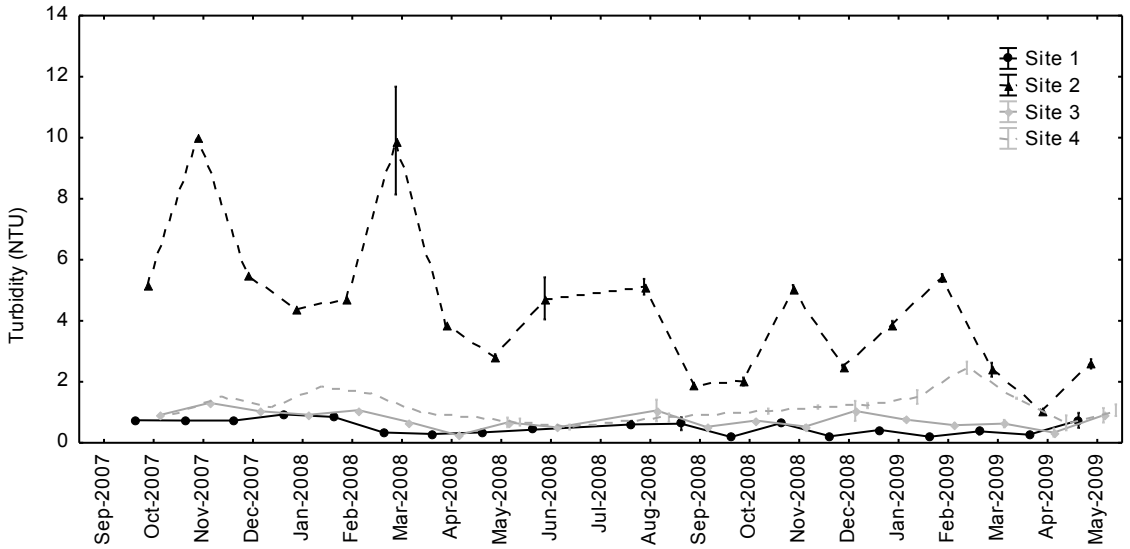


Figure 3.23 Turbidity measured monthly from all four sampling Sites on the Berg and Molenaars Rivers between September 2007 and May 2009. Error bars = ±1SE

pH

pH at Site 1 was generally lower than at the other three sites, with values between 3.7 and 5.7 (Figure 3.24), which is typical of acidic waters of pristine foothill rivers of the south-western Cape. With the exception of December 2008, pH at Site 2 was higher than that at Site 1, with a pH above 7 during November 2007, suggesting a considerable change in the water chemistry of the system relative to natural conditions. Similarly, the Molenaars River was generally less acidic than the Berg River under natural conditions, with pH values generally above 6 during the summer months. A comparison between Sites 3 and 4 shows that water at Site 4 below the trout farm was generally less acidic than water at Site 3 (Figure 3.24).

No clear seasonal pattern in pH was discernible at Sites 1 on 2 on the Berg River, whereas Sites 3 and 4 on the Molenaars River where characterised by lower pH values during the winter (May 2008 to July 2008) and high pH values during the summer of both 2007/08 and 2008/09 (Figure 3.24).

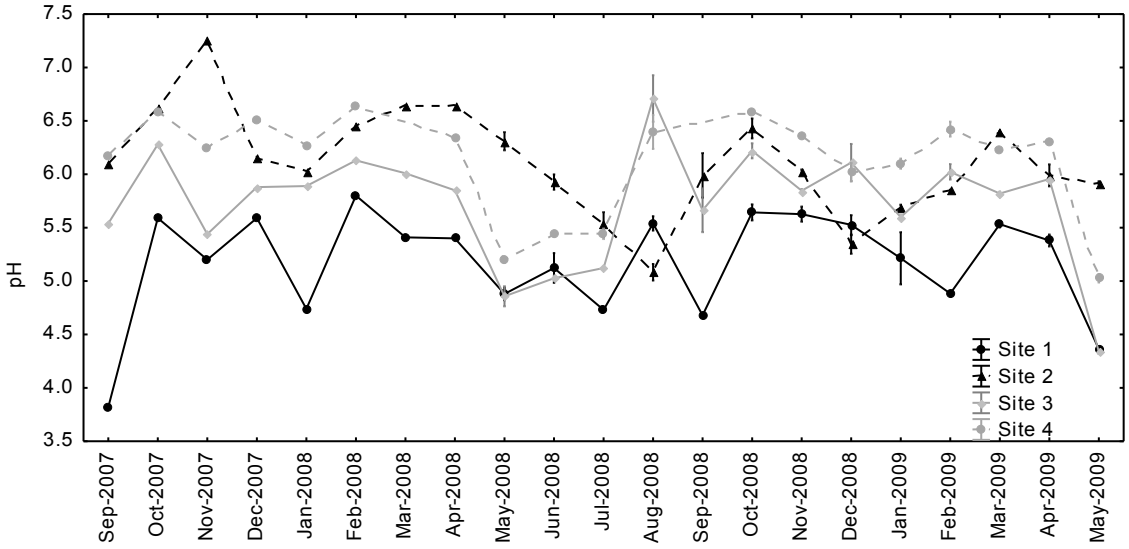


Figure 3.24 pH measured monthly from all four sampling sites on the Berg and Molenaars Rivers between September 2007 and May 2009. Error bars = ±1SE

Conductivity

No apparent seasonality in conductivity was observed at any of the four sites sampled between September 2007 and May 2009 (Figure 3.25). Lowest conductivity was recorded at all four sites at the onset of the dry season during November 2008 when values were around 10 µS cm^{-1}. A spike in conductivity was observed at all four sites during March 2009 when conductivity ranged between 38 and 48 µS cm^{-1}. This spike may simply be a result of equipment error, although this could not be confirmed. With the exception of December 2008 to January 2009 when conductivity was higher at Site 4, no difference in conductivity was evident between sites over the sampling period.

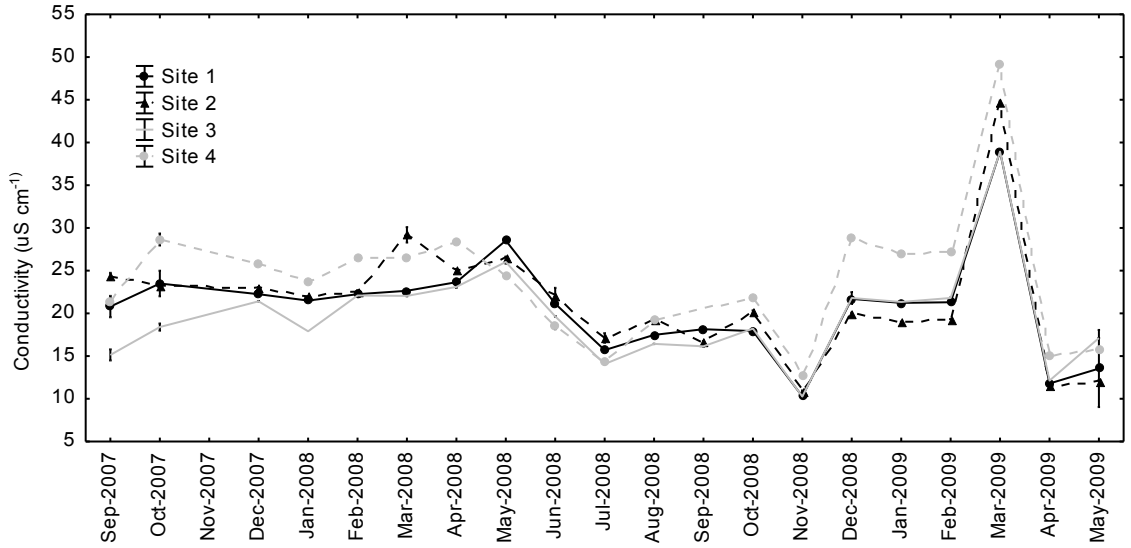


Figure 3.25 Conductivity (µS cm^{-1})measured monthly from all four sampling sites on the Berg and Molenaars Rivers between September 2007 and May 2009. Error bars = ±1SE

3.3.1.7 Patterns in mean water depth and near-bed velocity

As might be expected from the hydrological regime, Sites 1, 3 and 4 were shallower during the dry summer months compared with the wet winter period, particularly between February and April 2008 and 2009 respectively (Figure 3.26). Similarly, near-bed velocity was greatest during the wet winter months at Sites 1, 3 and 4 between May 2008 and November 2008 with the exception of August 2008 following a warm spell during the winter with no rainfall and thus a lack of flood events (Figure 3.27). By contrast, no clear seasonal pattern in water depth was observed at Site 2 downstream of the Berg Dam where the stream was usually deeper compared with the other three sites. Site 2 was significantly deeper than Site 1 between November 2008 and April 2009 reflecting unnaturally higher base flow discharge during this period. Also, near-bed velocity was significantly lower under altered flow conditions (Site 2) compared with Sites 1, 3 and 4 where flow conditions were natural. Site 2 therefore was deeper with slower flowing compared with the other sites.

Mean water depth was generally higher and near-bed velocities lower at Sites 3 and 4 on the Molenaars River, compared with Site 1 on the Berg River, which is expected considering that the Molenaars River is a larger system than the Berg River upstream of the dam.

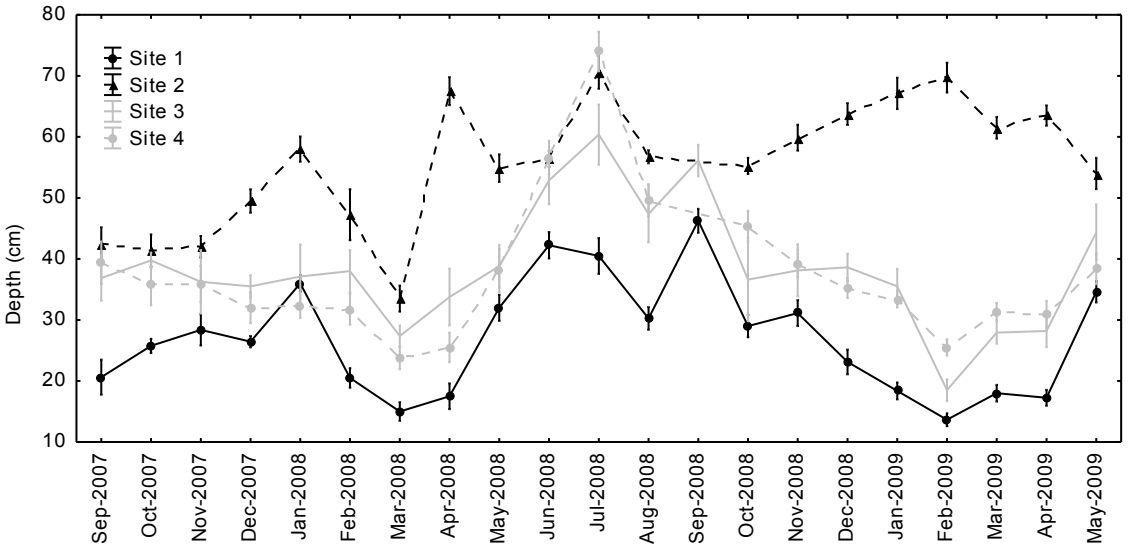


Figure 3.26 Mean monthly water depth (cm) for all four sampling sites on the Berg and Molenaars Rivers between September 2007 and May 2009. Error bars = ±1SE

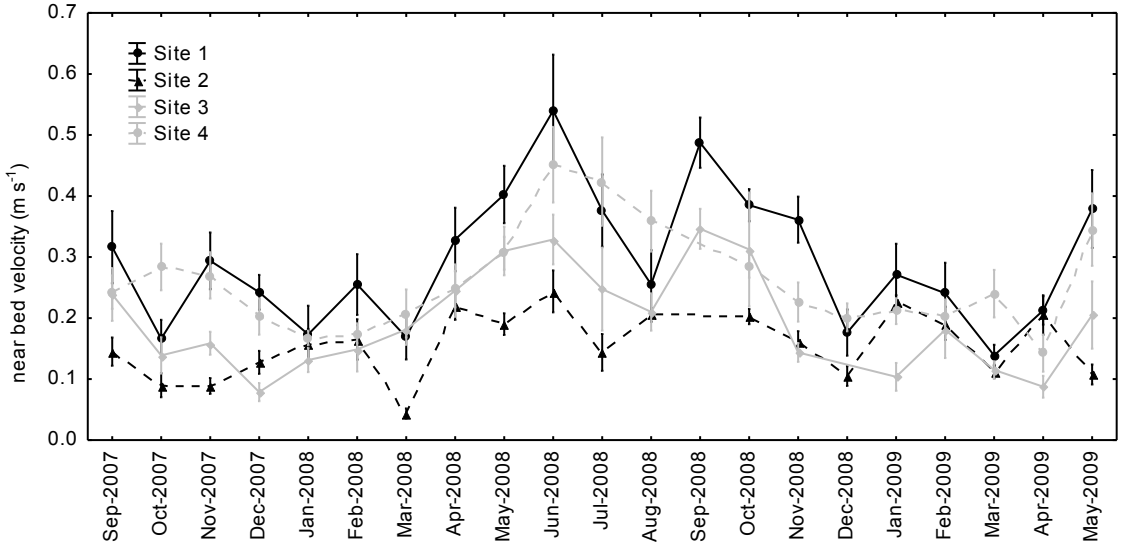


Figure 3.27 Mean monthly near-bed velocity for all four sampling sites on the Berg and Molenaars Rivers between September 2007 and May 2009. Error bars = ±1SE

3.3.2 Temporal patterns in grazers

The dominant aquatic invertebrate taxa classified as grazers for this study included Baetidae, Chironomidae, Elmidae, and Heptageniidae. A comparison of grazer biomass and densities between Sites 1 and 2 upstream and downstream of the Berg Dam are presented in Figure 3.28, while grazer

biomass and densities for the Berg River (Site 1) and the Molenaars River (Sites 3 and 4) are compared in Figure 3.29.

With a few exceptions, grazer biomass and density were higher at Site 1 above the dam compared with that below the dam at Site 2 (Figure 3.28). The greatest difference in total biomass and density of grazers between Sites 1 and 2 was during the summer months, particularly during the summer of 2008/2009 (Figure 3.28) By contrast, both biomass and density of grazers were similar at Sites 1 and 2 on the Berg River, between May 2008 and October 2008, which marks the wet winter and early spring. At Site1, grazer biomass and density were lowest during mid-winter (i.e. July 2008) and highest during late summer (i.e. February 2008) or autumn (i.e. April 2009) with a smaller peak in spring (i.e. October 2007 and 2008) suggesting a seasonal change in the abundance of grazers, although these differences are tentative.

Figure 3.30 shows that Baetidae appear to be the most abundant taxon and generally drive the overall pattern of the total abundance at Site 1 upstream of the Berg Dam. Chironomidae, however, did contribute significantly to the peak in biomass and density of grazers during the spring period at this site. By contrast, the most dominant grazer taxon at Site 2 downstream of the dam were the chironomids, which seemed to peak during the early spring of both 2007 and 2008, immediately following the cessation of flooding (Figure 3.31). Interestingly, relatively large peaks in chironomid densities at both Sites 1 and 2 (Figures 3.30b and 3.31b) were not reflected in the biomass data (Figures 3.30a ad 3.31a) suggesting that blooms of chironomids are dominated by very small individuals that do not affect the dominance of grazers with regard to biomass.

Under natural flow conditions at Sites 1, 3 and 4 (Figure 3.29), the biomass of grazers was similar with regard to winter minima but was greater in the Molenaars River (Sites 3 and 4) compared with the Berg River (Site 1) during the remainder of the year. Despite biomass differences between the Berg and Molenaars Rivers, grazer densities were similar between the Berg River at Site 1, and both sites on the Molenaars River (Figure 3.29b). A mid-winter low and summer high in grazer biomass at both Sites 3 and 4 in the Molenaars River suggests a seasonal pattern in grazer abundance similar to that observed in the Berg River under natural flow conditions (Figure 3.29). This pattern was not very clear from the density data in Figure 3.29b, however, which shows a peak in number during October 2007 with a secondary peak in autumn of 2008. Nevertheless, both Sites 3 and 4 show a small peak in density during November 2008, similar that at Site 1, with a summer peak in 2009.

Unlike the Berg River, the Heptageniidae at Sites 3 and 4 on the Molenaars River contributed significantly to the temporal pattern in overall grazer biomass, with a distinct seasonal pattern of low abundance throughout the winter and high density and biomass in summer (Figures 3.32 and 3.33). Interestingly, the spring peak in biomass and density in both 2007 and 2008 appears to be driven by a sharp increase in chironomid numbers at this time suggesting that this group of invertebrates recovers relatively rapidly following flood disturbance.

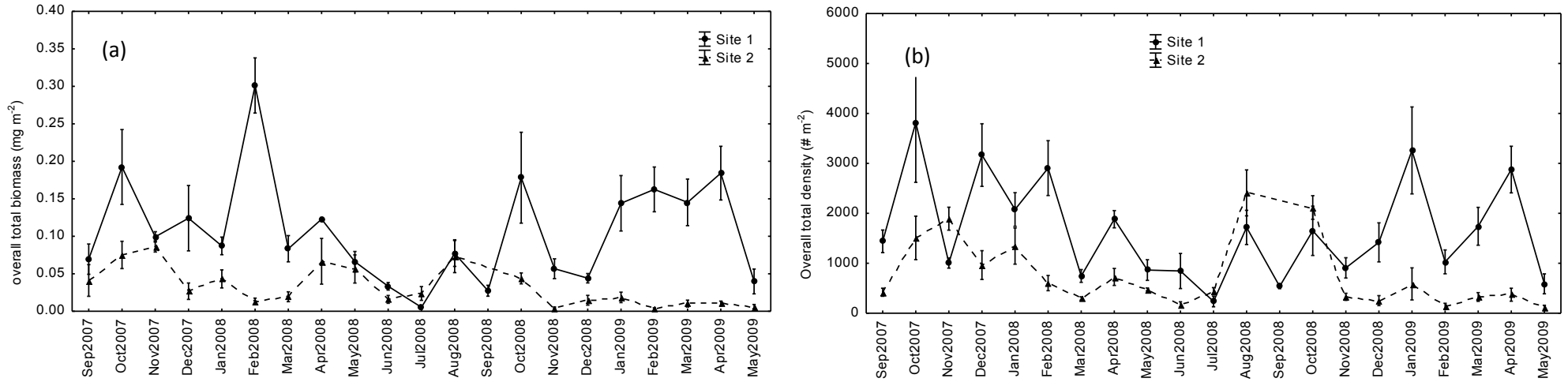


Figure 3.28 **Total a) biomass (mg m^{-2}) and b) density (# m^{-2}) of potential grazers in the Berg River upstream (Site 1) and downstream (Site 2) of the dam between September 2007 and May 2009. Error bars = ±1SE**

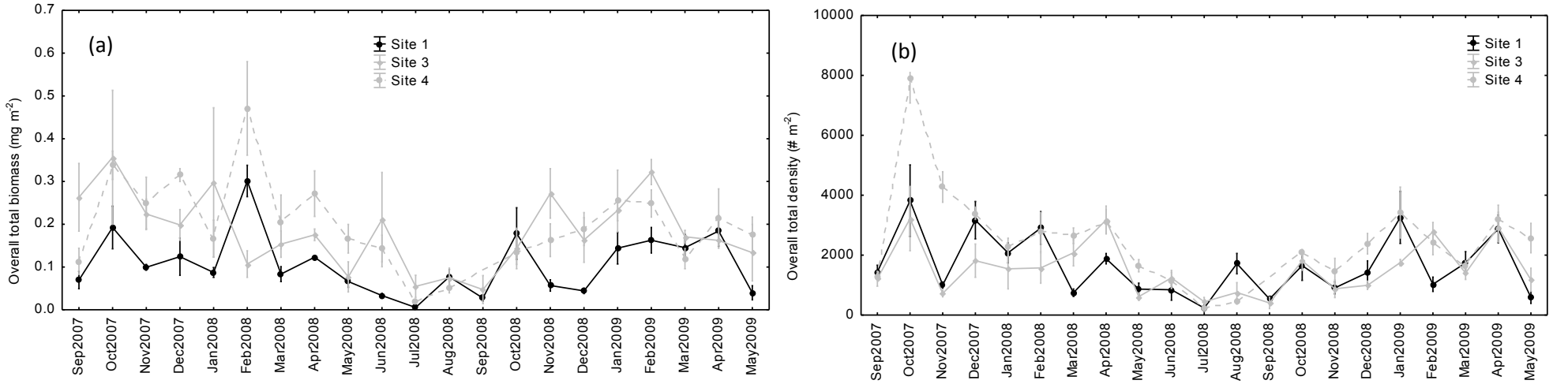


Figure 3.29 **Total a) biomass (mg m^{-2}) and b) density (# m^{-2}) of potential grazers in the Berg River at Site 1 and the Molenaars River at Sites 3 and 4 between September 2007 and May 2009. Error bars = ±1SE. Note the difference in biomass and density scales with those given for the Berg River sites alone in Figure 3.28.**

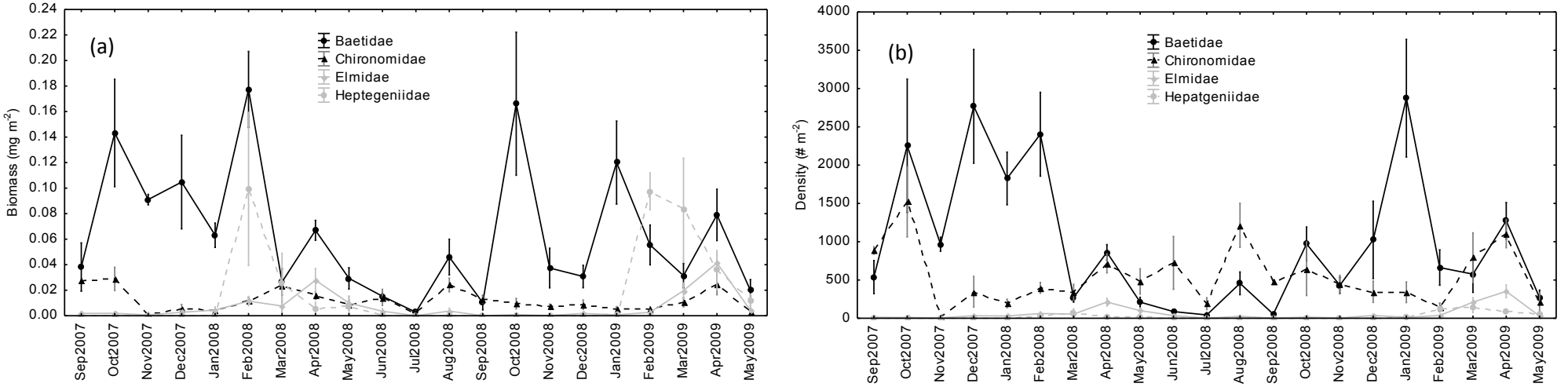


Figure 3.30 **a) Biomass (mg m^{-2}) and b) density (# m^{-2}) of Baetidae, Chironomidae, Elmidae and Heptageniidae at Site 1 between September 2007 and May 2009. Error bars = ±1SE. Note: the biomass and density scales are different from that given for the Molenaars River (Figures 3.32 and 3.33)**

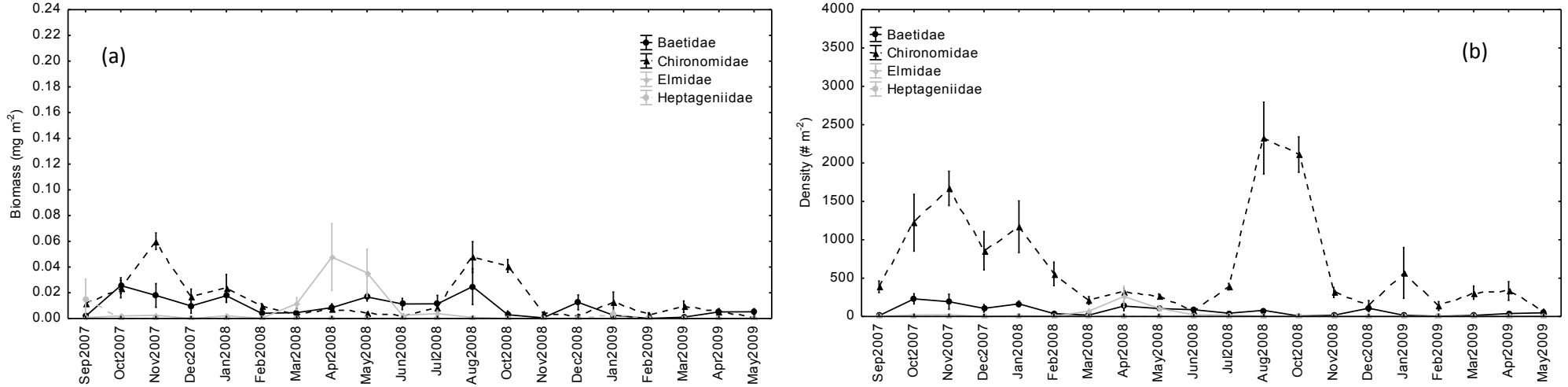


Figure 3.31 **a) Biomass (mg m^{-2}) and b) density (# m^{-2}) of Baetidae, Chironomidae, Elmidae and Heptageniidae at Site 2 between September 2007 and May 2009. Error bars = ±1SE. Note: the biomass and density scales are different from that given for the Molenaars River (Figures 3.32 and 3.33).**

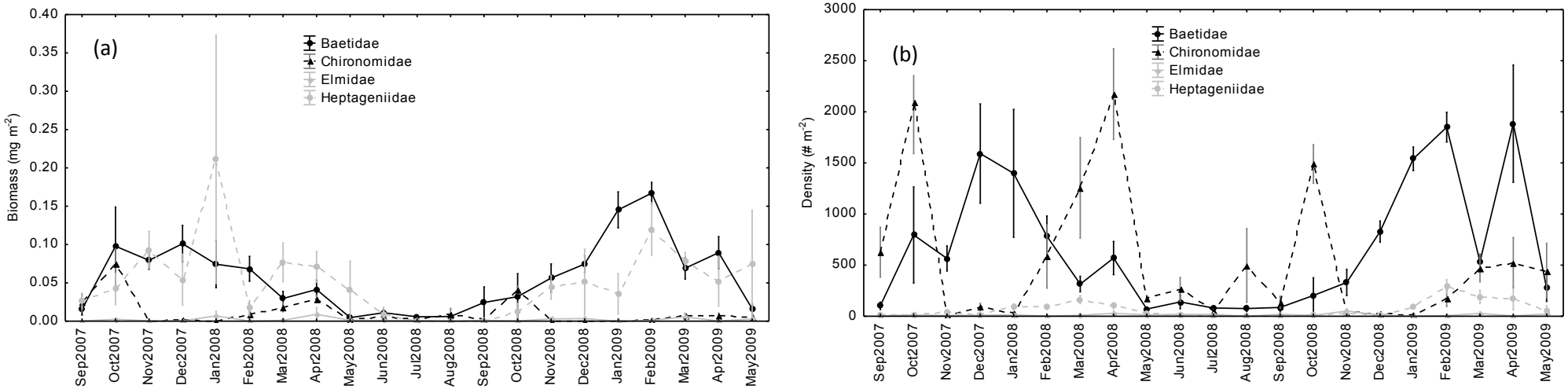


Figure 3.32 **a) Biomass (mg m^{-2}) and b) density (# m^{-2}) of Baetidae, Chironomidae, Elmidae and Heptageniidae at Site 3 between September 2007 and May 2009. Error bars = ±1SE. Note: the biomass and density scales are different from that given for the Berg River (Figures 3.30 and 3.31).**

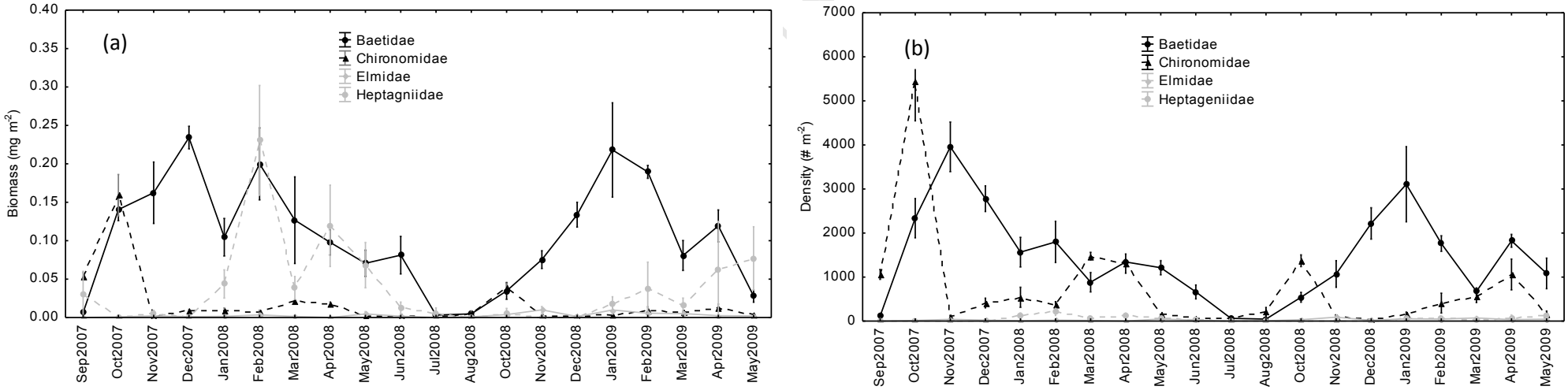


Figure 3.33 **a) Biomass (mg m^{-2}) and b) density (# m^{-2}) of Baetidae, Chironomidae, Elmidae and Heptageniidae at Site 4 between September 2007 and May 2009. Error bars = ±1SE. Note: the biomass and density scales are different from that given for the Berg River (Figures 3.30 and 3.31).**

3.3.3 Integration of environmental variables

Principle Component Analysis (PCA), together with correlation analysis was used to identify those environmental variables that that best explain a) temporal patterns in environmental conditions under natural flow and nutrient conditions and b) site differences under altered flow conditions and nutrient enrichment. Of the original set of environmental variables describing the six key environmental factors in Table 3.4, 29 were considered for the PCA analyses.

3.3.3.1 Temporal patterns based under oligotrophic, natural flow conditions

Of these 29 environmental variables, a total of 17 were derived from the correlation analyses that best described temporal patterns in environmental conditions at Site 1 over the sampling period. A principle components analysis (PCA) of these data indicates that 73 % of temporal variation could be explained by the first three eigenvectors (Table 3.8). The first eigenvector (PC1) explained 44.5 % of the variation in environmental variables, while PC2 and PC3 explained 16.6% and 11 % of the variation respectively. An ordination of these variables shows a continuum of seasonal change along the primary axis (PC1) with winter and late autumn on the left of Figure 3.34, spring near the centre and summer and early autumn to the far right. PC1 is driven by seven variables that represent the parameters of flow, temperature and physico-chemical conditions environmental variables (Table 3.8). In terms of flow, peak discharge, the frequency and duration of flooding and the length of the recovery period following the last flood event were the main drivers (Table 3.8). Cumulative water temperature, depth and velocity were the physico-chemical variables that describe temporal patterns at Site 1 (Table 3.8). PC2 shows some separation between early spring and late spring but the greatest distinction along PC2 is the separation of late spring and early autumn (Figure 3.34). Also, January of both years and February 2008 separate from the rest of summer and early autumn along PC2 (Figure 3.34). Whereas January and February 2008 and January 2009 group with late spring, the late summer months group with early autumn. These differences were driven largely by differences in grazer densities, nutrients, variability in flow conditions (Q_{cv}) and cumulative solar radiation (RS_{cum}). By contrast, flow and physico-chemical variables mostly describe the separation of winter/ late autumn from summer/early autumn.

Table 3.8 **Principle component loadings of the variables describing environmental factors measured monthly over the sampling period at Site 1 on the Berg River. The values highlighted in bold text explain the majority of the variation associated with each of the three primary axes (PC 1, 2 and 3). The acronyms in brackets are those given in the PCA plot in Figure 3.34.**

Environmental factor	Variable	PC1	PC2	PC 3
	Variation explained %	44.5%	16.6%	11.3%
Flow	peak discharge (Q_{max})	**-0.329**	-0.178	0.063
	CV discharge (Q_{cv})	-0.196	**-0.349**	0.109
	number of floods ≥ Class 2 (flood # >=2)	**-0.334**	0.033	-0.08
	number of day in flood ≥ Class 2 ($CL2_{dur}$)	**-0.35**	0.03	-0.097
	days since Class 2 flood (days since CL2)	**0.332**	0.184	-0.025
Solar radiation	RS_{cum}	0.277	**-0.304**	-0.04
Water temperature	Wt_{cum}	**0.297**	-0.168	-0.253
Physico-chemical	Depth	**-0.318**	-0.11	-0.117
	Velocity (Vel)	**-0.315**	0.044	-0.066
	Turbidity (Turb)	-0.084	-0.319	**0.381**
Nutrients	PO_4^{3+}-P (P04)	0.001	**-0.332**	-0.279
	SiO_4 (Si04)	0.167	-0.266	-0.229
	NH4+-N (NH4)	-0.132	**0.287**	**-0.385**
	TP	-0.006	0.112	**0.532**
Grazers	Scraper density (SC #)	0.052	**0.349**	0.337
	Brusher density (BR #)	0.171	**0.311**	-0.153
	Deposit feeder density (DF #)	0.244	**-0.281**	0.202

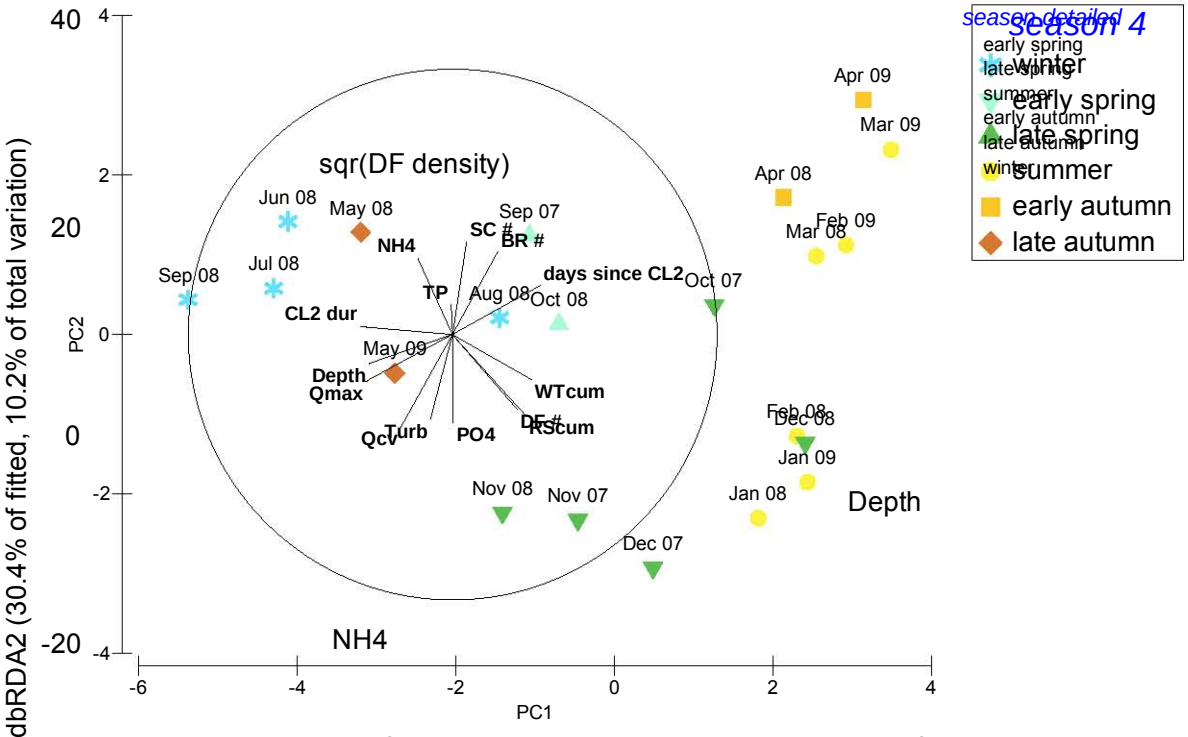


Figure 3.34 **Ordination of 17 variables describing 6 environmental factors showing temporal patterns over the 21–month sampling period at Site 1 on the Berg River using PCA on the basis of a Euclidean distance matrix. Acronyms for variables are given in Table 3.8.**

3.3.3.2 The effect of flow regulation on the temporal patterns in environmental variables (Sites 1 and 2)

A PCA analysis of environmental variables for both Sites 1 and 2 shows that 70 % of the variation could be explained by the first three axes (Table 3.9). Of these, PC1 and PC2 explained most of the variation, each contributing about a third of the overall variation. An ordination of these variables shows a strong distinction between Sites 1 and 2 along PC 2 (Figure 3.35). A seasonal signal along the primary axis (PC1) was apparent despite the inclusion of data downstream of the dam at Site 2. Nevertheless, the insert in Figure 3.42 shows that seasonal distinctions were more apparent among the data for Site 1 compared with that for Site 2, suggesting that PC 1 is driven largely by the natural environmental conditions at Site 1. As was the case for seasonal patterns at Site 1 alone (Section 3.3.3.1), flood parameters were the main drivers of seasonal differences along PC1 for both Sites 1 and 2 (Table 3.9). These included peak discharge, the number and duration of floods and the length of the recovery period following the last Class 2 flood event (Table 3.9). The distinction between Sites 1 and 2 along PC2 were driven by differences in the density of grazers, nutrient concentrations, depth and turbidity over the sampling period. This suggests that the distinction between unaltered and altered flow conditions was driven by conditions typical of the stable, low-flow period rather than differences in flood parameters.

Table 3.9 Principle component loadings for the variables describing environmental factors for Sites 1 and 2 on the Berg River. The values highlighted in bold text explain the majority of the variation associated with each axis. The acronyms in brackets are those given in the PCA plot in Figure 3.35.

Environmental factor	Variable	PC1	PC2	PC 3
	Variation explained %	**32.9%**	**26.5%**	**10.6%**
Flow	median discharge (Q_{med})	0.217	0.219	-0.111
	peak discharge (Q_{max})	**0.356**	-0.054	-0.281
	days since Class 2 flood (days since CL2)	**-0.322**	0.148	0.229
	number of floods ≥ Class 2 (flood # >=2)	**0.34**	-0.151	-0.094
	number of day in flood ≥ Class 2 ($CL2_{dur}$)	**0.35**	-0.145	-0.111
	CV discharge (Q_{cv})	0.198	-0.212	**-0.296**
Solar radiation	RS_{min}	-0.291	-0.039	**-0.378**
	RS_{cum}	-0.27	-0.056	**-0.462**
Water temperature	Wt_{range}	-0.271	**-0.284**	-0.194
	Wt_{cum}	-0.288	-0.024	**-0.354**
Physico-chemical	Depth	0.107	**0.386**	-0.136
	Velocity (Vel)	**0.275**	-0.25	0.053
	Turbidity (Turb)	-0.052	**0.351**	-0.114
Nutrients	NH_4^+-N (NH4)	0.011	**0.399**	0.075
	$N0_2^-$-N (N02)	0.049	**0.291**	-0.139
Grazers	Scraper density (SC #)	-0.046	0.01	**0.297**
	Brusher density (BR #)	-0.121	**-0.287**	0.259
	Deposit feeder density (DF #)	-0.149	**-0.302**	0.114

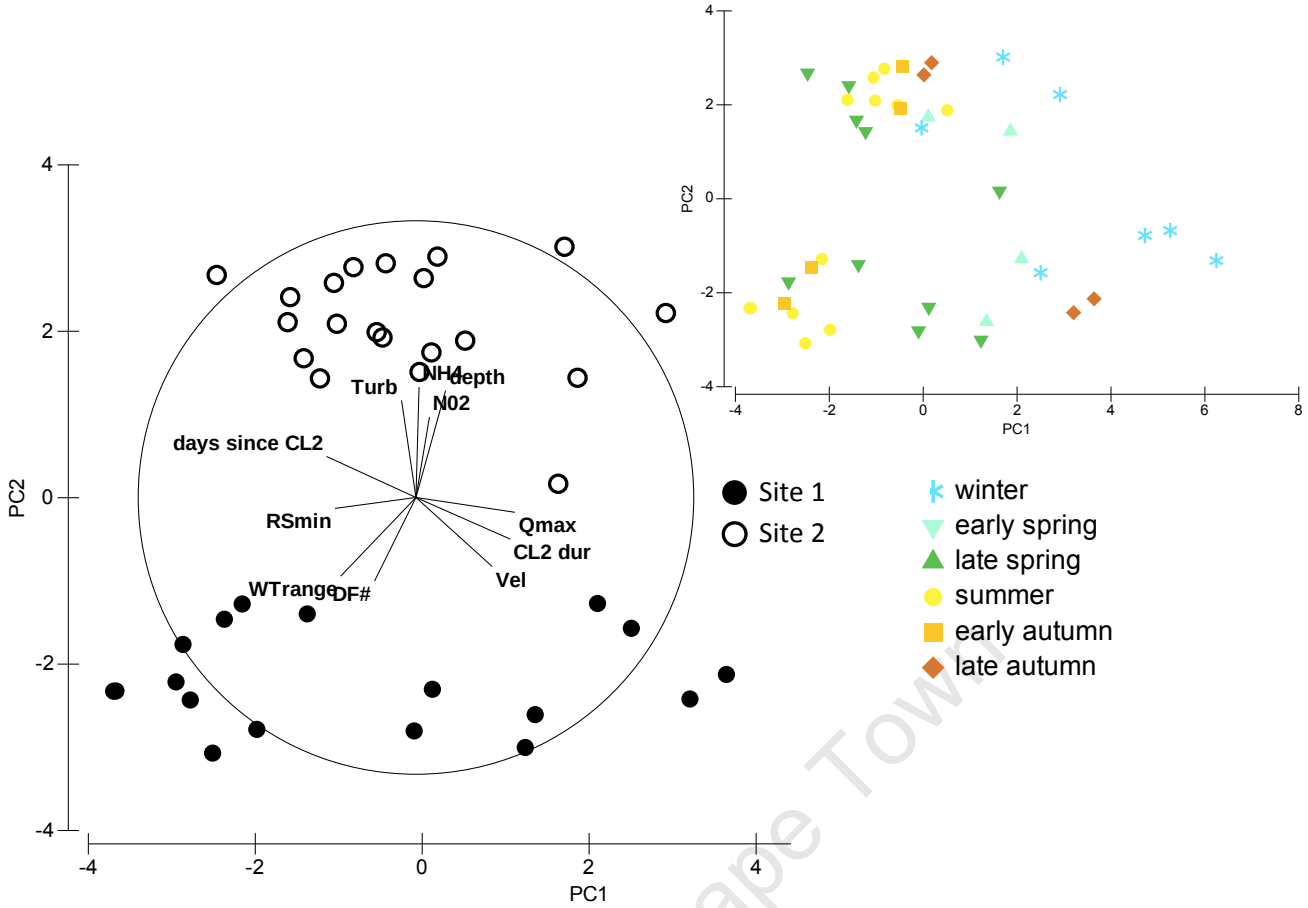


Figure 3.35 **Ordination of 18 variables characterizing 8 environmental factors on the Berg River at Sites 1 and 2 using PCA on the basis of a Euclidean distance matrix. The inserted graph represents the seasonal categorisation of these data, without the environmental variable vectors overlayed. Acronyms for variables are given in Table 3.9.**

3.3.3.3 The effects of nutrient enrichment on the temporal pattern of environmental conditions (Sites 1, 3 and 4)

A PCA ordination of 17 variables identified in the correlation analysis shows a clear distinction between seasons along the primary axis (PC1) based on data for Sites 1, 3 and 4 (Figure 3.36). This axis alone explained 40% of the variation in environmental conditions over these three sites and was driven mainly by flood characteristics and solar radiation (Table 3.10). These results reflect the natural flow regime characteristics of Sites 1, 3 and 4. PC2, however, shows a clear distinction between Site 1, which is oligotrophic, and Site 3 and 4, which are enriched (Figure 3.36). These site differences are explained largely by differences in nutrient concentrations and the grazer biomass (Table 3.10). These site differences are particularly distinct during the stable dry months characterising summer and autumn (see insert in Figure 3.36), suggesting that site differences are negligible during the wet season when flow characteristics are similar across all three sites. Nevertheless, PC2 explains only 17% of the variation in these data and thus the seasonal signal given by PC1 is far greater than the site differences in environmental conditions given by PC2 (Table 3.10 and Figure 3.36).

Although PC3 only explained 10% of the variability in environmental conditions, it shows some separation of Sites 3 and 4 during the summer and autumn months (Figure 3.37). Evidently, the

greater range in daily water temperature associated with a wider valley at Site 4, as well as higher turbidity and pH values, probably due to the trout farm effluent explained the differences in environmental condition between these two sites. Greater biomass of deposit feeding invertebrates at Site 4 compared with Site 3, where TP concentrations were higher, also contributed to the differences between these two sites (Table 3.10).

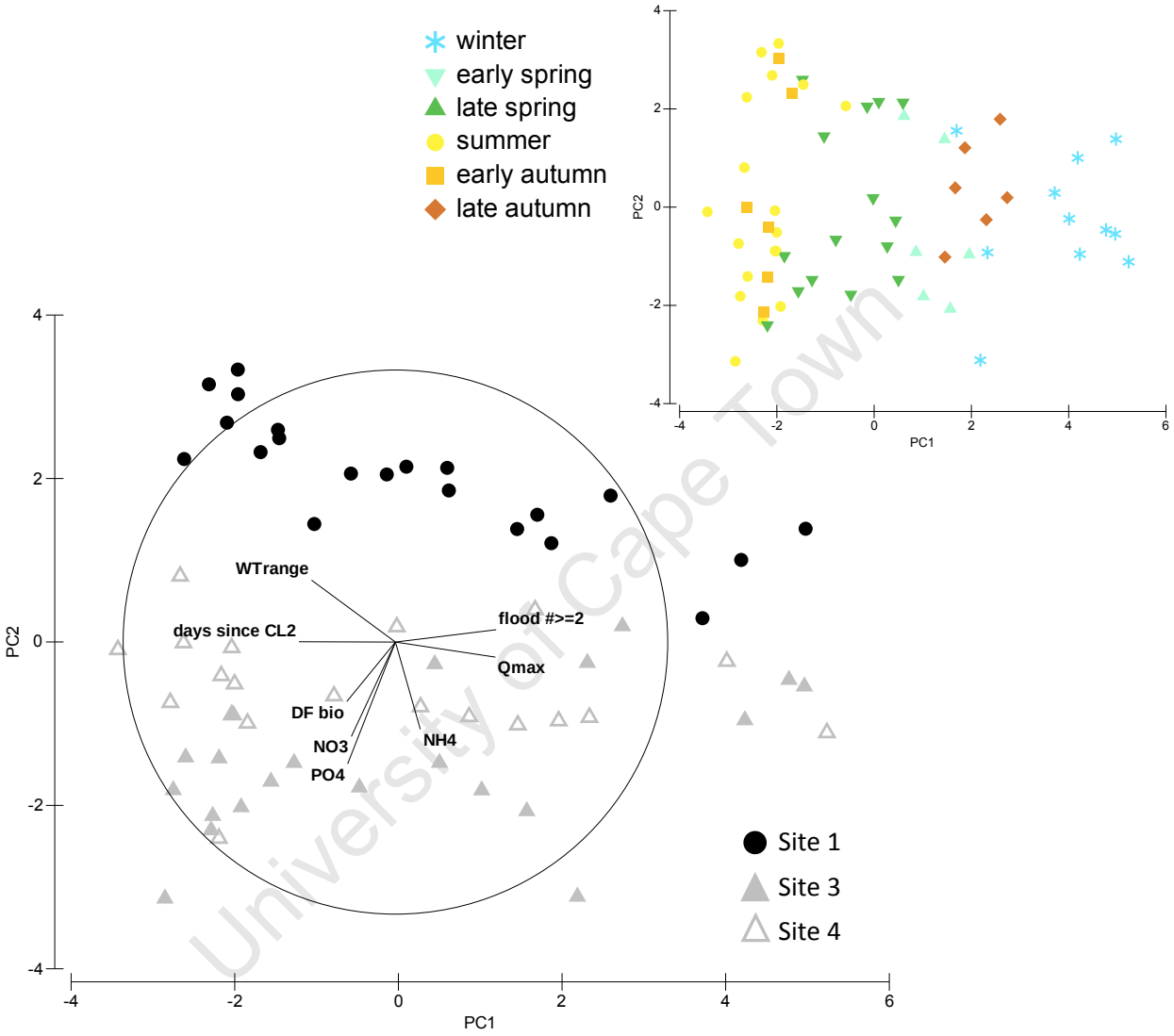


Figure 3.36 **Ordination of 14 variables characterizing 8 environmental factors on the Berg and Molenaars Rivers at Sites 1, 3 and 4 using PCA on the basis of a Euclidean distance matrix. The inserted graph represents the seasonal categorisation of these data, without the environmental variable vectors overlayed. Acronyms for variables are given in Table 3.10.**

Table 3.10 **Principle component loadings for the variables describing environmental factors for Sites 1, 3 and 4 on the Berg and Molenaars Rivers. The values highlighted in bold text explain the majority of the variation associated with each axis. The acronyms in brackets are those given in the PCA plot in Figures 3.36 and 3.37.**

Environmental factor	Variable	PC1	PC2	PC 3
	Variation explained %	39.7%	16.6%	9.9%
Flow	median discharge (Q_{med})	0.299	-0.239	0.16
	peak discharge (Q_{max})	**0.345**	-0.062	0.137
	days since Class 2 flood (days since CL2)	**-0.33**	0.008	-0.156
	number of floods ≥ Class 2 (flood # >=2)	**0.35**	0.039	0
	number of day in flood ≥ Class 2 ($CL2_{dur}$)	**0.361**	0.025	0.006
Solar radiation	RS_{cum}	**-0.304**	0.025	0.246
Water temperature	WT_{range}	-0.284	0.233	**0.343**
Physico-chemical	Depth	0.284	-0.297	0.127
	Turbidity (Turb)	-0.059	-0.249	**0.418**
	pH	-0.184	-0.265	**0.389**
Nutrients	PO_4^{3+}-P (P04)	-0.165	**-0.444**	-0.195
	NH_4^{+}-N (NH4)	0.08	**-0.323**	-0.125
	NO_2^{-}-N (N02)	0.087	-0.289	0.169
	NO_3^{-}-N (N03)	-0.149	**-0.343**	-0.214
	TP	-0.123	**-0.321**	**-0.354**
Grazers	Deposit feeder biomass (DF bio)	-0.171	-0.216	**0.354**
	Brusher biomass (BR bio)	-0.174	-0.098	-0.18

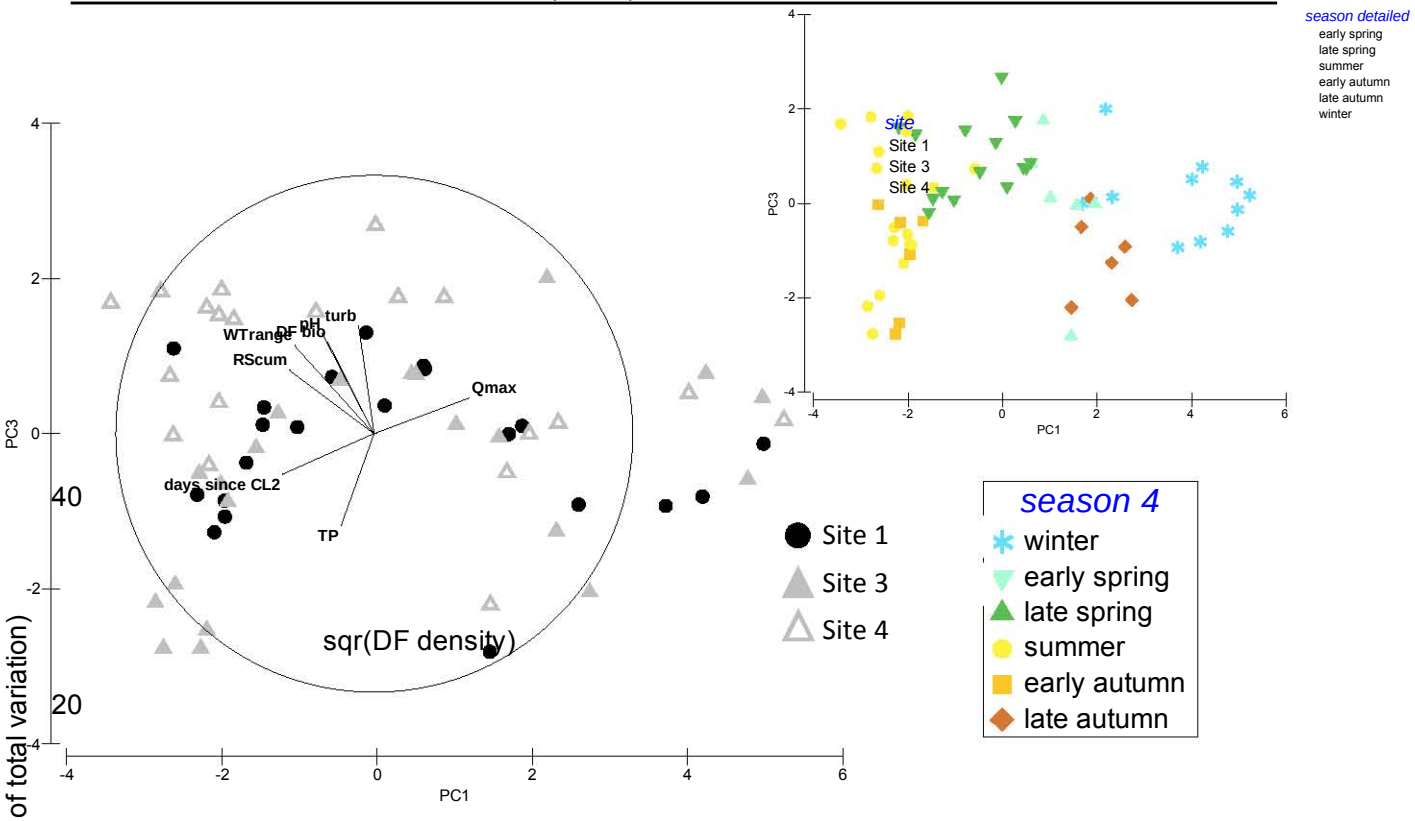


Figure 3.37 **Ordination of 14 variables characterizing 8 environmental factors on the Berg and Molenaars Rivers at Sites 1, 3 and 4 using the same PCA data plotted in Figure 3.36 but showing PC1 and PC3. Acronyms for variables are given in Table 3.10.**

3.4 SUMMARY AND CONCLUSIONS

There were two primary aims to this chapter. The first was to describe temporal changes in key environmental variables potentially responsible for patterns in periphyton communities under conditions typical of unimpacted south-western Cape rivers, such as low nutrient availability and natural flows. The second was to compare these patterns with a) those under altered flow conditions, by comparing conditions at Sites 1 and 2 on the Berg River and b) those that are moderately enriched, by comparing Site 1 with Sites 3 and 4 on the Molenaars River.

As expected under natural flow conditions, temporal patterns of water temperature and discharge in the Berg and Molenaars Rivers were consistent with those in Mediterranean climates, which characteristically have a distinct wet cool period when flooding is frequent and daily radiation is low, highlighted as predictions 1 and 2 in Table 3.1 This period is followed by a dry, warm period when daily radiation is high and flood disturbances are absent or infrequent. (Figures 3.4 to 3.15). The wet winters were separated from the dry summers by short seasons, characterised as early and late spring and early and late autumn following winter and summer respectively (Figures 3.9 and 3.10). This suggests that temporal changes in environmental conditions of the south-western Cape do not follow the conventional division of an annual cycle into four specific seasons (i.e. winter, spring, summer and autumn), comprised of an even number of months within each season. Six separate seasons were therefore identified during this study and the key environmental characteristics of each season are summarized in Table 3.11. These redefined seasons therefore provide a more precise indication of temporal changes in physical conditions that can be used in the interpretation of temporal changes in periphyton biomass and community structure in Chapters 4 and 5. Despite these seasonal categories, the PCA analyses suggested a separation of summer with some months characteristic of late spring and others characteristic of late autumn (Figure 3.34). Evidently, summer shares some characteristics with the late spring as well as early autumn (Table 3.11) and therefore the separation of summer months given by the PCA analyses was not surprising.

The abundance of grazers was generally low between late autumn and early spring. Low abundance in spring could have been due to a delay in recovery following the winter floods, whereas low autumn abundance in grazers could have been a result of aquatic invertebrate emergence prior to the onset of winter floods. Despite these general patterns, the density of grazers seemed highly variable, both within a site and between months, during the spring and autumn, particularly in the case of chironomids. As indicated by Ractliffe (2009), the wet season marks a period of serial loss and subsequent recruitment of grazers by flood disturbance. Depending on the frequency of floods in any given year, densities fluctuate due to increases and decreases in the number of small individuals over the wet season. However, grazer biomass remains constantly low over this period because most of the larger individuals are removed by flooding and the inter-flood period does not allow for significant growth of individuals (Ractliffe 2009).

Besides fluctuations in grazer densities, spring and autumn seasons seem transitional, signifying periods of significant shifts in light and temperature, with either stable flows or flood events. Changes during these seasons may be important for biological processes. In particular, increases in

the availability of resources for growth (light and temperature) during the early spring may provide a "window" where 'top down' control by grazers is limited due the lag in the invertebrate recovery following flood disturbance, and 'bottom up' conditions that promote primary productivity. This window of opportunity for development of periphyton during the spring was demonstrated in a study of *Cladophora* in a Mediterranean stream in Northern California (Power 1992).

It was predicted that, under conditions where seasonality of flow is reduced due to base flow reversals and a reduction in floods, no seasonality in other environmental conditions would be evident (Prediction 3, Table 3.1). Although seasonality was somewhat altered downstream of the dam, particularly during spring and autumn, the wet season was still differentiated from the dry season by a series of floods over later winter and early spring 2008 at Site 2 when larger floods overtopped the dam wall. There was also a clear cyclical change in mean water temperature due to controlled releases that endeavoured to minimise alteration in water temperature from the natural condition (Bertrand van Zyl, Department of Water Affairs, pers. comm.). Therefore, seasonal changes in environmental conditions below the dam were not completely disrupted, as predicted. Indeed, the PCA analysis showed that, despite a protracted wet season with smaller, less frequent floods than under natural conditions, flood characteristics did not separate Site 2 from Site 1. Rather, these sites were distinct from each other due to differences in low flow conditions described by habitat characteristics such as depth and turbidity, nutrients, particularly NH_4^+-N and NO_2-N, and grazer abundance. Besides grazers, other invertebrate taxa were also low in biomass (pers. obs.). Unnaturally high base flows resulting in deep turbid in-stream conditions during the summer period, coupled with slightly elevated nutrient concentrations relative to natural conditions, suggests that the Berg River downstream of the dam was affected more by an alteration of low flows and decomposition of organic matter in the dam, than high flows over the study period.

As expected, concentrations of N and P reflect the oligotrophic status of the Berg River with slight enrichment downstream of the dam, while the Molenaars River was far more enriched by effluent from trout farms in its foothill reaches. As predicted (prediction 4, Table 3.1), the PCA analysis showed that Site 1 was distinct from Sites 3 and 4 during the summer and early autumn period. Both nutrients and the biomass of grazers were greater in the Molenaars River compared with the Berg River at Site 1, thus reflecting the higher trophic status of the Molenaars River relative to the Berg River.

The ambient nutrient concentrations did not, however, reflect the difference in enrichment of Sites 3 and 4. Nutrient concentrations at Site 3 were consistently higher than that at Site 4. These results were unexpected, considering the position of Site 4 immediately downstream from the outfall of the trout farm. Nutrient concentrations are often considered inadequate indicators of the trophic state of a river because N and P are rapidly used up for the production of chlorophyll in benthic algae (Biggs 2000) characteristic of open canopied systems like the Molenaars River. In retrospect, a measure of nutrient loading as an indicator of nutrient availability, rather than ambient nutrient concentrations may have been more appropriate for this study.

In conclusion, this study has shown clear seasonal cycles in environmental conditions of the Berg and Molenaars River that are typical of rivers in Mediterranean climates elsewhere (Gasith and Resh 1999). Although water temperature and irradiance provide a clear indication of distinct seasonal shifts in environmental conditions over an annual cycle, a summary of environmental conditions typical of each season (Table 3.11) shows that there is considerable overlap between seasons in terms of flow characteristics, particularly flood disturbance. Differences in the nutrient concentrations and abundance of grazers between the Berg River upstream of the dam and the Molenaars River suggest that these two systems provide a useful vehicle for understanding temporal patterns in periphyton community structure under different trophic states. Nevertheless, the subtle differences in nutrient availability between the two Molenaars sites were not apparent in this study. It is possible that higher biomass of grazers at Site 4 reflects a higher trophic state at this site compared with that at Site 3. Considering the sensitivity of periphyton to even slight enrichment, it is probable that these differences will be detected by differences in periphyton biomass, which is explored further in Chapter 4.

Flood characteristics were not significant in separating sites with unaltered and altered flow conditions. Whereas Site 2 did not experience flooding over the winter of 2007 due to the dam, overtopping of the Berg Dam in late winter and spring of 2008 led to flooding at Site 2 during the study period. Therefore, the lack of significant site differences in terms of flood characteristics was probably because the Berg Dam did not severely curtail floods over the study period as expected. Understanding the role of floods as a driver of periphyton communities by comparing communities upstream and downstream of the Berg Dam may therefore not be warranted. Despite these findings, however, subtle changes in the flow regime such as the protracted wet season with less frequent, smaller floods compared with the upstream reach may affect periphyton communities. Indeed, the sensitivity of periphyton communities to even subtle changes in flow and water temperature would provide useful information for the management of dam releases. Evidently, comparisons of the drivers of temporal patterns in periphyton communities with those under altered flow or nutrients may be more complex than anticipated. These issues are revisited in Chapter 4 and again in Chapters 7, 8 and 9 following an understanding of the links between flow and periphyton in the Berg and Molenaars Rivers in Chapter 5.

Table 3.11 Summary characteristics of seasons detected in this study based on key environmental variables.

Season	Flood charactersistics	Low flow characteristics	Water temperature and solar radiation	Habitat characteristics of the run biotope	Nutrients	Grazer biomass and density
Winter	Floods ≥ CLASS 2 common and frequent	Base flows remain elevated during interflood periods which are short (days to weeks)	Lowest daily average and range in both water temperature and solar radiation over an annual cycle	Deep and fast flowing dominate	Oligotrophic: no pattern Mesotrophic: P and N low	Low
Early spring	Floods ≥ CLASS 2 common but infrequent	Base flows are lower than those in winter because interflood periods are longer (weeks)	Distinct increase in daily averages (WTave and RSave)	Deep and fast flowing conditions occur but there are extended periods when conditions are shallower and slower flowing than the winter	Oligotrophic: no pattern Mesotrophic: P and N low	biomass low but densities of scrapers can increase if the interflood period is long enough
Late spring	Floods ≥ CLASS 2 can occur but not common	Extended periods of low flow evident with base flows similar to summer	Increase in daily range (WT_{range}) and culumative conditions (WT_{cum} and Rs_{cum}). Daily averages similar to summer	Water depth drops and velocities are lower than early spring	Oligotrophic: no pattern Mesotrophic: P and N slightly elevated	Scraper density peaks
Summer	Floods ≥ CLASS 2 usually non-existent but can occur very rarely	Stable period with very low base flows	Daily averages similar to late spring but maximum temperatures are greater than those during the late spring	Water depth and velocities are at their lowest over an annual cycle. Conditions are similar to early autumn	Oligotrophic: no pattern Mesotrophic: P and N peak	Deposit feeder biomass and density peaks
Early autumn	Floods ≥ CLASS 2 uncommon	Similar to summer	Distinct decrease in solar radiation and water temperature	Similar to summer	Oligotrophic: no pattern Mesotrophic: P and N peak	Brusher biomass elevated (but only under conditions of slight enrichment)
Late autumn	Floods ≥ CLASS 2 common but infrequent	Similar to summer	Solar radiation and water temperature similar to early autumn and therefore higher than winter		Oligotrophic: no pattern Mesotrophic: P and N decrease but greater than winter	Low

CHAPTER 4 TEMPORAL PATTERNS IN PERIPHYTON BIOMASS AND COMMUNITY STRUCTURE: THE EFFECTS OF ENRICHMENT AND FLOW ALTERATION

4.1 INTRODUCTION

Several studies have demonstrated that the long term spatial and temporal dynamics of periphyton communities in open-canopied temperate streams show distinct patterns that generally reflect the outcome of flood disturbance and its interaction with nutrient supply (Biggs 1996; Uehlinger *et al.* 1996, Biggs *et al.* 1998b; Biggs *et al.* 1998c; Fayolle *et al.* 1998;Villeneuve *et al.* 2011). In some cases, particularly under stable conditions with few flood disturbances, grazing is also considered an important regulator of periphyton communities over time (Mulholland *et al.* 1991a; Mulholland *et al.* 1991b; Power 1992; Feminella and Hawkins 1995; Steinman 1996; Peterson *et al.* 2001; Opsahl *et al.* 2003; Hillebrand 2009). Medium- to long-term temporal patterns in these communities essentially describe variability in shorter-term accrual cycles (Figure 4.1), which are typical at the scale of less than one year. According to Biggs' (1996) idealised short-term periphyton post-flooding accrual cycle (Figure 4.1), biomass accrual starts with rapid colonisation of pioneer taxa and follows an exponential growth and succession towards a community dominated by slower colonising but competitive taxa, when peak biomass (P_B) is reached (Lamberti *et al.* 1989; Peterson and Stevenson 1990; McCormick and Stevenson 1991; Uehlinger 1991; Biggs and Thomsen 1995; Biggs and Stokseth 1996; Uehlinger *et al.* 1996). Thereafter, biomass decreases as a result of spontaneous sloughing, grazing and death, during the 'loss phase' (Figure 4.1). Flood disturbance can disrupt the accrual cycle and reset the community at any time. Also, time to P_B, the actual biomass at P_B and the taxa that contribute to the community are all affected by the level of enrichment of a stream (Pringle 1990; Mulholland *et al.* 1991a; Mulholland *et al.* 1991b; Peterson and Grimm 1992; Biggs 1996; Biggs *et al.* 1999; Molinos and Donohue 2010). Thus, depending on any combination of factors including the size, frequency and timing of flood disturbances, as well as the availability of nutrients and the effect of grazing during the accrual cycle, the culmination of a series of partial or complete accrual cycles over time can result in a range of different medium- to long-term temporal patterns in periphyton communities under natural conditions. It therefore follows that alteration of the availability of nutrients and/or the flow regime of a river can significantly alter the dynamics of a periphyton community, with direct and indirect feedback loops.

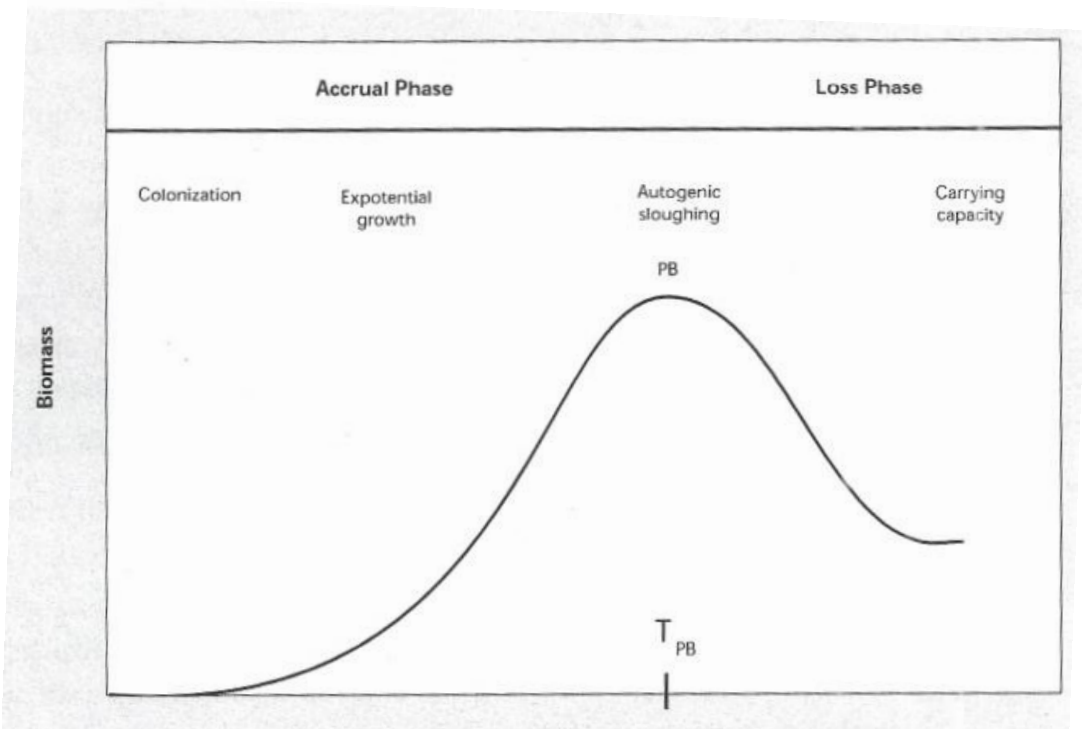


Figure 4.1 **An idealised short-term periphyton accrual cycle following a major flood event. P_B (Peak biomass) is the maximum accrual cycle biomass; T_{PB} is the time to P_B from the onset of colonisation (Biggs 1996).**

In their conceptual model for stream periphyton, Biggs *et al.* (1998a) described the predicted changes in periphyton biomass and community structure over time under different flow and nutrient conditions. Based on the outcome of numerous investigations of periphyton seasonality, their model predicted that rivers in Mediterranean climates, where flood disturbance is limited to a relatively short predictable portion of the year, would undergo a succession after disturbance that commences with rapidly colonising, fast growing diatoms that are relatively resistant to flood disturbance. The trajectory of succession is dependent on the level of enrichment but the climax or peak biomass community will be dominated by taxa ranging from tall-growing filamentous green algae and/or filamentous diatoms under enriched conditions, to those with smaller cells and simple branching filaments, or form mats of prostrate intertwined filaments embedded in mucilage (including some diatom taxa and cyanobacteria) in oligotrophic habitats. Succession is most significantly affected by grazing where enrichment is either low or moderate, provided that the accrual cycle is not disrupted by floods (Biggs *et al.* 1998b, c), because algal growth is slow in nutrient poor conditions.

Rivers of the south-western Cape, which experience a Mediterranean climate, have strongly seasonal flows with a distinct, predictable wet season characterised by flood flows and low temperatures and dry hot summers with stable low flow conditions (Chapter 3). These extreme conditions are separated by transitional seasons when physical conditions are variable, characterising spring and autumn respectively. While temporal studies of periphyton communities in Mediterranean climates elsewhere support the model proposed by Biggs *et al.* (1998b, c), it is unknown whether these predictions apply to foothill rivers of the south-western Cape because temporal patterns of these periphyton communities have yet to be studied.

The primary aim of this study therefore, was to investigate seasonal changes in periphyton biomass and community composition in foothill rivers of the south-western Cape and to determine the effects of enrichment and flow alteration on these patterns.

Four sites on two rivers representing a range of enrichment conditions and one with an altered flow regime were selected to address the following hypotheses:

1) There is a strong seasonal pattern in river periphyton of low biomass in the winter and high biomass in the summer in the Upper Foothill reaches of south-western Cape rivers, following natural seasonal cycles of hydrological disturbance.
2) The community composition of periphyton will change significantly over annual cycles with distinct communities characterising summer, autumn, winter and spring periods, which are defined by changes in physical conditions.
3) Peak periphyton biomass will be greater in enriched reaches and the community composition at peak biomass in enriched reaches will differ significantly from that in unenriched reaches, regardless of the flow regime.
4) The seasonal pattern of periphyton development as indicated by biomass and community composition changes will differ between conditions of natural and altered flow regimes.

4.2 METHODS

4.2.1 Sampling Strategy and experimental design

This study looked specifically at temporal patterns of periphyton biomass and species composition under natural conditions compared with those (a) that are enriched with nutrients and (b) where the flow regime is altered. Data were collected monthly from four study sites, two on the Berg River and two on the Molenaars Rivers between September 2007 and May 2009 so that changes over almost two annual cycles could be recorded. Section 2.2 provides a description of the four study sites. Differences in nutrient availability between sites were implicit in the study design such that Site 1 upstream of the Berg Dam is located in a natural catchment and is oligotrophic, Site 3 on the Molenaars River is slightly enriched by a trout farm some distance upstream and Site 4, immediately downstream of a trout farm on the Molenaars River is further enriched. Similarly, Site 2 is situated downstream of the Berg Dam where flows are artificially controlled by dam releases, although larger floods overtopped the dam wall in late winter 2008. During this period, flow could not be experimentally controlled and the hydrological regime was similar upstream and downstream of the dam.

Analyses of data collected during a pilot study in September 2005 (Ewart-Smith 2007) indicated that runs were the least variable of all the biotopes sampled and represented the largest available habitat type at each site in the Upper Foothill reaches of the Berg and Molenaars Rivers (see Section 2.3.2). These data indicated that six replicates in runs should be taken from each study site to ensure statistical rigour in the sampling protocol. Periphyton samples were therefore collected monthly from runs only to avoid variability associated with spatial differences in substratum and

flow within a site. To account further for spatial variability, however, eight replicates at each site were collected monthly. Samples and biophysical data were collected according to the sampling protocols outlined in Section 2.3.

Run biotopes at all four sites were generally characterised by large and small cobble overlying gravel material with surface flow being unbroken and ranging from barely perceptible flow to rippled surface flow, occasionally with undular standing waves during higher flow conditions (King and Schael 2001). Eight cobbles were randomly selected and processed from each site during each sampling occasion as replicates for the estimation of periphyton biomass and taxon composition. Where possible, each replicate was collected from hydrologically independent run biotopes. In cases where this was not possible, particularly during high base flow conditions, samples were collected more or less across the channel to ensure independence of each replicate. Details of sample collection and processing are provided in Section 2.3.2, together with the procedures followed for periphyton biomass determination and algal taxonomic analyses (Section 2.4).

Two descriptors of the periphyton community were used to assess biomass changes during this study, namely Chlorophyll *a* (Chl *a*), which measures the autotrophic (i.e. algae) component of the periphyton, and Ashfree Dry Mass (AFDM), which measures both the autotrophic and heterotrophic (i.e. bacteria, fungi, protozoa and organic debris) components of the periphyton. Details of the determination of these biomass descriptors are provided in Section 2.4.1. The Autotrophic Index (AI) was used to measure the balance between autotrophic and heterotrophic components of the periphyton and was calculated as AFDM/Chl *a* (Biggs and Kilroy 2000).

The composition of the periphyton community was determined by enumeration of algal taxa and calculation of the weighted cell densities per unit area of each replicate stone as described in detail in Section 2.4.2.

4.2.2 Data analysis

2-way ANOVAs between sites and months were conducted on two separate datasets of periphyton biomass. The first tested whether there were differences in periphyton biomass between sites that are oligotrophic (Site 1) and those with different levels of moderate enrichment (Site 3 and Site 4) as the main effects. The second consisted of monthly periphyton biomass data for Site 1 and Site 2 to test whether there were differences between a site with unaltered flow (Site 1) and that with altered flow (Site 2) as the main effects. Nutrient enrichment affects the autotrophic component of the periphyton and therefore mean monthly Chl *a* concentrations were used as an estimate of periphyton biomass to test the effects of enrichment. Both Chl *a* and AFDM were used to compare biomass differences between sites with and without flow alteration on the Berg River. Sites were considered fixed factors with months as random factors in the statistical analyses. All data were log (x+1) transformed to meet the assumption of normality and to reduce variance. Where significant differences were found ($p < 0.05$), Tukey HSD *post-hoc* multiple comparisons of differences between sites and months were used. The Factorial ANOVA option in STATISTICA version 10, 2011 was used for these analyses.

Ordination by non-metric multidimensional scaling (MDS) based on Bray-Curtis similarities was used to examine differences in benthic algal communities using all taxa between sites and over time (monthly). Length-weighted densities of each algal taxon (see Section 2.4.2) were averaged for each site on each sampling occasion and then square-root transformed prior to ordination to reduce variability among samples. Two-way crossed Permutational multivariate ANOVA (PERMANOVA) tests were conducted to compare the assemblage structure between months and among sites for each treatment (i.e. treatment one represents enrichment (Sites 1, 3, and 4) and treatment two represents flow alteration (Sites 1 and 2). PERMANOVA tests the dissimilarity values generated by the resemblance matrix on which permutations are based, thereby generating a test statistic value of pseudo-F and pseudo-t for pair-wise tests (Anderson *et al.* 2008). The PERMDISP routine in PRIMER 6.1 was used to test for the homogeneity of multivariate dispersions among samples to assess the validity of significant differences determined by PERMANOVA.

SIMPER analyses were undertaken to determine which periphyton taxa were most responsible for differences between sites and over time, comparing sequential months over the 21-month study period. The SIMPER routine computes dissimilarity (1-similarity) between each pair of samples in the two groups of samples being compared. All ordinations and subsequent multivariate analyses were performed using PRIMER version 6 and its add-on package PERMANOVA+ (Clarke and Warwick 2001, Clarke and Gorley 2006, Anderson *et al.* 2008).

4.3 RESULTS

4.3.1 General characteristics of periphyton biomass across all sites

An average of all periphyton biomass data collected monthly between September 2007 and May 2009 showed that Chlorophyll *a* (Chl *a*) concentrations were highest at Site 4, which returned a mean Chl *a* concentration of 5.28 mg m^{-2} and a maximun of 21 mg m^{-2}. Chl *a* was lowest at Site 1 on the Berg River upstream of the Berg Dam where the mean and maximum Chl *a* concentration was 2.17 mg m^{-2} and 9.73 mg m^{-2}, respectively (Table 4.1). By contrast, AFDM was on average highest at Site 2 downstream of the Berg Dam with a mean of 2.34 g m^{-2} reaching a maximum of 9.88 g m^{-2} , and lowest at Site 3 upstream of the trout farm on the Molenaars River with a mean AFDM of 0.85 mg m^{-2} and a maximim of 2.78 mg m^{-2}.

Despite these site differences in average periphyton biomass, clear temporal shifts in periphyton biomass were evident at all four sampling sites over the 21-month sampling period in terms of Chl *a* and AFDM (Figures 4.2 a – d). These temporal patterns of change in biomass differed considerably between sites at certain times of the year and the influence of nutrient enrichment and flow alteration are presented in the following sections by examining temporal patterns under natural conditions (i.e. Site 1) and comparing these under conditions of enrichment (i.e. Sites 3 and 4) and flow alteration (i.e. Site 2).

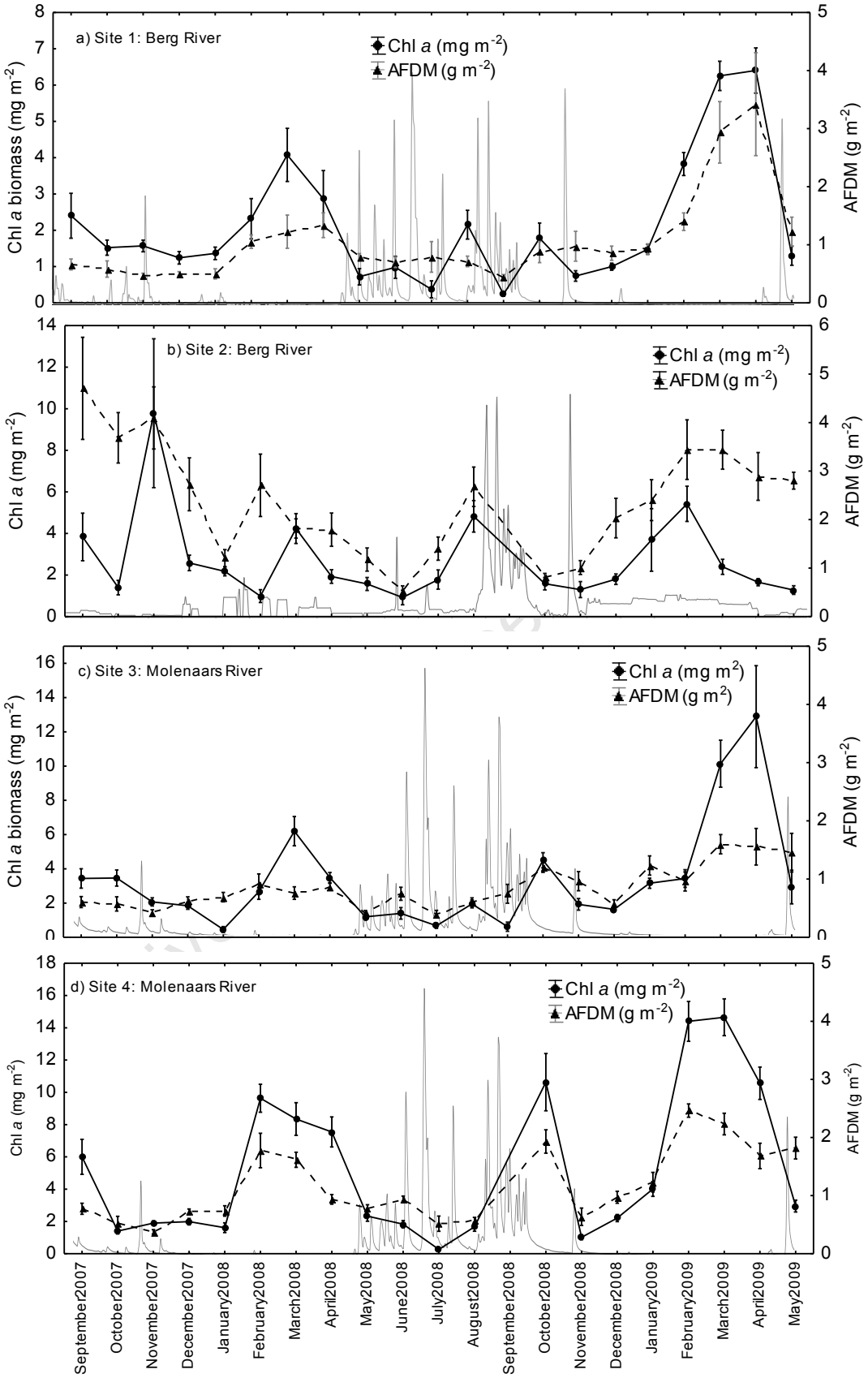


Figure 4.2 **Periphyton biomass plotted monthly between September 2007 and May 2009 for (a) Site 1 and b) Site 2 on the Berg River and (c) Site 3 and (d) Site 4 on the Molenaars River. Mean daily discharge is plotted in the background. Error bars indicated ± 1 Standard Error (SE). Note: different scales on the y-axes.**

Table 4.1 Summary statistics for Chlorophyll *a* biomass (Chl *a*) and Ash Free Dry Mass (AFDM) at each site on all sampling dates combined.

Variables	Chl a (mg m^{-2})				AFDM (g m^{-2})			
	Site 1	*Site 2*	*Site 3*	*Site 4*	*Site 1*	*Site 2*	*Site 3*	*Site 4*
Valid N	164	159	166	159	163	159	166	159
Mean	2.17	2.78	3.38	5.28	1.07	2.34	0.85	1.16
Median	1.53	2.05	2.33	2.78	0.76	1.94	0.70	0.97
Minimum	0.02	0.04	0.01	0.01	0.23	0.20	0.14	0.25
Maximum	9.73	34.07	23.75	21.00	7.76	9.88	2.79	3.49
Std.Dev.	1.98	3.35	3.73	4.95	1.04	1.63	0.53	0.73
p		***< 0.001***				***< 0.001***		
Tukey post-hoc		**S1 < S2 < S3 < S4**				**S2 > S1 > S3 < S4**		

4.3.1.1 Temporal patterns under oligotrophic, natural flow conditions

Temporal shifts in Chl *a* biomass observed at Site 1 (Figure 4.2a) reflect broad seasonal changes expected of oligotrophic rivers in Mediterranean climates. Highest biomass occurred in late summer and autumn with a peak in March 2008 and in March/April 2009. Lowest biomass occurred during mid-winter, dropping to near zero Chl *a* biomass in July 2008. Chl *a* biomass was elevated slightly in September 2007 compared to the period immediately after. Smaller spikes were also evident in August 2008 and again in October 2008, separated by very low Chl *a* biomass in September 2008 when sampling occured shortly after a series of flood events.

Monthly shifts in Chl *a* biomass in Figure 4.2a suggest that despite obvious differences between the winter and summer seasons, there were considerable changes from month to month, rather than between seasons as indicated by the significant differences in both Chl *a* biomass (p<0.001) and AFDM (p<0.001) at Site 1 (Table 4.2). The pair-wise comparisons in Table 4.2 show significant month-to-month increases or decreases in eight of the 20 comparisons of Chl *a* biomass whereas monthly changes in AFDM were not significant for the most part. Chl *a* biomass was particularly variable over the late winter and early spring of 2008 (July 2008 to October 2008). Biomass remained relatively constant over the dry season in both annual cycles, but significant month-to-month shifts were evident as biomass peaked in summer/ early autumn and then decreased rapidly (Figure 4.2a and Table 4.2).

Inter-annual differences in Chl *a* biomass at Site 1 were evident in early spring, with a significantly higher biomass in September 2007 compared to 2008, and in late summer/early autumn with a significantly higher biomass in the second growing season (March/April 2009) compared with the first (March/April 2008)(Table 4.2).

Table 4.2 **Tukey pair-wise comparisons of Chl *a* biomass (log x+1) mg m^{-2} and AFDM (log x+1) g m^{-2} between consecutive months and between months in different years over the sampling period for Site 1. Significant differences at p<0.05 are indicated in *bold italics* and at p< 0.1 in *italics* only.**

Chlorophyll a		AFDM	
Intra-annual		*Intra-annual*	
Months	**p**	**Months**	**p**
September2007, October2007	0.79	September2007, October2007	0.96
October2007, November2007	0.88	October2007, November2007	0.65
November2007, December2007	0.96	November2007, December2007	0.93
December2007, January2008	0.94	December2007, January2008	0.88
January2008, February2008	0.70	January2008, February2008	0.36
February2008, March2008	*0.06*	*February2008, March2008*	0.95
March2008, April2008	***0.02***	March2008, April2008	0.95
April2008, May2008	***0.01***	April2008, May2008	0.97
May2008, June2008	0.77	May2008, June2008	0.97
June2008, July2008	0.18	June2008, July2008	0.76
July2008, August2008	***0.00***	July2008, August2008	0.85
August2008, September2008	***0.00***	August2008, September2008	0.58
September2008, October2008	***0.00***	September2008, October2008	0.57
October2008, November2008	0.26	October2008, November2008	0.94
November2008, December2008	0.61	November2008, December2008	0.95
December2008, January2009	0.74	December2008, January2009	0.92
January2009, February2009	***0.00***	January2009, February2009	0.60
February2009, March2009	***0.02***	***February2009, March2009***	***0.04***
March2009, April2009	0.97	March2009, April2009	0.51
April2009, May2009	***0.00***	***April2009, May2009***	***0.00***
Inter-annual		*Inter-annual*	
September2007, September2008	***0.00***	September2007, September2008	0.78
October2007, October2008	0.95	October2007, October2008	0.68
November2007, November2008	0.30	November2007, November2008	*0.10*
December2007, December2008	0.56	December2007, December2008	0.59
January2008, January2009	0.76	January2008, January2009	0.38
February2008, February2009	*0.07*	February2008, February2009	0.57
March2008, March2009	***0.04***	***March2008, March2009***	***0.01***
April2008, April2009	***0.00***	***April2008, April2009***	***0.00***
May2008, May2009	0.46	May2008, May2009	0.98

4.3.1.2 The effects of enrichment

The two-way ANOVA of differences between sites and months (Table 4.3) at Sites 1, 3 and 4 indicate that Chl *a* biomass differed significantly between sites with varying levels of enrichment. On average, Chl *a* biomass at Sites 3 and 4 was greater than at Site 1 and Site 4 immediately below the trout farm had a signficantly higher Chl *a* biomass than Site 3 upstream of the trout farm on the Molenaars River (Table 4.1). Although similar temporal shifts in Chl *a* biomass were observed between the wet

and dry seasons as described previously for Site 1, the amplitude of these changes and the month-to-month shifts were different between these sites (Figure 4.2a,c and d). *Post hoc* analyses show that signficant month-to-month shifts in biomass at Site 1, where nutrient availability was low, were less frequent compared to Sites 3 and 4 where nutrients were more available (Table 4.4). In particular, a pronounced secondary peak in biomass was observed at Sites 3 and 4 in October 2008 that was less obvious at Site 1 under oligotrophic conditions (Figure 4.3). Also, a spring peak in biomass was signficant the previous season during September 2007, which was greatest at Site 4. Under conditions of enrichment (Sites 3 and 4) therefore, a rapid increase in periphyton biomass appeared to be characteristic of the early spring period when grazing invertebrates had not yet recovered after the winter floods but flows were more stable and solar irradiation and temperature had increased.

Table 4.3 **Two- way ANOVA resutls of Chl *a* biomass in mg m^{-2} between sites and months for Sites 1, 3 and 4 between September 2007 and May 2009. Significant differences at p<0.05 are indicated in *bold italics*.**

	SS	Df	MS	F	p
Site	4.2	2	2.09	99.6	***<0.001***
Month	25.7	19	1.36	64.5	***<0.001***
Site x Month	4.4	38	0.12	5.6	***<0.001***
Error	8.8	420	0.02		

As predicted, differences between Sites 1, 3 and 4 were only significant following a period of stability (≥ two weeks) when periphyton had had an opportunity to develop and accumulate biomass, such as late summer/autumn and spring (Table 4.5). These results reflect a response to the availablity of nutrients at these three sites and it is therefore not surprising that peak biomass at these times was highest at Site 4 followed by Site 3 on the Molenaars River and lowest in the Berg river upstream of the dam at Site 1 (Figure 4.3, Table 4.6).

Regardless of differences in the availability of nutrients, however, periphyton biomass declined signficantly during the winter months when the frequency and intensity of flooding was at its greatest and periphyotn biomass was similar between all three sites (Figure 4.2a, c and d, Table 4.5). Although the periphyton communities at Sites 3 and 4 were able to respond rapidly to the availabilty of resources under conditions of enrichment during October 2008, it was surprising that these communities did not respond by similarly rapid increases in biomass after losses induced by the late spring floods in October 2007 and November 2008 (Figure 4.2). No differences were observed between Sites 1 and 4 until February 2008 of the first accrual cycle and January 2009 of the second (Table 4.5 and Figure 4.3). This suggests that, despite the availabilty of resources over this period, environmental conditions other than flood disturbance prevented the accumulation of periphyton biomass during late spring and early summer.

Table 4.4 **Tukey *post-hoc* tests for Chl *a* biomass between monthly samples for each site with differing levels of enrichment (i.e. Sites 1, 3 and 4). Inter-annual differences between monthly samples are also given as an indication of medium- to long-term variability in periphyton biomass. Significant differences at p<0.05 are indicated in ***bold italics*** and at p< 0.1 in *italics* only.**

Site 1		Site 3		Site 4	
Within year					
Months	**p**		**p**		**p**
September2007, October2007	0.79	September2007, October2007	0.98	***September2007, October2007***	***<0.01***
October2007, November2007	0.88	October2007, November2007	0.40	October2007, November2007	0.67
November2007, December2007	0.96	November2007, December2007	0.91	November2007, December2007	0.88
December2007, January2008	0.94	***December2007, January2008***	***<0.01***	December2007, January2008	0.76
January2008, February2008	0.70	***January2008, February2008***	***<0.01***	***January2008, February2008***	***<0.01***
February2008, March2008	*0.06*	***February2008, March2008***	***<0.01***	February2008, March2008	0.57
March2008, April2008	***0.02***	***March2008, April2008***	***0.03***	March2008, April2008	0.56
April2008, May2008	***0.01***	***April2008, May2008***	***<0.01***	***April2008, May2008***	***<0.01***
May2008, June2008	0.77	May2008, June2008	0.86	May2008, June2008	0.85
June2008, July2008	0.18	June2008, July2008	0.19	***June2008, July2008***	***<0.01***
July2008, August2008	***<0.01***	***July2008, August2008***	***0.02***	***July2008, August2008***	***<0.01***
August2008, September2008	***<0.01***	***August2008, September2008***	***<0.01***		
September2008, October2008	***<0.01***	***September2008, October2008***	***<0.01***	***August2008, October2008***	***<0.01***
October2008, November2008	0.26	***October2008, November2008***	***0.01***	***October2008, November2008***	***<0.01***
November2008, December2008	0.61	November2008, December2008	0.71	***November2008, December2008***	***0.04***
December2008, January2009	0.74	December2008, January2009	0.18	***December2008, January2009***	***0.02***
January2009, February2009	***<0.01***	***January2009, February2009***	0.97	***January2009, February2009***	***<0.01***
February2009, March2009	***0.02***	***February2009, March2009***	***<0.01***	February2009, March2009	0.89
March2009, April2009	0.97	March2009, April2009	0.77	March2009, April2009	0.09
April2009, May2009	***<0.01***	***April2009, May2009***	***<0.01***	***April2009, May2009***	***<0.01***
Inter-annual					
Months	**p**		**p**		**p**
September2007, September2008	***<0.01***	***September2007, September2008***	***<0.01***	September2007, September2008	NS
October2007, October2008	0.95	October2007, October2008	0.43	***October2007, October2008***	***<0.01***
November2007, November2008	0.30	November2007, November2008	0.97	November2007, November2008	0.12
December2007, December2008	0.56	December2007, December2008	0.92	December2007, December2008	0.63
January2008, January2009	0.76	***January2008, January2009***	***<0.01***	***January2008, January2009***	***<0.01***
February2008, February2009	*0.07*	February2008, February2009	0.70	***February2008, February2009***	***0.03***
March2008, March2009	***0.04***	***March2008, March2009***	***0.02***	***March2008, March2009***	***<0.01***
April2008, April2009	***<0.01***	***April2008, April2009***	***<0.01***	April2008, April2009	0.21
May2008, May2009	0.46	May2008, May2009	0.33	May2008, May2009	0.24

Table 4.5 **Tukey *post-hoc* tests for Chl *a* biomass between sites for each month sampled at Sites 1, 3, and 4. Significant differences at p<0.05 are indicated in *bold italics* and at p< 0.1 in *italics* only.**

	Site 1 vs Site 3		*Site 1 vs Site 4*		*Site 3 vs Site 4*	
month		**p**		**p**		**p**
September2007	Site 1 = Site 3	0.66	***Site 1 < Site 4***	***<0.01***	Site 3 = Site 4	0.23
October2007	Site 1 = Site 3	0.14	Site 1= Site 4	0.96	*Site 3 = Site 4*	*0.07*
November2007	Site 1 = Site 3	1.00	Site 1= Site 4	0.99	Site 3 = Site 4	1.00
December2007	Site 1 = Site 3	0.98	Site 1= Site 4	0.96	*Site 3 < Site 4*	1.00
January2008	*Site 1 = Site 3*	*0.10*	Site 1= Site 4	1.00	***Site 3 < Site 4***	***0.05***
February2008	Site 1 = Site 3	0.98	***Site 1 < Site 4***	***<0.01***	***Site 3 < Site 4***	***<0.01***
March2008	Site 1 = Site 3	0.19	***Site 1 < Site 4***	***<0.01***	Site 3 = Site 4	0.52
April2008	Site 1 = Site 3	0.60	***Site 1 < Site 4***	***<0.01***	***Site 3 < Site 4***	***0.01***
May2008	Site 1 = Site 3	0.61	***Site 1 < Site 4***	***0.01***	Site 3 = Site 4	0.79
June2008	Site 1 = Site 3	0.89	Site 1= Site 4	0.48	Site 3 = Site 4	0.96
July2008	Site 1 = Site 3	0.49	Site 1= Site 4	0.77	Site 3 = Site 4	0.44
August2008	Site 1 = Site 3	0.95	Site 1= Site 4	1.00	Site 3 = Site 4	1.00
October2008	***Site 1 < Site 3***	***<0.01***	***Site 1 < Site 4***	0.00	***Site 3 < Site 4***	***<0.01***
November2008	Site 1 = Site 3	0.22	Site 1= Site 4	0.76	Site 3 = Site 4	0.81
December2008	Site 1 = Site 3	0.95	Site 1= Site 4	0.49	Site 3 = Site 4	0.99
January2009	Site 1 = Site 3	0.25	***Site 1 < Site 4***	***<0.01***	Site 3 = Site 4	0.97
February2009	Site 1 = Site 3	0.96	***Site 1 < Site 4***	***<0.01***	***Site 3 < Site 4***	***<0.01***
March2009	Site 1 = Site 3	0.22	***Site 1 < Site 4***	***<0.01***	Site 3 = Site 4	0.17
April2009	Site 1 = Site 3	0.12	Site 1= Site 4	0.14	Site 3 = Site 4	0.97
May2009	Site 1 = Site 3	0.85	Site 1= Site 4	0.16	Site 3 = Site 4	0.71

Table 4.6 **Mean, minimum and maximum periphyton biomass (Chl *a* concentrations in mg m^{-2}) at time of peak biomass (P_B) in 2008 and 2009.**

	P_B					
Site	**2008**			**2009**		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**
Site 1	4.1	1.3	7.4	6.4	3.8	9.7
Site 3	6.2	3.5	11.5	12.9	2.3	23.8
Site 4	9.6	7.4	14.4	14.7	11.6	21.0

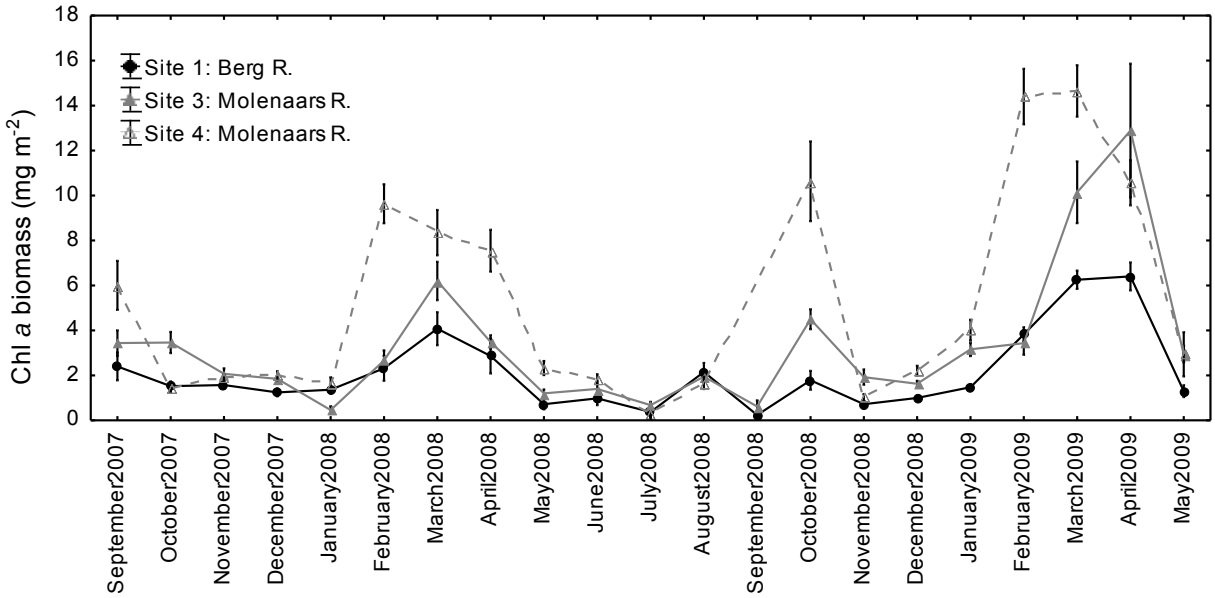


Figure 4.3 **Chl *a* biomass in mg m^{-2} plotted monthly between September 2007 and May 2009 for Site 1 (unenriched), Site 3 (slighlty enriched) and Site 4 (enriched). Error bars indicated ± 1 SE.**

4.3.1.3 The effects of flow alteration

Figure 4.2a and b show that while both measures of periphyton biomass (i.e. AFDM and Chl *a* concentrations) followed a similar pattern of change over time at Site 1, with peaks in late summer/autumn, the patterns of change in AFDM and Chl *a* concentrations at Site 2 were quite different from each other at certain times. Temporal differences in Chl *a* biomass at Sites 1 and 2 were therefore different from those for AFDM. A two-way ANOVA of differences in Chl *a* biomass showed significantly greater biomass at Site 2 compared to that for Site 1 (Table 4.6), but only during November 2007, and March and April 2009 (Table 4.7).

By contrast, AFDM was signficantly higher at Site 2 than at Site 1 throughout the spring of 2007 (i.e. September to December 2007; Table 4.7). Similarly, AFDM was signficantly higher at Site 2 during late summer (March 2008) and during winter (August 2008). Figure 4.4 shows that mean AFDM between December 2008 and February 2009 at Site 2 was somewhat higher than that at Site 1 over this period, although the differences were not significant (possibly due to large within-site variances at Site 2; Table 4.7). The frequency of flooding and variability in flow over this period was somewhat different between Sites 1 and 2 with frequent flooding until mid November 2007 at Site 1 when a class 3 flood was recorded at this site. By contrast, Site 2 had relatively high base flows with one small flood (Class 1) recorded during early December 2007. Thus, the differences in AFDM between Sites 1 and 2 during spring were probably due to the accumulation of periphyton at the latter site. Similarly, Site 1 experienced a series of large floods during July whereas no flooding occured downstream of the dam during this period. Both Chl *a* biomass and AFDM therefore increased at Site 2 reaching a peak in August 2008 that was signficantly greater than the biomass at Site 1 during this period. By contrast, both Chl *a* biomass and AFDM were particularly similar at Sites 1 and 2 during

June, October and November 2008 when sampling took place shortly after a period of intense, large floods at both sites.

Table 4.6 **Two-way ANOVA results of Chl *a* biomass in mg m^{-2} between sites and months for Sites 1 and 2 between September 2007 and May 2009. Significant differences at p<0.05 are indicated in *bold italics*.**

	SS	Df	MS	F	p
Site	0.2	1	0.25	8.8	***0.003***
Month	7.4	19	0.39	13.9	***<0.001***
Site x Month	3.7	19	0.19	6.9	***<0.001***
Error	7.8	280	0.03		

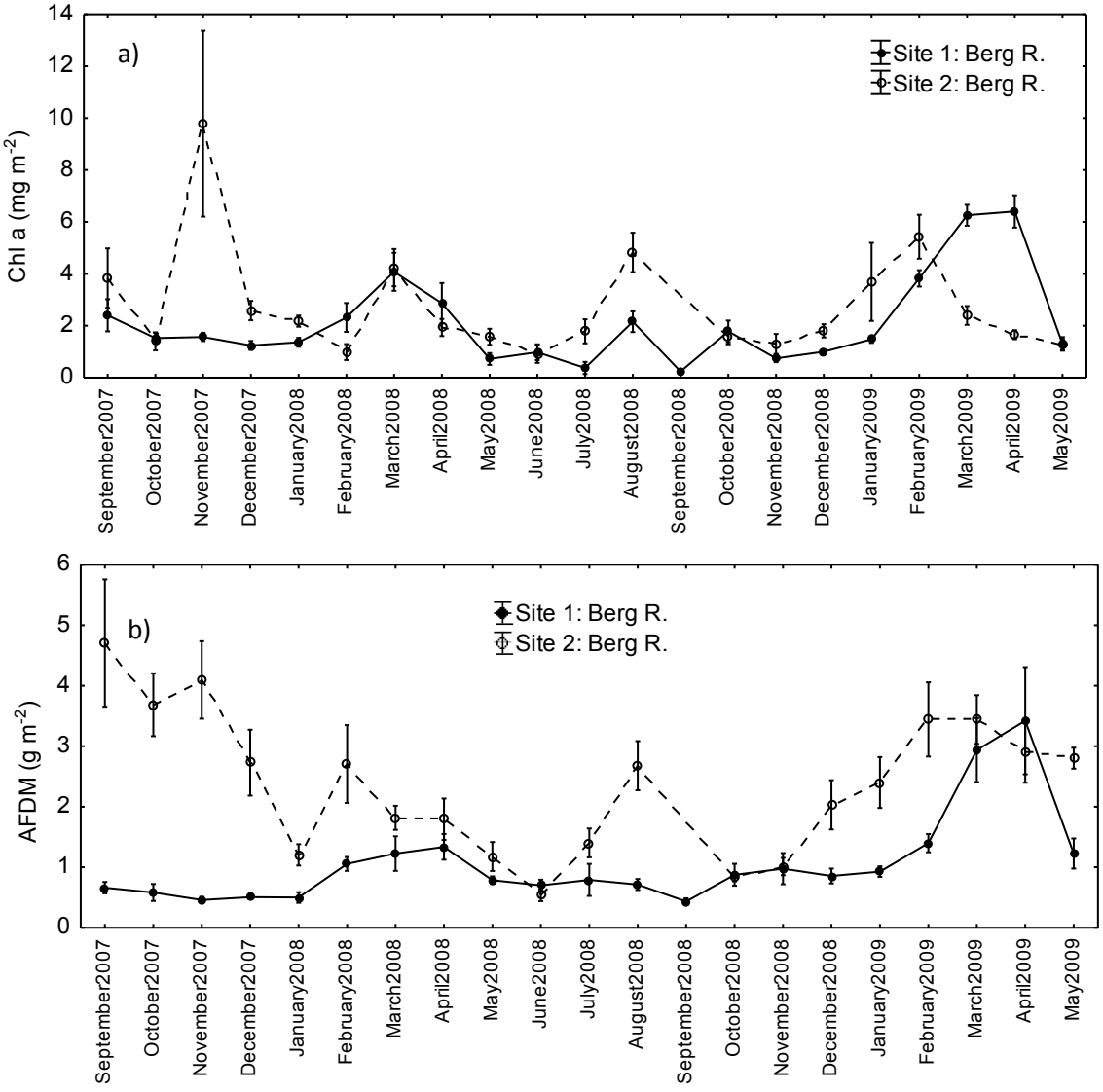


Figure 4.4 **a) Chl a biomass (mg m^{-2}) and b) AFDM (g m^{-2}) plotted monthly between September 2007 and May 2009 for Sites 1 and 2 on the Berg River . Error bars indicate ± 1 Standard Error (SE).**

Table 4.7 Tukey post-hoc tests for Chl *a* biomass between sites for each month sampled at Sites 1 and 2. Significant differences at p<0.05 are indicated in *bold italics*.

	Site 1 vs Site 2			
month	**Chl *a* biomass**	**p**	**AFDM**	**p**
September2007	Site 1 = Site 2	0.75	***Site 1 < Site 2***	***<0.01***
October2007	Site 1 = Site 2	1.00	***Site 1 < Site 2***	***<0.01***
November2007	***Site 1 < Site 2***	***<0.01***	***Site 1 < Site 2***	***<0.01***
December2007	Site 1 = Site 2	0.75	***Site 1 < Site 2***	***<0.01***
January2008	Site 1 = Site 2	0.97	Site 1 = Site 2	0.31
February2008	Site 1 = Site 2	0.52	Site 1 = Site 2	0.50
March2008	Site 1 = Site 2	1.00	***Site 1 < Site 2***	***<0.01***
April2008	Site 1 = Site 2	0.99	Site 1 = Site 2	0.81
May2008	Site 1 = Site 2	0.63	Site 1 = Site 2	0.97
June2008	Site 1 = Site 2	0.79	Site 1 = Site 2	0.77
July2008	***Site 1 < Site 2***	***0.04***	Site 1 = Site 2	0.47
August2008	***Site 1 < Site 2***	***0.05***	***Site 1 < Site 2***	***<0.01***
October2008	Site 1 = Site 2	1.00	Site 1 = Site 2	1.00
November2008	Site 1 = Site 2	0.87	Site 1 = Site 2	0.93
December2008	Site 1 = Site 2	0.97	Site 1 = Site 2	0.30
January2009	Site 1 = Site 2	0.73	Site 1 = Site 2	0.20
February2009	Site 1 = Site 2	0.60	Site 1 = Site 2	0.24
March2009	***Site 1 > Site 2***	***<0.01***	Site 1 = Site 2	0.98
April2009	***Site 1 > Site 2***	***<0.01***	Site 1 = Site 2	0.97
May2009	Site 1 = Site 2	0.98	Site 1 = Site 2	0.12

4.3.2 Changes in the temporal pattern of autotrophic and heterotrophic components of the periphyton across a flow and nutrient gradient

The Autotrophic Index (AI) can be used to measure the balance between autotrophic and heterotrophic components of the community (Hill and Knight 1987, Wellnitz *et al.* 1996). AI values between 100 and 400 generally represent a community with both autotrophic and heterotrophic components, while those with an AI over 400 consist mainly of heterotrophs and/or organic detritus (Biggs 2000). All four sites on the Berg and Molenaars Rivers had average AI values well over 400 (Table 4.9) suggesting that the communities in the foothill reaches of the south-western Cape are predominantly heterotrophic. Average AI values were significantly higher at Site 2 (AI = 1809), compared with the other sites which were not signficantly different from each other (Table 4.9).

Temporal patterns in the AI values at each site show that dominance by heterotrophs was not always the case (Figure 4.5 a-d). Monthly AI values varied significantly over the sampling period, and fell within the range of 100 to 400 around 75% of the time at Sites 3 and 4 on the Molenaars River and about 30% of the time at Site 1 upstream of the Berg Dam (Figure 4.5a, c and d). Thus, it would seem that the enriched sites were predominantly autotrophic while the oligotrophic Berg River upstream of the dam fluctuated between autotrophy and heterotrophy.

The periphyton community at Site 1 only shifted towards one dominated by heterotrophs over the wet season with a sharp rise in AI values from March 2008 to April 2008 (Figure 4.5a). AI values remained high but fluctuated over the wet season until November 2008 and were significantly higher in months when sampling was undertaken shortly after flooding in the Berg River, but much lower when sampling followed periods of no flooding. The high AI values in April 2008 were an exception to this pattern because there were no flood events prior to the April sampling. From March to April 2008, AFDM increased slightly, while Chl *a* biomass declined, possibly the result of autogenic sloughing of the algal community between March and April 2008, which shifted the community to one dominated by heterotrophs.

Sharp increases in AI values also followed periods of flood disturbance prior to sampling on the Molenaars River (Figure 4.5c) in July and September 2008 and May 2009 at Site 3. Similarly, AI values peaked at Site 4 in July 2008 with a slight shift towards a heterotrophic community in May 2009 (Figure 4.5d).

Table 4.9 Summary statistics for the Autotrophic Index (AI) at each site on all sampling dates combined.

Variables	Autotrophic Index (AI)			
	Site 1	*Site 2*	*Site 3*	*Site 4*
Valid N	162	159	166	159
Mean	1322	1809	923	841
Median	503	838	312	263
Minimum	82	132	63	43
Maximum	25745	37679	24906	29469
Std.Dev.	3186	3637	2974	3167
p		***< 0.004***		
Tukey post-hoc		**S2 >** S1 **=** S3 = S4		

Unlike Site 1 where the community was generally only dominated by heterotrophs following periods of flood disturbance during the winter (Figure 4.4a), monthly AI values at Site 2 were always above 400. This suggests that the community was predominantly heterotrophic throughout the year (Figure 4.9b). AI values were signficantly higher downstream of the dam compared with those upstream in almost all months, suggesting that environmental conditions other than nutrient availablity may be responsible for the imbalance between autotrophs and heterotrophs downstream of the dam. Despite high AI values throughout the sampling period at Site 2, the sudden increase in AI values during February 2008, June 2008 and November 2008 coincided with flooding in the period prior to sampling.

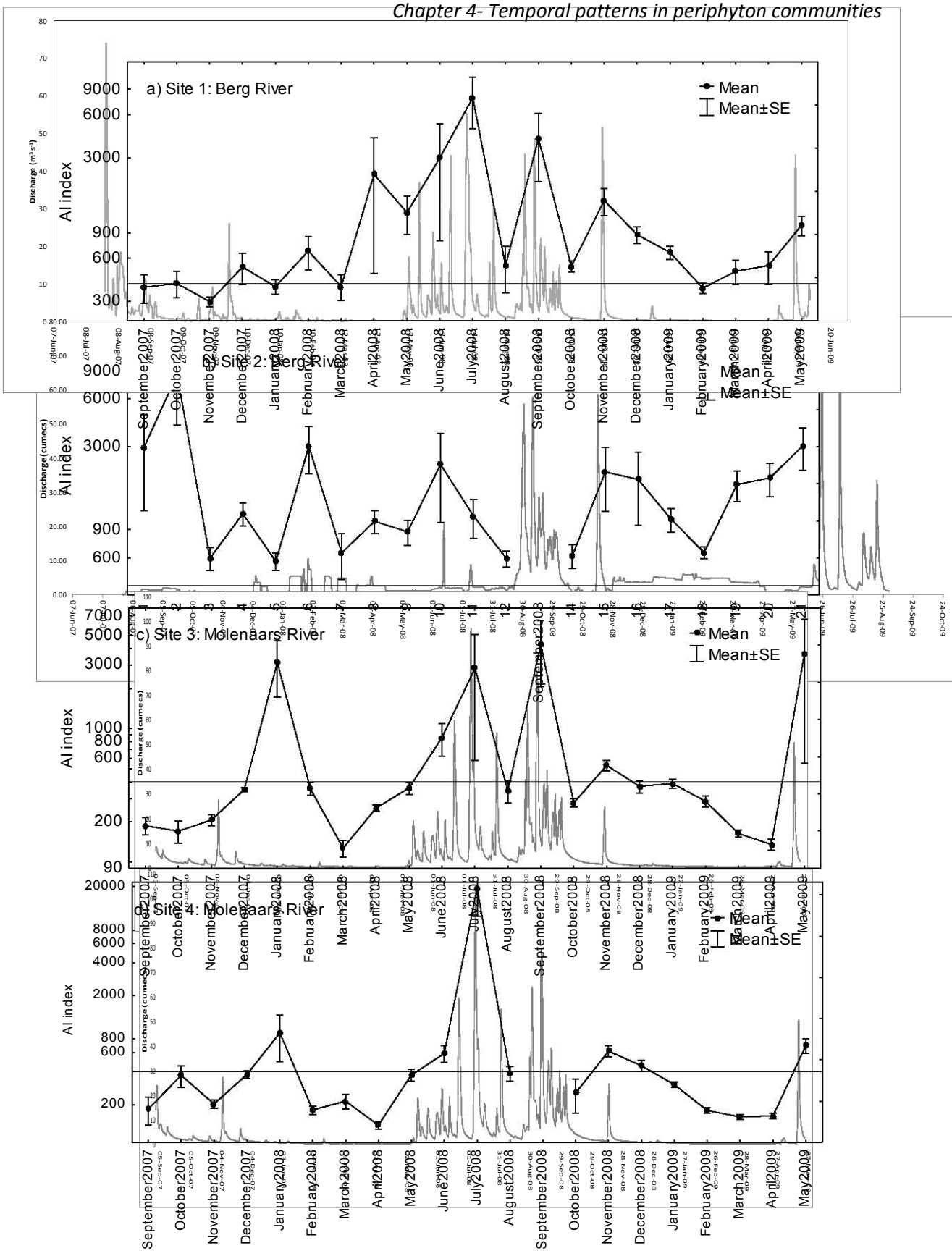


Figure 4.5 **Autotrophic Index representing the balance between autotrophic and heterotrophic components of the periphyton community plotted monthly between September 2007 and May 2009 for (a) Site 1 and b) Site 2 on the Berg River and (c) Site 3 and (d) Site 4 on the Molenaars River. Values between 100 and 400 represent balanced communities while those over 400 (dotted reference line) are dominated by heterotrophs. Error bars indicate ± 1 SE. Note: logarithmic scale on the y-axis.**

4.3.3 Benthic algal community composition

4.3.3.1 General characteristics

A total of 67 benthic algal taxa, comprising 30 diatom taxa (Bacillariophyta), 21 green algae (Chlorophyta), 14 cyanobacteria (Cyanophyta), 1 golden-brown alga (Chrysophyta) and 1 yellow-green alga (Euglenophyta) were recorded at the four study sites over the 21-month sampling period. Taxon richness was generally low but was similar at all sites except Site 2 downstream of the Berg Dam where the highest number of taxa was recorded (Table 4.10). Both the taxon composition and abundance varied considerably between sites and over time, suggesting that both nutrient enrichment (Sites 3 and 4) and flow alteration (Site 2) affect temporal dynamics in benthic algal communities in foothill rivers of the south-western Cape.

Table 4.10 Summary of the number of taxa recorded at each site over the 21-month study period.

Division	*Common name*	*Site 1*	*Site 2*	*Site 3*	*Site 4*
Bacillariophyta	diatoms	17	22	19	17
Chlorophyta	green algae	12	15	10	9
Cyanophyta	cyanobacteria	8	10	8	7
Chrystophyta	golden-brown algae	0	1	0	0
Euglenophyta	yellow-green algae	1	1	0	0
Total taxa		**39**	**50**	**39**	**35**

4.3.3.2 The benthic algal community composition under natural flow and nutrient conditions

The benthic algal community at Site 1 was, on average, dominated by three unicellular taxa that were common to all four sites, namely, the cyanobacterium, *Chamaesiphon* spp., the diatom *Eunotia rhomboidae* and the green alga, *Desmococcus*[7] spp. (Appendix 1). Five taxa, namely the diatoms *Encyonema* spp. and *Eunotia bilunaris*, the green algae *Palmella* spp. and *Spirogyra* sp.1, and the cyanobacterium *Merismopedia* sp., were found only at this site.

The pattern of temporal changes in densities (Figure 4.6a) generally reflect that given by Chl *a* biomass (Figure 4.2a) with lowest benthic algal densities in July 2008 and a clear peak during the summer of both 2008 and 2009 (Figure 4.6a). Although not particularly clear from the Chl *a* biomass patterns presented in Section 4.3.2, the benthic algal densities show a clear secondary spring peak during both October 2007 and October 2008 at Site 1 (Figure 4.6a). During the first annual growing season, the spring peak was dominated by diatoms, particularly the pollution sensitive diatom, *Eunotia rhomboida*e, (Figure 4.6b, Figure 4.7). By contrast, the summer peak in 2008 was dominated by *Mougeotia* spp. and *Spirogyra* spp., two unbranched filamentous green algae (Figure 4.6c; Figure

[7] The literature indicates that *Desmococcus* spp. is a 'terrestrial' taxon growing on damp surfaces (e.g. Bellinger 1980), rather than a fully aquatic taxon growing on submerged surfaces in rivers. Therefore the current identification of these small green colonial algae as *Desmococcus* spp. is questionable. Although there is some indication that this taxon may be a component of a crustose lichen (*Verrucaria*), molecular studies will be necessary to confirm its taxonomy (Prof. B. Whitton, School of Biological and Medical Sciences, Durham University, pers. comm.). Considering the proportional contribution of these algae to the periphyton, further molecular work is warranted and may reveal some interesting findings.

4.7) characteristic of late succession communities (Leland *et al.* 1986). By contrast, the spring peak during October 2008 was equally dominated by both diatoms (mainly *Eunotia rhomboidae*) and non-diatom taxa, particularly the single celled yellow-green alga, *Euglena* spp. and the colonial green alga, *Desmococcus* spp. (Figure 4.6c). A far greater proportion of single-celled and filamentous cyanobacteria were evident in the second cycle, relative to the first (Figure 4.7). Unlike the summer and early autumn months in 2008, summer and autumn 2009 were dominated by the single-celled cyanobacterium, *Chamaesiphon* spp. During late spring, however, the colonial cyanobacterium, *Aphanocapsa* spp., which typically occurs as a gelatinous mass, became dominant (Figure 4.6d and 4.7). While green filamentous taxa were an important component of the climax community at the time of peak biomass during the summer of 2008, these taxa appear to have been replaced by high densities of the cyanobacteria, *Chamaesiphon* spp. and *Aphanocapsa* spp. in 2009. It is noteworthy that *Aphanocapsa* spp. was completely absent from the benthic algal community throughout the summer of 2008 but was a dominant component of the community during the summer of 2009.

The number of taxa was generally greater during the first growing season relative to the second, when both benthic algal biomass and cell densities were far greater. From November 2007 to March 2008, between 9 and 17 taxa were recorded monthly at Site 1 with a peak in February 2008. During the second growing season, the number of taxa was greatest during October 2008, coinciding with the secondary peak in algal biomass and density with a total of 15 taxa recorded, but dropped sharply thereafter with a total of only 6 taxa recorded during March and April 2009 when biomass reached its highest peak throughout the sampling period.

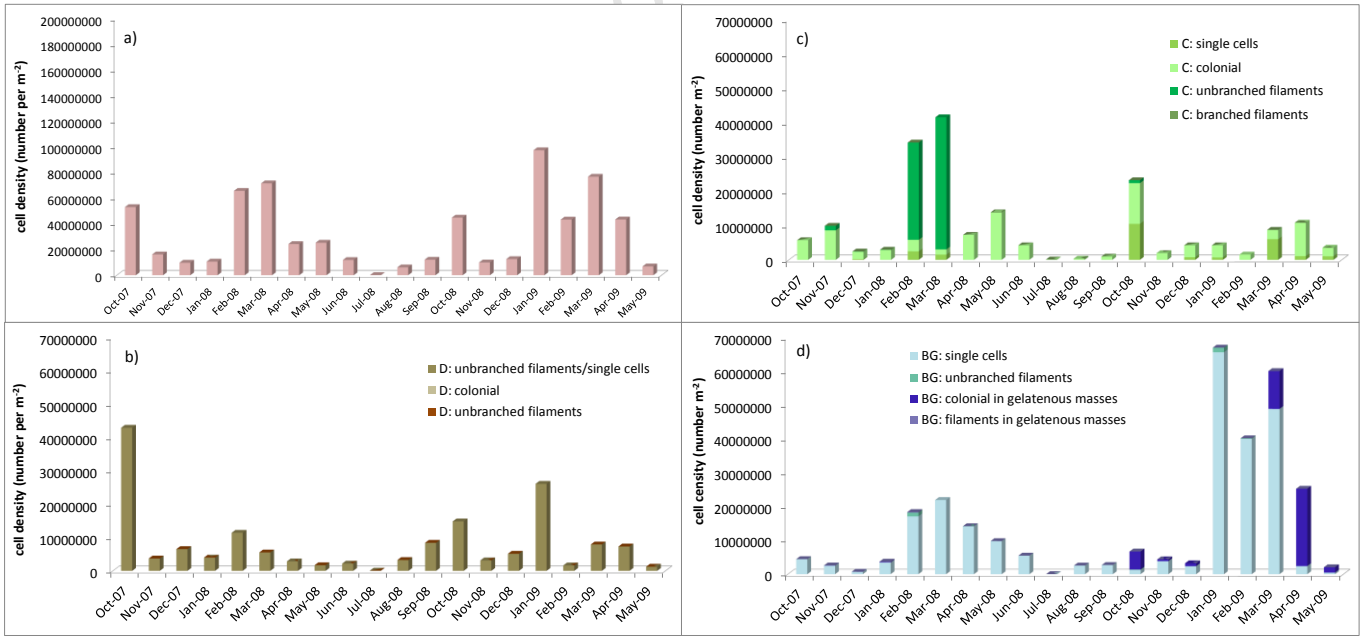


Figure 4.6 a-d Monthly cell densities at Site 1 for a) all benthic algal divisions together and separately for b) diatoms, c) green algae and d) cyanobacteria.

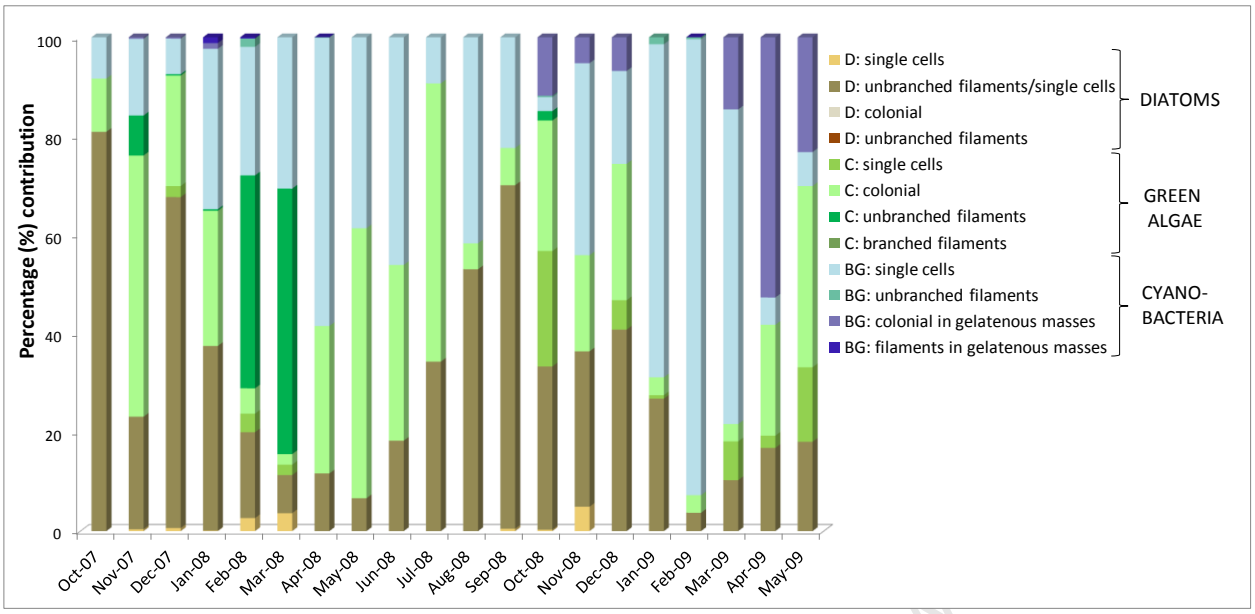


Figure 4.7 **Proportion of taxa per month at Site 1 by division and form. D= diatoms; C=green algae; BG=cyanobacteria.**

Despite clear temporal changes in the density and composition of benthic algal taxa over the 21-month sampling period, the benthic algal community composition at Site 1 did not separate clearly according to seasons as indicated in the cluster diagram and multi-dimensional scaling (MDS) plot (Figure 4.8). Both summer 2008 and summer 2009 were an exception to this and grouped together at 45% similarity (Figure 4.8). Nevertheless, a one-way nested PERMANOVA between seasons indicated that periphyton communities between both seasons and months (nested within seasons) were significantly different over the sampling period (Table 4.11). The post-hoc analyses showed that seasonal differences were driven largely by the differences between summer 2008 and winter 2008 and again between winter 2008 and the following summer 2009, which would be expected when comparing these seasonal extremes (Table 4.12). Also, the community structure towards the end of the dry season (i.e. summer, early autumn and late autumn) of 2008 was significantly different from that of 2009 when Chl *a* biomass was greater (Figure 4.2a). The community composition did not however, shift with the onset of spring in both years when no significant changes in the community were recorded between sequential seasons over the growing season (i.e. early spring to early autumn). The only sequential seasonal shift in the community was observed between early and late autumn of both years (Table 4.12) when a decline in Chl *a* biomass (Figure 4.2a) and algal abundance (Figure 4.6 a) was apparent.

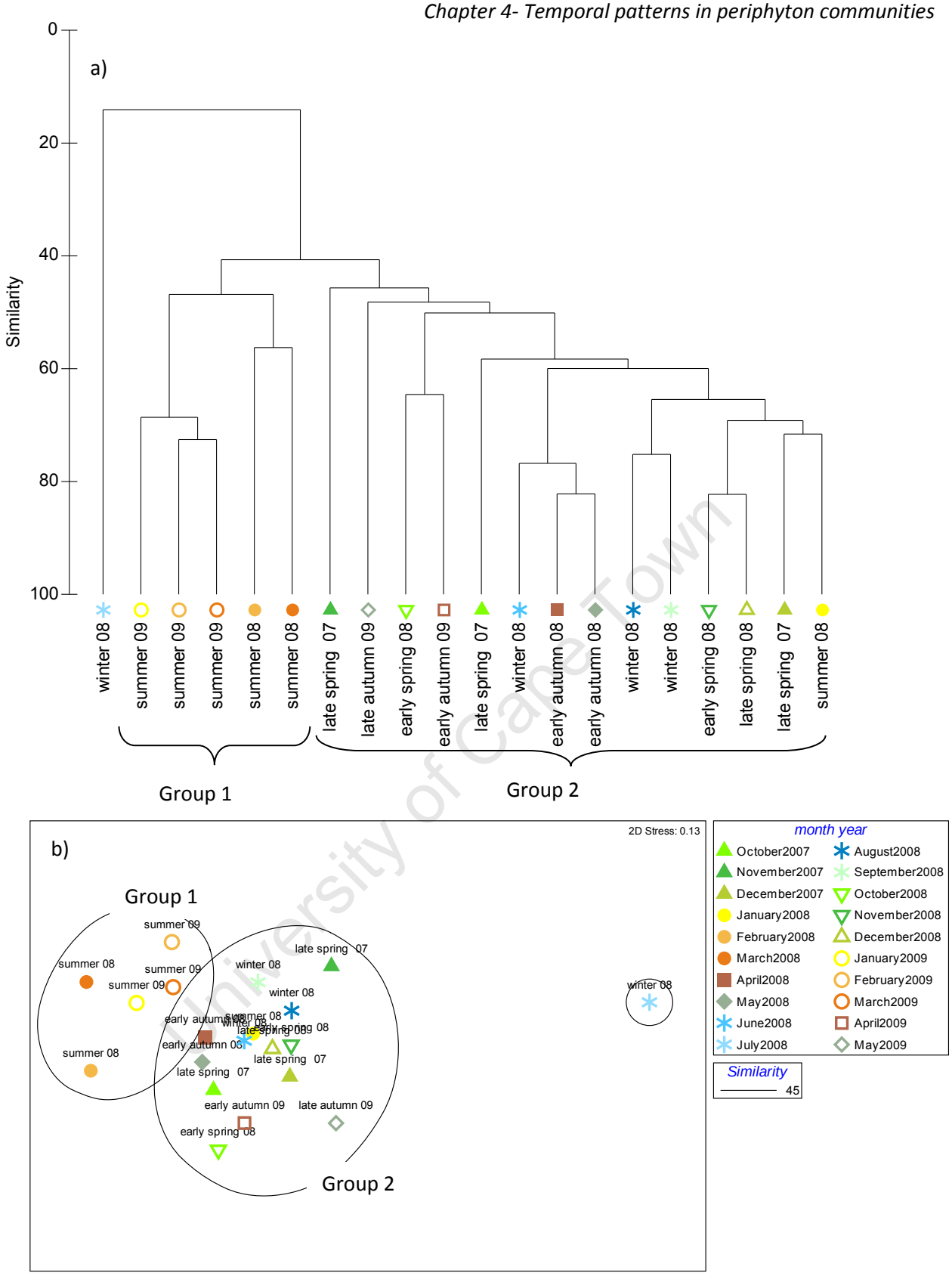


Figure 4.8 **a) Hierarchical cluster and b) MDS plot of the periphyton community composition at Site 1, upstream of the Berg Dam based on log (x+1) transformed taxon abundances averaged monthly between October 2007 and May 2009. Two disctinct groups separating the summer community from that during all other months was evident at 45% Bray-Curtis Similarity, with the July 2008 community as an outlier.**

Table 4.11 **One-way nested PERMANOVA results for seasonal comparisons of periphyton community composition at Site 1 sampled monthly over two annual cycles (21 months) between October 2007 and May 2009. Significant differences at p≤ 0.05 are indicated in *bold italics*.**

Groups	*df*	*SS*	*MS*	*Pseudo-F*	*p*
season	10	67626	6763	1.78	***0.014***
month(season)	9	34079	3787	4.99	***<0.001***
Residuals	60	45507	758		
Total	79	147160			

Table 4.12 **PERMANOVA pair wise comparisons of between seasons defined *a priori* within Site 1. Comparisons for both intra- and inter-annual differences are shown, as well as those for seasonal extremes. Only consecutive seasons for the intra-annual differences are shown. Significant differences at p≤0.05 are indicated in *bold italics.***

			% similarity		
Groups	***t***	***p***	**between seasons**	**within season 1**	**within season 2**
Intra annual (seasonal)					
early spring '07, late spring '07	1.26	0.33	41.86	60.71	55.06
late spring '07, summer '07/08	1.52	0.19	37.34	55.06	48.38
summer '07/08, early autumn '08	0.69	1.00	55.62	48.38	80.26
early autumn '08, late autumn '08	***1.88***	***0.03***	***77.25***	***80.26***	***85.20***
late autumn '08, winter '08	1.08	0.20	44.87	85.20	48.22
winter '08, early spring '08	1.13	0.33	39.03	48.22	46.59
early spring '08, late spring '08	0.54	1.00	50.62	46.59	54.73
late spring '08, summer '08/09	2.41	0.25	35.71	54.73	62.67
summer '08/09, early autumn '09	2.68	0.24	32.53	62.67	60.98
early autumn '09, late autumn '09	***1.80***	***0.03***	***47.72***	***60.98***	***54.07***
Seasonal extremes					
summer '07/08, winter '08	***1.39***	***0.05***	***38.90***	***48.38***	***48.22***
winter '08, summer '08/09	***2.43***	***0.00***	***34.23***	***48.22***	***62.67***
Interannual comparisons					
early spring '07, early spring '08	0.97	0.66	43.26	60.71	46.59
late spring '07, late spring '08	1.20	0.34	41.67	55.06	54.73
summer '07/08, summer '08/09	***1.67***	***0.05***	***42.94***	***48.38***	***62.67***
early autumn '08, early autumn '09	***2.47***	***0.03***	***52.98***	***80.26***	***60.98***
late autumn '08, late autumn '09	***3.16***	***0.03***	***38.44***	***85.20***	***54.07***

The MDS plot undertaken using all seasonal replicates at Site 1 (Figure 4.9), suggests that the community structure seems to shift *monthly* rather than seasonally. Indeed, a one-way PERMANOVA between the communities (pseudo F=7.05; p=<0.001) showed clear shifts in the community from one month to the next, regardless of seasons (Appendix 2). The average Bray Curtis similarities in Figure 4.10 provide an indication of the degree of change in community structure over time. Not surprisingly, the pair-wise comparisons indicate that the community was more stable during the summer months, particularly during the second annual cycle when no significant shift in the community was evident between February and March 2009 (Appendix 2, Figure 4.10). The most dramatic shift in community structure was evident during mid-winter (i.e. June to July 2008 and July to August 2008) suggesting that community change within the winter is particularly frequent (Figure 4.10). Nevertheless, the most distinct differences in community structure often coincided with the seasonal boundaries, particularly between early and late spring of both 2007 and 2008, late spring and summer 2009 and summer and early autumn 2009. Temporal patterns in community structure therefore, do not necessarily follow the seasonal categories defined by abiotic characteristics but seem to occur both *within* and *between* seasons.

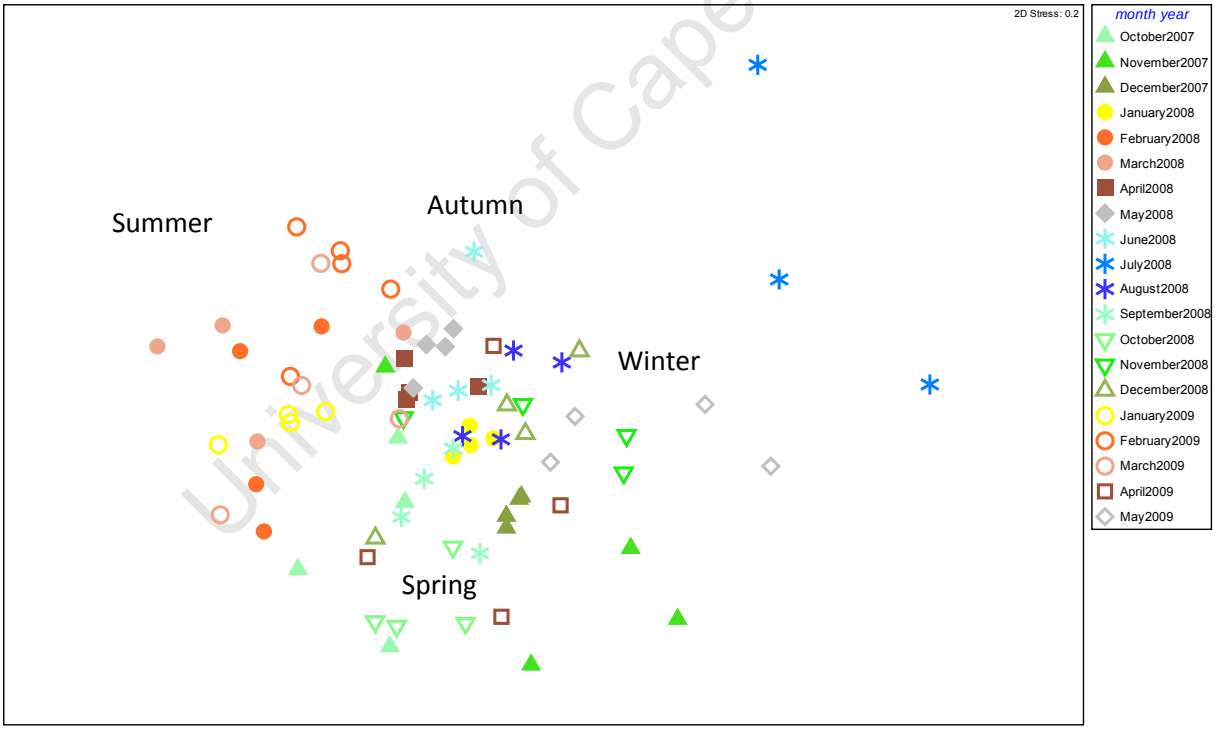


Figure 4.9 **MDS plot of the periphyton community composition at Site 1, upstream of the Berg Dam based on log (x+1) transformed taxon abundances of individual replicates collected monthly between October 2007 and May 2009.**

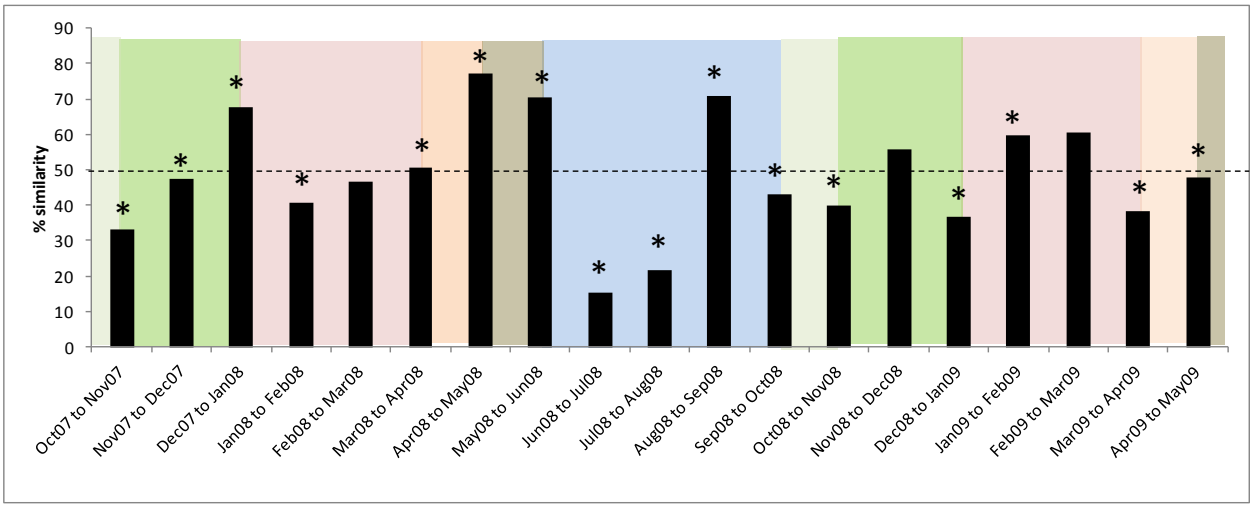


Figure 4.10 **The average Bray Curtis Similarity (%) among periphyton community structure from the PERMANOVA pair wise comparisons between consecutive sampling occasions (months) at Site 1. The asterisks indicate a significant change in community structure at p≤0.05 (see Appendix 4.1). The background colour depicts seasonal boundaries. Light green = early spring; green = late spring; pink = summer; orange = early autumn; brown = late autumn, blue = winter.**

Temporal changes in sample variability (or dispersion) can be used as an indicator of change in the patchiness of benthic algal communities over time (Anderson *et al.* 2008). During certain months, particularly December 2007 and January 2008, and again during January and February 2009, replicate samples grouped particularly closely together (Figure 4.9) suggesting lower patchiness in community structure during the growing season when conditions are stable and the community has recovered somewhat following disturbance. By contrast, the replicates taken during November 2007, July 2008 and November 2008 were spread widely (Figure 4.9) suggesting greater patchiness among communities during the wet season. These results were confirmed by the analysis of dispersion results based on the PERMDISP routine in PERMANOVA. The dispersion (or variability) within communities was indeed significantly different between months (F= 5.59; p= <0.001, Appendix 3). As a measure of the extent of dispersion among samples, a plot of the average Bray-Curtis distance-to-centroid percentages provided by the analysis clearly show a pattern of much greater variability among samples taken after flood disturbance (Figure 4.11). In particular, variability was relatively high among samples in November 2007, July 2008 and November 2008, compared with that during the stable period prior to peak biomass (i.e. December 2007 and January 2008 and again during January and February 2009) (Figure 4.11). Although not evident from the MDS plot in Figure 4.9, these data also suggest greater patchiness in the distribution of communities in March 2008 and March 2009, at the time of peak biomass in both years (Figure 4.11). Essentially these data suggest that communities remaining after a flood event or during the early stages of succession following disturbance are highly patchy, whereas those that have recovered significantly from disturbance under stable conditions prior to the time of peak biomass are far more uniform. As the community reaches peak biomass the community shows greater patchiness again, despite the absence of flooding. The March samples of both years may represent a period immediately following peak biomass when the process of autogenic sloughing, which acts differentially across the stream

habitat in response to localized differences in flow conditions, has already begun, thus resulting in a more patchy community.

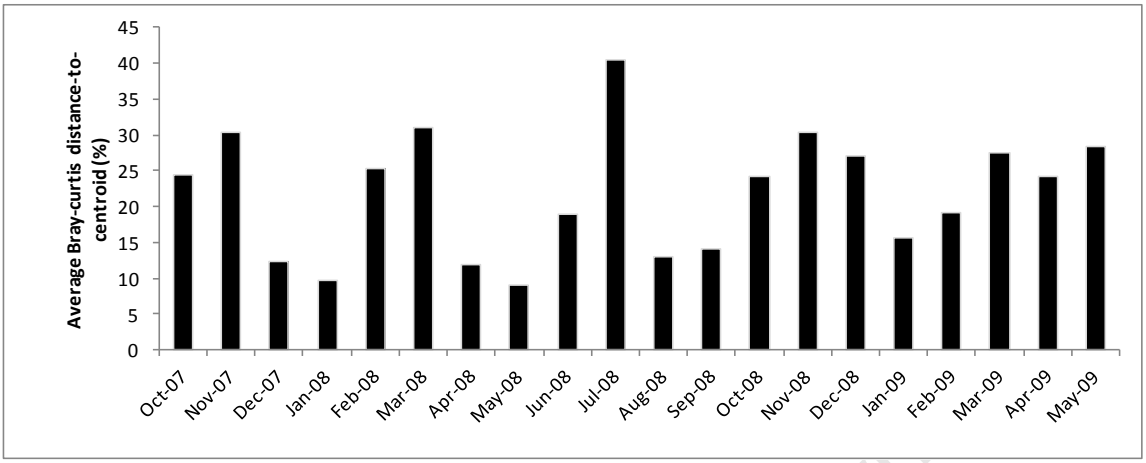


Figure 4.11 **Average Bray-curtis distance-to-centroid percentage per month at Site 1 as a measure of the dispersion of benthic algal communities among samples in each month. High percentages indicate large dissimilarities among samples.**

SIMPER showed that the majority of sequential monthly shifts in community composition at Site 1 were driven largely by changes in the abundance of at least one of the three dominant taxa, namely *Eunotia rhomboidae, Desmococcus* spp. and *Chamaesiphon* spp., rather than a change in algal composition (Appendix 4). This was most apparent during the winter months when dramatic shifts in the abundance of these three taxa explained the high dissimilarity between June and July 2008 and again between July and August 2008 (Figure 4.12a; Appendix 4). Nevertheless, certain taxa demonstrated clear seasonal patterns of occurrence and thus their presence or absence contributed to monthly shifts in community structure at certain times. For example, the shift from September 2008 to October 2008 was partly due to the presence of the colonial cyanobacterium, *Aphanocapsa* spp. and the yellow-green alga, *Euglena* spp. in October 2008, both of which were absent in September 2008 (Figure 4.12 c, Appendix 4). Also, M*ougeotia* spp. and *Spirogyra* spp., both characteristic of late succession communities, were absent during the spring months but dominant in February 2008 and March 2008 respectively. These two taxa therefore contributed significantly to changes in the community over the growing season in 2008 (Figure 4.12d and 4.12e, Appendix 4). *Mougeotia* spp. and *Spirogyra* spp. were absent from the community at the end of the growing season in 2009, however, when the colonial cyanobacterium, *Aphanocapsa spp*., was relatively dense. Indeed, *Aphanocapsa spp.* contributed significantly to the shift in community composition between March 2009 and April 2009 when it reached a peak, and again between April 2009 and May 2009 when densities dropped considerably.

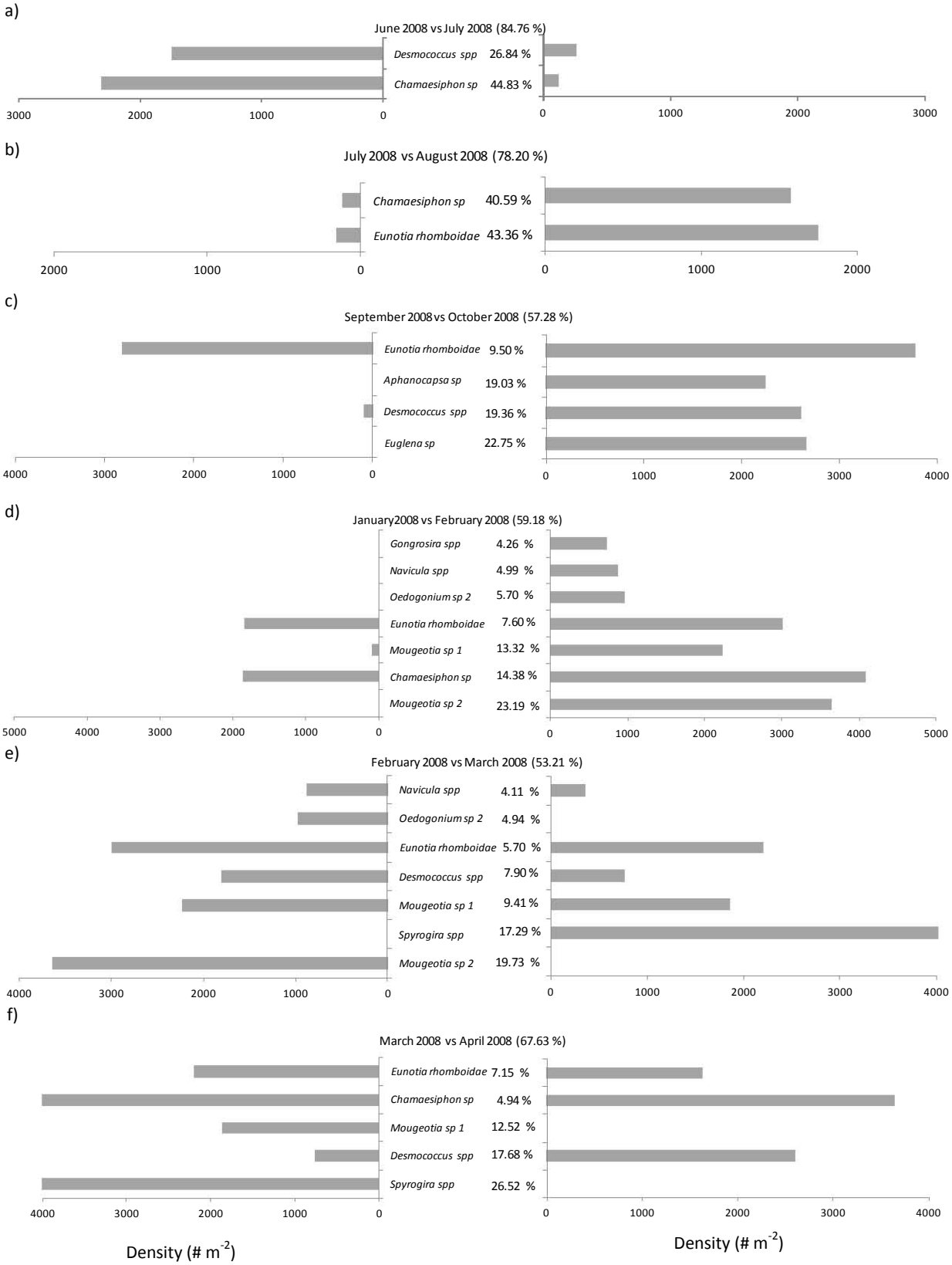


Figure 4.12 **SIMPER results of benthic algal taxa densities at Site 1 that together contributed at least 70% to the overall differences between certain consecutive months after square root transformation and Bray-Curtis dissimilarity. Average dissimilaries between months (in brackets) well as the contributions given by each taxon to the average dissimilarities are presented as percentages (%).**

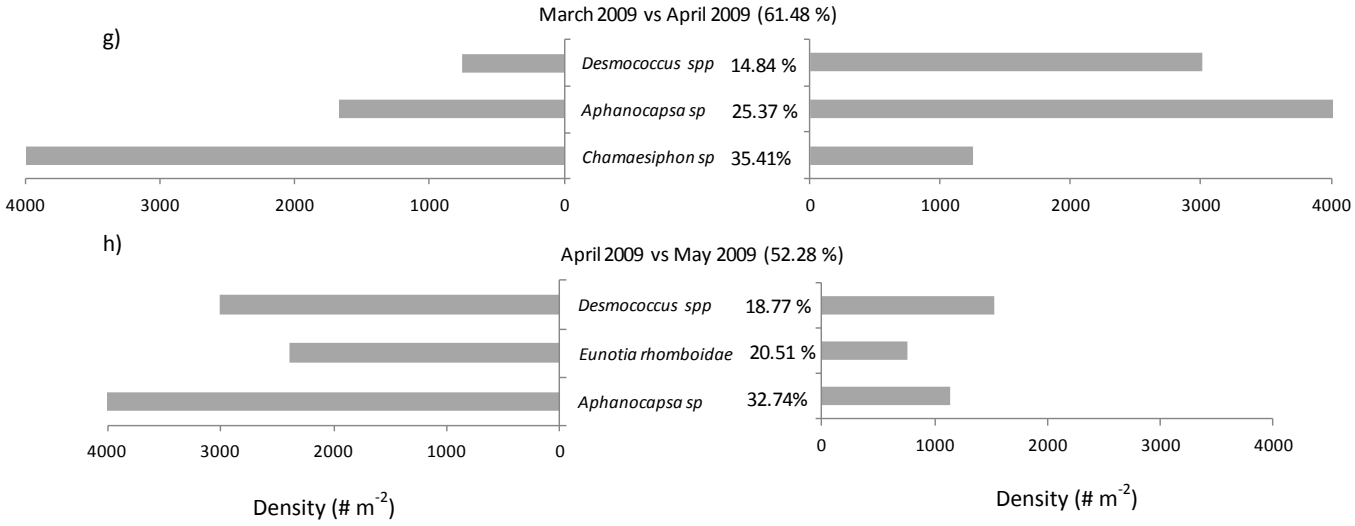


Figure 4.12 *continued* SIMPER results of benthic algal taxa densities at Sit 1 that together contributed at least 70% to the overall differences between certain consecutive months after square root transformation and Bray-Curtis dissimilarity. Average dissimilaries between months (given in brackets) as well as the contributions given by each taxon to the average dissimilarities are presented as percentages (%).

Besides these within-year differences in benthic algal community structure, inter-annual differences were also evident. In particular, the summer community of 2008 was significantly different from that the following summer, due largely to the absence of green filamentous taxa and an increase in cyanobacteria in 2009. This is evident from the SIMPER results at the time of peak biomass (Figure 4.13, Appendix 5) which shows the absence of the green filamentous taxa, *Mougeotia* spp. and *Spirogyra* spp. and the presence of the cyanobacterium, *Aphanocapsa* spp. in March 2009.

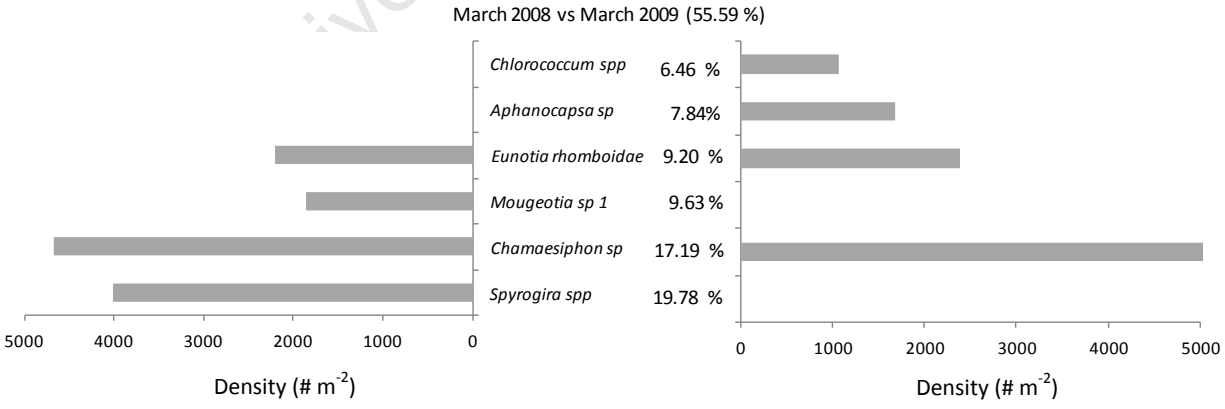


Figure 4.13 SIMPER results of benthic algal taxa densities at Sit 1that together contributed at least 70% to the overall differences between March 2008 and March 2009 after square root transformation and Bray-Curtis dissimilarity. The average dissimilary between these two communities (in brackets) as well as the contributions given by each taxon to the average dissimilarities are presented as percentages (%).

4.3.3.3 The effects of enrichment

Hierarchical clustering and MDS analysis of benthic algal communities at Site 1 and the two enriched sites on the Molenaars River (Sites 3 and 4) did not clearly separate sites from each other (Figure 4.14). Nevertheless, algal communities representing certain months at Site 1 appear to separate from Sites 3 and 4 (Figure 4.14). A two-way PERMANOVA analysis of benthic algal community composition at Sites 1, 3 and 4 showed that there were clear differences between the benthic algal communities among sites and months (Table 4.13). All three sites were significantly different from each other (Table 4.14) but the distinction between Sites 3 and 4 was far less clear (Figure 4.14). The distinction between sites was not evident in all months, however.

Table 4.13 **Two-way PERMANOVA results for comparisons between the community structure of Site 1 (unenriched) and Sites 3 and 4 (enriched) sampled monthly over two annual cycles (21 months) between October 2007 and May 2009. Significant differences at p≤ 0.05 are indicated in bold italics.**

Groups	*df*	*SS*	*MS*	*Pseudo-F*	*p*
site	2	40709	20354	25.6	***<0.001***
month	19	233070	12267	15.5	***<0.001***
site x month	37	141000	3811	4.8	***<0.001***
residuals	178	141270	794		
Total	236	556000			

Table 4.14 **PERMANOVA pair wise comparisons between sites that differ in their enrichment (i.e. Sites 1, 3 and 4). Significant differences at p≤0.05 are indicated in *bold italics.***

Groups	t	p	*Average similarity*
Site 1 vs Site 3	2.489	***<0.001***	37
Site 1 vs Site 4	2.484	***<0.001***	34
Site 3 vs Site 4	1.732	***0.004***	36

The importance of temporal changes in communities as a factor implicit in the separation of communities between these sites is borne out by the PERMANOVA pair-wise comparisons given in Table 4.15 and the hierarchical cluster and MDS diagrams for selected months given in Figure 4.15. Table 4.15 shows that Site 1 was significantly different from both Sites 3 and 4 in all months except November 2007 and June 2008 (Figure 4.15a). Sites 3 and Site 4 were similar in all months except those representing summer and early autumn, and isolated winter and spring months (i.e. August 2008 and October 2008, respectively). Considering the similarity in flow characteristics between these sites, it is likely that the differences between all three sites during these periods were a response to differences in the availability of nutrients, as expected. Essentially, resource availability affects the trajectory of accrual and the succession of benthic taxa following stable flow conditions. Indeed the summer 2008 and summer/early autumn 2009 communities at all three sites represent the climax communities at peak biomass when site differences were distinctly clear (Figure 4.15 d). Also, October 2008 presented a period when all three sites were particularly different from each other (Figure 4.14b), following a period of stability during the spring when light was no longer

limiting, temperatures were favourable, but nutrient availability differed, allowing distinct communities to develop. Considering the complexity of temporal changes as a factor determining the importance of nutrients for the development of algal communities, it is not surprising that the site differences presented in Figure 4.14 are not clear.

A comparison between the relative contributions of different benthic algal divisions and growth forms at Sites 1, 3 and 4 (Figures 4.7, 4.16 and 4.17, respectively) shows that, in general, single celled diatoms contribute far more to the community composition at Site 1, relative to Sites 3 and 4. Nevertheless, diatoms dominated the spring community at both Sites 3 and 4 in October 2007 and again in October 2008 at Site 4. Unlike Site 1, however, where pollution sensitive taxa such as *Eunotia exigua, E. minor* and *E. rhomboidae* characterised the diatom community during October 2007 and 2008, *Gomphonema parvulum*, a pollution tolerant diatom, was the key contributor to the community at Site 4 during October 2007 and 2008.

SIMPER analysis was used to determine which taxa were most responsible for the distinction between Sites 1, 3 and 4 during certain months. The distinction between all three sites during the spring period, particularly during October 2008 (Figure 4.14b), was driven largely by the presence or absence of certain taxa. For example, the highly significant dissimilarity of 80.45% between Site 1 and 3 at this time was due to relatively low densities of *Eunotia rhomboidae*, and the absence of *Euglena* spp., *Desmococcus* spp. and *Aphanocapsa* spp. at Site 3 that all contributed significantly to the October 2008 community at Site 1. Also, *Lyngbya* sp. 1, a filamentous cyanobacterium, was present in relatively high densities at Sites 3 and 4 during October but was completely absent from the community at Site 1 during the spring of 2008 (Figure 4.18a). Site 1 was even more dissimilar to Site 4 during October 2008 (Figure 4.18a). The absence of the diatoms *Gomphonema parvulum* and *Fragilaria capucina* from Site 1 and their presence in high numbers at Site 4 were the main drivers for the distinction between these two sites in October 2008 (Figure 4.18a). Similarly, these two taxa were the main contributors to the distinction between Site 3 and 4 at this time (Figure 4.18a). As with Site 1, *Aphanocapsa* spp. became a conspicuous component of the community towards the end of the growing season in 2009 but was only present in small numbers during the growing season in 2008. At the time of peak biomass, the communities at both Sites 3 and 4 were dominated by colonial green algae, mostly Chl*orococcum* spp., whereas Site 1 was dominated by green filamentous taxa during this period. The distinction between Sites 1 and 3 and Sites 1 and 4 in March 2009 (Figure 4.15 e) was driven largely by the high abundance of Chl*orococcum* spp. and *Aphanocapsa* spp. and a lower abundance of *Chamaesiphon* spp. at Site 3 and 4, relative to Site 1 (Figure 4.18b). Sites 1 and 4 were further distinguished from each other by the absence of *Eunotia rhomboidae*, a diatom typical of oligotrophic conditions, and the presence of *Mougeotia* spp. 2 at Site 4 (Figure 4.18b).

Table 4.15 PERMANOVA pair wise comparisons of monthly periphyton communities between Site 1 (unenriched) and Sites 3 and 4 (enriched). Significant differences at p ≤ 0.05 are indicated in bold italics and at p≤ 0.1 in italics only.

month	Site	Average similarity					
		between sites 1 and 3	between sites 1 and 4	between sites 3 and 4	within site 1	within site 3	within site 4
October 2007	*Site 1 ≠ (Site 3 = Site 4)*	***24***	***38***	30	61	35	34
November 2007	Site 1 = Site 3 = Site 4	38	44	58	50	54	55
December 2007	*Site 1 ≠ (Site 3 = Site 4)*	***32***	***49***	63	80	66	70
January 2008	***Site 1 ≠ Site 3 ≠ Site 4***	***47***	***43***	***55***	84	72	77
February 2008	***Site 1 ≠ Site 3 ≠ Site 4***	***42***	***37***	***38***	58	60	75
March 2008	*Site 1 ≠ (Site 3 = Site 4)*	***33***	***30***	75	50	79	74
April 2008	*Site 1 ≠ (Site 3 = Site 4)*	***63***	***57***	63	80	63	70
May 2008	***Site 1 ≠ Site 3 ≠ Site 4***	***51***	***67***	***55***	85	55	82
June 2008	Site 1 = Site 3 = Site 4	63	64	68	70	64	81
July 2008	*Site 1 ≠ (Site 3 = Site 4)*	***20***	***16***	68	28	48	72
August 2008	***Site 1 ≠ Site 3 ≠ Site 4***	***48***	***55***	***47***	79	42	68
September 2008	***Site 1 ≠ Site 3***	***51***	NS	NS	77	63	NS
October 2008	***Site 1 ≠ Site 3 ≠ Site 4***	***20***	***6***	***18***	61	81	63
November 2008	*Site 1 ≠ (Site 3 = Site 4)*	***21***	***19***	58	50	49	65
December 2008	*Site 1 ≠ (Site 3 = Site 4)*	***30***	***30***	69	55	64	74
January 2009	***Site 1 ≠ Site 3 ≠ Site 4***	***38***	***51***	***58***	75	70	70
February 2009	***Site 1 ≠ Site 3 ≠ Site 4***	***47***	***44***	***28***	70	53	64
March 2009	***Site 1 ≠ Site 3 ≠ Site 4***	***32***	***42***	***57***	54	70	63
April 2009	***Site 1 ≠ Site 3 ≠ Site 4***	***33***	***37***	***40***	61	49	49
May 2009	*Site 1 ≠ (Site 3 = Site 4)*	***42***	***37***	53	54	59	62

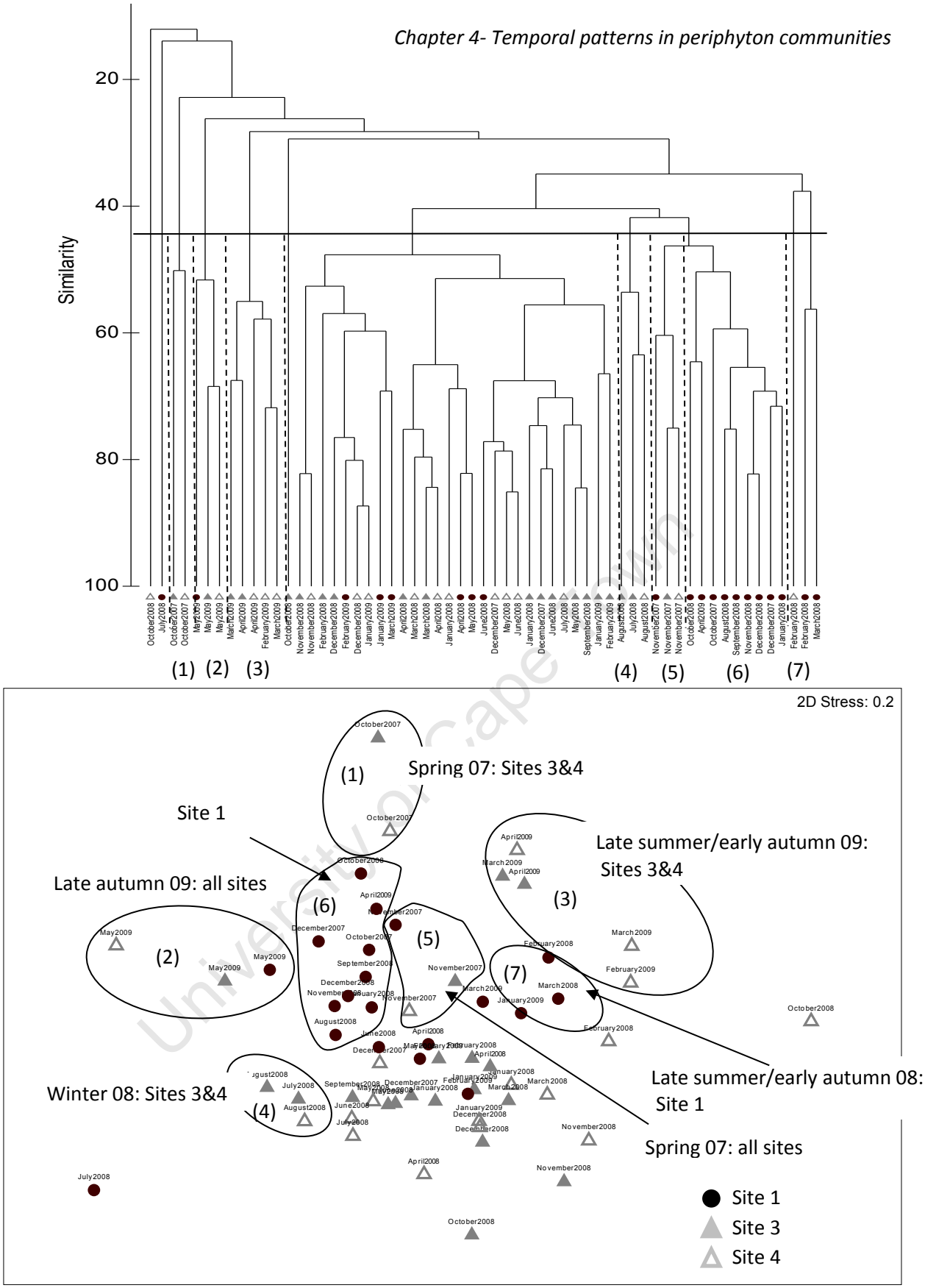


Figure 4.14 **Hierarchical clustering and 2-D MDS plot of square root transformed and weighted periphyton densities from individual replicates pooled per monthly sampling period at Sites 1, 3 and 4. The ellipses represent the Bray-Curtis similarity between samples defined by hierarchical clustering at 45%.**

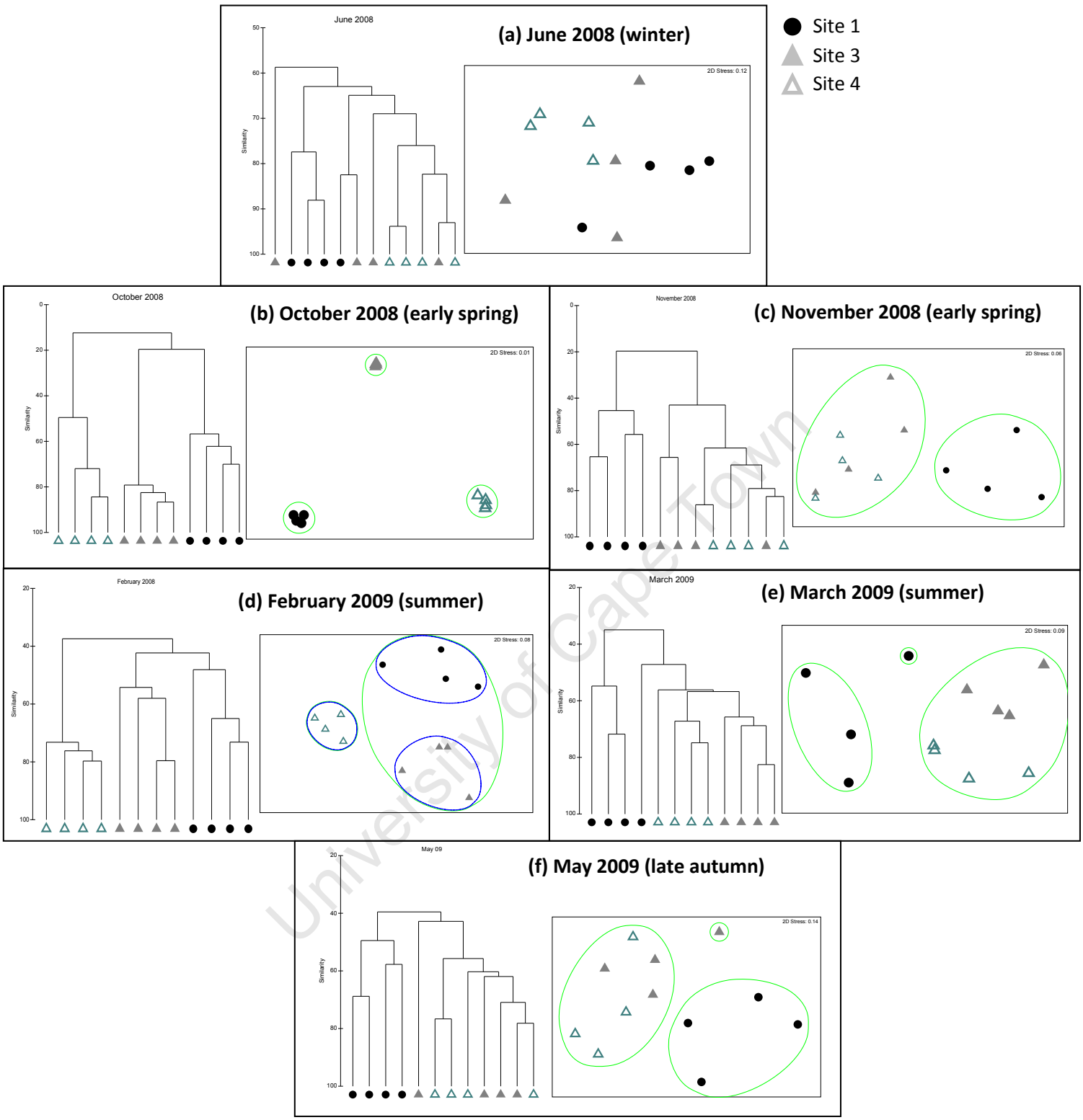


Figure 4.15 **2-D MDS plot of square-root transformed and weighted periphyton densities from individual replicates at Site 1 on the Berg River and Sites 3 and 4 on the Molenaars River. The ellipses represent the major groupings of samples defined by hierarchical clustering using Bray-Curtis similarities. October 2008 represents the secondary peak biomass (P_B) in spring while February and March 2009 represent the primary P_B in late summer.**

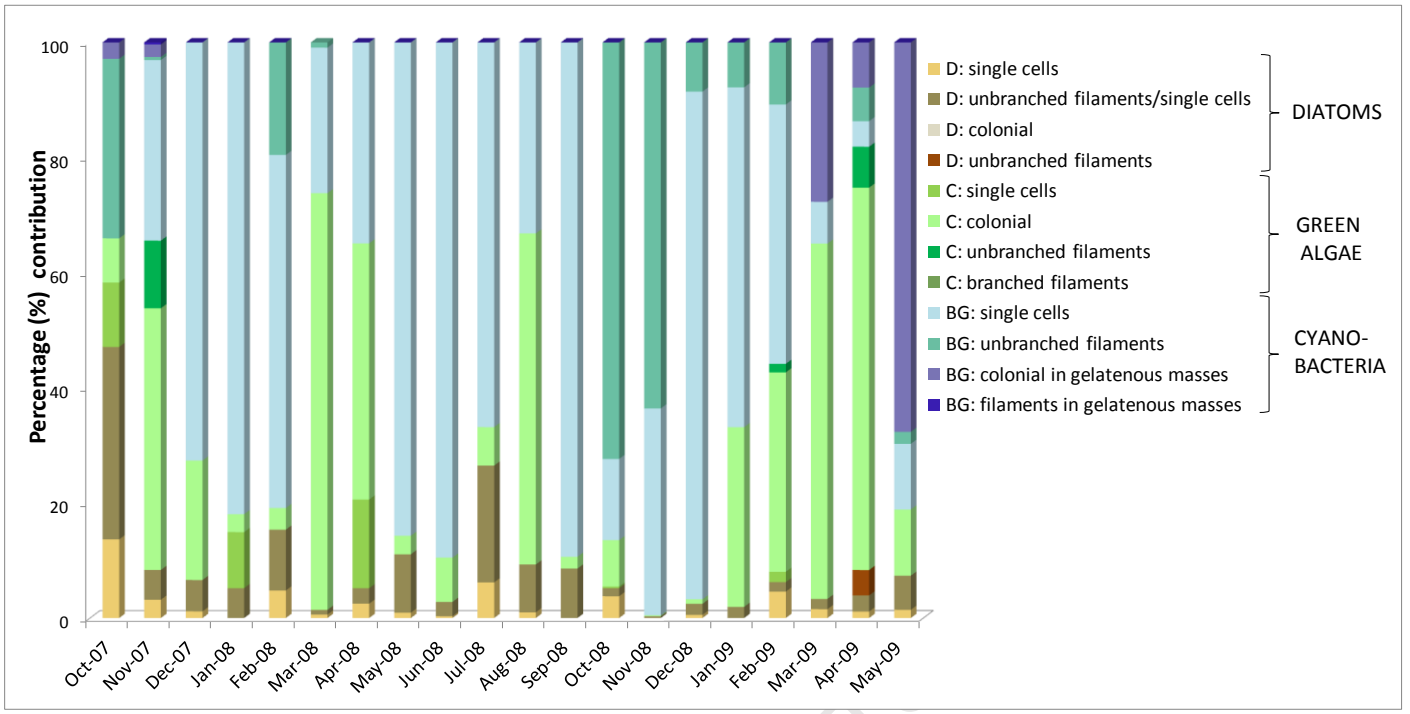


Figure 4.16 **Proportion of taxa per month at Site 3 by division and form. D= diatoms; C=green algae; BG=cyanobacteria.**

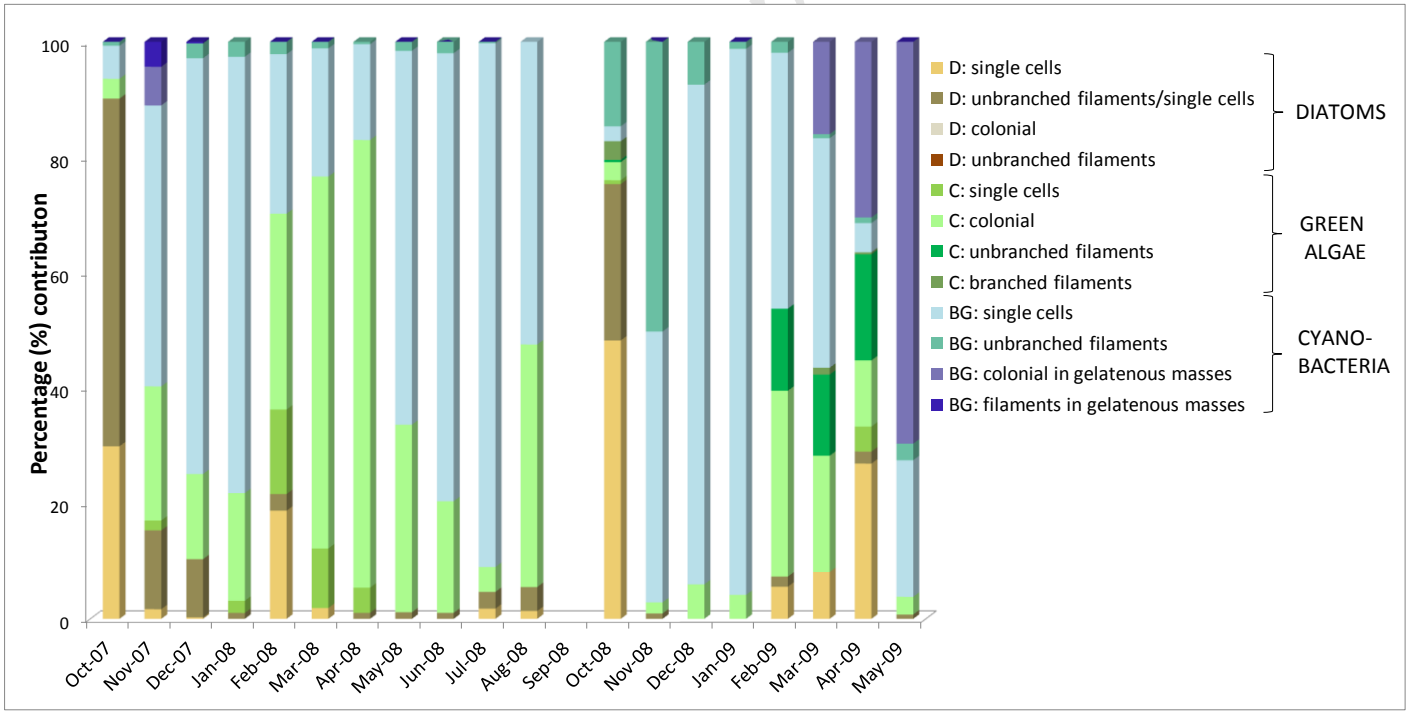


Figure 4.17 **Proportion of taxa per month at Site 4 by division and form. D= diatoms; C=green algae; BG=cyanobacteria.**

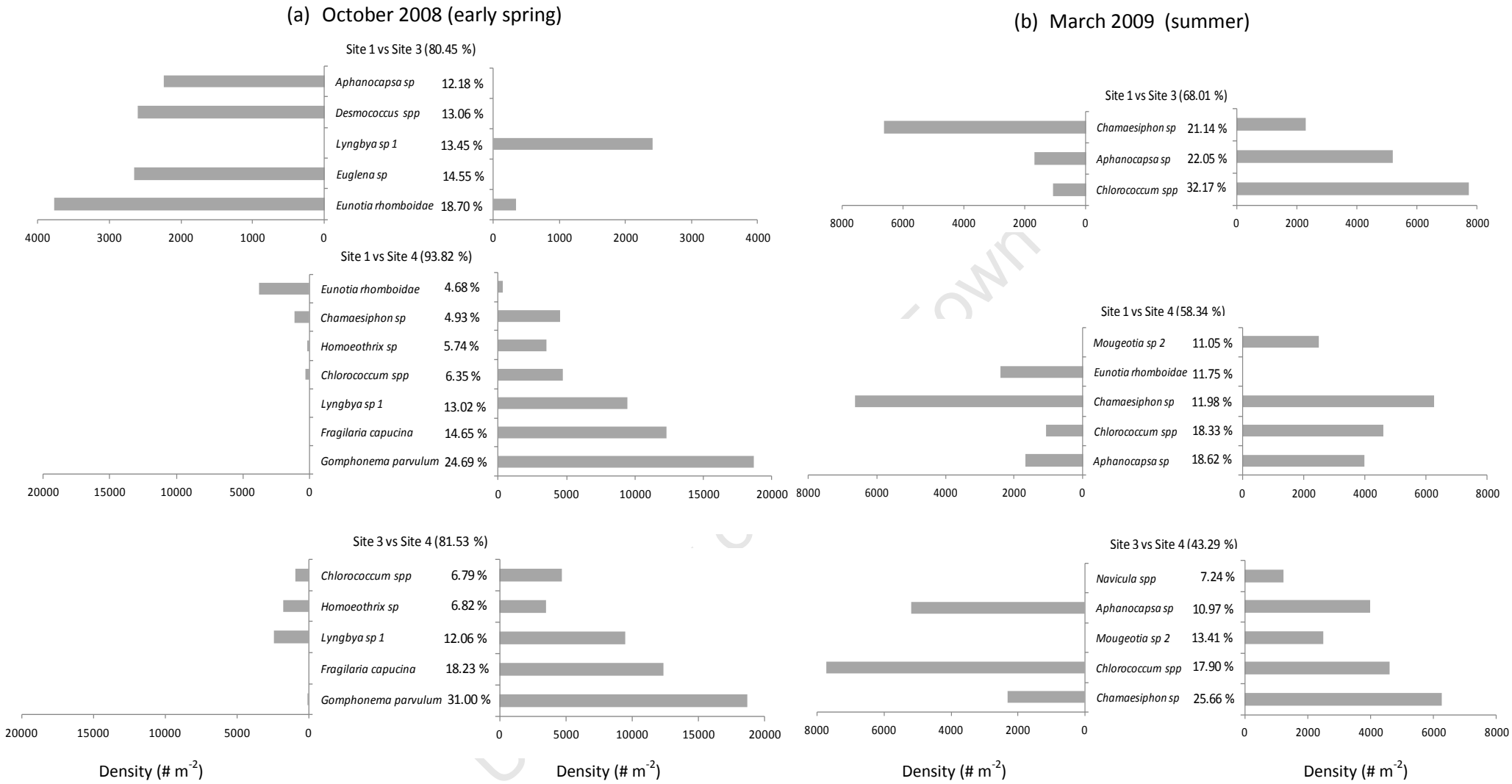


Figure 4.18 **SIMPER results of benthic algal taxa densities that together contributed at least 70% to the overall differences between Sites 1, 3 and 4 during (a) October 2008 (spring) and (b) March 2009 (summer) after square-root transformation and Bray-Curtis dissimilarity. The average dissimilary between communities (in brackets) and the the contribution of of each taxon to dissimilarity are presented as percentages.**

4.3.3.4 Periphyton community differences upstream and downstream of the Berg Dam

The hierarchical cluster diagram and MDS plot in Figure 4.19 show some distinction between Site 1, and Site 2 that is clearly influenced by seasonal changes in benthic algal communities at both sites. These two sites were largely distinct from each other at a Bray Curtis similarity of 39%, although there was overlap in the community structure during certain months (Figure 4.19). Firstly, the community structure at Site 1 during late summer 2008 (i.e. February and March 2008) as well as summer 2009 (i.e. January, February and March 2009) grouped with the majority of the Site 2 samples (Figure 4.19). By contrast, the benthic algal community at Site 2 during June and August 2008 (i.e. winter), grouped with Site 1. The overall distinction between Sites 1 and 2 was confirmed by a two-way PERMANOVA analysis which showed significant differences between the benthic algal communities among sites and months (Table 4.16). With the exception of June 2008, when no differences in community structure were evident following flooding, these sites were significantly different from each other in all months over the sampling period (Table 4.15). Besides these two larger groups representing Sites 1 and 2 respectively, three smaller groups were evident (Figure 4.19). The summer 2009 and late spring 2007 at Site 2 formed two distinct groups while mid-winter (July 2008) at Site 1 was distinct from the other groups.

Table 4.16 **Two-way PERMANOVA results for comparisons between the community structure of sites 1 (upstream of the Berg Dam) and Site 2 (downstream of the Berg Dam) during different seasons sampled over two annual cycles (21 months) between October 2007 and May 2009. Significant differences at p≤ 0.05 are indicated in bold italics.**

Groups	*df*	*SS*	*MS*	*Pseudo-F*	*p*
site	1	44578	44578	51.6	***<0.001***
month	19	151440	7971	9.2	***<0.001***
site x month	18	75738	4208	4.9	***<0.001***
residuals	117	101090	864		
Total	155	373680			

A comparison between the relative contributions of different taxa in each month provides an indication of the general differences between these two sites (Figures 4.7 and Figure 4.20). Whereas Site 1 showed a clear cycle of dominance by diatoms and colonial green algae in the spring and winter, diatoms were almost completely absent from the winter and spring samples in 2008 at Site 2 (Figure 4.7 and Figure 4.20). Also, green filamentous taxa were only characteristic of the late summer community at Site 1 during 2008 but were frequently a significant component of the community at Site 2 (Figure 4.7 and Figure 4.20). In particular, two filamentous green algal taxa were equally dominant during November 2007 and were therefore a significant component of the spring 2007 community at Site 2. Unlike the winter months at Site 1, particularly between June and August 2008 when diatoms dominated the community, the winter at Site 2 was dominated by the single-celled cyanobacterium, *Chamaesiphon* spp., which peaked in abundance during July 2008.

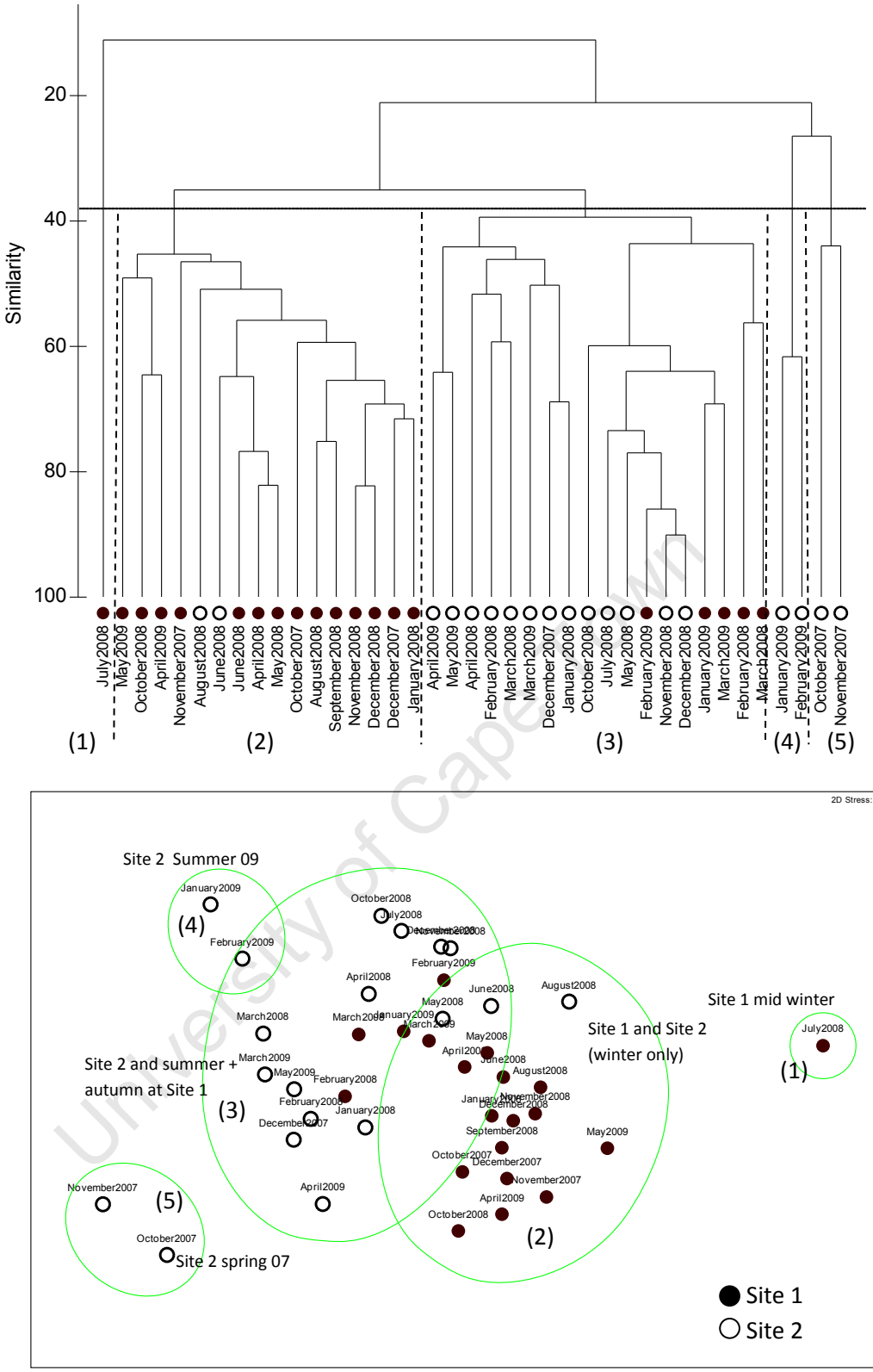


Figure 4.19 **2-D MDS plot of square-root transformed and weighted periphyton densities from individual replicates pooled per monthly sampling period at Sites 1 and 2. The ellipses represent the Bray-Curtis similarity between samples defined by hierarchical clustering at 39%.**

A SIMPER analysis of all monthly algal density data shows that differences in the abundance of *Chamaesiphon* spp., *Eunotia rhomboidae* and *Desmococcus* spp. between Sites 1 are 2 were largely responsible for their overall distinction (Figure 4.21). Whereas *Chamaesiphon* spp. was more dominant at Site 2 than at Site 1, both *Eunotia rhomboidae* and *Desmococcus* spp. were more abundant at Site 1. Also, both *Navicula cryptotenella* and *Fragilaria* spp. were present at Site 2 but were never encountered at Site 1. Other taxa that contributed to the overall distinction between these sites were *Mougeotia* spp., *Cosmarium* spp. and Chl*orococcum* spp., which were all more dominant at Site 2.

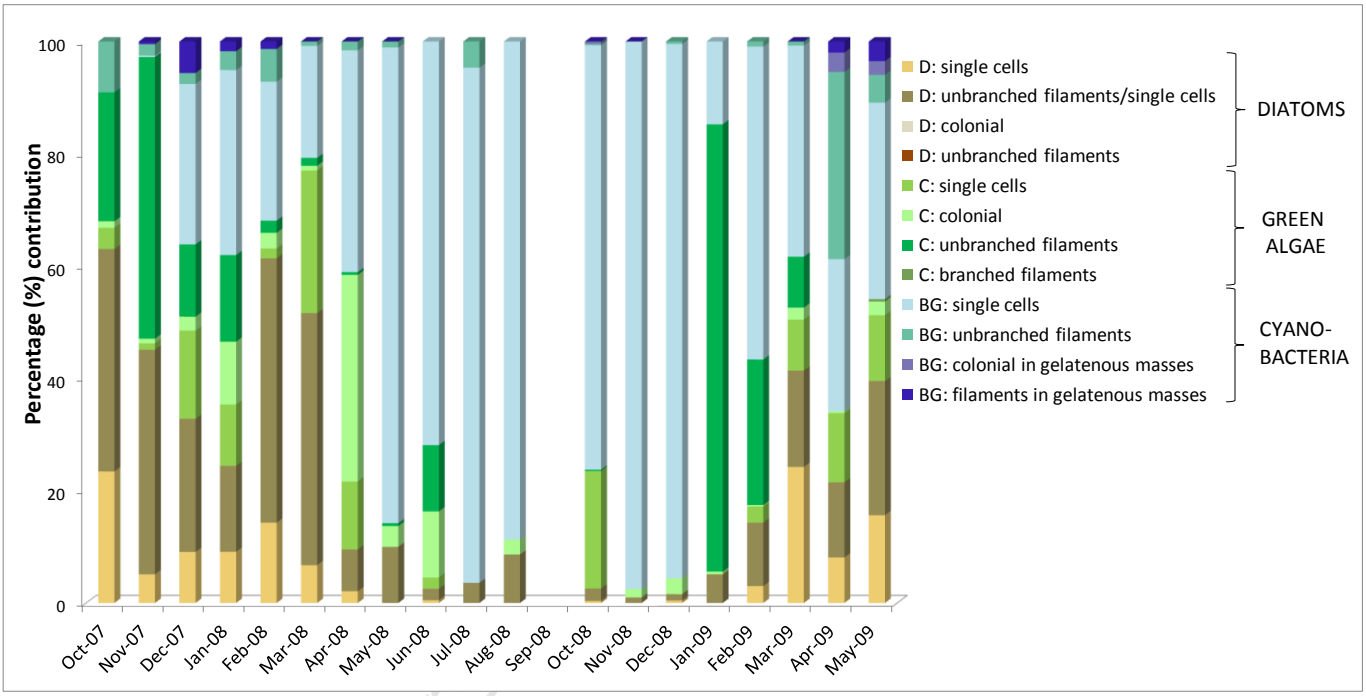


Figure 4.20 **Proportion of benthic algal taxa per month at Site 2 by division and form. D= diatoms; C= Chlorophytes (green algea) and BG = Cyanobacteria.**

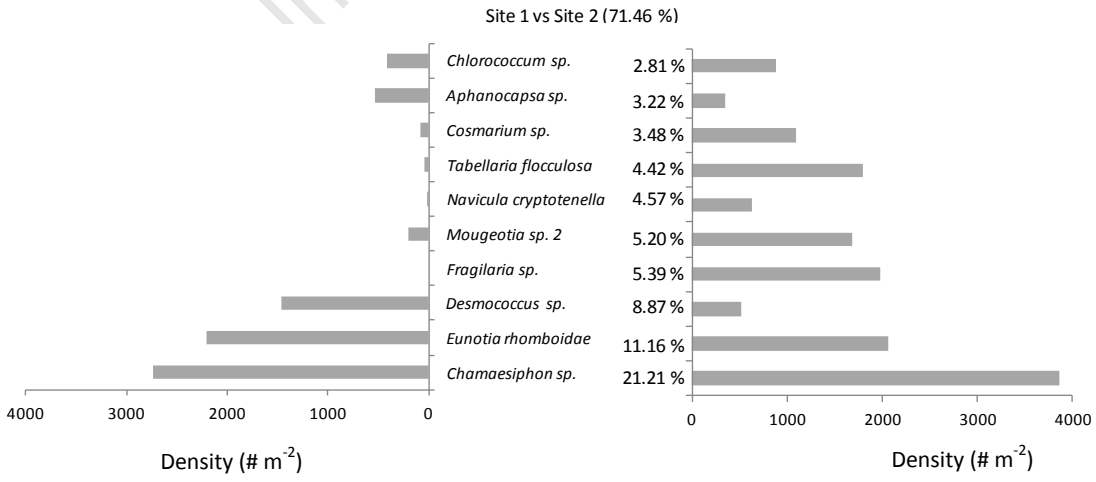


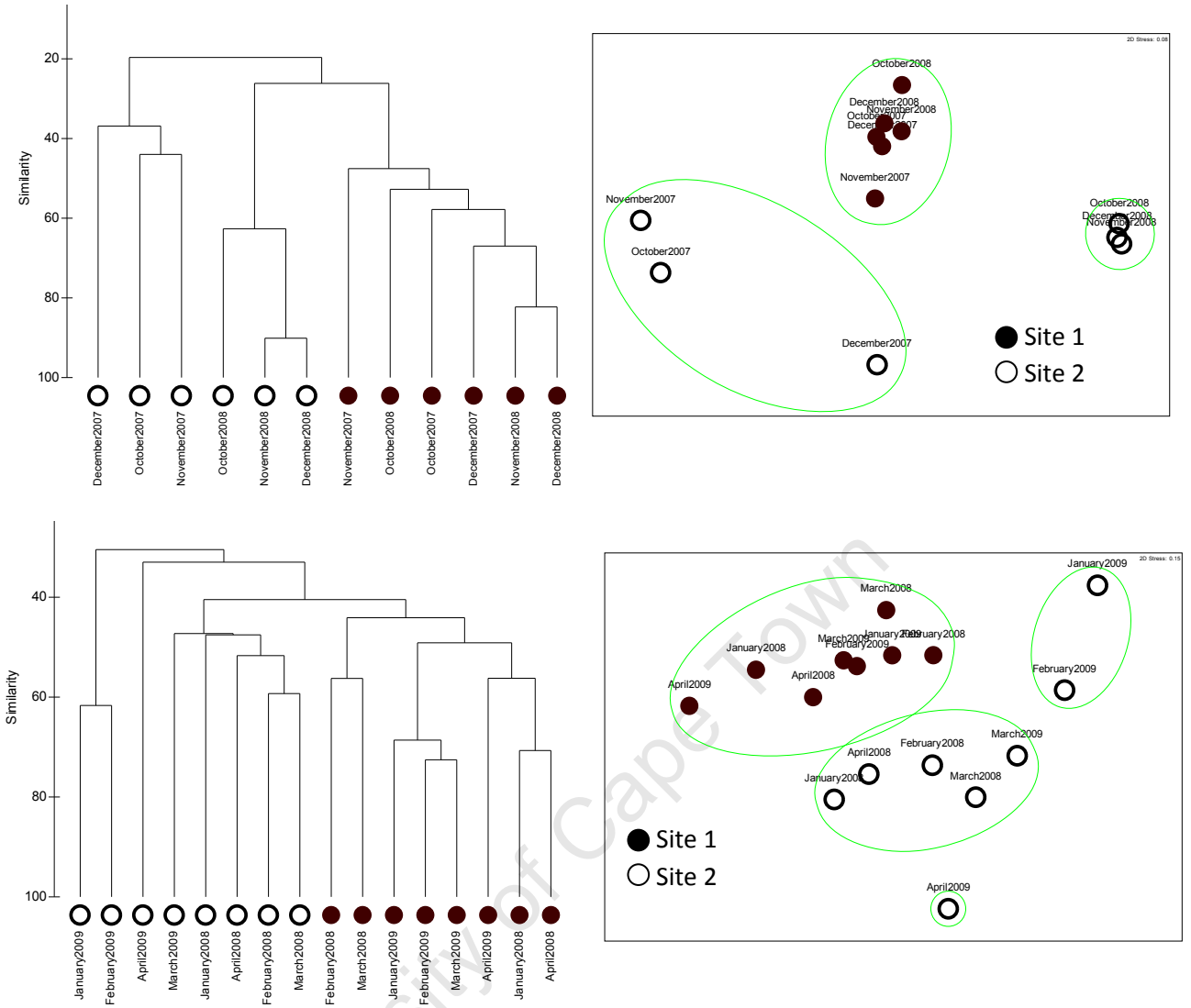
Figure 4.21 **SIMPER results of benthic algal taxa densities that together contributed at least 70% to the overall differences between Sites 1 and 2 after square-root transformation and Bray-Curtis dissimilarity. The average dissimilary between communities (in brackets) and the the contribution of of each taxon to dissimilarity are presented as percentages .**

Although Figure 4.19 shows temporal changes in community structure at both Sites 1 and 2 to some extent, the effect of season on the distinction between Sites 1 and 2 is clearly demonstrated by the cluster diagrams and MDS plots given in Figure 4.22. During the spring period (October, November and December) benthic algal communities at Sites 1 and 2 were completely distinct from each other (Figure 4.22a). Similarly, Sites 1 and 2 were distinct during the summer and early autumn of both years (Figure 4.22b). During late autumn and winter however, no clear site distinction was evident (Figure 4.22c), although PERMANOVA showed that Sites 1 and 2 were still significantly different from each other during all months representing late autumn and winter except June 2008 (Table 4.17). Indeed, only 3% of the community at Sites 1 and 2 were similar during July 2008 (Table 4.17).

A SIMPER analysis of communities at both sites during spring and summer/early autumn respectively shows the taxa which were most responsible for the clear separation of sites during these two seasons (Figure 4.23). During the spring period, the clear distinction between Sites 1 and 2 was driven primarily by the dominance of *Chamaesiphon* spp. at Site 2 at this time. By contrast, the clear distinction between Sites 1 and 2 towards the end of the growing season was due to high densities of *Mougeotia* sp. 2 and *Chamaesiphon* spp. at Site 2 and the absence of *Fragilaria* spp. at Site 1.

Table 4.17 PERMANOVA pair wise comparisons of monthly periphyton communities between Site 1 and 2, upstream and downstream of the Berg dam respectively. Significant differences at p ≤ 0.05 are indicated in bold italics and at p≤ 0.1 in italics only.

			Average similarity		
month	**Site**	**p**	**between sites**	**within site 1**	**within site 2**
October 2007	***Site 1 ≠ Site 2***	***0.028***	***31***	***61***	***49***
November 2007	***Site 1 ≠ Site 2***	***0.030***	***14***	***50***	***65***
December 2007	***Site 1 ≠ Site 2***	***0.028***	***58***	***80***	***33***
January 2008	***Site 1 ≠ Site 2***	***0.030***	***41***	***84***	***57***
February 2008	***Site 1 ≠ Site 2***	***0.029***	***39***	***58***	***58***
March 2008	***Site 1 ≠ Site 2***	***0.028***	***37***	***50***	***60***
April 2008	***Site 1 ≠ Site 2***	***0.029***	***52***	***80***	***53***
May 2008	***Site 1 ≠ Site 2***	***0.028***	***57***	***85***	***70***
June 2008	Site 1 = Site 2	0.168	58	70	55
July 2008	***Site 1 ≠ Site 2***	***0.030***	***3***	***28***	***70***
August 2008	*Site 1 ≠ Site 2*	*0.057*	*63*	*79*	*68*
September 2008	Site 1 ≠ Site 2	NS			
October 2008	***Site 1 ≠ Site 2***	***0.032***	***31***	***61***	***45***
November 2008	***Site 1 ≠ Site 2***	***0.030***	***28***	***50***	***76***
December 2008	***Site 1 ≠ Site 2***	***0.029***	***30***	***55***	***54***
January 2009	***Site 1 ≠ Site 2***	***0.028***	***39***	***75***	***56***
February 2009	***Site 1 ≠ Site 2***	***0.008***	***39***	***70***	***70***
March 2009	***Site 1 ≠ Site 2***	***0.028***	***33***	***54***	***59***
April 2009	***Site 1 ≠ Site 2***	***0.028***	***19***	***61***	***30***
May 2009	***Site 1 ≠ Site 2***	***0.026***	***20***	***54***	***53***



(c) Late autumn and winter 2008 and 2009

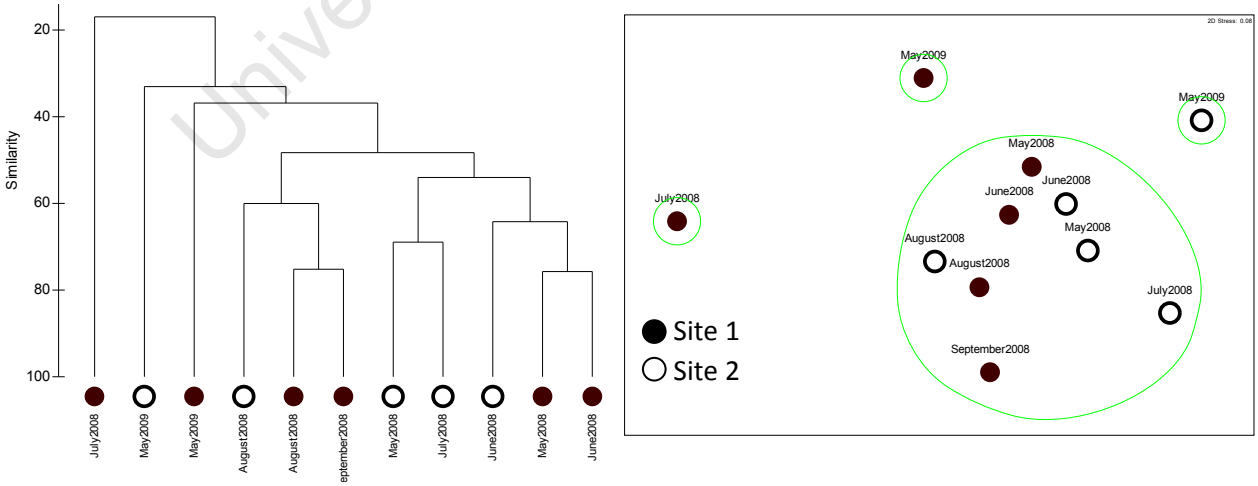


Figure 4.22 **2-D MDS plot of square-root transformed benthic algal densities from individual replicates pooled per monthly sampling period at Site 2. The green and blue ellispses represent the Bray-Curtis similarity between samples defined by hierarchical clustering at 40%.**

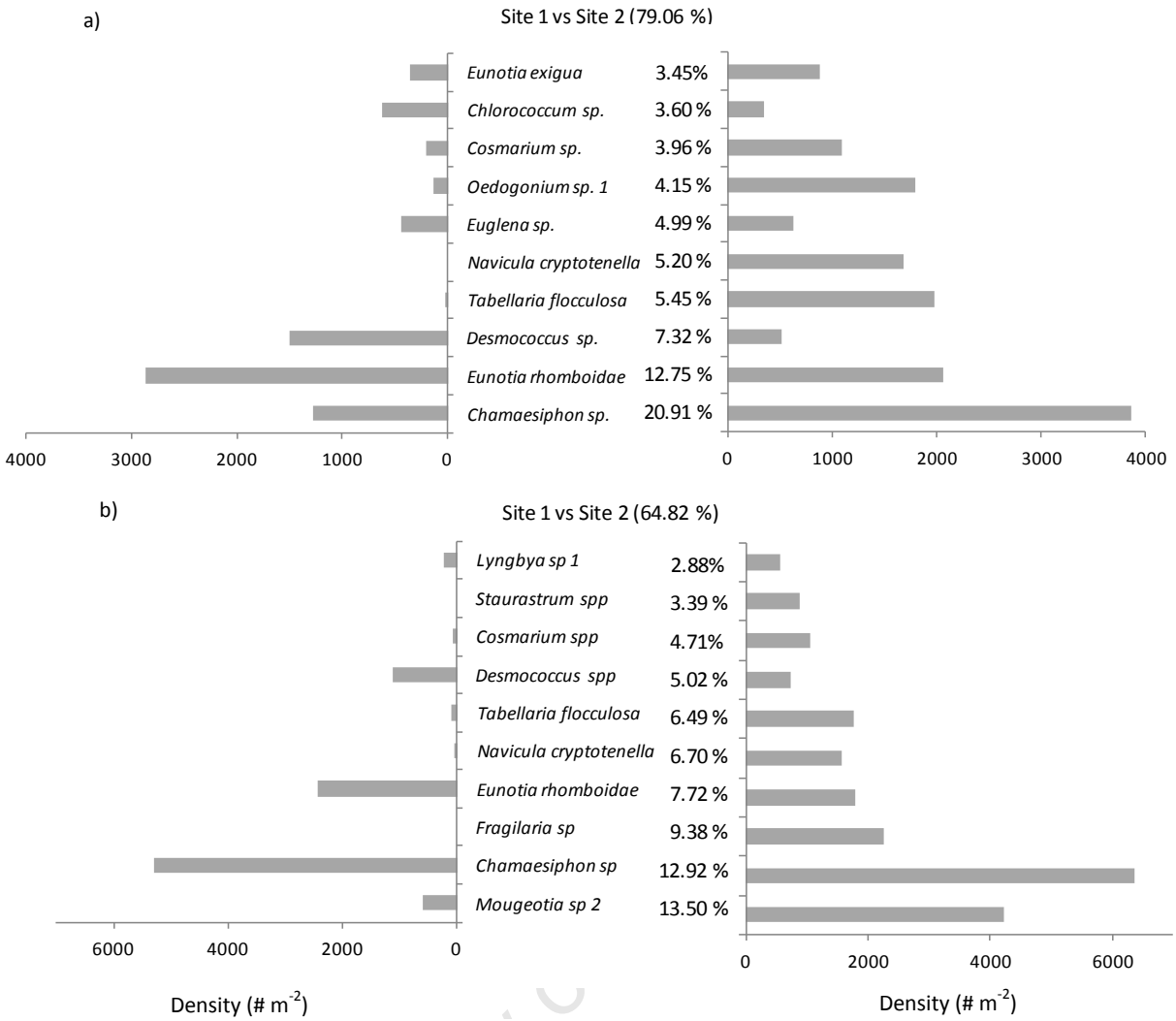


Figure 4.23 **SIMPER results of benthic algal taxa densities that together contributed at least 70% to the overall differences between Sites 1 and 2 after square root transformation and Bray-Curtis dissimilarity. a) spring 2007 and 2008 b) summer 2007 and 2008. The average dissimilary between communities are presented as percentages.**

Unlike Site 1, where community composition was variable both within and between seasons (see Section 4.3.3.1), the communities at Site 2 were not always significantly different as suggested by the PERMANOVA pair-wise comparisons (Appendix 6 and Figure 4.24). This suggests that the benthic algal community downstream of the Berg Dam was less variable than that at Site 1 over time, particularly between December 2007 and June 2008 (Figure 4.24). Also, monthly shifts in community structure downstream of the dam seldom coincided with seasonal shifts, particularly during the first growing season. In particular, there were no community changes from late spring to summer 2007/2008, summer to early autumn 2008 or late autumn to winter 2008 Figure 4.24, Appendix 6). The community at Site 2 however, shifted considerably during the spring 2007 when Bray-Curtis similarities between October and November 2007 and between November and December 2007 were low (Figure 4.24). Community changes were far greater during the second growing season at Site 2 (Figure 4.24) with the greatest dissimilarities between monthly communities coinciding with the change in season. Indeed, the shifts from early spring to late spring 2008, late spring to summer 2008/2009 and summer to early autumn 2009 were quite distinct from

monthly shifts in community structure, despite significant differences within the summer 2009 (Figure 4.24, Appendix 6).

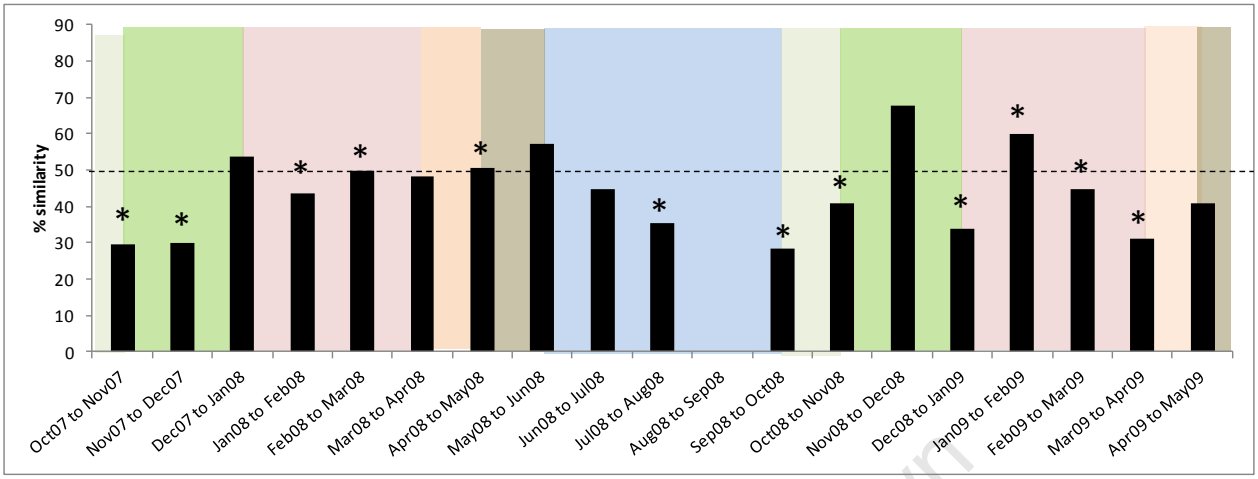


Figure 4.24 **Average Bray Curtis Similarity (%) among periphyton community structure between consecutive sampling occasions (months) at Site 2 which provide an indication of the degree of change in community structure over time. The asteriks indicate a significant change in community structure at p≤0.05 (see Appendix 4.4). The background colour depicts seasonal boundaries. Light green = early spring; green = late spring; pink = summer; orange = early autumn; brown = late autumn, blue = winter.**

Inter-annual differences in community structure at Site 2 were far greater than those at Site 1 (Figure 4.25). Indeed, there was only 7% similarity between the October communities and 4% similarity between the November communities at Site 2 (Figure 4.25). Unlike the winter of 2007 when Site 2 did not experience flooding (Figure 3.15), overtopping of the dam in late winter and spring led to flooding at Site 2. The clear difference in communities during the spring period may therefore be a consequence of distinct differences in flow conditions between 2007 and 2008. This will be dealt with in more detail in Chapter 5.

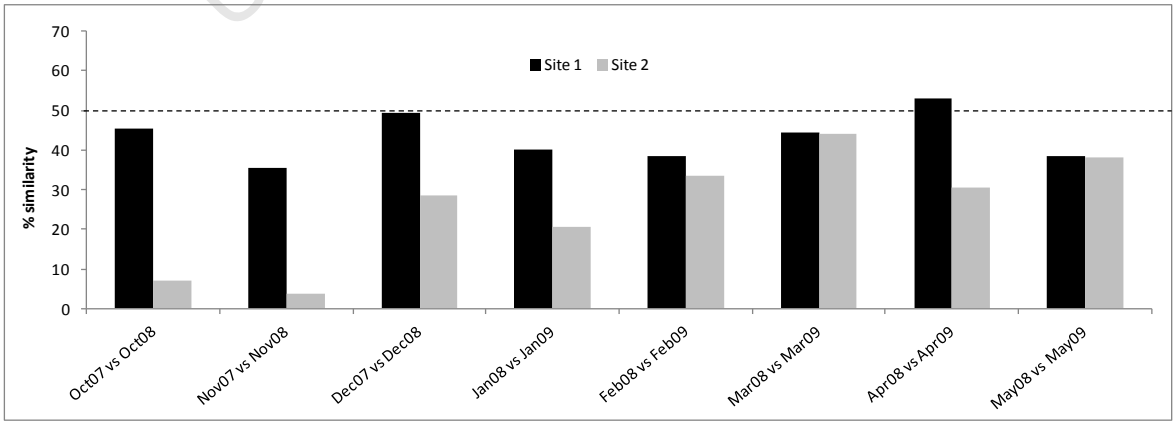


Figure 4.25 **Inter-annual differences in the average Bray Curtis Similarity (%) among benthic algae community structure at Sites 1 (black) and 2 (grey)**

4.4 DISCUSSION

The primary aim of this study was to investigate seasonal changes in periphyton biomass and community composition in foothill rivers of the south-western Cape and to test the hypothesis that there is a strong seasonal pattern in periphyton of low biomass in the winter and high biomass in the summer. As predicted, this study has demonstrated that, under natural flow regimes, periphyton communities in south-western Cape foothill rivers follow a cycle of peak biomass towards the end of the growing season in late summer/early autumn, followed by a biomass minimum during mid-winter (Figure 4.2). Such seasonal dynamics support similar cycles of accrual and loss typical of Mediterranean climates elsewhere (Power 1992, Sabater and Sabater 1992). Many studies attribute winter minima and summer maxima to seasonal differences in the disturbance regime of Mediterranean streams with stable low flows during the dry hot summer promoting biomass accrual and frequent, intense flooding drastically reducing biomass to very low levels during winter.

A median Chl *a* concentration of 1.53 mg m^{-2} and AFDM of 1.08 g m^{-2} recorded at Site 1 was similar to that reported by Biggs (2000) for defining oligotrophic conditions. Using data from several streams in New Zealand (Biggs 1995), Biggs (2000) defined oligotrophic streams as those with a median monthly Chl *a* concentration of ≤ 1.7 mg m^{-2} and AFDM of ≤ 1.5 gm^{-2}. The boundary between oligotrophic and mesotrophic streams had median Chl *a* concentrations of 21 mg m^{-2} and AFDM of 4.8 gm^{-2}. Mean monthly Chl *a* concentrations at Sites 2, 3 and 4 (i.e. 2.78 mgm^{-2}, 3.38 mgm^{-2} and 5.28 mg m^{-2} respectively), suggest that all sites in this study were oligotrophic, regardless of relative enrichment in the Molenaars River. Nevertheless, the categorisation of trophic state using periphyton biomass data is premature considering the limited spatial extent of this study.

4.4.1 Temporal dynamics and scale (frequency) of change

Besides the seasonal extremes in hydrological conditions in the Berg and Molenaars Rivers between winter and summer, flooding is not confined to the winter but often extends into the early spring and late autumn (Ractliffe 2009) and seasonality in these Mediterranean systems is defined by the suite of environmental conditions indicated in Chapter 3.

Accordingly, it was expected that temporal shifts in periphyton biomass and community composition would reflect broad seasonal shifts in abiotic conditions that characterise the winter, early and late spring, summer, and early and late autumn as identified in Chapter 3. However, significant differences in community composition and biomass between sequential months suggest that periphyton communities shifted more frequently than at the seasonal level. Indeed, dissimilarities between monthly communities were often greater than dissimilarities in communities representing a transition from one season to the next, particularly during the winter and early spring (Figure 4.10). This temporal pattern suggests that periphyton communities are highly variable over the winter and early spring, with less frequent changes that usually coincided with seasonal shifts during the dry season. Considering that floods operate over a period of days while temperature and light change gradually with the seasons, it is not surprising that periphyton communities in foothill rivers of the south-western Cape are highly dynamic both *within* and *between* seasons with a shift in the

frequency of change over an annual cycle. The hypothesis that distinct communities characterise the seasons over an annual cycle can therefore only be partially accepted as true.

During periods of intense frequent floods, abiotic control by flood disturbance is the dominant process structuring benthic communities in streams (Hildrew and Giller 1994). Indeed, frequent shifts in the community observed in both the Berg and Molenaars Rivers over the winter and early spring represent a period when the community is in a state of perpetual recovery from frequent disturbance. This state has been phrased the 'clinging to the wreckage' model of community organisation (Hildrew and Giller 1994) and in the case of periphyton communities describes communities that are continuously reset by floods before they reach their climax state.

4.4.2 Temporal shifts in abiotic (flood disturbance) and biotic (grazer) control of the community

Besides the primary peak in biomass at the end of the growing season under natural flow conditions, rapid biomass accrual resulted in either one or two secondary biomass peaks under both oligotrophic and enriched conditions in late winter and early spring (Figure 4.2, Figure 4.26). Similarly, in a study of the seasonal cycle of algal blooms in northern California rivers, Power (1992) found that blooms of *Cladophora* were evident during the spring period immediately following the cessation of floods. She hypothesised that the spring peak in periphyton biomass reflects a window of time following flood disturbance when periphyton can develop unchecked by grazing because invertebrate algal feeder densities are slower to recolonise and reproduce following floods. Evidence in support of this hypothesis was provided by Riseng *et al.* (2004) in a study of 97 mid-western US streams. They found that flood disturbance had a strong indirect positive effect on algal biomass that was mediated by a reduction in grazer densities following disturbance events. Also, Rutherford *et al.* (2000) developed a model to investigate grazer control of benthic algae in New Zealand streams. They showed that floods reduced diatom and mayfly biomass but mayfly recovery lagged behind algal growth, which resulted in a post-flood diatom bloom. Their model also showed that, under favourable temperatures (10^0C to 20^0C), mayflies maintain diatom biomass at low levels (Rutherford *et al.* 2000). Indeed, grazer abundance, dominated by baetids was low throughout early spring but increased considerably in October (Figure 4.26) when Chl *a* biomass peaked suggesting that an early spring release from grazing pressuring may have permitted periphyton to grow unchecked. This pattern was particularly evident at Site 4 where nutrients were not limiting (Figure 4.6c). When unrestrained by grazing, the rate of recovery of periphyton communities is rapid, as implied by this and other studies (Hillebrand 2002, Hillebrand *et al.* 2002). If unimpeded by grazers, therefore, a rapid increase in periphyton biomass and density would be expected during these stable conditions characterised by an increase in water temperature and solar radiation that mark the transition from early to late spring. Nevertheless, monthly sampling did not provide a clear indication of changes in either periphyton biomass or grazer abundances over the spring and therefore it is unclear whether the recovery of baetids lagged algal growth immediately prior to sampling in October 2007 or 2008. Following the last wet season flood in November 2007 and 2008,

however, Chl *a* biomass and algal cell densities maintained a low steady-state akin to the predictions of Rutherford *et al.* (2000) that grazing by mayflies can suppress diatom growth. Although baetid abundance was reduced by the November flood events in this study, densities remained higher than those recorded in early spring and increased considerably over the summer (Figure 3.30; Figure 4.26). Also, the periphyton community had shifted from one dominated by palatable diatoms in early spring, towards one with fewer diatoms and a higher abundance of less palatable green algae and cyanobacteria in summer. It is possible therefore that grazing activity, primarily by baetid mayflies in late spring and summer, may have exerted control over periphyton biomass accrual thus preventing a significant increase in periphyton biomass during this time. Similarly, a number of studies have shown that grazing activity can restrict the accrual of biomass and thus reduce temporal variability (Peterson and Grimm 1992; Rutherford *et al.* 2000; Yang *et al.* 2009), or change the composition of the community thus affecting the trajectory of succession over time (Steinman *et al.* 1987; DeNicola and McIntire 1991; Steinman *et al.* 1991; Rosemond 1994 Rosemond *et al.* 2000; Hillebrand 2005). Essentially, if grazer densities are sufficient to reduce periphyton biomass, 'top-down' control of periphyton communities can shift from abiotic control (flood disturbance) to biotic control (grazing) between floods in late winter and early spring.

4.4.3 Top-down versus bottom-up control of periphyton communities

It is well known that periphyton communities are able to exploit nutrient resources such that higher nutrient availability will (a) increase the rate of regeneration of the community following disturbance (Dodds *et al.* 2004, Murdock *et al.* 2011), (b) result in a greater peak biomass (Biggs and Close 1989, Biggs 2000) and c) change the composition of periphyton communities (Steinman 1996). Consequently, the third hypothesis addressed in this study was that peak biomass of the periphyton community would be greater in enriched reaches, regardless of the flow regime, and that the community structure at the time of peak biomass would differ between reaches with different levels of enrichment. As predicted, significant differences in Chl *a* biomass in this study were evident at times of peak biomass in both spring and late summer with the highest biomass at Site 4, followed by Site 3 and lowest at Site 1 during both growing seasons. Periphyton can only respond to nutrient availability under stable low flows when current speeds are low enough to prevent removal by shear stress and thereby permit algal biomass accrual. It was not surprising therefore that periphyton biomass and composition between Sites 1, 3 and 4 were particularly different following periods of flow stability when the community had had sufficient time to develop. By contrast, during the wet, unstable winter season, no biomass differences were evident between sites with different levels of enrichment and community differences were less clear. Similar effects of nutrients on periphyton communities have been reported in the literature (e.g. Biggs and Close 1989; Francoeur *et al.* 1999; Biggs *et al.* 1998b, c; Molinos and Donohue 2010; Sabater *et al.* 2011). In a study of the relationships between dissolved nutrient concentrations and Chl *a* biomass in systems with different flood frequencies, Biggs (2000) reported that increases in nutrients lead to high Chl *a* biomass only in streams with infrequent floods and long accrual periods, whereas no nutrient effects were evident in

streams with a high frequency of floods. Biomass reached a peak during March of both growing seasons at Site 1 while peak biomass at Site 4 was reached a month earlier, during February in both 2008 and 2009. This result supports others which have demonstrated that regeneration rates and the time to peak biomass under stable flow conditions are promoted under enriched conditions (e.g. Grimm and Fisher 1989; Uehlinger *et al.* 1996). These results emphasise the importance of accounting for temporal change in periphyton biomass and community composition when addressing the effects of flow and nutrients alterations.

Similarly, differences between the periphyton taxa of Sites 1, 3 and 4 were indicative of their differences in the availability of nutrients. For example, the spring peak at Site 1 was dominated by diatoms sensitive to enrichment such as *Eunotia exigua, E. minor* and *E. rhomboidae,* whereas *Gomphonema parvulum,* a diatom tolerant of organic pollution was dominant at Site 4 during the same period. This supports the hypothesis that differences between Sites 1, 3, and 4 during the accrual phase are driven by differences in the availability of nutrients which result in significantly different communities in terms of both community composition and biomass.

Whereas flood disturbance as a 'top-down' abiotic control of periphyton communities may have been an important overriding factor responsible for resetting cycles of periphyton growth and accrual, nutrient availability was an important 'bottom-up' determinant of temporal changes in periphyton biomass during inter-flood periods. The 'bottom-up' control of periphyton, which promoted algal accrual, may therefore work antagonistically with the 'top-down' biotic control by algal feeders, particularly during the late spring and early summer. However, the 'bottom-up' effects of nutrient availability seemed to outrun grazing effects towards the late summer period when peak biomass was significantly different among sites with different levels of enrichment. A number of investigations focused on successional changes in periphyton communities have shown that conditions during late summer favour dominance by taxa that are tolerant of high water temperatures and extreme low flow conditions (Steinman 1996). These late stage periphyton taxa are often either unpalatable to invertebrate algal feeders or the cells are too large for effective grazing by invertebrates (Lamberti *et al.* 1995; Wellnitz and Ward 1998; Villanueva and Modenutti 2004). Thus periphyton can develop unchecked by grazing where communities are dominated by such taxa. Late summer communities in both the Berg and Molenaars Rivers were indeed dominated by either filamentous green algae such as *Mougeotia* spp. and *Spirogyra* spp. that are typical of stable late successional communities (Biggs *et al.* 1998b, c) or cyanobacteria (i.e. *Chamaesiphon* spp. and *Aphanocapsa* spp.). Although basal cells of *Mougeotia* spp. are considered palatable (Holomuzki and Biggs 2006), *Spirogyra* spp. are known to be particularly unpalatable to aquatic invertebrates and cyanobacteria such as *Chamaesiphon* spp. are less nutritious than diatoms (Feminella and Resh 1991; Rosemond *et al.* 1993). Considering the high densities of invertebrate grazers at the time of peak biomass, however, it is probable that epiphytic diatoms such as *Eunotia rhomboidae* provided a source of food for these grazers late in the accrual cycle.

Rapid biomass loss in the absence of either floods or high algal feeder densities was observed at Sites 1, 3 and 4 following peak biomass in late summer 2008. Bouletreau *et al.* (2006) observed a

similar pattern during summer low flow conditions in a stream in south-west France and were able to demonstrate that sudden large losses of periphyton were the result of self-generated detachment process. Essentially, as the periphyton mat thickened under stable conditions, both nutrients and light may eventually have become limiting to benthic cells at the base of the mat. Die off of algal cells may therefore have resulted in autogenic sloughing of the algal mat and thus significant decreases in periphyton biomass can be observed even in the absence of flooding (Fisher *et al.* 1982, Bouletreau *et al.* 2006). Based on their findings, Bouletreau *et al.* (2006) suggested that models to determine the factors important for periphyton biomass dynamics should consider sloughing during typical summer low flow conditions when algal mats become detached from the substrata in the absence of physical disturbance.

4.4.4 The effects of flow regulation

Chapter 3 showed that flood characteristics did not significantly separate Sites 1 and 2, which represented unaltered and altered flow conditions. Rather, differences in low flow conditions seemed to differ considerably between these two sites. Given the importance of flood disturbance as a key determinant of periphyton dynamics in temperate streams (e.g. Biggs and Close 1989; Biggs *et al.* 1998b, c; Fayolle et al. 1998; Biggs *et al.* 1999; Riseng *et al*. 2004; Bergey and Resh 2006; Bouletreau *et. al.* 2008), however, it was predicted that even subtle changes in flood characteristics such as flood frequency, magnitude and timing would alter the seasonal trends in periphyton biomass observed under natural flow regimes. The hypothesis that seasonal patterns of periphyton development would differ between conditions of natural and altered flow regimes was therefore addressed in this study. Indeed, Chl *a* minima at Site 2 were not confined to the winter or early spring months, while biomass peaks were generally erratic and aseasonal compared with the pattern observed upstream of the dam. During months when both Sites 1 and 2 experienced flood disturbance in the period prior to sampling, both Chl *a* biomass and AFDM were similar between these sites. Conversely, when floods occurred naturally upstream of the dam but were absent downstream of the dam in July 2008, the cycle of biomass accrual was not reset at Site 2 as it was at Site 1. The community downstream of the dam therefore continued to accrue biomass until it reached a peak in August 2008. Furthermore, the biomass minimum in February 2008 at Site 2 followed a series of aseasonal small floods. This suggests that aseasonality in periphyton dynamics downstream of the dam may, in part, have been a consequence of an altered flow regime with aseasonal flooding during the low flow season, and extended stable inter-flood periods in winter that allowed the development of aseasonal biomass peaks. Other studies have demonstrated that the time since the last flood disturbance, reflecting the frequency of flooding is one of the key factors affecting temporal pattern of periphyton communities in streams (Uehlinger 1991, Uehlinger *et al.* 1996, Fayolle *et al.* 1998, Biggs 2000). Similarly, Chester and Norris (2006) showed differences in periphyton communities downstream of dams with and without environmental flows in Australia. Similar to this study, they found a higher Chl *a* biomass driven by a greater proportion of green filamentous algae in reaches with fewer floods and concluded that these differences were probably a result of a reduction in the frequency of floods with a return period less than two years.

Besides an altered flood regime downstream of the dam, elevated low flows during the dry season and low grazer densities typical of Site 2 may have altered both 'top down' and 'bottom up' control of biomass accrual following floods. While increased base flow velocities may have promoted the mass transfer of nutrients to algal mats thus enhancing the "bottom up" effect on periphyton biomass (Biggs *et al.* 1998b, c), low algal feeder densities would have reduced the "top down" control of biomass postulated under natural flow conditions (Rosemond *et al.* 1993). Under these conditions, both the rate of biomass accrual and thus the time to peak biomass as well as the size of the peak should have increased (Grimm and Fisher 1986, Biggs 1996). Indeed, following the Class 4 flood in November 2008, biomass accrued rapidly during the late spring and summer of 2009 when base flows were elevated, algal feeders were scarce and resources such as light, nutrients and temperature were favourable. Nevertheless the positive effect of increased nutrient availability and absence of grazing did not seem to promote peak biomass. Thus, it may be that higher near-bed velocities associated with elevated base flows counteracted the advantages of greater nutrient availability, which led to premature loss of biomass through sloughing (Biggs *et al.* 1998b, c).

4.4.5 Temporal changes in community patchiness

Considering that flow is described as " the major architect of physical patchiness in steams" (Hildrew and Giller 1994) it is not surprising that heterogeneity in periphyton biomass and community structure among samples, as an indicator of patchiness, was generally higher immediately following flood events, particularly in July 2008 and November 2007 and 2008 at Site 1. Floods generally act differentially across the stream bed because "flow refugia" (Fuller *et al.* 2011) create areas where shear stress is lower (Matthaei *et al.* 2003) and others create a mosaic of patches defined by heterogeneity of the stream bed (Cardinale *et al.* 2002; Bergey 2004). Patchiness also increased following peak biomass when autogenic sloughing led to biomass loss in late summer 2008 and 2009 at Site 1. As the periphyton mat dies, differences in velocities across the stream bed may have differentially removed dead material from substrata (Bouletreau *et al.* 2006), resulting in greater patchiness of remaining algal mats at the time of sampling. Interestingly, patchiness was lowest during the stable late spring and early summer period when grazing was implicated as the major control on periphyton communities.

4.4.6 The balance between heterotrophy and autotrophy

Whereas the autotrophic index is often used as an index of enrichment (Biggs 2000), temporal shifts in the balance between heterotrophy and autotrophy under different levels of nutrient availabilty and altered flow regimes provided valuable insight into the structure of periphtyon communities in south-western Cape Rivers. While the periphyton community at Site 1 was predominantly autotrophic over the stable dry season, the community shifted towards heterotrophy following both flood disturbance in the wet season and autogenic sloughing following the summer peak in biomass, typical of extented periods of stablity (Bouletreau *et al.* 2006). Regardless of whether a decline in Chl *a* biomass was a result of sloughing or flood disturbance however, the shift towards a heterotrophic community shortly thereafter might be a reflection of early colonization by bacteria and fungi which

are generally the first to arrive at the onset of temporal succession in periphyton communities (Korte and Blinn 1983, Robson 2000, Dodds *et al.* 2004, Hodoki 2005). Similarly, Villanueva and Modenutti (2004) found that Chl *a* levels were low relative to AFDM during spring and winter at the onset of succession in their experimental analysis of grazing in Andean streams.

Higher nutrient availability in the Molenaars River may have favoured the growth of algae resulting in a greater proportion of autotrophs, hence lower AI values in the Molenaars River relative to the oligotrophic Berg River upstream of the dam. This suggests that under low nutrient conditions, an imbalance between autotrophic and heterotrophic communities might develop because benthic algal production is limited by nutrient availability. By contrast, where nutrients are not limiting, the community represents a balance between autotrophic and heterotrophic components of the periphyton.

At certain times, particularly during February 2008 and again between February and May 2009 when a steady increase in AFDM was not accompanied by an increase in Chl *a* biomass at Site 2 (Figure 4.2b), it might be that environmental conditions, possibly high, constant base flows over the summer when flows would naturally be at there lowest, favoured the development of heterotrophs over benthic algae.

These data essentially suggest that where nutrients are not limiting (e.g. the Molenaars River), the community might shift towards one that is balanced between autotrophs and heterotrophs but where nutrients are limiting, autotrophs might increase in biomass over the accrual period but heterotrophs remain dominant as was the case in the Berg River usptream of the dam. Under altered flow conditions, the imbalance between heterotrophs may be exacerbated by conditions which either favour the dominance of bacteria, fungi or protozoa, or promote the accumulation of allochthnous detritus which might restrict the development of Chl *a* biomass.

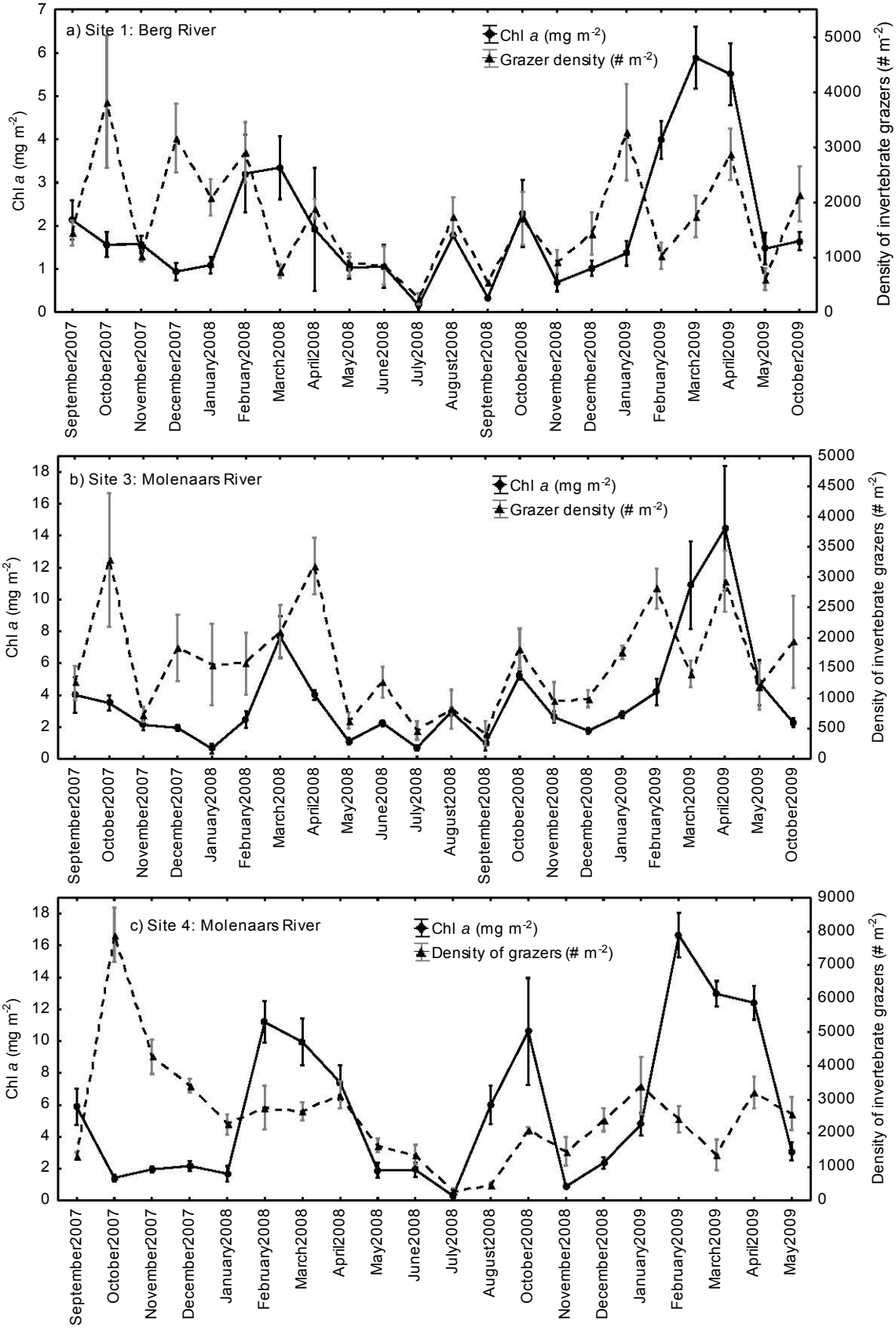


Figure 4.26 **Mean monthly Chl *a* biomass (mg m^{-2}) and total density (# m^{-2}) of grazers (including scrapers, deposit feeders and brushers) at a) Site 1, b) Site 3 and c) Site 4.**

4.5 CONCLUSIONS

This study provides clear evidence of temporal changes in periphyton biomass and community structure that are linked to environmental factors acting both within and between seasons. While many studies on the seasonality of periphyton dynamics in rivers focus on seasonal extremes, or aggregate data into broad seasonal categories, this study shows that many of the factors that drive periphyton communities operate within seasons and even within months. This study therefore demonstrates the need to account for temporal changes in periphyton biomass and community composition when addressing the effects of flow and nutrients on these communities. Also, the highly dynamic temporal patterns evident in this study suggest that the frequency of sampling is particularly important to establishing an understanding of the temporal pattern of periphyton dynamics in rivers. Considering the frequent shifts in periphyton communities observed over the late winter and early spring period in this study, it is likely that bi- weekly or even weekly sampling over this period would strengthen our understanding of the factors that drive periphyton dynamics in rivers of the south-western Cape.

A shortcoming of this study is the lack of spatial replication of both natural and altered flow and nutrient scenarios. Locating replicates of natural foothill sites with similar geomorphological and hydrological features in the south-western Cape proved challenging because most foothill systems are impacted to some extent by anthropogenic activities. Nevertheless, the patterns evident in this study with its limited spatial representation corroborate many of the patterns in periphyton dynamics observed in other ecosystems. It is likely that the oligotrophic Berg River site upstream of the dam is a relatively good representation of natural conditions in these systems and thus provides a sound baseline for understanding the structure of periphyton communities representative of this region.

This study was designed to assess periphyton dynamics under natural conditions and to compare these with patterns under altered flow and nutrient conditions. Temporal patterns of periphyton community structure and biomass under these different conditions provided evidence to support the large body of research that implicates flood disturbance as the primary driver of periphyton dynamics in rivers, with grazing and nutrients altering the pattern of change between flood events as secondary determinants of periphyton biomass. The outcome of these patterns therefore provided a basis for linking temporal changes in periphyton biomass and community structure with specific determinants of both abiotic and biotic controls potentially important in foothill rivers of the south-western Cape. Empirical evidence for the relative importance of these environmental determinants is therefore the subject of Chapter 5.

CHAPTER 5 IDENTIFICATION OF THE KEY ENVIRONMENTAL VARIABLES RESPONSIBLE FOR TEMPORAL PATTERNS IN PERIPHYTON

5.1 INTRODUCTION

Of the multitude of factors known to influence periphyton in streams, flood disturbance and its interaction with nutrient supply are considered the primary drivers of biomass and community structure, particularly in Mediterranean climates where floods are limited to a short, predictable period ((Biggs 1996; Uehlinger *et al.* 1996; Biggs *et al.* 1998b; Biggs *et al.* 1998c; Fayolle *et al.* 1998; Villeneuve *et al.* 2011). Consequently, Chapter 4 explored temporal changes in periphyton biomass and community structure under natural flow and oligotrophic conditions and compared these to the situation firstly at enriched sites and secondly, at a site with regulated flows. The results of that study demonstrated a link between periphyton dynamics and both flood disturbance and nutrient availability and therefore supported the prediction that these factors are important drivers of periphyton communities in foothill rivers of the south-western Cape. Furthermore, there was evidence to suggest that grazers, mainly baetid mayflies, may regulate periphyton communities, particularly during the dry, stable months following the winter high flow period. Nevertheless, based on the results presented in Chapter 4, the significance of specific variables responsible for the temporal changes in periphyton biomass and community structure could not be identified. The ability to identify the relative importance of factors that control periphyton dynamics under natural conditions is one of the most critical aspects of assessing the impact of enrichment and flow alteration on periphyton communities in streams (Villeneuve *et al.* 2011).

Besides nutrient availability and grazers, other key factors that potentially affect periphyton between floods include habitat features such as low flows, water depth and chemistry, water temperature and light (Larned 2010; Law 2011) Chapter 3 identified seasonal changes in a range of hydrological, nutrient, grazer, temperature, irradiance and physico-chemical variables under natural and altered flow conditions and between sites with different levels of nutrient enrichment. This provided some insight into the suite of variables representing environmental factors that potentially affect periphyton under both natural and altered conditions.

Using the suite of measured variables that were described in Chapter 3, the objective of this chapter is to identify the relative importance of some of the key abiotic and biotic variables that regulate periphyton biomass and community structure in foothill rivers of the south-western Cape. Based on temporal patterns identified in Chapter 4, hydrological variables that describe flood disturbance are predicted to be the primary factors regulating temporal dynamics of periphyton communities, whereas other environmental factors such as nutrient availability, temperature, light, grazers and low flows may be important regulators of periphyton during the stable dry season.

This study therefore aims to address the following questions:

1. Are hydrological variables that describe flood characteristics the best predictors of temporal patterns in periphyton biomass and community structure under natural nutrient and flow conditions in south-western Cape Rivers?
2. Do nutrients, temperature, light, habitat and grazers play a role in regulating periphyton community structure and biomass in south-western Cape Rivers?
3. Which environmental variables are the key drivers of periphyton community structure and biomass under altered flow and enrichment conditions?

These questions were addressed by identifying which of the measured abiotic and biotic variables considered both separately and in combination, best explain differences in periphyton communities under natural conditions compared with those under a) enriched and b) altered flow conditions.

5.2 METHODS

5.2.1 Environmental variables

A total of 50 environmental variables (Chapter 3, Table 3.4) describing different components of flow, light, temperature, nutrients, physico-chemistry and grazing pressure were measured during this study and described fully in Chapter 3. The PCA and correlation analyses in Chapter 3 provided a means of eliminating redundant variables and identifying those most likely to influence periphyton biomass and community structure in this study. To avoid the inclusion of redundant information (i.e. collinearity) in these analyses, only those variables with a spearman correlation coefficient (r) < 0.9 were included as described in Section 3.2.5.2. Where strong collinearity was observed, only one of these could be selected for further analyses. Deciding which of the correlated variables to omit was based on the PCA analysis in Section 3.3.3, Chapter 3.

The selection of variables that would best represent grazing pressure as a potential predictor of periphyton biomass and community structure was complicated by the contrasting relationships between periphyton biomass and the three functional feeding groups (FFG) of invertebrate grazers. Grazers were separated into FFGs according to their mode of feeding, namely 'brushers' (dominated by heptageniids), 'deposit feeders' (dominated by baetids) and 'scrapers' (dominated by chironomids) as described in Chapter 2, Section 2.4.3. Based on the analyses detailed in Appendix 7, deposit feeder densities were selected as the most appropriate indicator of grazing pressure on temporal changes in periphyton biomass under natural conditions. By contrast, the abundance of grazers might reflect differences between sites with different trophic status (Appendix 7; Figure 5.A2). The density of both deposit feeders and brushers were therefore included in the dataset of environmental variables for site comparisons.

A total of 21 variables representing six environmental factors were therefore identified as potential predictor variables for investigating the factors that drive periphyton dynamics under natural flow condition, regardless of enrichment (Table 5.1). Considering that irradiance, temperature and flood characteristics were similar between Sites 1, 3 and 4 as identified by the environmental diagnostics

of differences between sites with varying levels of nutrient enrichment, only four variables were included in the analysis which tested the effects of enrichment between sites (Table 5.2). Twelve potential predictor variables were used for analyzing site differences between those that have unaltered flows and those that have modified flow conditions, based on the outcome of the environmental diagnostics in Chapter 3, which identified the environmental variables that best separate Sites 1 and 2, based on environmental conditions alone (Table 5.3).

Table 5.1 List of potential predictor variables characterising five environmental variables used to identify the key drivers of temporal change in periphyton under natural flow and nutrient conditions

Environmental factor	Variable
Irradiance	Minimum daily solar radiation during the inter-sampling period (RS_{min})
	Cumulative solar radiation during the inter-sampling period (RS_{cum})
Temperature regime	Minimum daily water temperature during the inter-sampling period (WT_{min})
	Average daily range in temperature of the inter-sampling period (WT_{range})
	Cumulative degree days during the inter-sampling period (WT_{cum})
Flow regime	Median of inter-sampling discharge (FLW median)(Q_{med})
	Peak discharge in inter-sampling period (Q_{max})
	Cv of inter-sampling discharge (Q_{cv})
	Number of floods ≥ Class 1, 2, 3 or 4*
	Number of days in flood ≥ Class 1,2,3 or 4 flood (FLW duration)($Class_{dur}$)*
	Days since flood ≥ Class1, 2, 3 or 4 *
Physico-chemical	Water depth
	Near bed velocity
	Turbidity
	pH
Nutrients	PO_4^{3+}-P
	SiO_4
	NH_4^+-N
	NO_2^--N
	NO_3^--N
Grazing	Total density of deposit feeders

*Flood size classes were highly correlated but the option of including any one of the flood class sizes was dependant on the initial analyses in Section 5.3.1

Without prior knowledge of the flood size that most affects periphyton biomass and community structure change over time selection of the most appropriate DRIFT flood class for inclusion in the set of predictor variables was difficult and therefore the option of including any one of the four flood classes was retained for further analyses within this chapter (Section 5.2.2).

5.2.2 Statistical analyses

Simple linear regressions in STATISTICA v10, 2011 were used to explore the relationship between periphyton biomass and the four flood categories defined in Chapter 3. The results of these analyses were used to select the most ecologically relevant flood category for inclusion in the data set of potential predictor variables.

The relationship between both univariate (i.e. biomass) and multivariate (taxon composition) measures of periphyton and environmental variables were investigated using Distance Based Linear Modelling (DistLM) and distance-based redundancy analysis (dbRDA) (McArdle and Anderson 2001; Anderson *et al.* 2008). DistLM partitions the variation in data distribution based on predictor variables and is analogous to multiple linear regression. dbRDA is used for visualizing the DistLM results as a principal component ordination (PCO) (Anderson *et al.* 2008). Unlike traditional multiple linear regression analyses, DistLM uses permutations to test hypotheses and therefore does not require normality and homoscedasticity of the dataset. However, data were either log (x+1) or square-root transformed where necessary prior to analyses to reduce variance in the data set. In the case of univariate analysis, the comparison between periphyton biomass and the environmental variables was based on Euclidean distance matrices, while Bray-Curtis dissimilarity matrices were used for all multivariate analyses of periphyton community structure.

Table 5.2 List of potential predictor variables characterising five environmental variables used to identify the factors that best explain nutrient enrichment effects between sites

Environmental factor	Variable
Nutrients	PO_4^{3+}-P
	NH_4^{+}-N
	NO_3^{-}-N
Grazing	Total density of brushers

Different models were run to establish which variables best describe temporal patterns in biomass and community structure under (a) unaltered flow and nutrient conditions (i.e. Site 1) (Table 5.1), (b) nutrient enriched conditions compared with natural conditions (i.e. Sites 1, 3 and 4) (Table 5.2) and (c) flow alteration compared with natural conditions (Sites 1 and 2)(Table 5.3). In the case of (a) 'month' was the main effect and was therefore considered a fixed variable in the statistical analyses. For models (b) and (c), 'site' was the main effect considered and was therefore included as a fixed variable with 'month' as a random factor in the statistical analyses. The contribution of each environmental variable was assessed in two ways. First, 'marginal tests' were performed to assess the statistical significance and percentage contribution of each variable alone, ignoring all other variables (Anderson *et al.* 2008). Secondly, 'sequential tests' were used to explain the cumulative variation attributed to each variable fitted to the model, taking into account the variability of all other variables (Anderson *et al.* 2008). The distribution for the pseudo-F value was obtained from a maximum of 9999 random permutations of sample data amongst the factor groups.

In all cases, the step-wise procedure and adjusted R^2 criterion of the environmental variables were used to determine the best model for all possible combinations of environmental variables. All univariate and multivariate analyses were performed using the PRIMER v.6 computer program (Clarke and Gorley 2006) with the additional add-on package PERMANOVA+ (Anderson *et al.* 2008).

Table 5.3 List of potential predictor variables characterising five environmental variables used to identify the factors that best explain site differences under natural and altered flow conditions between sites

Environmental factor	Variable
Temperature regime	Average daily range in temperature of the inter-sampling period (WT_{range})
Flow regime	Median of inter-sampling discharge (FLW median)(Q_{med})
	Cv of inter-sampling discharge (Q_{cv})
	Number of floods ≥ Class 1, 2, 3 or 4*
Physico-chemical	Water depth
	Near bed velocity
	Turbidity
Nutrients	NH_4^+-N
	NO_2^--N
Grazing	Total density of brushers
	Total density of deposit feeders

*Flood size classes were highly correlated but the option of including any one of the flood class sizes was dependant on the initial analyses in Section 5.3.1

Finally, simple linear regression analyses in STATISTICA v10, 2011 were used to explore the relationships established by the DistLM analyses. Analyses of periphyton biomass patterns thus employed both traditional multiple linear regression and its multivariate analogue, DistLM. In most cases, the results were similar, regardless of the analytical approach used but the results presented here are those based on DistLM because it produced the clearest understanding of the links between periphyton and the environment.

5.3 RESULTS

5.3.1 Selection of the DRIFT flood class for further analysis

Simple linear regressions of periphyton biomass and the recovery period (i.e. days since the last flood) following floods of differences sizes at Sites 1, 3 and 4 showed that Chl *a* biomass was most strongly correlated with the recovery period following the last flood greater than or equal to a Class 2 at Sites 1 and 4 and to a Class 1 flood at Site 3 (Table 5.2). Considering the insubstantial difference between the R^2 values for Class 1 and Class 2 flood categories, however, all further analyses were based on the Class 2 flood category as the best single predictor of biomass change among the different flood categories considered in this study.

Table 5.4 **R^2 values for the correlations between the length of time (days) since the last class 1, 2, 3 or 4 flood and Chl *a* biomass at Sites 1, 3 and 4. Those highlighted in bold italics are significant at p=0.05 and the grey shading highlights the category with the strongest relationship at each site. Note: only sites with unregulated flows were used in this analysis**

Flood category	Site 1	Site 3	Site 4
Class 1	***0.73***	***0.46***	***0.51***
Class 2	***0.75***	***0.43***	***0.52***
Class 3	***0.68***	0.08	0.15
Class 4	***0.46***	0.1	0.14

5.3.2 Temporal patterns of periphyton biomass in relation to environmental variables under natural flow and nutrient conditions (Site 1)

The DistLM results show that flood size, represented by peak discharge in the inter-sampling period (Q_{max}) (variation explained: 61 %) and the length of the recovery period (variation explained: 75 %), as well as water depth (variation explained: 64 %) contributed substantially to the observed temporal variation in Chl *a* biomass at Site 1 (Table 5.5: marginal tests). The recovery period following flood disturbance (i.e. days since the last Class 2 flood) alone described 75% of the observed variation and was the single best predictor of temporal variation in periphyton biomass under nutrient poor, unregulated flow conditions (Table 5.5: marginal tests). Flood size (Q_{max}), however, described 61% of the observed variation and is therefore a relatively good predictor of temporal patterns in Chl *a* biomass under natural conditions. Habitat characteristics such as depth and velocity represent temporal changes in base flow conditions and thus provided a good proxy for understanding the importance of base flow conditions to periphyton dynamics. Depth contributed 64 % to the observed variation (Table 5.5: marginal tests). Although nutrients, water temperature and solar radiation contributed significantly to the total variation, their contributions were relatively small. In other words, both high flow disturbance events that disrupt periphyton accrual as well as temporal changes in low flow conditions were the most important factors regulating periphyton development in this study.

Only two co-variates contributed significantly to the overall pattern of Chl *a* biomass. These were $N0_2^-$-N (variation explained: 8.4%) and minimum daily solar radiation (RS_{min})(variation explained: 6.6%) (Table 5.5: sequential tests).

Table 5.5 **Relationship between monthly Chl *a* biomass and environmental variables at Site 1 based on a Euclidean Distance matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.**

Environmental factor	Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum. % var
	MARGINAL TESTS [1]						
Flow	FLW median (Q_{med})	-	1.56	9.36	0.005	33.0	-
	FLW peak (Q_{max})	-	2.86	29.27	≤0.001	60.6	-
	Cv of discharge (Q_{cv})	-	1.74	11.09	0.005	36.9	-
	No. floods ≥ Class 2 (Class 2_{freq})	-	2.21	16.82	0.001	47.0	-
	FLW duration (Class 2_{dur})	-	2.29	17.96	0.001	48.6	-
	Days since Class 2 flood	-	3.52	56.10	≤0.001	74.7	-
Solar radiation	RS_{min}	-	0.92	4.63	0.044	19.6	-
Water temperature	WT_{range}	-	0.95	4.78	0.041	20.1	-
	WT_{cum}	-	1.03	5.29	0.033	21.8	-
Physico-chemical	Water depth	-	3.02	33.99	≤0.001	64.1	-
	Near-bed velocity	-	1.87	12.52	0.003	39.7	-
Nutrients	NO_2^--N	-	1.56	9.44	0.008	33.2	-
	SEQUENTIAL TESTS [2]						
Flow	+Days since Class 2 flood	0.73	3.52	56.10	≤0.001	74.7	74.7
Nutrients	+NO_2^--N	0.81	0.39	8.87	0.010	8.4	8.4
Solar radiation	+RS_{cum}	0.88	0.31	10.79	0.005	6.6	6.6

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Scatter plots with simple linear regression results provide a means of exploring general trends and possible temporal shifts in the relationship between periphyton biomass and key environmental variables identified by the DistLM (Figures 5.1 and 5.2). Both the length of the recovery period following the last disturbance flood and the peak discharge in the inter-sampling period (Q_{max}) correlated significantly with Chl *a* biomass at Site1 (Figures 5.1 and 5.2). The trend line in Figures 5.1a and 5.2a depict data points along the regression line that deviate from the linear regression and therefore indicate which data are weakly represented by the linear plot. Although Chl *a* biomass correlated significantly with the recovery period (Figure 5.1a) and peak discharge in the inter-sampling period (Figure 5.2a), the trend lines depicted in these figures indicate that the relationship was weak at certain times of the year. In particular, periphyton biomass was lower than predicted by the length of the recovery period during October 2007 and January 2008 of the first growing season, and December 2008 and January 2009 of the second (Figure 5.1a). Conversely, periphyton biomass was higher than predicted by the length of the recovery period during September 2007 and again during late winter, in August 2008 (Figure 5.1a). The same pattern was evident from the regression of peak discharged with Chl *a* biomass (Figure 5.2a), particularly during December 2008 when Chl *a* biomass was well below the regression line. Also, Chl *a* biomass was well above the regression line in September 2007 and August 2008 (early spring) and March 2008 and 2009 (late summer), coinciding with both the spring and summer Chl *a* maxima at Site 1. Despite the importance of flood characteristics such as the recovery period and peak discharge as predictors of Chl *a* biomass

patterns, these results suggest that variables other than these may be more important drivers of Chl *a* biomass, particularly at the time of peak biomass and over the late spring and early summer.

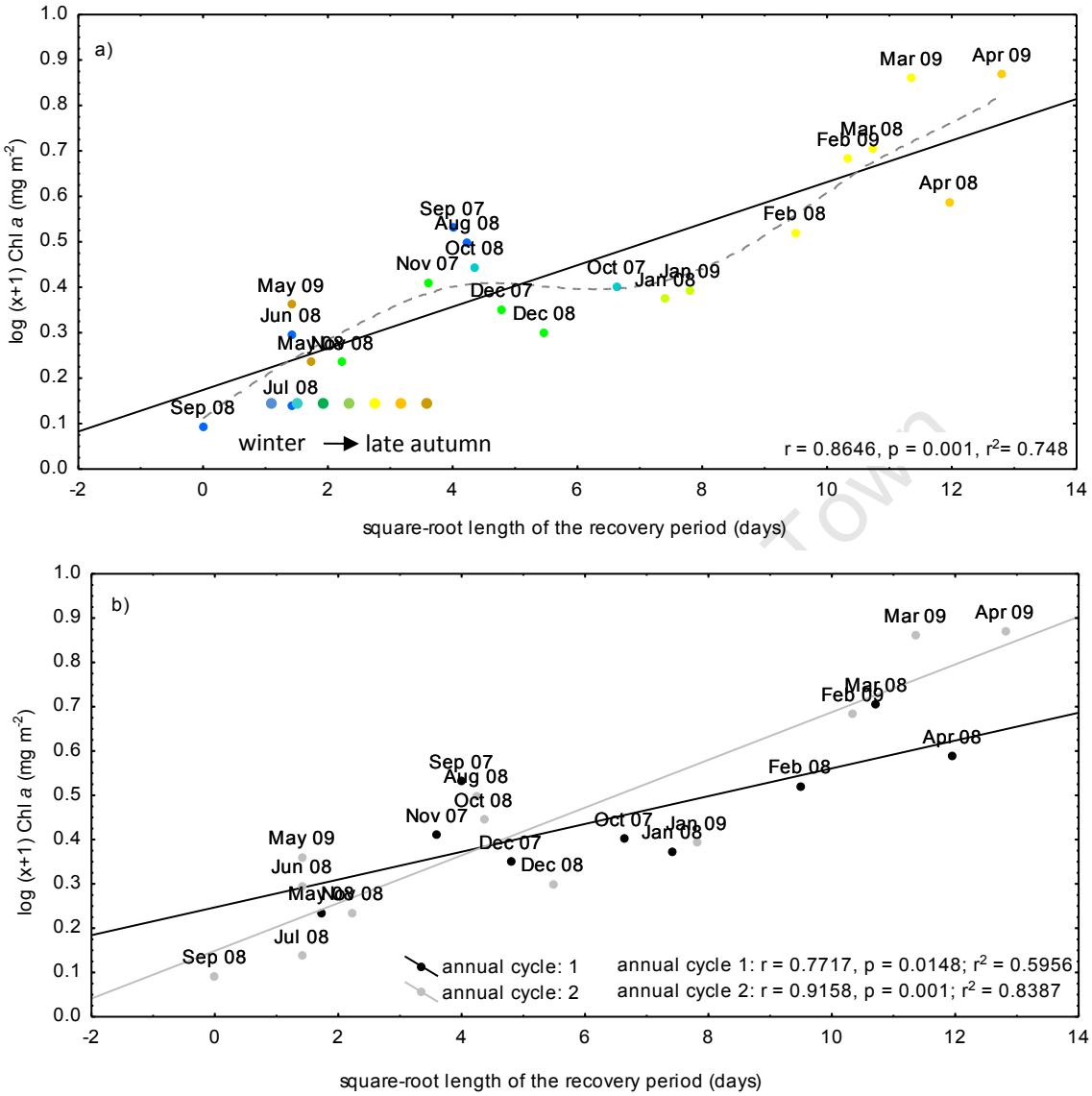


Figure 5.1 **Relationship between Chl *a* biomass and days since the last Class 2 flood a) for the total 21-month sampling period showing the linear regression (solid line) and the trend (broken line) based on a distance-weighted least square fit and b) for each annual cycle showing the linear regression fit for each separately.**

Although solar radiation and NO_2-N concentration contributed to the overall variability in biomass (Table 5.5: sequential tests), their contribution was small and no clear relationship between these variables and Chl *a* biomass could be established during periods when flow variables were poor predictors of temporal variability.

As suggested in Chapter 4, it was expected that the abundance of deposit feeders (mainly baetids) would contribute to the temporal pattern of Chl *a* biomass. However, no significant relationship between Chl *a* biomass and deposit feeder densities were identified by the DistLM (Table 5.5). Deposit feeder densities peaked during late spring/early summer (Figure 5.3), which coincided with

the period when Chl *a* biomass was not adequately accounted for by flood variables as shown in Figures 5.1 and 5.2.

Differences in the slope of the regression lines for different annual cycles in Figures 5.1b and 5.2b indicate inter-annual differences in the relationship between Chl *a* biomass and flood disturbance. These inter-annual differences, which essentially indicate a difference in the rate of biomass accrual over the stable, dry season, further suggest that periphyton biomass was affected by environmental variables other than flood disturbance and recovery time. This was particularly so during the late summer/early autumn when inter-annual differences in periphyton biomass was significant (Chapter 4, Table 4.2).

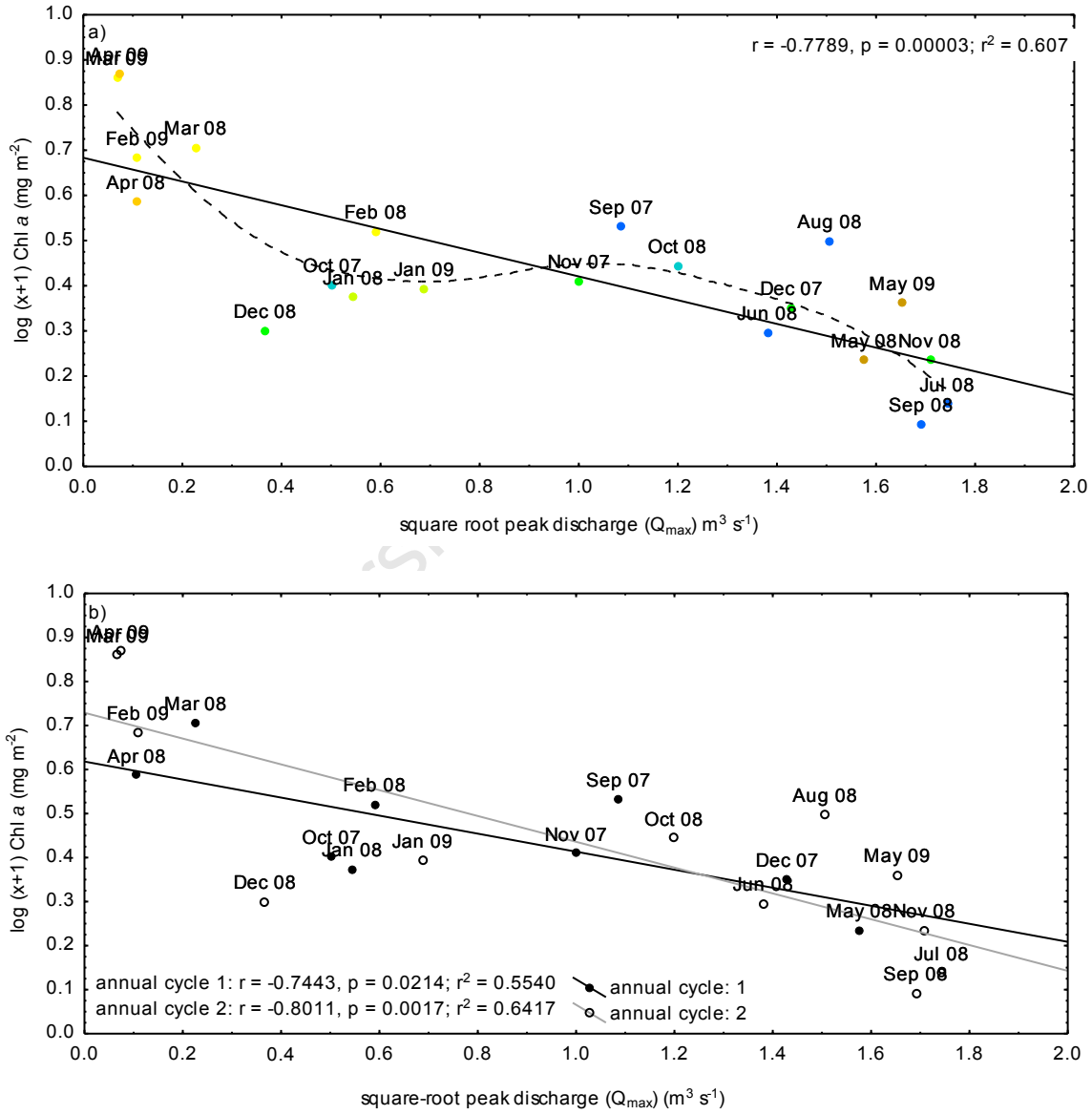


Figure 5.2 **Relationship between Chl *a* biomass and peak discharge in the inter-sampling period at Site 1 a) for the total 21-month sampling period showing the linear regression and the trend based on a distance-weighted least square fit and b) for each annual cycle showing the linear regression fit for each separately.**

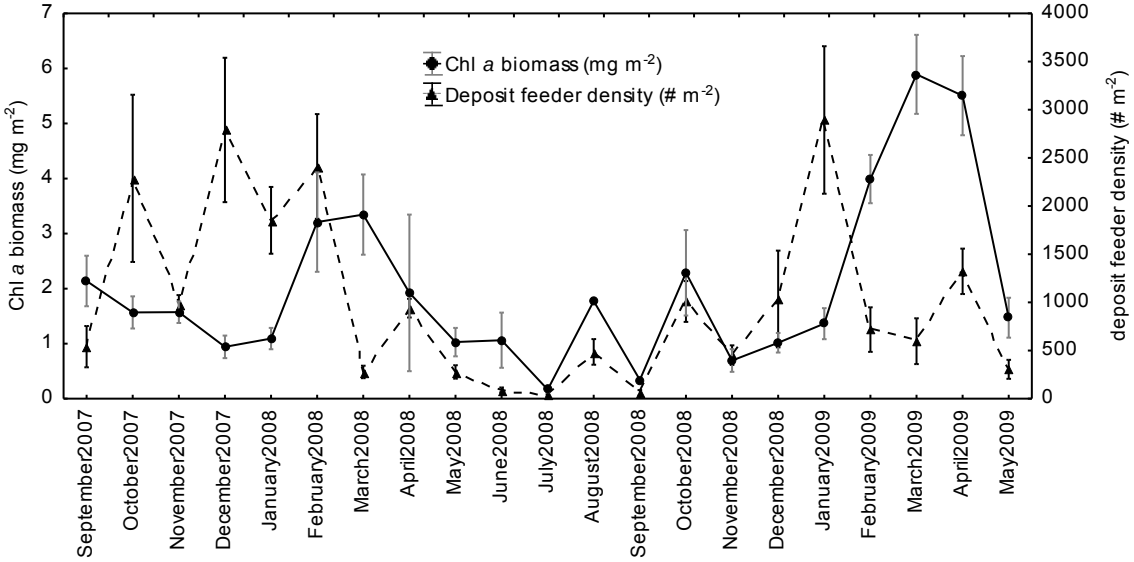


Figure 5.3 **Mean monthly Chl *a* biomass (mg m^{-2})and deposit feeder density (dominated by baetids)(# m^{-2}) at Site 1.**

The DistLM analysis in Table 5.5 did not provide a clear indication of what factors account for these inter-annual differences in Chl *a* biomass at Site 1. Although bottom-up factors such as resource availability are usually important controls of Chl *a* biomass during the dry stable season (Chapter 4), only NO_2-N, accounted for a significant proportion of the temporal changes in Chl *a* biomass at Site 1 (Table 5.5). Nevertheless, the relationship was unclear because Chl *a* biomass was highest when NO_2-N concentrations were negatively correlated with biomass and the relationship was only significant in the second annual cycle (Figure 5.4). The relative importance of nutrient availability as a factor driving periphyton biomass was, however, investigated further by analyzing the factors that drive temporal variability at the two enriched sites on the Molenaars River (Sites 3 and 4) and comparing these to Site 1 in the following section.

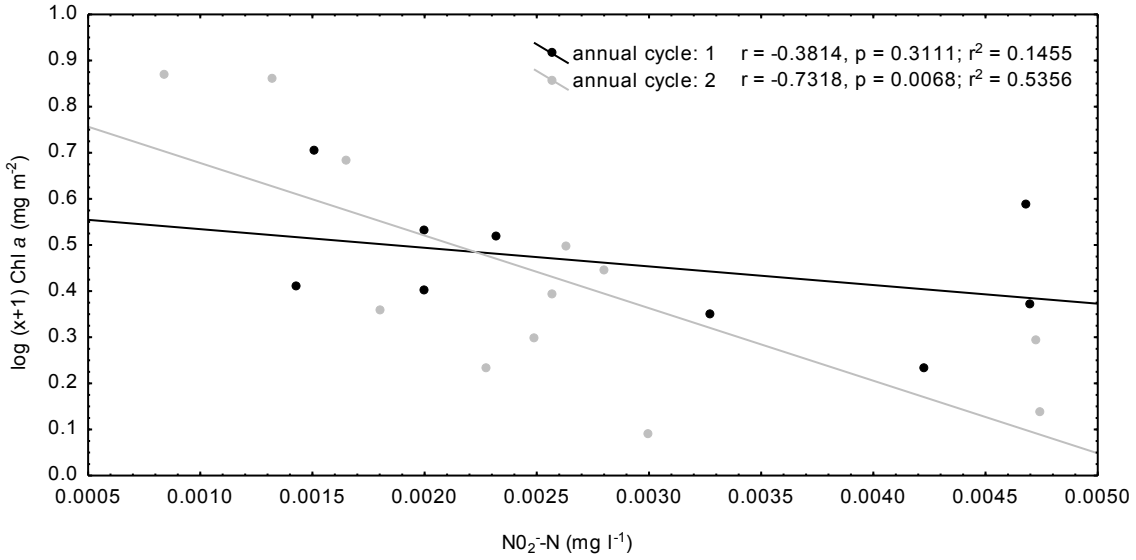


Figure 5.4 **Relationship between Chl *a* biomass and monthly NO_2^--N concentations at Site 1 for each annual cycle showing the linear regression fit for each separately.**

5.3.3 The effects of enrichment on the links between periphyton biomass and the environment

Using the same set of environmental variables as used in the DistLM analysis of Site 1, the DistLM for Sites 3 and 4 showed that the length of the recovery period (%) remained the single most important predictor of periphyton biomass over time under enriched conditions (Table 5.6: marginal tests). Nevertheless, compared with Site 1 where the recovery period accounted for 75% of the observed temporal variability, the recovery period at Sites 3 and 4 only accounted for 45% of the variation (Table 5.6: marginal tests). These differences are quite clearly represented by comparing the scatter plots and linear regression results of the relationship between Chl *a* biomass and the recovery period for Sites 1, 3, and 4 in Figures 5.1a, 5.5 and 5.6 respectively. Firstly, the linear regression slope for Site 4 (Figure 5.6, slope = 0.062) is steeper than that for Sites 1 and 3 (Figure 5.1a; slope = 0.045 and Figure 5.5; slope = 0.043), indicating that under enriched conditions (Site 4), the rate of periphyton accrual is greater compared with unenriched or only slightly enriched conditions (Sites 1 and 3). Secondly, the temporal trend at all three sites was similar, with values above the regression line during peak biomass in late summer/autumn and values below the line in late spring/early summer when deposit feeders may suppress algal growth. Like Site 1, deposit feeder abundances peaked in the late spring and early summer (Figures 5.7 and 5.8) when Chl *a* biomass was relatively low, providing further support for the suggestion that deposit feeder densities were able to regulate Chl *a* biomass accrual over the late spring/ early summer period at sites with natural flow conditions (i.e. Sites 1, 3 and 4). Thirdly, the relationship between Chl *a* biomass and length of the recovery period at both Sites 3 and 4 was weaker with increased enrichment, as indicated by the greater difference between the trend line and the linear regression line under enriched conditions (Figures 5.5 and 5.6) relative to unenriched conditions (Figure 5.1a). This was driven largely by greater peak biomass between sites in both late winter/early spring and late summer/early autumn. These results suggest that conditions during periods of stability between flood events promoted faster biomass accrual under enriched conditions, despite the probable regulation of accrual by grazing over the late spring and early summer months.

PO_4^{3+}-P (variation explained: 5.2%), water depth (variation explained: 6.6%), water temperature (variation explained: 6.9%), SiO_4 (variation explained: 6.6%) and peak discharge (variation explained: 5.1%) were co-variates that, together with days since the Class 2 flood, explained 75.6% of the total variation (Table 5.3: sequential tests). Nevertheless, the percentage contribution of each of these co-variates was relatively small.

Table 5.6 **Relationship between monthly Chl *a* biomass and environmental variables at Sites 3 and 4 based on a Euclidean Distance matrix. The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.**

Enviromnetal factor	Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	FLW median (Q_{med})	-	4.454	12.686	0.002	24.5	-
	FLW peak (Q_{max})	-	5.789	18.271	<0.001	31.9	-
	Cv of discharge (Q_{cv})	-	4.945	14.609	0.001	27.3	-
	Number of Floods ≥ Class 2 (Class 2 $_{freq}$)	-	5.822	18.420	<0.001	32.1	-
	FLW duration (Class 2 $_{dur}$)	-	4.102	11.391	0.002	22.6	-
	days since Class 2 flood	-	8.207	32.203	<0.001	45.2	-
Solar radiation	RS_{min}	-	2.493	6.210	0.015	13.7	-
Water temperature	WT_{range}	-	1.868	4.475	0.044	10.3	-
	WT_{cum}	-	3.180	8.286	0.006	17.5	-
Physico-chemical	Depth	-	7.335	26.458	<0.001	40.4	-
Nutrients	pH	-	2.988	7.688	0.009	16.5	-
	SiO_4	-	4.932	14.556	0.001	27.2	-
	NO_2^--N	-	2.302	5.665	0.024	12.7	-
	SEQUENTIAL TESTS [2]						
Flow	days since Class 2 flood	0.438	8.207	32.203	<0.001	45.2	45.2
Nutrients	$+PO_4^{3+}$-P	0.478	0.946	3.995	0.054	5.2	50.4
Physico-chemical	+Depth	0.535	1.194	5.663	0.023	6.6	57.0
Water temperature	$+WT_{range}$	0.600	1.261	6.943	0.014	6.9	64.0
Nutrients	$+SiO_4$	0.663	1.192	7.803	0.010	6.6	70.5
Flow	+FLW peak (Q_{max})	0.713	0.924	7.100	0.011	5.1	75.6

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

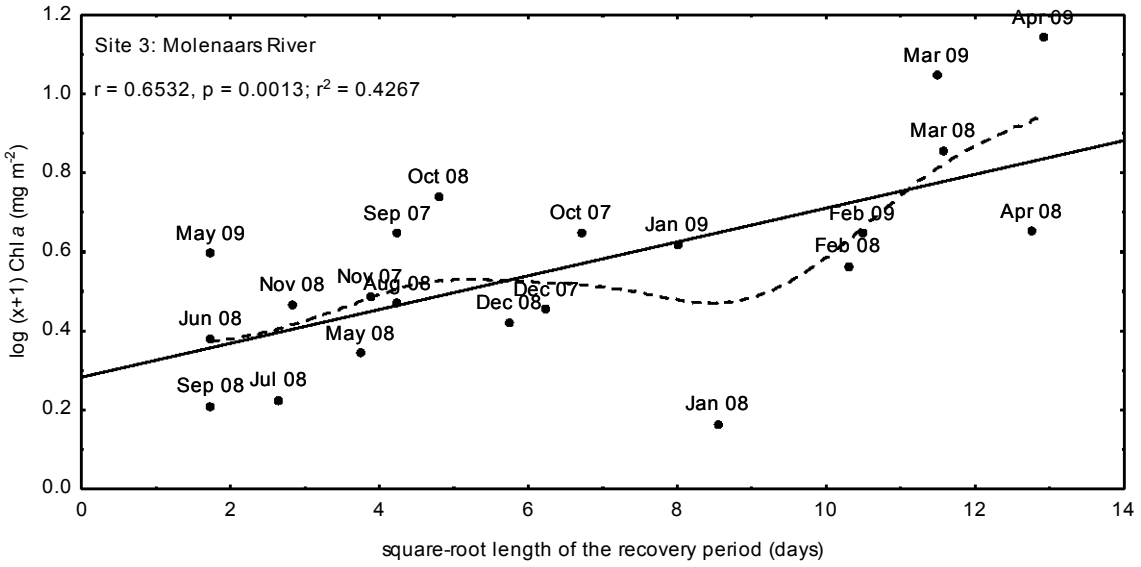


Figure 5.5 **Relationship between monthly Chl *a* biomass and days since the last Class 2 flood at Site 3 showing the linear regression (solid line) and the trend (broken line) based on a distance-weighted least square fit.**

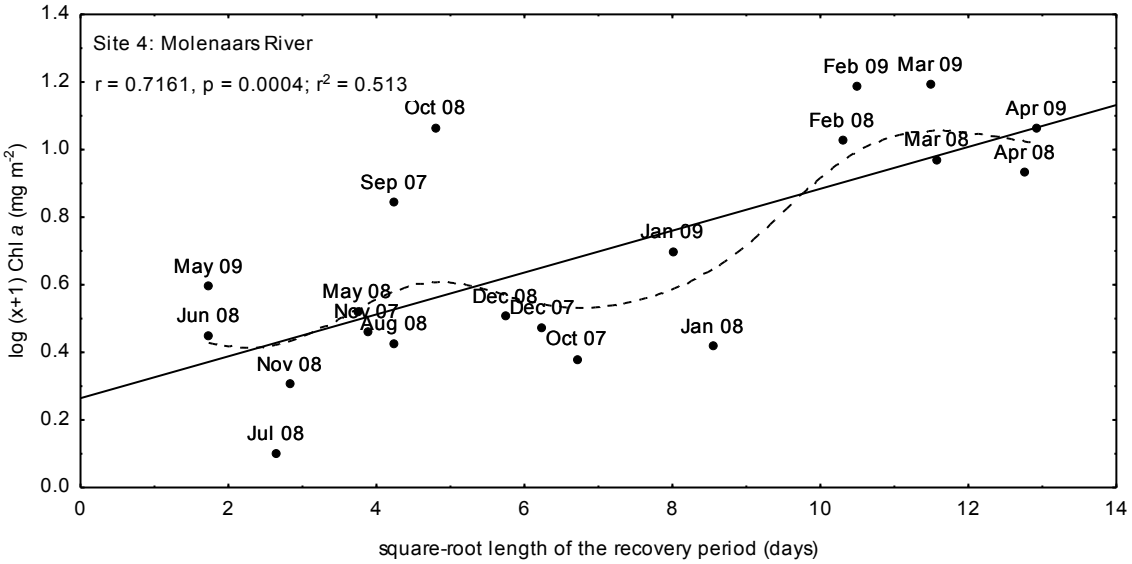


Figure5.6 Relationship between monthly Chl *a* biomass and days since the last Class 2 flood at Site 4 showing the linear regression (solid line) and the trend (broken line) based on a distance- weighted least square fit.

Chl *a* biomass values from Sites 1, 3 and 4 were combined in a separate DistLM analysis in an attempt to establish the factors that separate sites under different levels of enrichment (Table 5.2), regardless of their temporal pattern. Only PO_4^{3+}-P concentrations and grazer densities accounted for differences in Chl *a* biomass between these three sites with grazer densities accounting for 32.5% of the observed variation (Table 5.7). A scatter plot of Chl *a* biomass and PO_4^{3+}-P and in Figure 5.9 shows no apparent relationship between them. In fact, PO_4^{3+}-P concentrations were generally higher at Site 3 although Chl *a* biomass was higher at Site 4. By contrast, brusher densities increased with increasing Chl *a* biomass and densities of these grazers were greater at Site 4, followed by Site 3, then Site 1 (Figure 5.10). This suggests that nutrient concentrations *per se* did not provide a good indicator of trophic status. Greater brusher densities, possibly in response to greater Chl *a* biomass, reflected differences in the trophic status among these three sites. A relatively large portion of the variability is unexplained by the measured environmental variables in this model (i.e. 78% of the total observed variability), however, suggesting that other, unmeasured environmental variables also contributed to the observed temporal pattern of periphyton changed across a nutrient gradient.

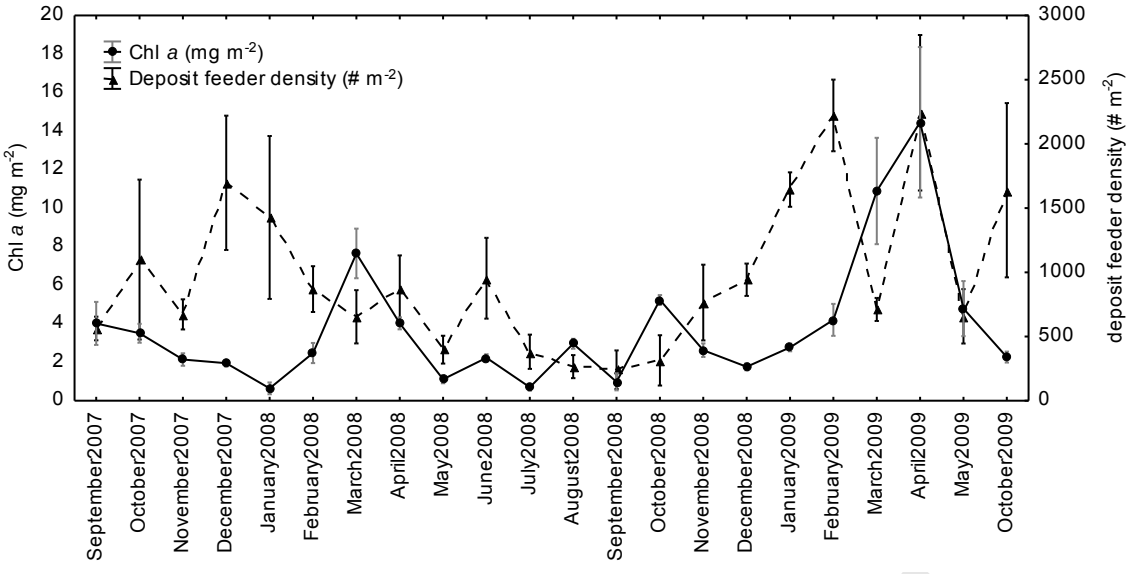


Figure 5.7 **Mean monthly Chl *a* biomass (mg m^{-2}) and deposit feeder density (dominated by baetids) (# m^{-2}) at Site 4**

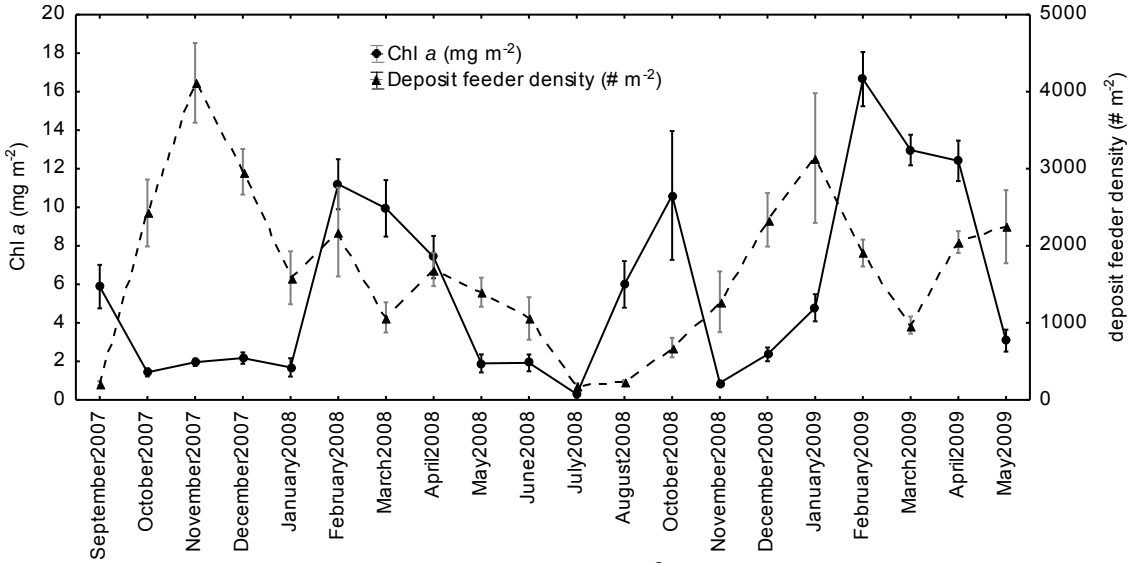


Figure 5.8 **Mean monthly Chl *a* biomass (mg m^{-2}) and deposit feeder density (dominated by baetids) (# m^{-2}) at Site 3**

Table 5.7 **Relationship between monthly Chl *a* biomass and environmental variables at Sites 1, 3 and 4 based on a Euclidean The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.**

Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
PO_4^{3+}-P	-	2.40	6.33	0.012	9.5	-
brusher density	-	8.17	28.83	0.001	32.5	-
SEQUENTIAL TESTS [2]						
brusher density	0.31	8.17	28.83	0.001	32.5	32.5

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Overall, flood disturbance (i.e. the length of the recovery period) was the best predictor of temporal patterns in Chl *a* biomass at Sites 3 and 4 , reflecting a similar scenario to that for Site 1 (Section 5.3.2). By contrast, brusher densities, as and indicator of trophic state, was important in explaining site differences

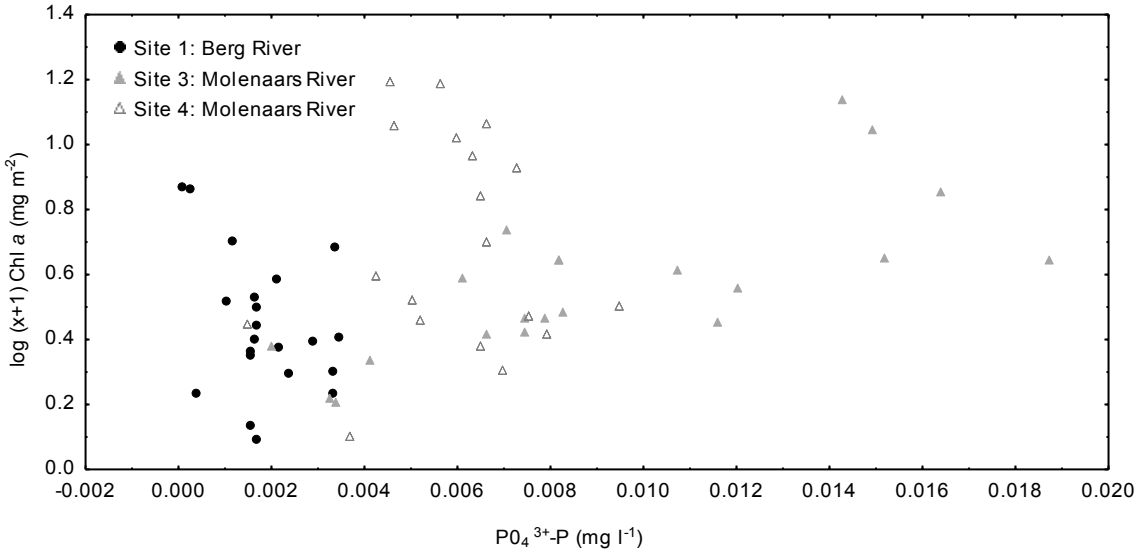


Figure 5.9 A comparison of the relationship between monthly Chl *a* biomass (mg m^{-2}) and PO_4^{3+}-P (mg l^{-1}) at Sites 1, 3 and 4.

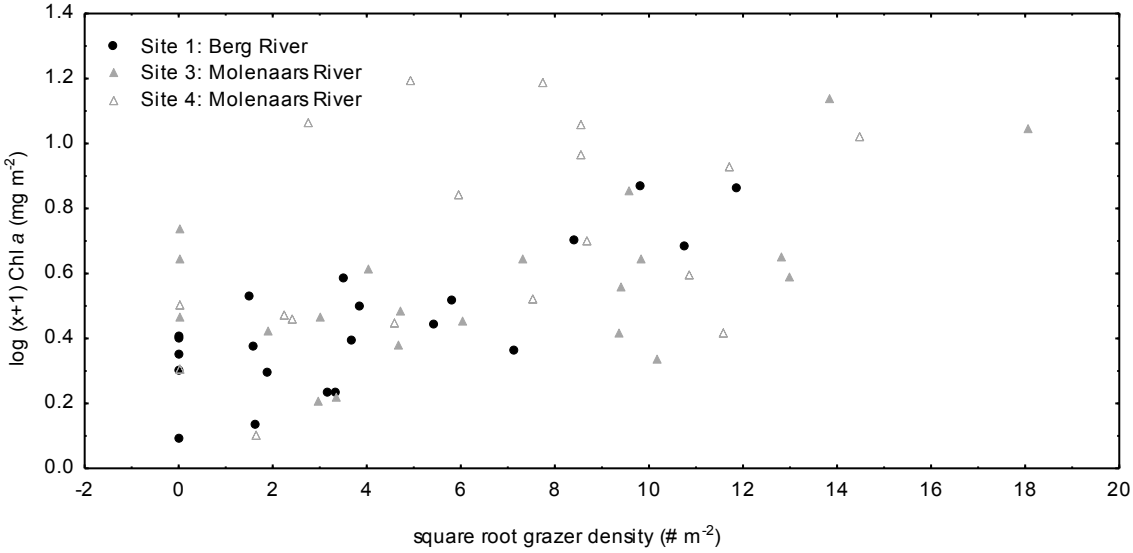


Figure 5.10 A comparison of the relationship between monthly Chl *a* biomass (mg m^{-2}) and brusher densities at Sites 1, 3 and 4.

The relationship between nutrients and Chl *a* biomass, both temporally and between sites, was not particularly apparent from these analyses. Nevertheless, there was a strong relationship between Chl *a* biomass maxima in late summer and NH_4^+-N (Figure 5.11) at Sites 1, 3 and 4. Nutrient availability may indeed be an important factor that affected the rate of accrual and the absolute biomass of the 'climax' community (Figure 5.11).

Although these data suggest that flood disturbance (given by the length of the recovery period) was an important driver of temporal changes in periphyton biomass under natural flow conditions, flood disturbance was less important under enriched conditions. Grazing activity, most probably by deposit feeders may have been an important regulator of periphyton growth in the late spring and early summer months, despite enrichment, although the evidence is circumstantial. While nutrient availability, possibly PO_4^{3+}-P and NH_4^+-N may be important factors that promote rapid accrual of biomass during inter-flood periods, ambient nutrient concentrations in the water column did not adequately represent nutrient loading in this system. Grazer densities on the other hand, seemed to provide a good indicator of differences in trophic status. Nevertheless, the difficulty of linking biomass with ambient nutrient concentrations measured in this study meant that the relative importance of different nutrients as a key factor affecting Chl *a* biomass could not be quantified.

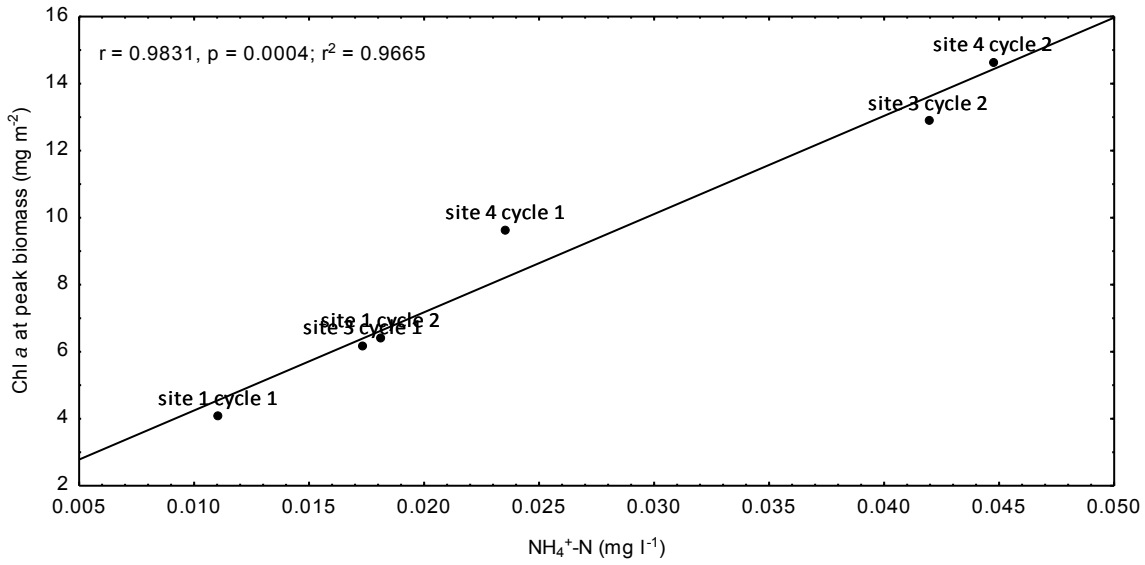


Figure 5.11 Relationship between peak Chl *a* biomass (mg m^{-2}) and cumulative NH_4^+-N concentrations over two accrual periods at Sites 1, 3 and 4. Linear regression results describing the relationship are included.

5.3.4 The effects of altered flow conditions on the links between periphyton biomass and the environment

As expected, the suite of environmental variables that accounted for the observed variation in Chl *a* biomass at Site 2 differed considerably from that under unregulated flow conditions (Table 5.8). Whereas the temporal pattern in biomass for Sites 1, 3 and 4 were best explained by the length of the recovery period following flood disturbance, turbidity accounted for the greatest percentage of observed variability (31.8%) at Site 2 below the Berg Dam (Table 5.8). Nevertheless, determinants describing flood disturbance such as size (variation explain: 23.6%), frequency (variation explained: 25.5%) and duration of floods (variation explained: 15.8%) each individually accounted for a proportion of the variability in Chl *a* biomass (Table 5.8: marginal tests). This was not surprising considering that Site 2 did experience floods in winter and early spring 2008, despite flow regulation downstream of the dam.

Table 5.8 Relationship between monthly Chl *a* biomass and environmental variables at Site 2 based on a Euclidean Distance matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.

Enviromnetal factor	Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	FLW peak (Q_{max})	-	0.723	5.553	0.025	23.6	-
	Cv of discharge (Q_{cv})	-	0.666	4.994	0.041	21.7	-
	Number of Floods ≥ Class 2 (Class 2_{freq})	-	0.782	6.172	0.019	25.5	-
	FLW duration (Class 2_{dur})	-	0.485	3.385	0.070	15.8	-
Physico-chemical	Turbitdity	-	0.973	8.377	0.011	31.8	-
	SEQUENTIAL TESTS [2]						
Physico-chemical	+Turbitdity	0.280	0.973	8.377	0.008	31.8	31.8
Flow	+Cv of discharge (Q_{cv})	0.632	1.083	18.252	0.001	35.3	67.1
Physico-chemical	+pH	0.695	0.221	4.498	0.052	7.2	74.3

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.
[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Co-variates that contributed to the overall variation explained by the DistLM (74.3%), included variability in daily flow (Q_{cv}) (variation explained: 35.3%) and pH (variation explained: 7.2%) (Table 5.8: sequential tests). Flow variability (Q_{cv}), as an indicator of low flow conditions, and turbidity therefore contributed equally to the overall model (Table 5.8: sequential tests).

In fact, flow variability was indicated as the single best predictor of differences in Chl *a* biomass (variation explained: 32.9%) between Sites 1 and 2 (Table 5.9: marginal tests). Grazer densities (variation explained: 17.7%) and median flow (variation explained: 16%), and to a lesser extent turbidity (variation explained: 7.3%) and $NH4^+$-N (variation explained: 3.3%) contributed to the overall variation explained by the DistLM (77.2%) for both Sites 1 and 2. This suggests that alteration of low flow conditions together with habitat differences, rather that high flow conditions explained the differences in Chl *a* biomass between Sites 1 and 2.

Essentially, flood releases and spillage from the dam during the winter of 2008 adequately reset the accrual cycle over the wet season such that small differences in biomass were evident between Sites 1 and 2 at this time. Nevertheless, conditions associated with aseasonally high, constant base flows in summer accounted for the differences in biomass, particularly at the end of the growing season.

Table 5.9 Relationship between monthly Chl *a* biomass and environmental variables for Sites 1 and 2 based on a Euclidean Distance matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.

Enviromnetal factor	Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	Cv of discharge (Q_{cv})	-	2.7	19.09	0.001	32.9	-
Physico-chemical	Near-bed velocity	-	1.9	12.36	0.001	24.1	-
	Turbidity	-	1.0	5.36	0.021	12.1	-
Algal feeders	brusher density	-	1.3	7.13	0.007	15.5	-
	SEQUENTIAL TESTS [2]						
Flow	+Cv of discharge (Q_{cv})	0.31	2.66	19.09	0.001	32.9	32.9
	+FLW median (Q_{med})	0.46	1.30	11.92	0.002	16.0	48.9
Physico-chemical	+Turbidity	0.53	0.59	6.15	0.013	7.3	56.2
	brusher density	0.71	1.43	24.35	0.001	17.7	73.9
Nutrients	+NH_4^+-N	0.74	0.27	5.12	0.030	3.3	77.2

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

5.3.5 The relationship between periphyton community composition and environmental variables

5.3.5.1 The case for nutrient-poor, unregulated flow conditions: Site 1

The temporal pattern of periphyton community composition was best explained by water depth (13.1%) and the length of the recovery period (13%), which contributed similarly as individual predictors (Table 5.10: marginal tests). Nevertheless, the measured environmental parameters only accounted for 23.1% of the overall observed variability in community composition (Table 5.10: sequential tests), compared with 89% for Chl *a* biomass (Table 5.5). Temporal differences in water depth (as an indicator of base flow conditions) and $NH4^+$-N were the key variables that accounted for temporal changes in composition (Table 5.10: sequential tests). An ordination of the distance-based redundancy analysis (dbRDA) in Figure 5.12a shows that these variables drove a distinction between summer and winter communities at Site 1. Considering that depth and the recovery period contributed similarly to the observed variation in the marginal tests, the length of the recovery period was overlaid on the dbRDA ordination (Figure 5.12b). From these data, summer communities with a long recovery period grouped together on the left, while winter, autumn and spring communities with a short recovery period were distinct (bottom right)(Figure 5.12). Interestingly, deposit feeder densities did not significantly contribute to the observed variability, but this variable was highly correlated with axis 2 of the dbRDA ($\rho = 0.733$) (Figure 5.12). Indeed, the spring and early summer communities with variable recovery periods, clustered together (top right). This supports the suggestion that deposit feeders, at least in part may regulate periphyton dynamics by suppressing biomass accrual and affecting the community composition during the late spring/ early summer.

Table 5.10 **Relationship between monthly periphyton composition and environmental variables for Sites 1 and 2 based on a Bray-Curtis dissimilarity matrix. The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.**

Environmental variable	Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	FLW median (Q_{med})	-	3124	2.09	0.036	10.4	-
	FLW peak (Q_{max})	-	3648	2.48	0.010	12.1	-
	FLW duration (Class 2 $_{dur}$)	-	3085	2.06	0.033	10.2	-
	Days since Class 2 flood	-	3898	2.68	0.005	13.0	-
Water temperature	WT_{range}	-	3085	2.06	0.034	10.3	-
	WT_{cum}	-	3483	2.36	0.010	11.6	-
Physico-chemcial	Depth	-	3928	2.70	0.009	13.1	-
	SEQUENTIAL TESTS [2]						
Physico-chemcial	+Depth	0.082	3928	2.70	0.004	13.1	13.1
Nutrients	$+NH_4^+$-N	0.141	3032	2.23	0.011	10.1	23.1

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

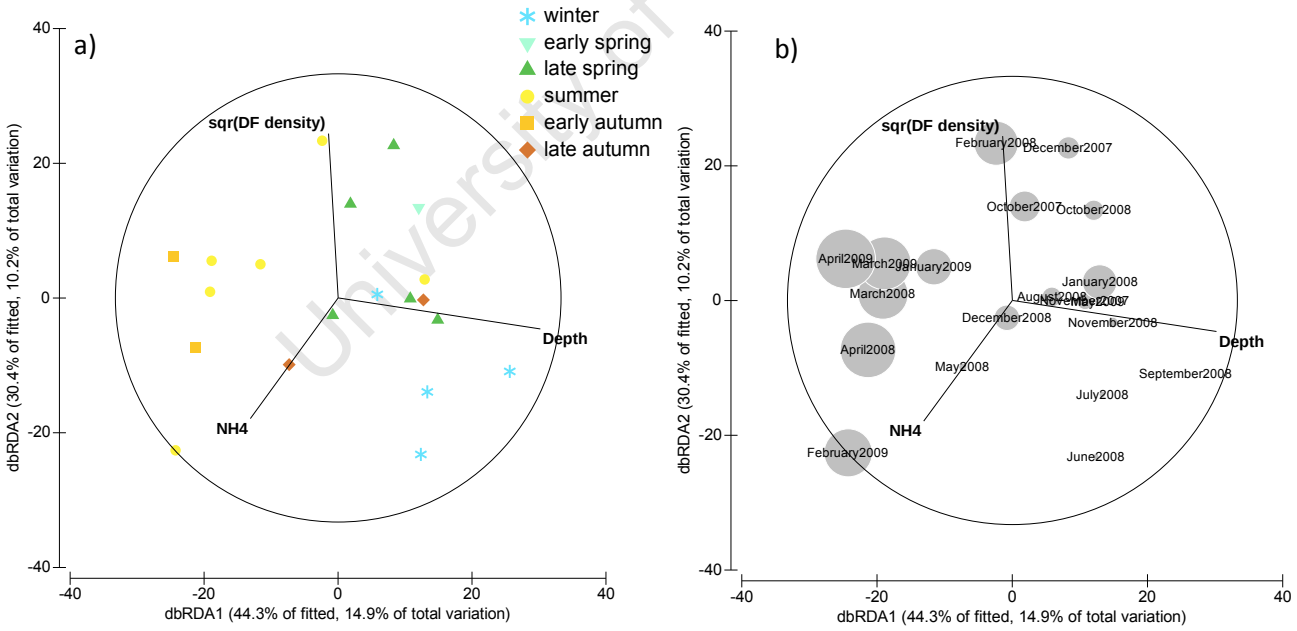


Figure 5.12 **dbRDA ordinations of monthly benthic algal abundances for individual taxa with vectors showing the Spearman correlation between environmental variables and the dbRDA axes at Site 1. a) gives the distributon of data represented by different seasons while b) provides an indication of the relationship between algal composition and the length of the recovery period – the larger the bubble, the greater the time since the last Class 2 (or larger) flood event. DF = deposit feeder.**

5.3.5.2 The effects of enrichment

Besides flow and temperature variables, habitat characteristics (depth, near-bed velocity and pH) and nutrients ($Si0_4$, $NH4^+$ -N and NO_2^--N) contributed between 5 and 12% to the observed variability in community composition at Sites 3 and 4 (Table 5.11). Similar to Site 1, flow and temperature variables all contributed between 10 and 13% to the observed variability in community composition under enriched conditions (Tables 5.10 and 5.11). The dbRDA ordination in Figure 5.13a shows no distinction in the data set between Sites 3 and 4 but, like Site 1, a clear temporal pattern separating summer and early autumn from winter and late autumn communities along axis 1 was evident (Figure 5.13b). Cumulative water temperature (WT_{cum}) and water temperature range (WT_{range}) were the primary drivers of this distinction (Table 5.11 and Figure 5.13). The length of the recovery period and NO_2^--N concentrations correlated with dbRDA2 (Figure 5.13b). These two variables separated the spring period into two groups with October 2007 and 2008 at the bottom of the ordination and November and December near the top (Figure 5.13b).

Table 5.11 Relationship between monthly periphyton composition and environmental variables for Sites 3 and 4 based on a Bray-Curtis dissimilarity matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.

Environmental variable	Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	FLW median (Q_{med})	-	8478	4.299	0.001	10.4	-
	FLW peak (Q_{max})	-	10898	5.716	0.001	13.4	-
	Cv of discharge (Q_{cv})	-	8902	4.541	0.001	10.9	-
	Number of Floods ≥ Class 2 (Class 2_{freq})	-	9115	4.663	0.001	11.2	-
	FLW duration (Class 2_{dur})	-	8775	4.469	0.001	10.8	-
	days since Class 2 flood	-	10773	5.641	0.001	13.2	-
Solar radiation	RS_{min}	-	9414	4.836	0.001	11.6	-
	RS_{cum}	-	9307	4.774	0.001	11.4	-
Water temperature	WT_{range}	-	9312	4.777	0.001	11.4	-
	WT_{cum}	-	11050	5.809	0.001	13.6	-
Physico-chemcial	Depth	-	9590	4.939	0.001	11.8	-
	Velocity	-	5256	2.553	0.008	6.5	-
	pH	-	8034	4.050	0.001	9.9	-
Nutrients	SiO_4	-	7937	3.995	0.002	9.7	-
	NH_4^+-N	-	3672	1.747	0.082	4.5	-
	$N0_2^-$-N	-	6784	3.362	0.001	8.3	-
	SEQUENTIAL TESTS [2]						
Water temperature	+WT_{cum}	0.112	11050	5.809	0.001	13.6	13.6
Flow	+days since Class 2 flood	0.180	7135	4.061	0.001	8.8	22.3
Nutrients	+$N0_2^-$-N	0.241	6285	3.862	0.002	7.7	30.0
Solar radiation	+RS_{min}	0.288	5066	3.319	0.001	6.2	36.3
Flow	+Number of Floods ≥ Class 2 (Class 2_{freq})	0.311	3172	2.148	0.029	3.9	40.2
Water temperature	+WT_{range}	0.334	3033	2.124	0.024	3.7	43.9
Flow	+FLW duration (Class 2_{dur})	0.357	2969	2.154	0.029	3.6	47.5
Nutrients	+PO_4^{3+}-P	0.373	2418	1.799	0.078	3.0	50.5

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

A plot of dbRDA 2 against dbRDA 3 (Figure 5.13c) shows that some of the variation in the data set could be attributed to inter-annual variation in the composition of benthic algae at Sites 3 and 4. Minimum irradiance (RS_{min}), flood duration and frequency, the length of the recovery period and NH_4^+-N all correlated with dbRDA3 (Figure 5.13c). In particular, RS_{min} was far greater during the spring 2008 (mean RS_{min} = 8.4 MJ m^{-2}) compared with spring 2007 (mean RS_{min} = 15.8 MJ m^{-2}) but flooding was more frequent and floods had a longer duration in spring 2008 compared with spring 2007 (Chapter 3, Section 3.3.1.4 and Figure 3.16). Also, NH_4^+-N concentrations were higher during the summer of 2008 relative to 2009 (see Figure 5.11). In other words, inter-annual differences in light availability and flood disturbance during the spring, as well as greater nutrient availability during the second growing season (2008/2009) compared with the first (2007/2008) may be responsible for inter-annual differences in periphyton community composition observed during this study.

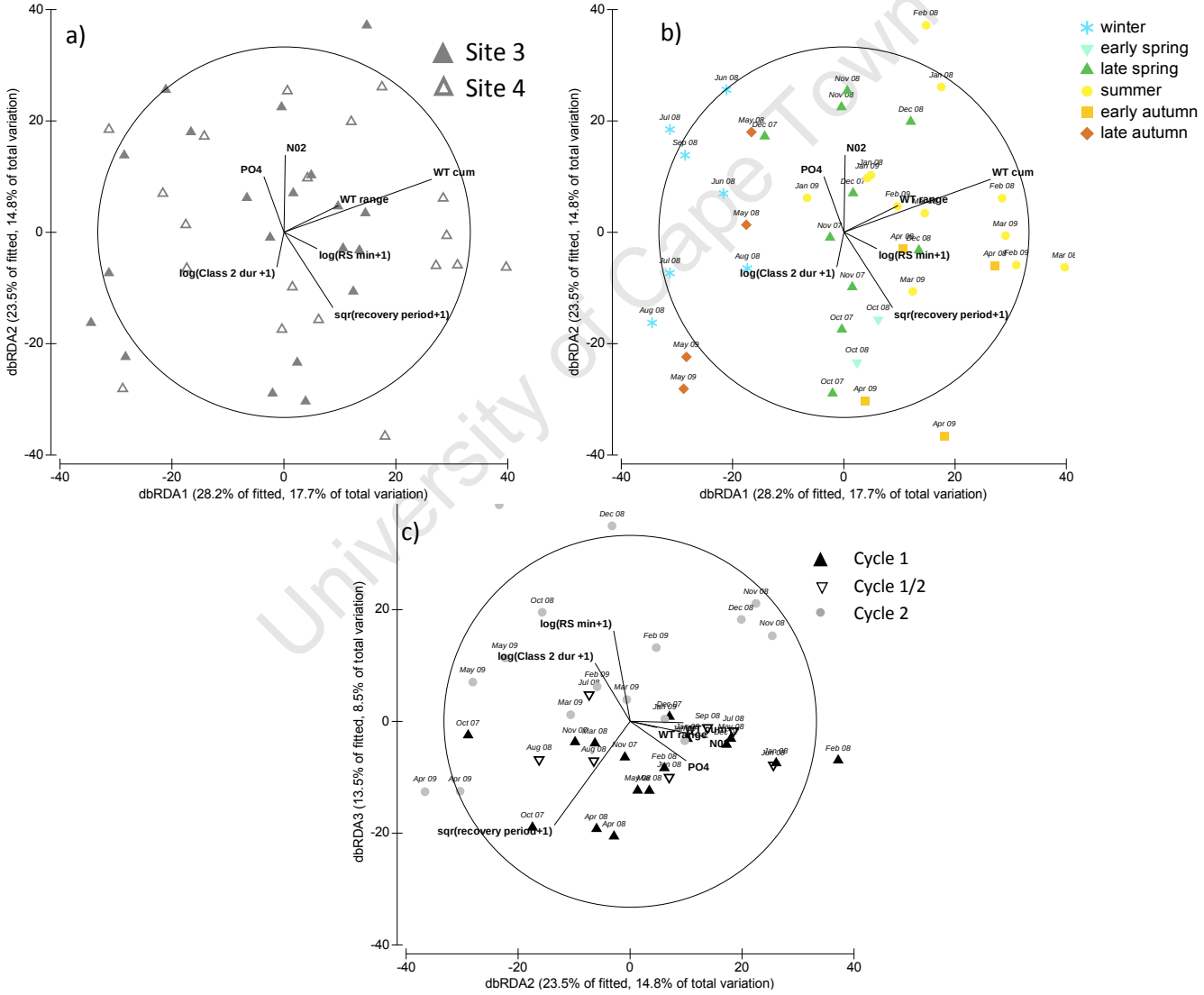


Figure 5.13 **dbRDA ordinations of monthly benthic algal abundances for individual taxa with vectors showing the Spearman correlation between environmental variables and the dbRDA axes for Sites 3 and 4 together. The distribution of data represented by different a) sites and b) different seasons are presented. c) is a plot of dbRDA 2 and dbRDA 3 for the same data set showing the inter-annual variation in community composition. Cycle 1/2 represents the transition (winter months) between the first and the second cycle.**

DistLM analysis of the data set combining Sites 1, 3 and 4 provided a means of assessing the key drivers that separate sites that are differentially enriched. The DistLM results (Table 5.12) and dbRDA ordination (Figure 5.14) show that only brusher densities (variation explained: 6.3%), NH_4^+-N (variation explained: 5.2%) and PO_4^{3+}-P concentrations (variation explained: 5.2%) were considered important in separating Sites 1 from Sites 3 and 4. Only 15 % of the total observed variation between these sites could, however, be explained by these variables (Table 5.12: sequential tests). Based on the assumption that brusher abundance reflected trophic status (see Section 5.3.2), it was not surprising that both nutrients and brusher abundance separated Site 1 from Sites 3 and 4. Brusher densities and PO_4^{3+}-P correlated relatively strongly with dbRDA 1, which shows the distinction between sites (Figure 5.14a). By contrast, NH_4^+-N correlated strongly with dbRDA2, which clearly shows a seasonal trend evident at all three sites (Figure 5.14b) Nevertheless, only a small percentage of the variation in community composition was explained by these three variables (Table 5.12). It should be emphasized that dbRDA1 and dbRDA2 explained only 6.9% and 6.1%, respectively (Figure 5.14) and therefore these results are inconclusive.

Table 5.12 Relationship between monthly periphyton community composition and environmental variables at Sites 1, 3 and 4 based on a Bray-Curtis dissimilarity matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.

Environmental variable	Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Nutrients	PO_4^{3+}-P	-	6291	3.11	0.006	5.2	-
	NH_4^+-N	-	6333	3.13	0.002	5.2	-
	Brusher density	-	7640	3.83	0.001	6.3	-
	SEQUENTIAL TESTS [2]						
Grazers	Brusher density	0.05	7640	3.83	0.001	6.3	6.3
Nutrients	$+NH_4^+$-N	0.08	6372	3.32	0.002	5.2	11.5
	$+PO_4^{3+}$-P	0.10	4294	2.29	0.018	3.5	15.1

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Of the total variation in community composition between Sites 1, 3 and 4, 75% remained unexplained by these variables. Therefore, the subtle difference in trophic status between Sites 3 and 4 could not be explained by the measurements of environmental conditions in this study. As suggested earlier, it is likely that nutrient loading measurements, rather than nutrient concentrations may have provided a better indicator of differences in the availability of nutrients between these three sites.

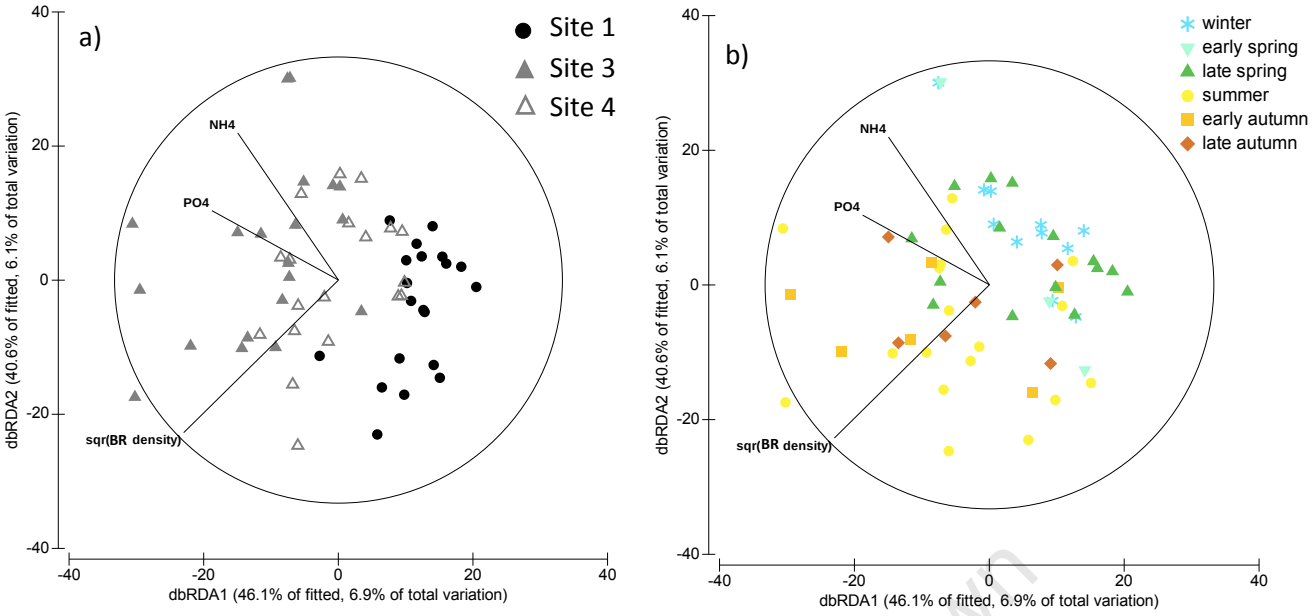


Figure 5.14 **dbRDA ordinations of monthly benthic algal abundances for individual taxa with vectors showing the Spearman correlation between environmental variables and the dbRDA axes for Sites 1, 3 and 4 together. The distribution of data represented by different a) sites and b) different seasons are presented.**

5.3.6 The effects of flow regulation

Unlike sites with natural flow regimes where flood disturbance was the primary predictor of temporal changes in periphyton community structure, a DistLM analysis shows that pH (variation explained: 16.2%) accounted for the largest proportion of observed variability in community composition at Site 2 (Table 5.13: marginal tests). Nevertheless, flood size (Q_{max}), length of the recovery period, depth and velocity as well as water temperature range (WT_{range}) each contribute more than 10% to the observed variability in community composition downstream of the dam (Table 5.13: marginal tests). Together pH (variation explained: 16.2%), length of the recovery period (variation explained: 10.7%), grazer density (variation explained: 10%) and turbidity (variation explained: 7.5%) accounted for 44.5 % of the total variation in community structure (Table 5.13: sequential tests). Despite nearly half of the variation explained by these environmental variables, the dbRDA ordination in Figure 5.15a shows no specific intra-annual pattern in community structure at Site 2. This was not surprising considering that flow conditions, particularly low flows, were considerably altered in certain months. The length of the recovery period correlated with dbRDA1 reflecting a continuum from pioneer communities following flood reset on the far right to climax communities on the left of the ordination (Figure 5.15). Although flow variability did not contribute significantly to the community structure at Site 2, it correlated strongly with dbRDA 2, as did depth (as an indication of low flow conditions)(Figure 5.15b). This axis seemed to separate communities with variable flow during the hot summer months from those that had constant elevated flows associated with irrigation releases during the summer, although dbRDA2, however, only accounted for a small proportion of the total variation (15.2%)(Figure 5.15).

Table 5.13 **Relationship between monthly periphyton community composition and environmental variables at Site 2 based on a Bray-Curtis dissimilarity matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.**

Environmental variable	Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	FLW peak (Q_{max})	-	5025	2.59	0.022	13.2	-
	Days since Class 2 flood	-	4926	2.53	0.025	13.0	-
	WT_{range}	-	4595	2.34	0.032	12.1	-
	Depth	-	5525	2.89	0.006	14.5	-
	Velocity	-	4125	2.07	0.041	10.9	-
Physico-chemcial	Turbidity	-	4532	2.30	0.037	11.9	-
	pH	-	6171	3.30	0.003	16.2	-
Nutrients	SiO_4	-	4359	2.20	0.037	11.5	-
	SEQUENTIAL TESTS [2]						
Physico-chemcial	+pH	0.11	6171	3.30	0.011	16.2	16.2
Flow	+Days since Class 2 flood	0.18	4069	2.35	0.022	10.7	26.9
Algal feeders	+Brusher density	0.24	3801	2.38	0.032	10.0	37.0
Nutrients	+Turbidity	0.29	2855	1.89	0.040	7.5	44.5

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

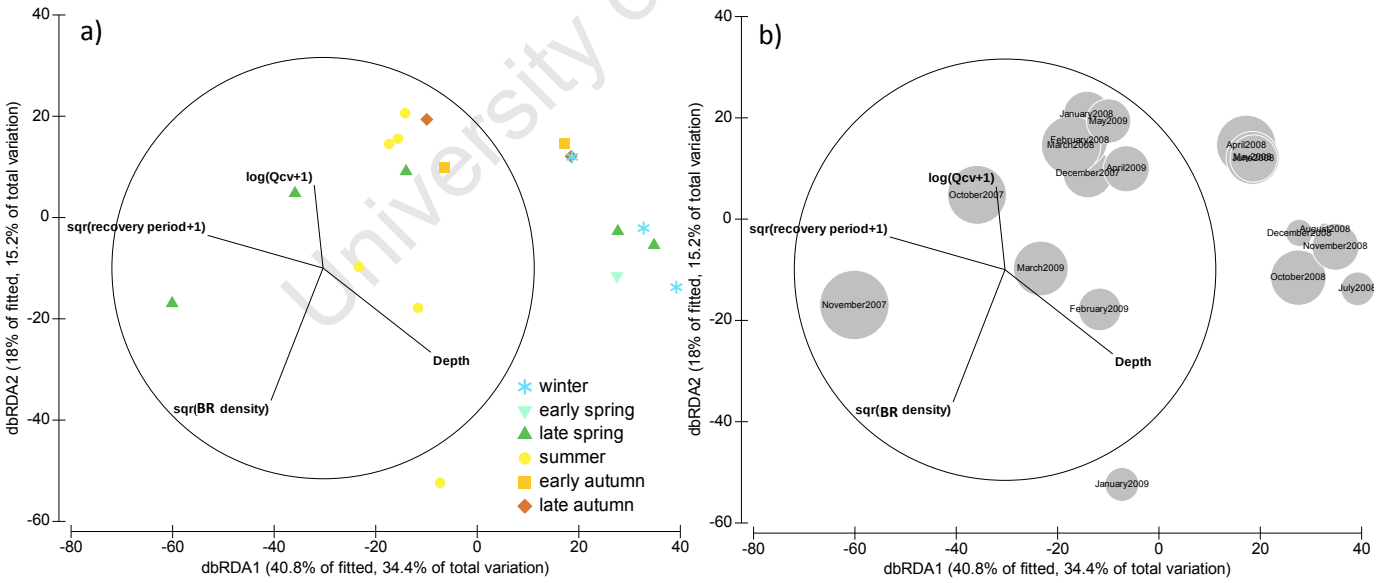


Figure 5.15 **dbRDA ordinations of monthly benthic algal abundances for individual taxa with vectors showing the Spearman correlation between environmental variables and the dbRDA axes for Site 2 only. The distribution of data represented by different a) seasons are presented while b) provides an indication of the relationship between algal composition and flow variability (Q_{cv}) – the larger the bubble, the greater the flow variability.**

Water quality, particularly turbidity was the key factor that accounted for differences in periphyton community structure between Sites 1 and 2, but this variable explained only 13.5% of the total variation (Table 5.14, Figure 5.16 a and b). Nevertheless, even when flows were similar upstream and downstream of the dam (e.g. September and October 2008), turbidity was always greater at Site 2, compared with Site 1 (Figure 5.16b). Similarly, $NH4^{+}$-N concentrations downstream of the dam were greater in all months relative to that upstream (Figure 5.16c). These data reflect changes in periphyton community structure downstream of the dam that were associated with water quality and nutrient availability. Although alteration in these environmental conditions was probably a consequence of damming, these results suggest that flow alteration *per se* was not a key factor driving periphyton community differences upstream and downstream of the dam. Nevertheless, depth and flood frequency too accounted for a portion of the variability in community structure between Sites 1 and 2 (Table 5.14). Despite spillage from the dam at the end of winter and early spring in 2008, which resulted in flood disturbance downstream of the dam, the frequency of flood events over the wet season was far greater upstream of the dam. Also, Site 2 was far deeper during most summer months relative to Site 1 because of artificially elevated low flow releases during this time.

Table 5.14 Relationship between monthly periphyton community composition and environmental variables at Sites 1 and 2 based on a Bray-Curtis dissimilarity matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Only significantly different ($p \leq 0.05$) relationships are shown.

Environmental variable	Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
	MARGINAL TESTS [1]						
Flow	Cv of discharge (Q_{cv})	-	4635.7	2.23	0.031	5.7	-
	Number of Floods ≥ Class 2 (Class 2_{freq})	-	5625.1	2.74	0.010	6.9	-
Physico-chemcial	Depth	-	8393.7	4.24	0.002	10.3	-
	Turbitdity	-	10982.0	5.76	0.001	13.5	-
Nutrients	NH_4^+-N	-	8398.7	4.25	0.001	10.3	-
	NO_2^--N	-	5225.6	2.53	0.014	6.4	-
						0.0	
	SEQUENTIAL TESTS [2]					0.0	
Physico-chemcial	+Turbitdity	0.11	10982	5.76	0.001	13.5	13.5
	+Depth	0.15	5151	2.83	0.009	6.3	19.8
Grazers	+Brushers density	0.19	4410	2.53	0.008	5.4	25.2

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

In essence, the DistLM of temporal changes in community composition at Site 2 highlighted the importance of flood disturbance as a reset mechanism for the development of periphyton communities over the wet season. Furthermore, communities during the stable summer period were separated by differences in their low flow conditions (Table 5.13, Figure 5.15). Flood frequency and elevated low flows accounted for the distinction in periphyton community structure between Sites 1 and 2 (Table 5.14, Figure 5.16). Nevertheless, alteration in water quality conditions and nutrient availability downstream of the dam may also be important environmental factors explaining differences in community composition between Sites 1 and 2.

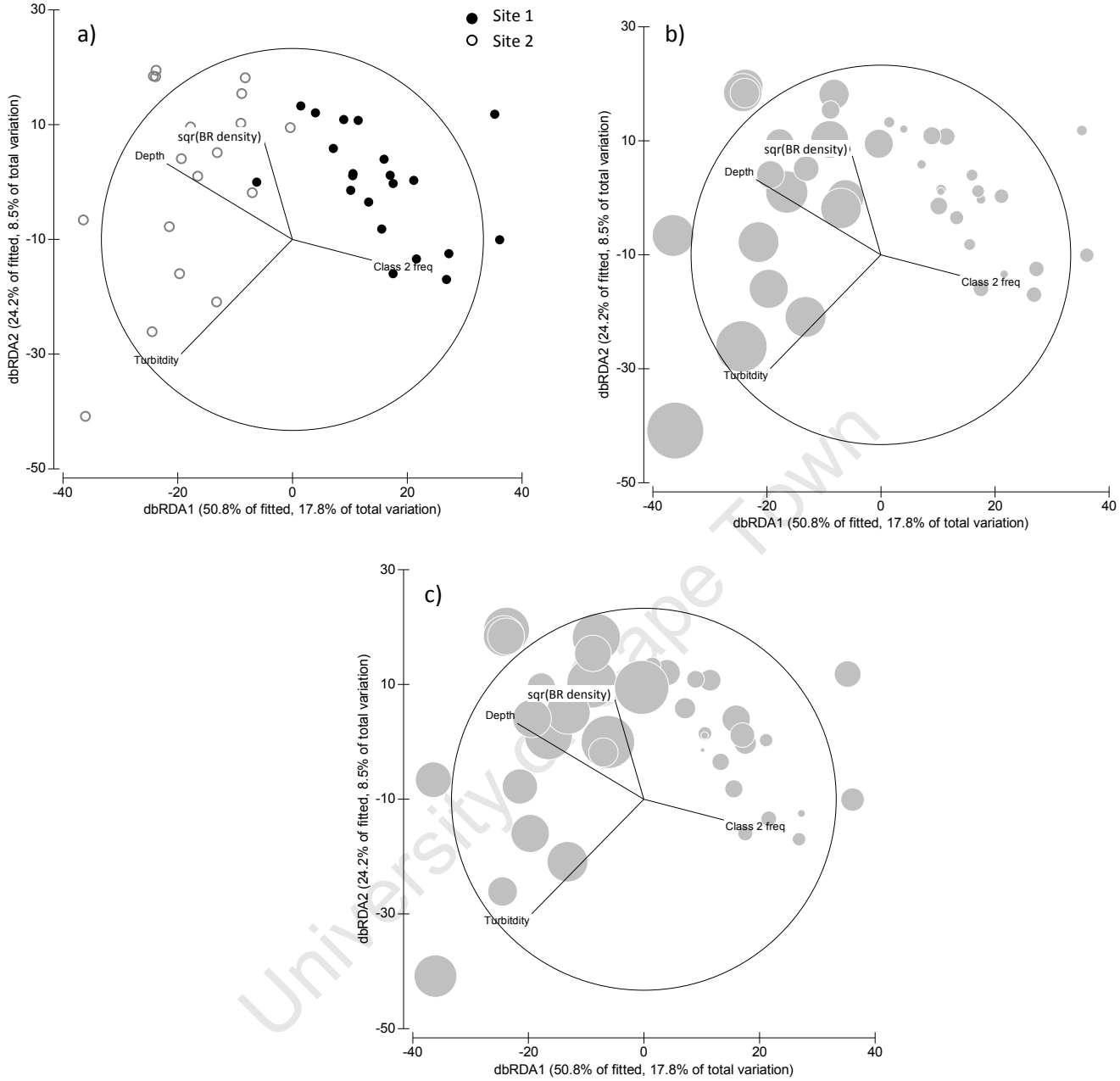


Figure 5.16 **dbRDA ordinations of monthly benthic algal abundances for individual taxa with vectors showing the Spearman correlation between environmental variables and the dbRDA axes for Sites 1 and 2. a) represents the distributon of data represented by different sites, while a visual representation of the relationship between algal composition and b) turbidity and c) NH_4^{3+}-N concentriatons are also provided - the larger the bubble, the greater the turbidity and NH_4^{3+}-N concentrations respectively.**

5.4 DISCUSSION

The main objective of this chapter was to analyze the relative importance of potential environmental drivers of periphyton biomass and community composition. Based on 21 months of data covering almost two annual cycles, the results from this study suggest that hydrological conditions, particularly flood disturbance are the primary regulators of periphyton biomass and community

composition in foothill rivers of the south-western Cape. These results confirm the hypothesis that periphyton communities were driven largely by hydrodynamics and support the findings of other such studies in temperate streams (Biggs and Close 1989, Biggs and Thomsen 1995, Bouletreau *et al.* 2006, Bouletreau *et al.* 2008).

In this study, disturbance events were defined as "episodic events that remove organisms at rates faster than rates of accrual or recruitment" (Larned 2010). Of the different DRIFT flood size categories considered in this study, those with within a Class 2 or larger correlated the best with temporal changes in Chl *a* biomass (Table 5.1) suggesting that floods within this category or larger are able to change the biomass of periphyton communities abruptly. Ractliffe (2009) found that significant decreases in invertebrate densities in the Molenaars and Berg Rivers were only observed at discharges that moved 33% or more of the bed, which translates into DRIFT Class 3 floods in these systems. While Class 3 floods define the threshold of disturbance for aquatic invertebrates, disturbance of the periphyton mat most likely occurs at a lower discharge than that defining bed movement because shear stress and sediment scour can remove considerable biomass, prior to movement of cobbles (Biggs and Thomsen 1995, Jowett and Biggs 1997, Francoeur and Biggs 2006, Webb *et al.* 2006). Nevertheless, defining the disturbance threshold for any given biotic community is complicated by the differential response of communities to floods with similar levels of physical force. Indeed, biotic communities can develop different responses to physical force, particularly resistance (i.e. the ability to withstand displacement by disturbance) and resilience (the ability to return to the pre-disturbance state) to such events (Peterson 1996, Ractliffe 2009).The relationship between flood size and resistance and resilience of periphyton communities are addressed in more detail in Chapter 7.

The length of the recovery period following the last disturbance event defines the length of the accrual cycle (Biggs 1996), which is essentially determined by the frequency of disturbance events over the wet season in Mediterranean climates. Under natural flow conditions, the length of the recovery period accounted for almost 75% of the total variation and was the single best predictor of Chl *a* biomass. Similarly, Biggs and Close (1989) found that 63.3% of the variance in Chl *a* biomass across nine gravel bed rivers in New Zealand could be explained by hydrological variables, with flood duration being the most important of these. Peak discharge, flood frequency and duration within the interval between sampling also accounted for a large percentage of the observed variation in Chl *a* biomass (Table 5.2) under natural flow and nutrient conditions.

Seasonally shifting variables such as water temperature and light availability also accounted for some variability in periphyton dynamics, while NO_2-N and NH_4^+-N concentrations too contributed substantially to explaining temporal variability. These variables, however, explained a far smaller proportion of the variation relative to flow variables, particularly in terms of Chl *a* biomass. This suggests that temporal dynamics in periphyton communities were controlled primarily by disturbance with the secondary effects of seasonal determinants and nutrients affecting the trajectory of change between flood events. Indeed, these results support the findings in Chapter 4 which showed that temporal shifts in periphyton communities are not confined to seasons but occur

over shorter time intervals. Similarly, McCormick and Stevenson (1991), suggested that generation times of benthic algae were much shorter than seasonal periodicities in the abiotic environment and therefore seasonally defined variables may not be the primary controllers of periphyton dynamics in the short term. Also, temporal shifts in periphyton communities did not always coincide with seasonal boundaries, particularly those defining autumn and spring (Chapter 4). Although flood events in the Berg and Molenaars Rivers generally occur in the winter, they were not confined to this period (Chapter 3, Figures 3.12 to 3.14) and often occur less predictably in both spring and autumn that are considered transitional between winter and summer (Ractliffe 2009). Considering the primary role of flow, and secondary role of seasons, it is not surprising that periphyton communities did not always shift predictably with seasonal changes, particularly spring and autumn.

Even under enriched conditions, the length of the recovery period was the best predictor of Chl *a* biomass over time but the proportion of the variation explained was considerably smaller (45%). Under these conditions, it is likely that enrichment accelerated the growth of algae during inter-flood periods, whereas biomass accrual would have been far slower under oligotrophic conditions. The relative importance of nutrients therefore increases and that of flow variables decreases with enrichment because temporal changes in the availability of resources and their effects on periphyton growth between flood events become more important (Guasch *et al.* 1995, Francoeur *et al.* 1999, Carr *et al.* 2005, Stevenson *et al.* 2006). In Chapter 4, it was suggested that nutrient availability was an important 'bottom up' determinant that resulted in peak biomass differences between unenriched and enriched reaches. The relationship between temporal biomass dynamics and ambient nutrient concentrations in streams is, however, complex and does not always reflect nutrient availability (Welch *et al.* 1988, Biggs and Close 1989, Biggs 2000, Stevenson *et al.* 2006, Larned 2010). Nevertheless, peak Chl *a* biomass in two growing seasons at Sites 1, 3 and 4 correlated significantly (R^2=0.97, p≤ 0.05) with the mean NH_4^{3+}-N concentration over the previous growing season. Similarly, Biggs and Close (1989) found that peak Chl *a* biomass recorded on 26 sampling occasions correlated significantly with mean accrual cycle DRP. The limited spatial extent of the present assessment (i.e. only three unregulated sites), however, precluded the possibility of using this variable in the DistLM analysis. It does however provide a strong case for exploring this relationship further by collecting peak biomass data over a broader spatial scale in future assessments. Nevertheless, establishing links between periphyton and nutrients for this study was not possible. Grazer density seemed to provide a good proxy for trophic status, however, and was the single best predictor of differences between sites that differed in levels of enrichment. Comparisons between sites with known differences in enrichment therefore provided evidence to suggest that nutrient availability was an important driver of periphyton dynamics during inter-flood periods but the importance of nutrient availability could not be quantified. Despite the effects of enrichment however, periphyton communities appear to be controlled primarily from the top down by flood disturbance.

Flood disturbance, however, may not always account for the top down-control of periphyton over an annual cycle. Under conditions favourable for growth following the last disturbance events in spring, one might expect that periphyton would accrue rapidly over the late spring and early summer. In

both oligotrophic and enriched sites, Chl *a* biomass maintained a steady-state low biomass that did not show a sizeable increase until February in most cases. The relationship between seasonal patterns in Chl *a* biomass and deposit feeder densities in this study suggest that high densities of deposit feeders during spring and early summer may suppress algal growth during this time. Indeed, following the last wet season floods, grazing activity can become the dominant factor controlling patterns of accrual (Feminella and Hawkins 1995, Steinman 1996). In a meta-analysis of grazer control on periphyton biomass using data from 865 experimental studies world-wide, Hillebrand (2009) concluded that grazers exert an exceptionally strong negative impact on stream periphyton, removing an average of 70% of the standing stock. Also, Stevenson *et al.* (2006) argued that disturbance regimes with high predictability support the development of high grazer densities that may constrain periphyton growth during certain periods. Despite the tentative links between Chl *a* biomass and grazing pressure in this study, it is plausible that grazing, particularly by baetids may be an important top-down control on periphyton during late spring and early summer in south-western Cape rivers.

Conditions that largely define seasonal boundaries, particularly solar radiation and average daily temperature, were not altered by the dam and yet no clear seasonal patterns in periphyton community structure or biomass were evident at Site 2. The role of these seasonal variables in affecting temporal dynamics in periphyton communities downstream of the dam, however, may have been overridden by changes in water quality at Site 2, particularly pH and turbidity. Besides water quality, base flow condition was also identified as a key factor that explained upstream and downstream differences in periphyton communities in this assessment. In particular, changes in flow variability and median discharge as a result of unseasonal releases from the dam for irrigation during summer explained the altered state of the periphyton community downstream of the dam.

In summary, this study provides evidence that flow variables, particularly flood disturbance are the primary controllers of periphyton communities in south-western Cape rivers, together with nutrients and seasonal changes in light and temperature affecting the growth and development of communities between flood events. There is some evidence to suggest that grazing too may affect the pattern of accrual over the late spring and early summer period. These findings support those of similar studies on temperate rivers elsewhere but the complex interaction between these variables and the temporal shift in the relative importance of each complicates the assessment of causality. This study was conducted over a limited spatial range and thus its applicability is spatially limited. Nevertheless, it provides a basis for more focused assessments of the key factors that may be important drivers of periphyton dynamics in foothill rivers of the south-western Cape.

CHAPTER 6 PATTERNS IN PERIPHYTON COMMUNITIES BETWEEN HYDRAULIC BIOTOPES

6.1 INTRODUCTION

Biotic patterns and processes in riverine ecosystems are not only temporally variable as discussed in Chapters 4 and 5, but vary considerably over a wide range of spatial scales (e.g. Palmer and Poff 1997a, b, Winemiller *et al.* 2010). Within the hierarchical organisation of rivers, hydraulic biotopes define morphological features at a micro-scale that are governed largely by flow (velocity), depth and substratum characteristics (Wadeson and Rowntree 1998). Differences in periphyton community structure between hydraulic biotopes reflect spatial differences in shear stress, substratum size and disturbance history, as well as nutrient mass transfer through the algal mat (Tett *et al.* 1978; Biggs 1996; Biggs *et al.* 1998; Matthaei *et al.* 2003). In rivers where cobbles and boulders are the dominant substratum type, it is believed that micro-scale patchiness in periphyton communities is driven largely by spatial heterogeneity in water velocities across the river bed (Horner *et al.* 1990; Peterson and Stevenson 1990; Biggs 1996; Biggs *et al.* 1998).

Both shear stress and nutrient mass transfer are affected by local velocity, and work antagonistically to create variability in periphyton community structure (Borchardt 1996; Stevenson 1996). Biggs *et al.* (1998) described these two counteracting processes as the 'subsidy-stress' response. Essentially, increasing velocity promotes nutrient uptake by the periphyton mat because of increasing turbulent flux of nutrients through the periphyton mat and a reduction in the thickness of the boundary layer (Dodds 1989, Biggs and Hickey 1994, Biggs and Stokseth 1996), thus 'subsiding' periphyton accrual. Conversely, velocity increases lead to shear stress levels that reduce colonisation by new cells or increase loss through sloughing (Biggs and Thomsen 1995). Evidently, the optimum current for benthic algae depends on the interaction of flow conditions with nutrient enrichment (Horner and Welch 1981), and growth form (Horner *et al.* 1983; Horner *et al.* 1990; Biggs *et al.* 1998). A review of the literature pertaining to the subsidy-stress response and its interaction with other variables is provided in Section 1.4 of this study. Considering the variation in local velocities within a river reach and the subsidy-stress response of periphyton communities, it is not surprising that periphyton communities differ between hydraulic biotopes within similar river reaches (Biggs and Gerbeaux 1993; Stevenson 1996). The difference between these habitats is however dependent on the level of enrichment (Biggs 1996). Periphyton biomass in enriched streams tends to be higher in biotopes with lower velocity such as pools and runs and lower in riffles because biomass is restricted by higher shear stress (Peterson and Stevenson 1990). By contrast, periphyton biomass in oligotrophic streams is generally highest at velocities of characteristic of runs (0.1 to 0.2 ms^{-1}) (Biggs and Gerbeaux 1993; Biggs and Hickey 1994; Biggs and Stokseth 1996) because accrual is limited by lower nutrient mass transfer in pools and by increased shear stress at higher velocities in riffles (Stevenson 1996).

Differences in periphyton communities between hydraulic habitats may only differ under different stages of accrual. Biggs and Stokseth (1996), for example, showed that no differences in periphyton

biomass were evident across a range of flow habitats during the early stages of colonisation but distinct differences became apparent during later stages of development. It is likely therefore that distinctions in periphyton communities between different hydraulic habitats will be greater during summer, following an extended period of flow stability, compared with spring, immediately following the wet season floods.

The primary objective of this study is to develop an understanding of differences in periphyton community structure between different hydraulic biotopes within foothill reaches of south-western Cape rivers. Similar-sized cobbles were present across a range of flow (velocity) and depth categories within the foothill reaches in these and therefore biotope differences should be governed largely by flow (velocity) and depth, rather than substratum characteristics. Three hypotheses are addressed in this study in order to understand spatial heterogeneity in periphyton communities within a river reach under different levels of enrichment and at different stages of accrual as follows:

1) During the late summer following a prolonged period of stability:

    a. Periphyton biomass will be greatest in runs compared with both riffles and slack-waters in nutrient limited streams (Figure 6.1);

    b. Periphyton biomass will be greatest in slack-waters compared with both riffles and runs in moderately enriched streams (Figure 6.1).

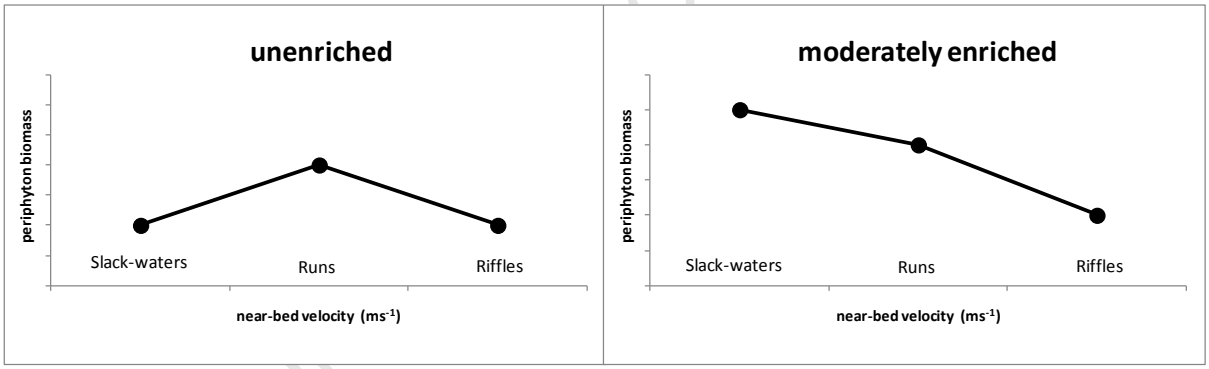


Figure 6.1 Schematic representation of expected differences in periphyton biomass between different hydraulic biomass during late summer

2) During early spring following the winter disturbance period:

    a. There will be no differences in periphyton biomass between runs, riffles and slack-waters in nutrient limited streams (Figure 6.2);

    b. Periphyton biomass will be greater in slack-waters compared to riffles and possibly runs in moderately enriched streams (Figure 6.2).

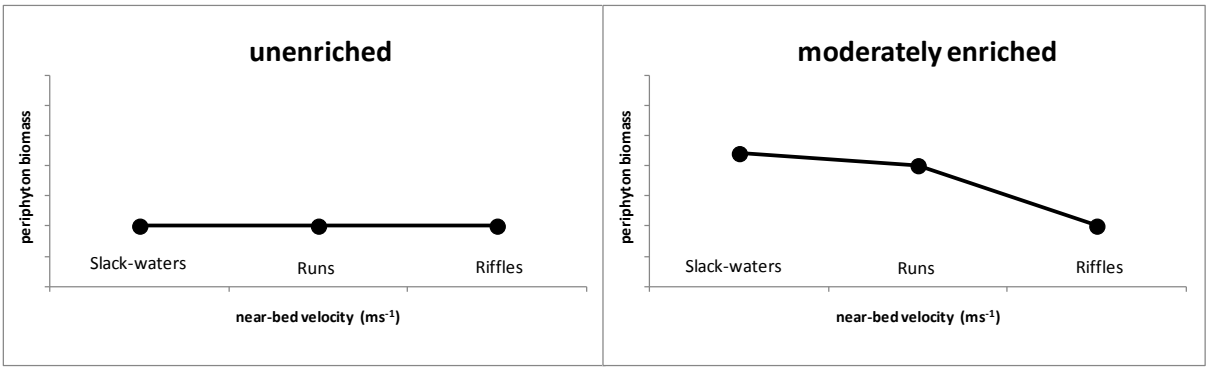


Figure 6.2 Schematic representation of expected differences in periphyton biomass between different hydraulic biomass during spring

3) Periphyton community composition will differ significantly between biotopes at both sites during the late summer low-flow period whereas few, if any differences in community composition will be evident during the early spring period

6.2 METHODS

6.2.1 Experimental design and sampling strategy

In order to test differences in periphyton biomass and community structure across a nutrient gradient, Site 1, representing oligotrophic conditions and Site 3, representing moderately enriched nutrient conditions, were selected. These two sites are described in Chapter 2, Section 2.2.

Seasonal differences in periphyton community structure and biomass over a range of hydraulic biotopes were investigated by sampling in late summer (March 2009), and early spring (October 2009). Late summer represents a time towards the end the growing season with a long recovery period following the last flood disturbance event. Early spring represents a period at the beginning of the growing season when the recovery period following disturbance is far shorter than that at the end of the growing season. Although the spring biotope sampling was originally scheduled for September 2009, an analysis of the time-series temperature data presented in Chapter 4, as well as the late rains experienced that wet season meant that base flows did not permit access to these sites until late October 2009. Spring sampling was therefore shifted from September 2009 to late October 2009.

Three hydraulic biotopes, *viz.* riffles, runs and slack-waters were identified *a priori* for the collection of samples representing each biotope and thus a stratified random sampling design was employed. These three hydraulic biotopes were indentified using the definitions given in Table 6.1 according to the flow type and substrata categories described in Tables 6.2 and 6.3. During each of the two sampling occasions, 10 replicates (represented by individual stones) were collected from each of the three biotopes. Therefore 30 samples were collected at each site in March 2009 and again in October 2009. Besides the collection of periphyton samples, invertebrates representative of each replicate were also collected in each biotope. Physico-chemical data represented by three replicates

within each biotope were also collected in the field. Sampling procedures and analytical methods are described fully in Chapter 2.

Table 6.1 Definitions of hydraulically defined biotopes using visually assessed flow type and substratum conditions defined in Tables 6.2 and 6.3 respectively (from King and Schael 2001). Each biotope was rated from 1 to 3 ranging from slow flowing to fast flowing biotopes.

Hydraulic biotope	Definition	Typical flow types	Typical substatum	rating
slack water	slow flowing margins over a range of substrata which are hydraulically linked to the the main flow. Flow is often not visually perceptible	NF, BPF	sand - bedrock (any substrata)	1
run	areas of unbroken, visibly flowing water over a range of substrata that are often transitional between riffles and pools	RSF, SBT	sand - bedrock (any substrata)	2
riffle	areas of broken water which usually occur over potentially mobile, coarse alluvial substata such as cobbles and pebbles	FRF, SRF, BSW	pebbles - large cobble	3

Table 6.2 Categories of visually distinct flow types present in this study (from King and Schael 2001). Each flow type was rated from 0 to 6 ranging from no flow to fast flowing broken standing waves.

Flow type		Definition	rating
no flow	NF	no water movement	0
barely perceptible flow	BPF	smooth surface flow; only perceptible through the movement of floating objects	1
rippled surface flow	RSF	the water surface has regular smooth disturbances which form low transverse ripples across the direction of flow	2
slow riffle flow	SRF	very shallow, slower, flickering flow, still covering most of the substrata	3
fast riffle flow	FRF	verly shallow, fast, flickering flow, still covering most of the substrata	4
smooth boundary turbulent flow	SBT	the water surface remains smooth; medium to slow streaming flow takes place throughout the water profile, turbulence can be seen as the upward ovement of fine suspended particles.	5
broken standing waves	BSW	standing waves present which break at the crest	6

Table 6.3 Categories of substrata present in this study (from King and Schael 2001, based on the Wentworth scale). Each substratum category was rated from 1 to 4 with 1 representing the largest and 4 representing the smallest particle size. All replicate samples were either large or small cobble.

Substratum category		size range (mm)	rating
boulder	B	>256	1
large cobble	LC	128-256	2
small cobble	SC	64-128	3
pebble	P	16-64	4

6.2.2 Analytical methods

Two-way ANOVAs between sites (representing enrichment differences) were used to detect periphyton biomass differences between the three hydraulic biotopes. ANOVAs were undertaken separately for data collected in March 2009 and October 2009, thus representing seasonal differences in the relationship between enrichment and biotopes. 'Sites' and 'biotope' were therefore included as fixed factors in the statistical analysis of periphyton biomass for each of the two sampling dates. Tukey tests were used for post-hoc pair-wise comparisons between multiple groups. Chl *a* concentrations, as the best available indicator of periphyton biomass, were log (x+1) transformed prior to analysis to meet the assumptions of normality and homoscedasticity necessary for employing parametric statistical analyses. These analyses were undertaken in STATISTICA, version 10 (2011).

Hierarchical clustering and MDS analyses based on Bray-Curtis similarity matrices were used to explore differences in periphyton assemblage structure between sites that differed in their availability of nutrients, and seasons that differed in the time since the last disturbance flood event. Two-way crossed PERMANOVA tests were conducted to compare the assemblage structure between Sites 1 and 3 and among biotopes, with both 'site' and 'biotope' as fixed factors in the analyses. SIMPER analyses were undertaken to determine which periphyton taxa were most responsible for differences between biotopes within each site and during each sampling occasion separately.

Relations among potential predictor variables likely to explain spatial variation in periphyton characteristics at a local scale were determined using Principle Components Analysis (PCA) in PRIMER v6 (Clarke and Gorley 2006). Measured variables were those describing habitat characteristics and included depth of each stone from the bed to the water surface, near-bed velocity, and the density of invertebrate grazers separated into three functional feeding groups (i.e. scrapers, brushers and deposit feeders). Semi-quantitative predictors included biotope, which ranked according to typical flow speed (Table 6.1), flow type, which was ranked from slow-shallow flow to fast flow (Table 6.2) and substratum size, which was ranked from large to small (Table 6.3). Ambient nutrient concentrations could not be used to separate hydraulic biotopes because no

replicate data were collected per biotope. These data, however, were used in the set of habitat variables to separate sites during difference seasons. All data were log (x+1) transformed where necessary and Pearson's correlations were used to detect redundant predictors. Parameters with ρ > 0.9 were considered strongly correlated and thus only one of a number of highly correlated predictor variables that contain biologically and statistically redundant information was retained and normalised prior to PCA analysis. The set of relevant predictor variables was further refined by eliminating those with eigenvalues (i.e. explained variances) between -0.4 and 0.4 in the first three axes of the PCA.

In the case of both univariate (i.e. biomass) and multivariate (i.e. assemblage structure) data sets, distance based linear models (DistLM) and redundancy analysis (dbRDA) were used to evaluate the relationship between either Chl *a* biomass or assemblage structure and a set of predictor variables. These tools are described in more detail in Chapter 5, Section 5.2. Although nutrients could not be included in the set of environmental predictors, the relative affect of nutrient availability was implicit in the selection of study sites, which represented different levels of enrichment. These analyses were performed using PRIMER 6 (Clarke and Gorley 2006) with the additional add-on package PERMANOVA (Anderson *et al.* 2008).

Once potentially important predictor variables were identified, the relationship between these variables and Chl *a* biomass was further explored using either simple linear or non-linear regression analyses in STATISTICA, version 10 (2011).

6.3 RESULTS

6.3.1 Biophysical characterisation of sites and hydraulic habitats

PCA and correlation analysis were used to identify the best set of measured habitat characteristics that separated hydraulic biotopes at Sites 1 and 3. Besides abiotic factors such as depth, near-bed velocity, flow type, substratum size and nutrients (for site comparisons only), habitat characterisation included grazer abundances separated into scraper, brusher and deposit feeder densities because of their potential affect on periphyton biomass accrual.

During late summer (March 2009) a total of 90% of the overall variation between sites and among hydraulic biotopes was explained by the first three axes of the PCA (Table 6.4, Figure 6.3). Table 6.4 shows that 50% of the variation was explained by PC1 alone, with 27 % explained by PC2 and 13% explained by PC3. PC1 therefore explained most of the overall variation in predictor variables that separated Site 1 from Site 3 (Table 6.4, Figure 6.3a). A greater density of deposit feeders and higher nutrient concentrations at Site 3 relative to Site 1 were the main factors driving the variation explained by PC1. PC2 (27%) was driven largely by differences in near-bed velocity, as well as scraper and brusher densities and explained differences among hydraulic biotopes (Table 6.4, Figure 6.3b).

During spring (October 2009), PC1 also accounted for 50% of the overall variation in habitat characteristics between sites and hydraulic biotopes (Table 6.5, Figure 6.4). Unlike March 2009,

however, biophysical differences between hydraulic biotopes were greater than site differences as indicated by the separation of biotopes on PC1 (Table 6.5 and Figure 6.4b. PC1 was driven by differences in scraper densities, near-bed velocity and flow type (Table 6.5). PC2 accounted for 24% of the overall variation, which separated Sites 1 and 3 (Figure 6.4a, Table 6.5). Substratum size and NH_4^{3+}-N were the key factors responsible for the variation explained by PC2. In other words, the majority of variation in biophysical or habitat characteristics in late summer was given by site differences, whereas differences among hydraulic biotopes were greater than site differences during spring 2009.

Despite the influence of substratum size in the data set, this factor explained site, rather than biotope differences during October 2009 and did not even feature as an important factor during March 2009. It can therefore be assumed that substratum size, as one of the defining criteria for categorising hydraulic biotopes was negligible during this study. Also, depth did not contribute to the explained variability during either sampling occasion as an important discriminator between biotopes or sites. Near-bed velocity and flow type therefore appear to be the key factors driving physical differences between biotopes in this study.

Nevertheless, velocity and depth measurements taken at the position of each stone sample provided a means of describing biotope differences based on hydraulic criteria specifically for this study. A plot of depth and velocity for each replicate sampled (Figure 6.5) provided an indication of the spread and overlap of velocity and depth measurements characteristic of each hydraulic biotope. In this study, riffles were generally shallow (0.04 m to 0.32 m) and fast flowing, although a wide range in velocities was recorded (0.2 to 1.7 ms^{-1}). Runs were generally slow flowing but ranged considerably in depth (0.11 m to 0.60 m), while slack-waters exhibited either no flow or very slow flow (<0.12 ms^{-1}) and were generally shallow (0.06 m to 0.28 m s^{-1}). It is noteworthy that there is considerable overlap in depth and velocity measurements between these three hydraulic biotopes.

Table 6.4 PCA results for Sites 1 and 3 in March 2009.

	Eigenvectors		
% variation explained	50.2%	26.90%	13.00%
Variable	*PC1*	*PC2*	*PC3*
deposit feeder density	**-0.52**	0.11	**0.70**
scraper density	-0.30	**-0.56**	0.14
brusher density	-0.25	**0.53**	0.20
velocity	-0.12	**-0.55**	0.18
NO_3^--N	**-0.50**	0.26	-0.35
TP	**-0.56**	-0.15	**-0.54**

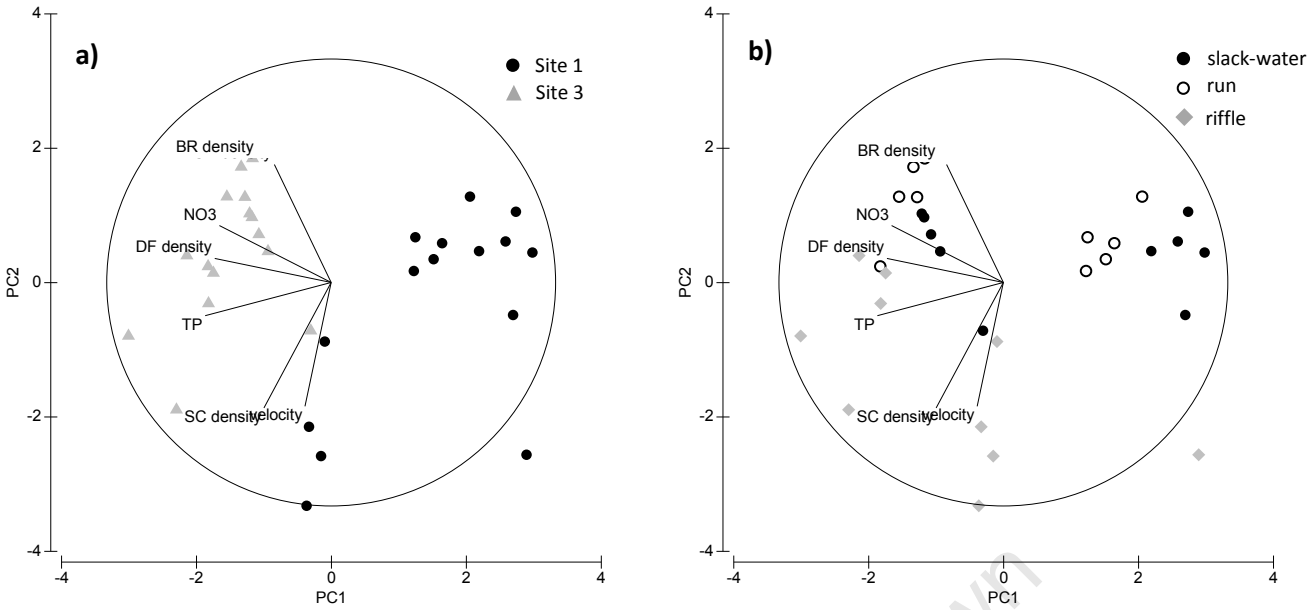


Figure 6.3 **PCA ordination of samples collected during March 2009 at both Sites 1 and 3 showing both (a) site and (b) biotope differences based on environmental variables.**

Table 6.5 **PCA Results for Sites 1 and 3 in October 2009.**

	Eigenvectors		
% variation explained	50.1%	23.80%	15.70%
Variable	*PC1*	*PC2*	*PC3*
scraper density	**-0.50**	0.03	0.00
near-bed velocity	**-0.65**	-0.07	0.10
flow type	**-0.55**	0.03	0.17
substratum	-0.13	**0.62**	**-0.77**
NH_4^{3+}-N	0.09	**0.78**	**0.61**

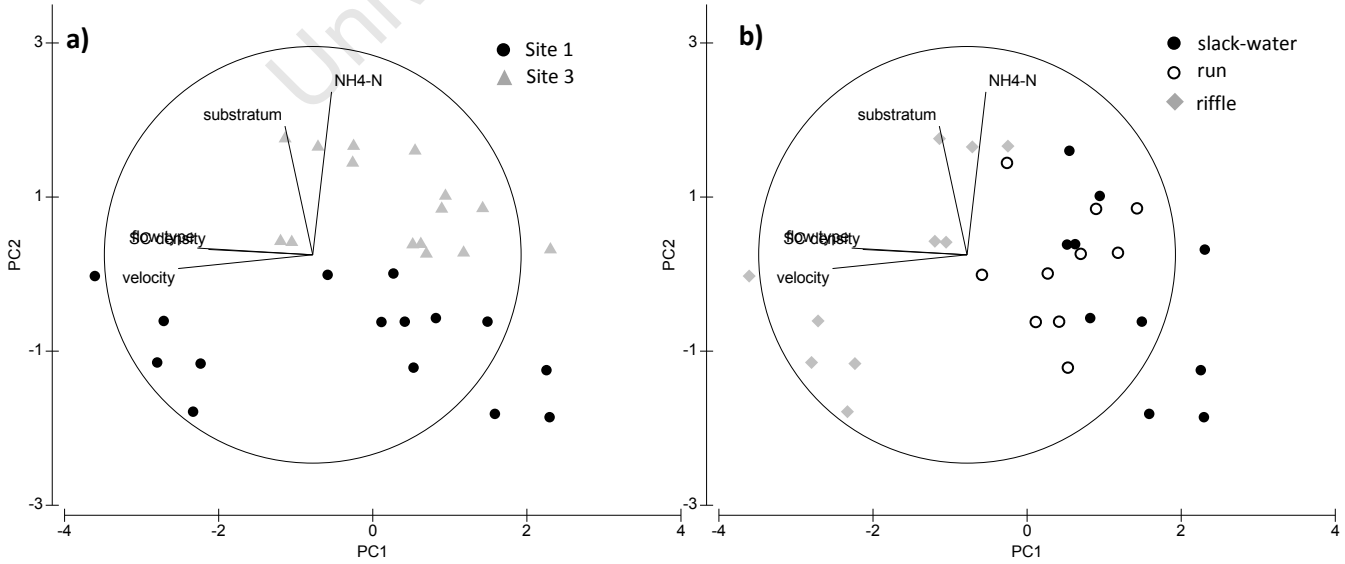


Figure 6.4 **PCA ordination of samples collected during October 2009 at both sites 1 and 3 showing both (a) site and (b) biotope differences based on environmental variables**

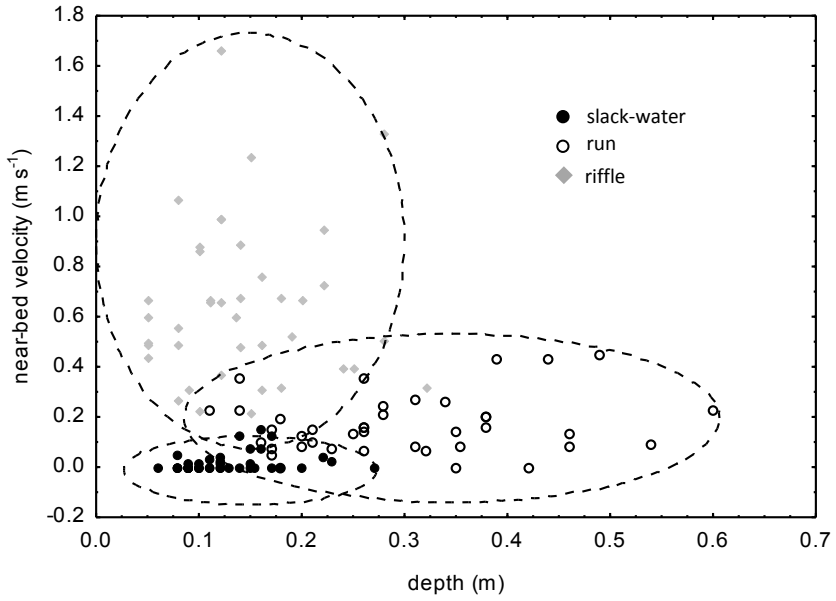


Figure 6.5 **Scatter plot of depth and near-bed velocity measured at each stone for the biotope study and characterised by the visual categorisation of samples into hydraulic habitats.**

6.3.2 Differences in periphyton biomass between biotopes under different levels of nutrient availability and different seasons

No significant site or biotope differences in Chl *a* biomass were observed during March 2009 (Table 6.6). This suggests that Chl *a* biomass was not particularly patchy across the range of habitats at either Site 1 on the Berg River or Site 3 on the Molenaars River late in the accrual cycle when flows were at a minimum over an annual cycle. By contrast, significant biotope differences in Chl *a* biomass were observed during the spring (October 2009)(Table6.7). The interaction between sites and biotopes during October 2009 was also significantly different, and a *post-hoc* pair-wise comparison between biotopes was undertaken within each site separately. The results of the Tukey tests (Table 6.7) show that riffles and slack-waters were significantly different from each other at both Site 1 ($p=0.003$) and Site 3 ($p<0.001$) during October 2009. While no significant difference was observed between runs and riffles at Site 1 ($p=0.85$) during October 2009, a significant difference between these two biotopes was observe at Site 3 ($p<0.001$). Figure 6.6 shows clearly that Chl *a* biomass increased progressively from slow flowing slack-waters to faster riffles at Site 1, while the opposite trend between biotopes was observed at Site 3.

Table 6.6 **A two-way ANOVA of Chl *a* biomass with site and biotope during March 2009, representing summer conditions.**

	SS	Df	MS	F	p
site	0.03	1	0.03	0.97	0.33
biotope	0.16	2	0.08	2.37	0.10
site x biotope	0.01	2	0.01	0.17	0.84
Error	1.71	52	0.03		

Table 6.7 **A two-way ANOVA of Chl *a* biomass with site and biotope during October 2009, representing spring conditions. Post-hoc comparisons of differences between biotopes representing each site are also given for those that are significantly different. Significant values at p ≤ 0.05 are given in *bold italics*.**

	SS	Df	MS	F	p
site	0.001	1	0.001	0.16	0.693
biotope	0.118	2	0.059	6.42	***0.003***
site x biotope	0.608	2	0.304	33.02	***<0.001***
Error	0.497	54	0.009		

Post-hoc tukey tests

Site 1	Run	Riffle	*Site 3*	Run	Riffle
Run			Run		
Riffle	0.850		Riffle	***<0.001***	
SW	*0.072*	***0.003***	SW	0.816	***<0.001***

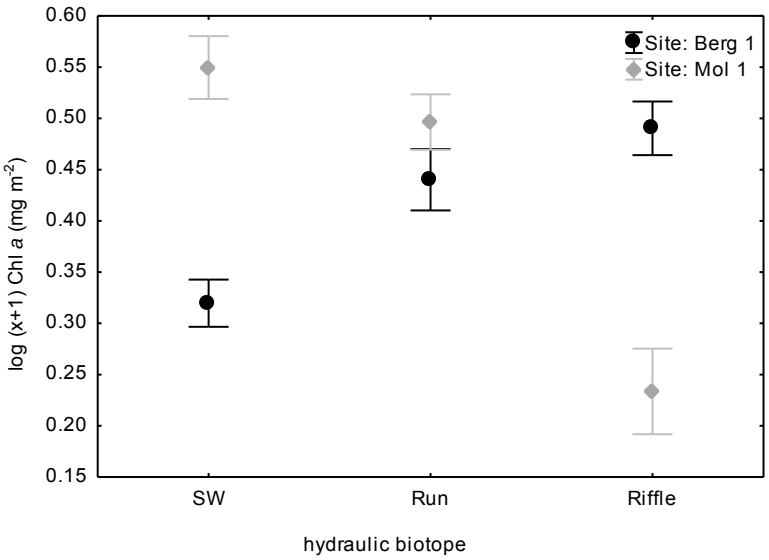


Figure 6.6 **Mean (± 1 SE) Chl *a* biomass log (x+1) transformed (mg m^{-2}) in each biotope at Sites 1 and 3 during October 2009.**

6.3.3 Linking periphyton biomass differences with biophysical characteristics

DistLM was used to investigate the relationship between Chl *a* biomass and a number of biophysical predictor variables potentially explaining differences between hydraulic biotopes under different enrichment conditions and seasons. These included velocity, depth and grazing pressure represented by grazer, scraper and deposit feeder densities. Semi-quantitative predictor variables such as biotope, flow type and substratum (Section 6.3.1) were also included in the analysis.

6.3.3.1 Late summer (March 2009)

Whereas the ANOVA did not detect any significant differences in Chl *a* biomass between biotopes during March 2009 under both enriched (Site 3) and unenriched (Site 1) conditions (Table 6.6), the

DistLM analyses (Tables 6.8 and 6.9) showed that some of the spatial variation across both sites could be explained by these variables.

Table 6.8 **Relationship between Chl *a* biomass and environmental variables at Site 3 in March 2009 based on a Euclidean Distance matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at p ≤ 0.05 is given in *bold italics* and at p≤ 0.1 in *italics* only.**

Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
depth	-	***0.21***	***4.82***	***0.05***	***27.0***	-
near-bed velocity	-	*0.16*	*3.23*	*0.09*	*19.9*	-
substratum	-	0.08	1.52	0.23	*10.4*	-
brusher density	-	0.08	1.38	0.28	9.6	-
scraper density	-	0.03	0.51	0.50	3.7	-
flow type	-	0.01	0.25	0.63	1.9	-
biotope	-	0.01	0.13	0.72	1.0	-
deposit feeder density	-	0.00	0.00	0.96	0.0	-
SEQUENTIAL TESTS [2]						
+depth	***0.21***	***0.21***	***4.82***	***0.05***	***27.0***	***27.0***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Depth alone explained nearly a third (27 %) of the variation at Site 3, and correlated positively with Chl *a* biomass (Figure 6.7) as the single best predictor of Chl *a* biomass at Site 3 at the end of the growing season. Velocity explained a fifth (20 %) of the variability but the significance of this result is questionable (p=0.09; Table 6.8: marginal tests). The sequential tests (Table 6.8: sequential tests) showed that no co-variates contributed to explaining the observed variation in and thus depth was the only factor that could reliable explain variability in Chl *a* biomass across the river bed under moderately enriched conditions in March 2009 . As expected, however, biotope did not contribute significantly to the observed spatial variation described in the DistLM (Table 6.8). In other words, depth (and possibly near-bed velocity) may have explained within-reach spatial variation in Chl *a* biomass during late March 2009 at Site 3, but these characteristics were not represented by specific hydraulic biotopes.

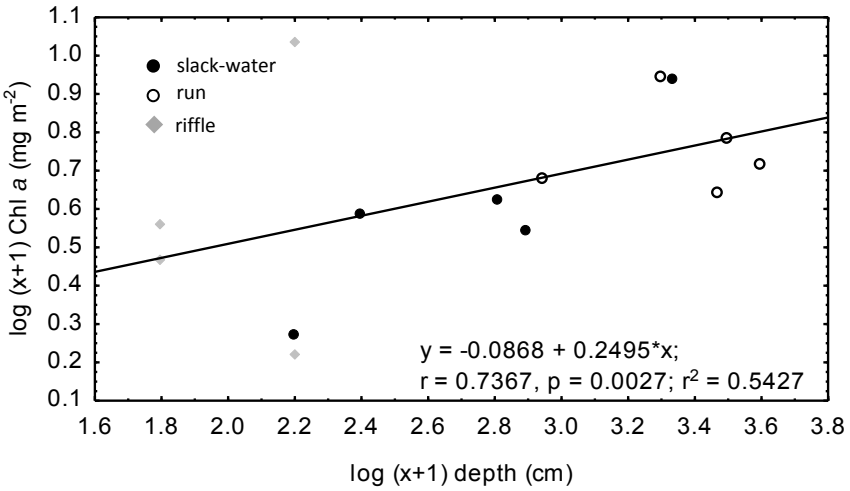


Figure 6.7 **Relationship between Chl *a* biomass and water depth at Site 3 (Molenaars River) during March 2009. Orange dots = riffles, blue dots = slack-waters, green dots = runs.**

Table 6.9 **Relationship between Chl *a* biomass and environmental variables at Site 1 in March 2009 based on a Euclidean Distance matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at p ≤ 0.05 is given in *bold italics* and at p≤ 0.1 in *italics* only.**

Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
scraper density	-	***0.07***	***7.40***	***0.02***	***38.1***	-
deposit feeder density	-	***0.07***	***7.08***	***0.02***	***37.1***	-
velocity	-	***0.07***	***7.07***	***0.02***	***37.1***	-
biotope	-	***0.06***	***6.98***	***0.02***	***36.8***	-
flow type	-	***0.06***	***6.47***	***0.03***	***35.0***	-
brusher density	-	0.03	2.17	0.17	15.3	-
depth	-	0.02	1.26	0.29	9.50	-
substratum		0.00	0.02	0.91	0.10	
SEQUENTIAL TESTS [2]						
+scraper density	***0.33***	***0.07***	***7.40***	***0.02***	***38.2***	***38.2***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

By contrast, biotopes explained 36.8% of the variability in Chl *a* biomass across the river bed at Site 1 during March 2009 (Table 6.9: marginal tests). Scraper densities (38.1%), deposit feeder densities (37.1%), velocity (37.1%) and flow type (35%) also individually explained some of the spatial variation observed at Site 1 in March 2009 (Table 6.9: marginal tests). Nevertheless, scraper density was the single best predictor of variation overall with no contribution from co-variates, and thus only 38.2% of the total variation was accounted for by measured variables (Table 6.7: sequential

test). Figure 6.8 shows a significant negative relationship between Chl *a* biomass and both scraper densities and deposit feeder densities. In other words, lower Chl *a* biomass in riffles may have been a consequence of higher grazing pressure by both scrapers (mostly Chironomidae) and deposit feeders (mostly Baetidae) in these biotopes under nutrient poor conditions at the end of the growing season.

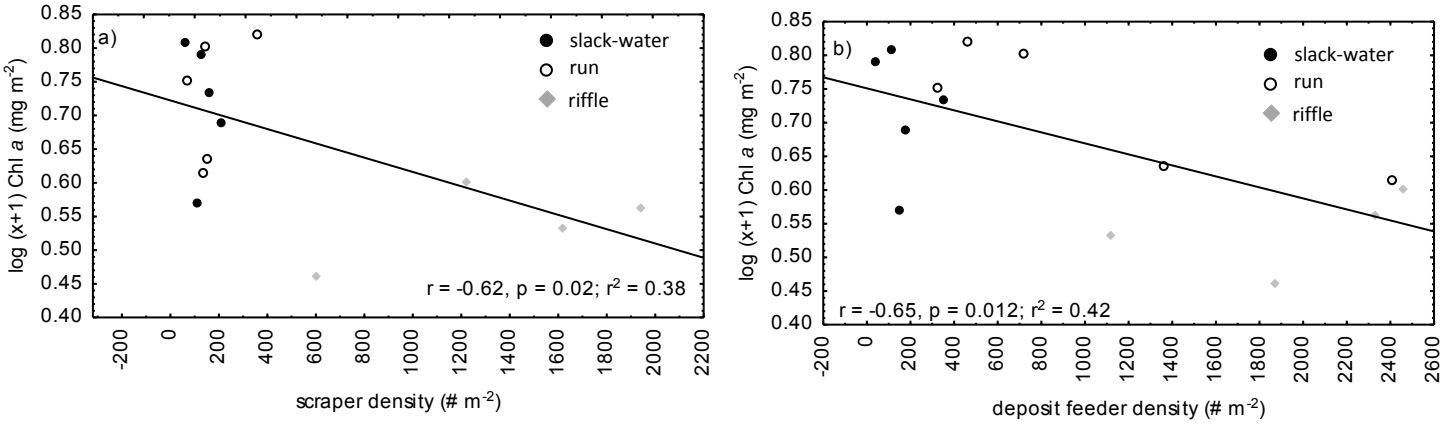


Figure 6.8 Relationship between Chl *a* biomass and (a) scraper density and (b) deposit feeder density at Site 1 (Berg River) during March 2009.

Only a small proportion of within-reach variation in Chl *a* biomass was explained by near-bed velocity at both Sites 1 and 3 during March 2009 (Tables 6.8 and 6.9). Nevertheless, simple non-linear regression analyses were used to explore the relationship further. Figure 6.9 shows a unimodal relationship between near-bed velocity and Chl *a* biomass at both Sites 1 and 3 during March 2009. That is, Chl *a* biomass was greater in areas with moderate near-bed velocities, relative to areas with no flow, or slow velocities and those with high near-bed velocities around the stream bed (Figure 6.9). Interestingly, this relationship was not significant over the full range of velocities at Site 1 (0 - 1.2 ms^{-1}), but was significant between zero and 0.6 ms^{-1}, which encompassed the full range of velocities recorded at Site 3 during March 2009. Nevertheless, this relationship explained 35% (r=0.59) and 39.8% (r=0.63) of the spatial variation observed at Sites 1 and 3 respectively. Based on the regression in Figure 6.9a, Chl *a* biomass was highest at velocities associated with runs and relatively slow riffles (≈ 0.2-0.3 m s^{-1}) under enriched conditions at Site 3. Similarly, Chl *a* biomass appears to have peaked in runs at Site 1 (Figure 6.9b) but at a lower velocity (≈0.18-0.2 ms^{-1}) compared with that at Site 3. It is noteworthy however, that these non-linear relationships are driven by very few data points and velocities associated with maximum Chl a biomass are tentative.

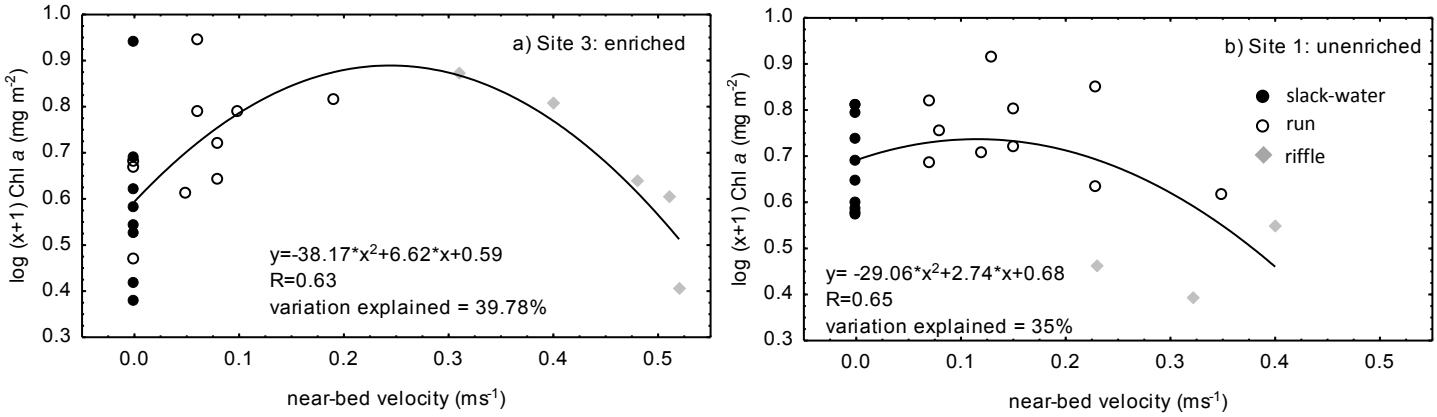


Figure 6.9 **Relationship between Chl *a* biomass and near-bed velocity at a) Site 1 (Berg River) and b) Site 3 (Molenaars River) during March 2009.**

6.3.3.2 Spring (October 2009) under oligotrophic conditions (Site 1)

Not surprisingly, flow type, velocity and biotope significantly explained a large proportion of the variation at both sites during October 2009 (Tables 6.10 and 6.11: marginal tests). Biotope accounted for 79% of the variability at Site 3 during October 2009 and was the single best predictor of spatial variation in Chl *a* biomass across the site (Table 6.10). Flow type (70.3%) and velocity (70.1%) were also particularly good predictors of spatial differences in Chl *a* biomass but were strongly correlated with biotope. Flow type and velocity were therefore excluded from the sequential tests to remove redundant information. After the variability explained by biotope, the two co-variates, depth and grazer density, explained an additional 7 % and 4 % of the variation in the data set respectively. A total of 91 % of within-reach variation at Site 3 could therefore be explained by these variables (Table 6.10: sequential test).

Table 6.10 **Relationship between Chl *a* biomass and environmental variables at Site 3 in October 2009 based on a Euclidean Distance matrix. The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at $p \leq 0.05$ is given in *bold italics***

Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
biotope	-	***0.45***	***49.04***	***0.00***	***79.0***	-
flow type	-	***0.40***	***30.71***	***0.00***	***70.3***	-
velocity	-	***0.40***	***30.44***	***0.00***	70.1	-
depth	-	***0.08***	***2.26***	***0.15***	14.8	-
brusher density	-	0.06	1.52	0.24	10.5	-
substratum	-	0.04	1.11	0.30	7.9	-
deposit feeder density	-	0.03	0.85	0.37	6.1	-
scraper density	-	0.01	0.19	0.66	1.5	-
SEQUENTIAL TESTS [2]						
biotope	***0.77***	***0.45***	***49.04***	***0.00***	***0.79***	***0.79***
depth	***0.84***	***0.04***	***6.17***	***0.03***	***0.07***	***0.86***
brusher density	***0.88***	***0.03***	***5.13***	***0.04***	***0.04***	***0.91***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Biotope explained half (50.6%) of the observed variation in Chl *a* biomass at Site 1 during October 2009 (Table 6.11: marginal tests) and therefore contributed substantially to explaining variability in biomass. Nevertheless, flow type (6.2%) was the single best predictor of spatial variation in Chl *a* (Table 6.11: marginal tests). Despite the exclusion of redundant variables in the sequential tests (i.e. velocity and biotope), no other variables contributed significantly to the sequential model for explaining variability in the data set. Thus only 62% of the total variation, given by flow type alone, was accounted for at Site 1 in October 2009 (Table 6.11: sequential tests).

A comparison between the DistLM results for Site 3 (Table 6.10) and Site 1 (Table 6.11) therefore suggests that a far greater proportion of the observed spatial variation in Chl *a* biomass was explained by measured biophysical variables Site 3, compared with those at Site 1 during October 2009. In other words, within-reach patchiness in Chl *a* biomass is greater under enrichment, compared with that in nutrient poor conditions.

Table 6.11 **Relationship between Chl *a* biomass and environmental variables at Site 1 in October 2009 based on a Euclidean Distance matrix, using the multivariate F-statistic (i.e. Pseudo-F). The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage of Chl *a* biomass variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at p ≤ 0.05 is given in *bold italics***

Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
flow type	-	***0.10***	***21.23***	***0.00***	***62.0***	-
near-bed velocity	-	***0.09***	***18.17***	***0.00***	***58.3***	-
scraper density	-	***0.08***	***14.31***	***0.00***	***52.4***	-
biotope	-	***0.08***	***13.33***	***0.00***	***50.6***	-
depth	-	0.00	0.32	0.58	50.6	-
substratum	-	0.00	0.17	0.69	2.4	-
brusher density	-	0.00	0.06	0.80	1.3	-
deposit feeder density	-	0.00	0.01	0.91	0.5	-
SEQUENTIAL TESTS [2]						
+flow type	***0.59***	***0.10***	***21.23***	***0.00***	***62.02***	***62.02***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

Simple linear regressions of Chl *a* biomass and near-bed velocity at Sites 1 and 3 clearly showed a difference in the relationship between Chl *a* biomass and flow under different levels of enrichment Figure 6.10). Indeed, while Chl *a* biomass responded *positively* to near-bed velocity at Site 1, Chl *a* biomass was *negatively* correlated with near-bed velocity at Site 3 (Figure 6.10) during October 2009. Near-bed velocity had a much smaller range at Site 3 (0-0.55 m s^{-1}) relative to Site 1 (0-1.0m s^{-1}) but a positive correlation was still evident at Site 1, even within a comparable range (0-0.08 m s^{-1}) (Figure 6.10).

Interestingly, the density of scrapers correlated positively with Chl *a* biomass during October 2009 at Site 1 with the highest densities in the riffles at high velocities and lowest densities in the slack-waters and runs (Figure 6.11). This relationship contrasts with that during March 2009 when a negative relationship between Chl *a* biomass and scraper densities was observed (Figure 6.8a). This suggests that during the late summer, consumption of periphyton by scrapers in riffles may be high enough to affect the accrual of biomass in this biotope relative to the slack-waters and runs, which supported lower densities of scrapers (Figure 6.9a). By contrast, higher scraper densities in riffles, relative that in runs and slack-waters during October 2009 (Figure 6.11), suggest that scrapers may have responded positively to greater periphyton biomass in the riffles, compared with the runs and slack-waters where periphyton was less abundant. These relationships are correlative, however and do not necessarily imply a cause-and effect relationship between spatial variability in Chl *a* biomass and grazing activity. The interpretation of these results should therefore be treated with caution.

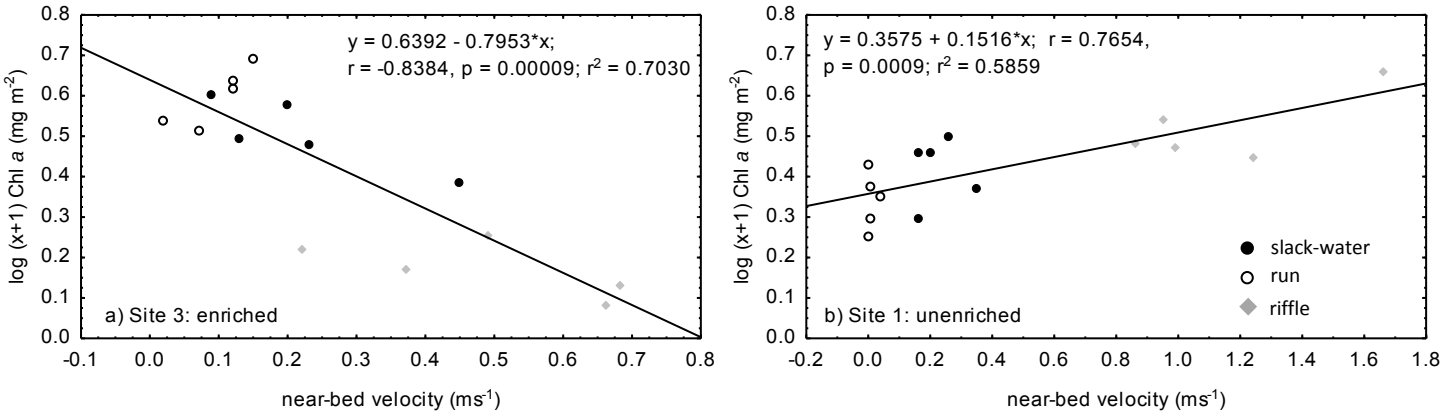


Figure 6.10 Relationship between Chl *a* biomass and near-bed velocity at a) Site 3 (Molenaars River) and b) Site 1 (Berg River) during October 2009.

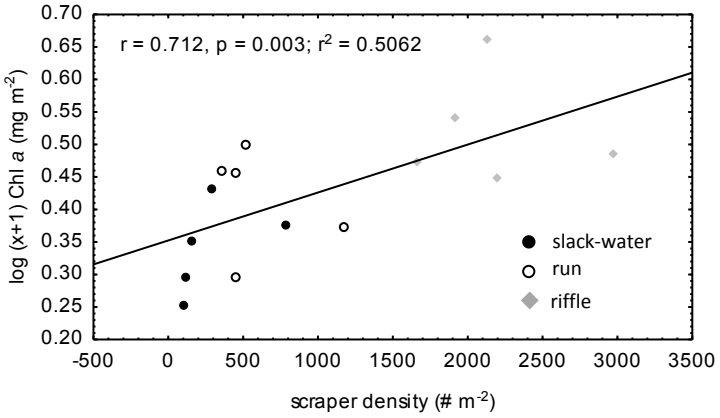


Figure 6.11 Relationship between Chl *a* biomass and scraper densities at Site 1 during October 2009.

6.3.4 Differences in benthic algal composition between biotopes under different levels of nutrient availability and links with biophysical characteristics

6.3.4.1 Late summer towards the end of the growing season (March 2009)

A cluster analysis of periphyton community composition data during March 2009 showed distinct site differences (Figure 6.12) but no clear biotope differences (Figure 6.12). These results were similar to those for Chl *a* biomass data in Section 6.3.3, which implies that periphyton communities were not distinct between habitats at the end of summer. Nevertheless, a PERMANOVA of these data showed that the community composition was distinct both between sites and between biotopes in March 2009 and that the interaction between site and biotope was also significant (Table 6.12). Considering the interaction effect, a pair-wise comparison of biotope differences within each site showed distinct differences in community composition between biotopes at Site 1, with only marginally significant differences between biotopes at Site 3. That is, riffles and runs supported distinct periphyton communities at Site 1 but the community composition in riffles and runs was not different at Site 3 during summer (March 2009). Also distinct differences between slack-waters and riffles were evident at Site 1, with a questionable difference between these biotopes at Site 3 (Table 6.12). By separating the data sets for each site, it was possible to show the biotope differences evident at Site 1 (Figure 6.13a), and the lack thereof at Site 3 (Figure 6.13b).

Table 6.12 Results of a two-way PERMANOVA of algal taxa between site and biotope during March 2009, representing late summer conditions at the end of the growing season. *Post hoc* pair-wise comparisons of differences between biotopes representing each site are also given. Significant values at p ≤ 0.05 are given in bold italics and at p ≤ 0.1 in italics only.

Groups	*df*	*SS*	*MS*	*Pseudo-F*	*p*
site	1	6104	6104	5.34	***<0.001***
biotope	2	5894	2947	2.58	***0.001***
site x biotope	2	6577	3289	2.87	***<0.001***
Residuals	24	27458	1144		
Total	29	46032			

Site 1

	Run	Riffle
Riffle	***0.024***	
sw	0.120	***0.041***

Site 3

	Run	Riffle
Riffle	0.131	
SW	*0.098*	*0.065*

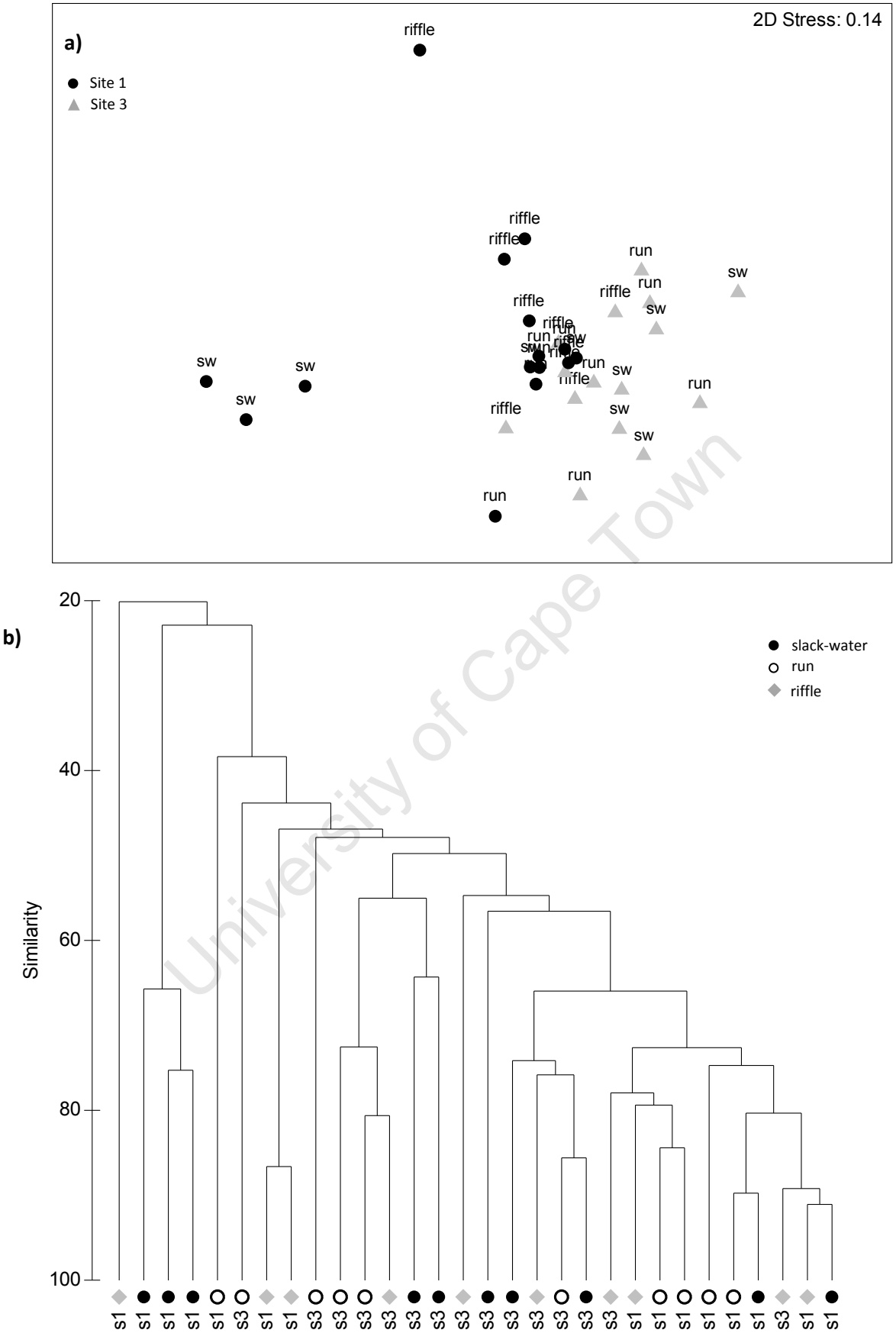


Figure 6.12 **MDS plot (a) and hierarchical clustering dendrogram (b) of Bray-Curtis similarities between sites and biotopes during March 2009. Benthic algal densities are square root transformed.**

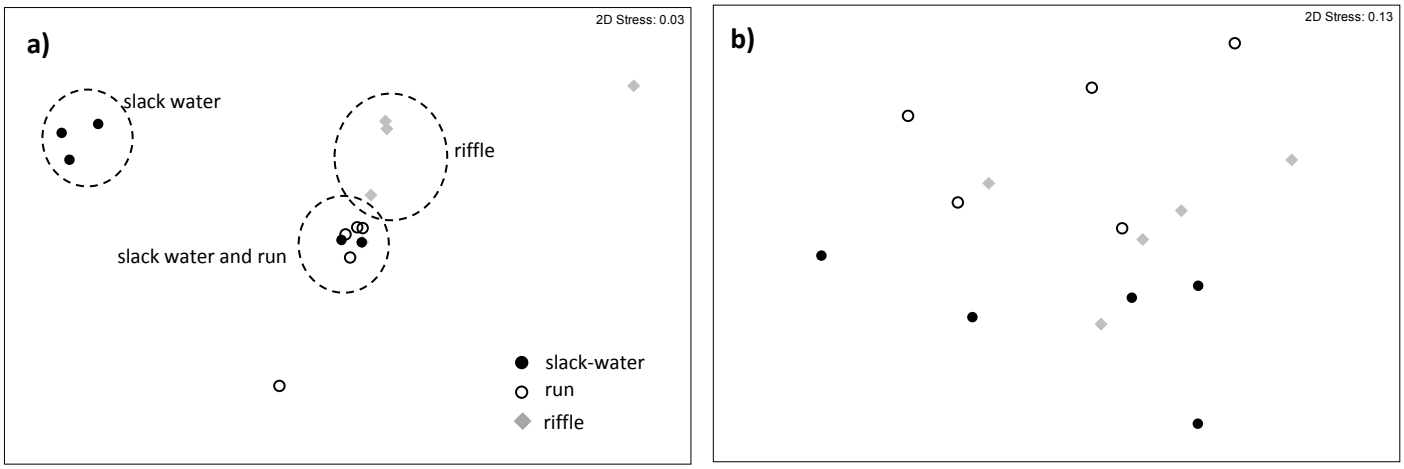


Figure 6.13 **MDS plots of Bray-Curtis similarities between biotopes at a) Site 1 and b) Site 3 during March 2009. Benthic algal densities are square root transformed.**

Differences in the structure of the communities between Sites 1 and 3 in March 2009 are clearly evident in the stacked bar graph of taxa given by division and form (Figure 6.14). Essentially, Site 3 was dominated by a community of adnate single celled taxa (mostly the cyanobacterium *Chamaesiphon* spp.) with few filamentous taxa and some colonial taxa (mostly colonial green algae such as *Desmococcus* spp.). Similarly, the runs at Site 1 were dominated by *Chamaesiphon* spp. but the diatom, *Eunotia rhomboidae* was abundant. By contrast, unbranched filamentous cyanobacteria dominated the community in the riffles and slack-waters at Site 1. In particular, riffles were dominated by *Phormidium* spp. while *Lyngbya* sp 1. and *Rivularia* spp. dominated the slack-waters.

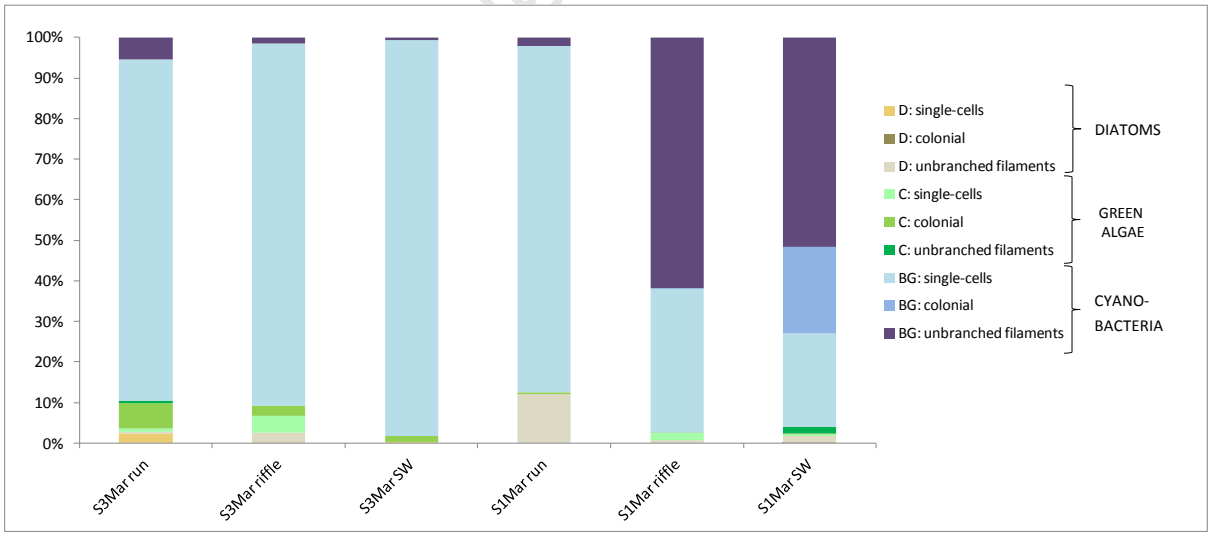


Figure 6.14 **Proportion of taxa by division and form (i.e. structural types) between biotopes and sites during March 2009.D=diatoms; C=green algae; BG=cyanobacteria.**

According to the SIMPER analysis of the data set for Site 1 during March 2009, the distinction between riffles and runs was driven largely by the high abundance of the filamentous cyanobacterium, *Phormidium* spp. in the riffles, which was completely absent from the runs (Figure 6.15a). Also, the filamentous diatom, *Tabellaria flocculosa*, occurred in the runs but was absent from the riffles (Figure 6.15a). *Phormidium* spp. was also absent from the slack-water biotope, which contributed to the distinction between slack-waters and riffles. The key taxon driving the difference between slack-waters and riffles was the high abundance of *Lyngbya* sp. 2 in the slack-waters relative to riffles where it was completely absent (Figure 6.15b). *Rivularia* sp., a filamentous cyanophyte that occurs in gelatinous masses, also occurred in the slack-waters but was absent from the riffles (Figure 6.15b). Despite these biotope distinctions at Site 1, the overlap between replicates suggested that factors other than biotope may also contribute to the spatial pattern of periphyton communities at this time.

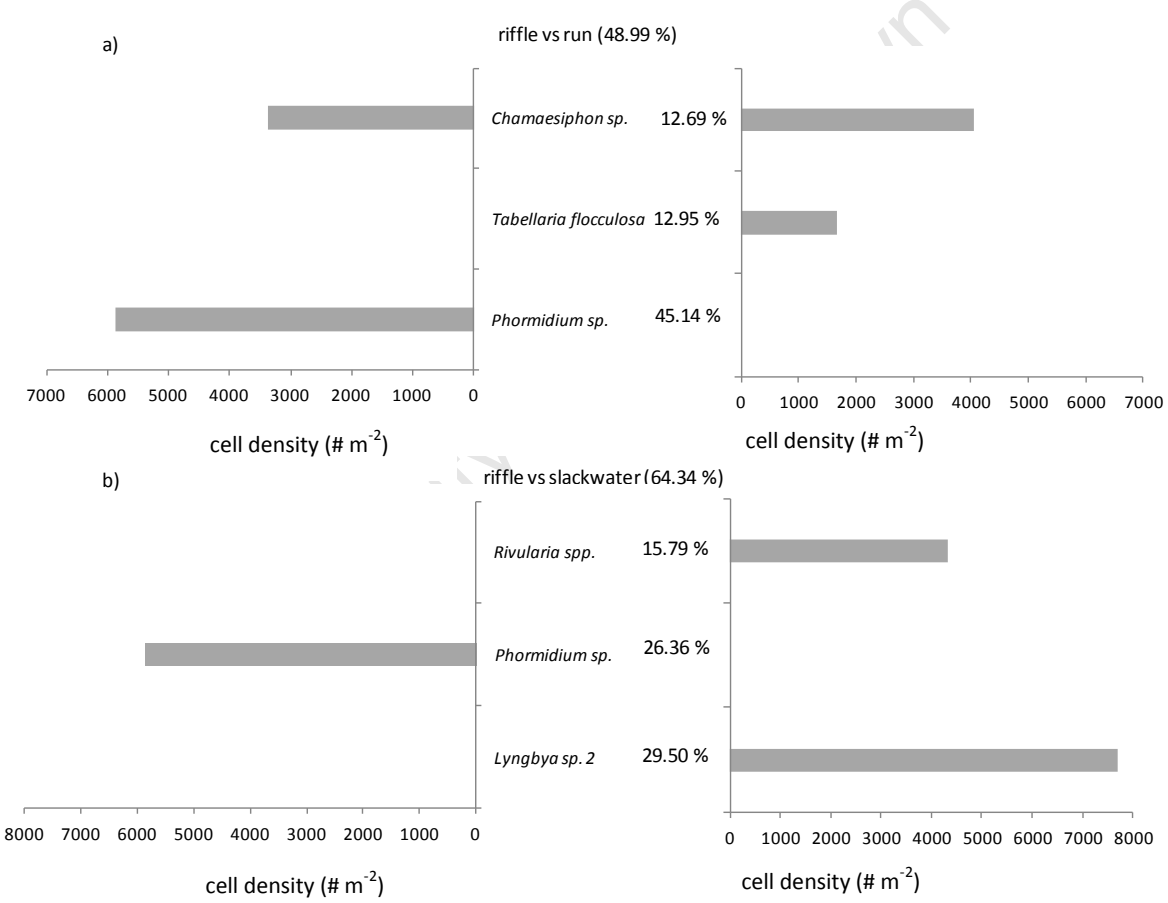


Figure 6.15 SIMPER results of benthic algal taxa densities for Site 1 during March 2009 that together contributed at least 70% to the overall differences between a) riffles and runs and b) riffles and slack-waters after square root transformation and Bray-Curtis dissimilarity. The average dissimilary between these two communities (in brackets) as well as the contributions given by each taxon to the average dissimilarities are presented as percentages (%).

A DistLM analysis was undertaken to investigate which biophysical variables best explained the pattern of periphyton community composition during late summer at Site 1 only. The results show that the three flow-related variables, i.e. biotope, flow type and near-bed velocity each explained

around 20% of the variability (Table 6.13: marginal tests). Biotope was the best single predictor of observed variability (23.4%) with grazer density contributing a further 15.4% in the sequential analyses (Table 6.13: sequential tests). These differences are indicated in the dbRDA plot in Figure 6.16 with biotope differences evident along the dbRDA 1 axis. Differences in brusher densities seemed to separate slack-waters from runs along dbRDA 2 but only 19% of the total variation was given by this axis (Figure 6.16). A total of 38.8% of the observed variation in the data set was explained by biotope and brusher densities together, leaving a large portion (about 60%) of the variability unexplained by the variables measured in this study (Table 6.13 sequential tests).

Table 6.13 **Relationship between periphyton community composition and environmental variables at Site 1 during March 2009 based on a Bray-Curtis dissimilarity matrix. The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at p ≤ 0.05 is given in *bold italics.***

Variable	Adjusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
biotope	-	***5497***	***3.96***	***0.00***	***23.4***	-
flow type	-	***5406***	***3.88***	***0.00***	***23.0***	-
near-bed velocity	-	***4651***	***3.20***	***0.01***	***19.8***	-
brusher density	-	*3197*	*2.04*	*0.08*	*13.6*	-
deposit feeder density	-	2826	1.77	0.16	12.0	-
scraper density	-	2742	1.72	0.14	11.7	-
depth	-	2051	1.24	0.28	8.7	-
substratum	-	1370	0.80	0.52	5.8	-
SEQUENTIAL TESTS [2]						
biotope	***0.17***	***5497***	***3.96***	***0.00***	***23.4***	***23.4***
brusher density	***0.29***	***3633***	***3.03***	***0.03***	***15.4***	***38.8***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.
[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

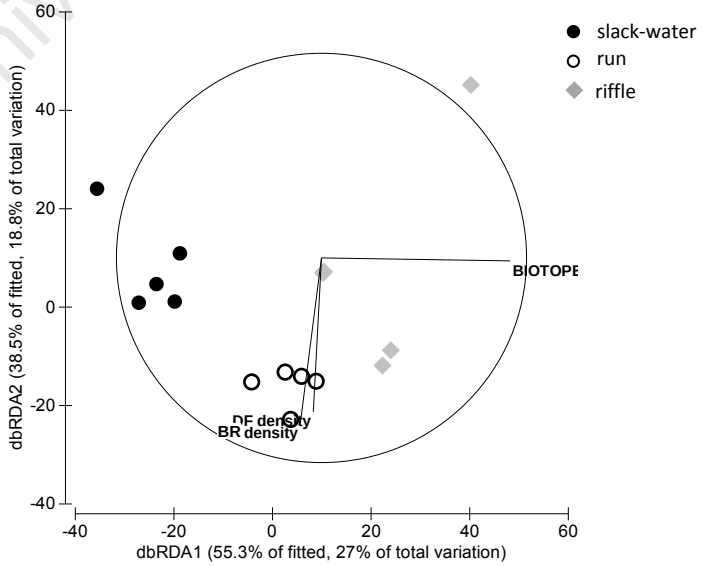


Figure 6.16 **dbRDA ordinations of benthic algal abundances with vectors showing the Spearman correlation between environmental variables and the dbRDA axes at Site 1 during March 2009. Only variables with ρ > 0.5 are shown. The distributon of data represented by different biotopes are shown. DF=deposit feeder; BR=brusher.**

Despite the marginally significant differences between runs and slack-waters, and riffles and slack-waters indicated by the pair-wise results in the PERMANOVA analysis (Table 6.12), none of the predictor variables, including biotope, explained variation in taxon composition observed at Site 3 in March 2009.

6.3.4.2 Differences in community composition during spring: the beginning of the growing season (October 2009)

Unlike the spatial patterns observed in March 2009, distinct site and biotope differences in periphyton community composition were observed at both Sites 1 and 3 during October 2009 (Table 6.14; Figure 6.17). The pair-wise PERMANOVA comparisons within each site show that all biotopes were clearly distinct with the exception of runs and riffles at Site 1 where p>0.05 (Table 6.14). These results support the visual representation of the data given by the MDS plots for Site 1 (Figure 6.18a) and Site 3 (Figure 6.18b). In particular, the MDS plot in Figure 6.18a shows the overlap between runs and riffles at Site 1. Although biotopes were more distinct at Site 3 during October 2009, the MDS plot in Figure 6.18b shows some overlap between runs and slack-waters.

Table 6.14 Results of a two-way PERMANOVA of algal taxa between site and biotope during October 2009, representing spring conditions. Post hoc pair-wise comparisons of differences between biotopes representing each site are also given. Significant values at p ≤ 0.05 are given in bold italics and at p ≤ 0.1 in italics only

Groups	*df*	*SS*	*MS*	*Pseudo-F*	*p*
site	1	12719	12719	17.8	***<0.001***
biotope	2	9310	4655	6.5	***<0.001***
site x biotope	2	9455	4727	6.6	***<0.001***
Residuals	24	17143	714.3		
Total	29	48627			

Site 1

	Run	Riffle
Riffle	*0.071*	
sw	***0.009***	***0.009***

Site 3

	Run	Riffle
Riffle	***0.010***	
sw	***0.021***	***0.008***

Differences in the structure of the communities between Sites 1 and 3 in October 2009 are clearly seen in the stacked bar graph of algal division and growth form (Figure 6.19). Similar to the community in March 2009, Site 3 was dominated by a community of adnate single celled taxa (mostly the cyanobacterium *Chamaesiphon* spp.). Site 1, particularly in runs and slack-waters, was dominated by the diatom, *Eunotia rhomboidae*, typical of naturally acid streams. Riffles were, however, dominated by the filamentous cyanobacterium, *Lyngbya* sp.2 during October 2009 (Figure 6.19).

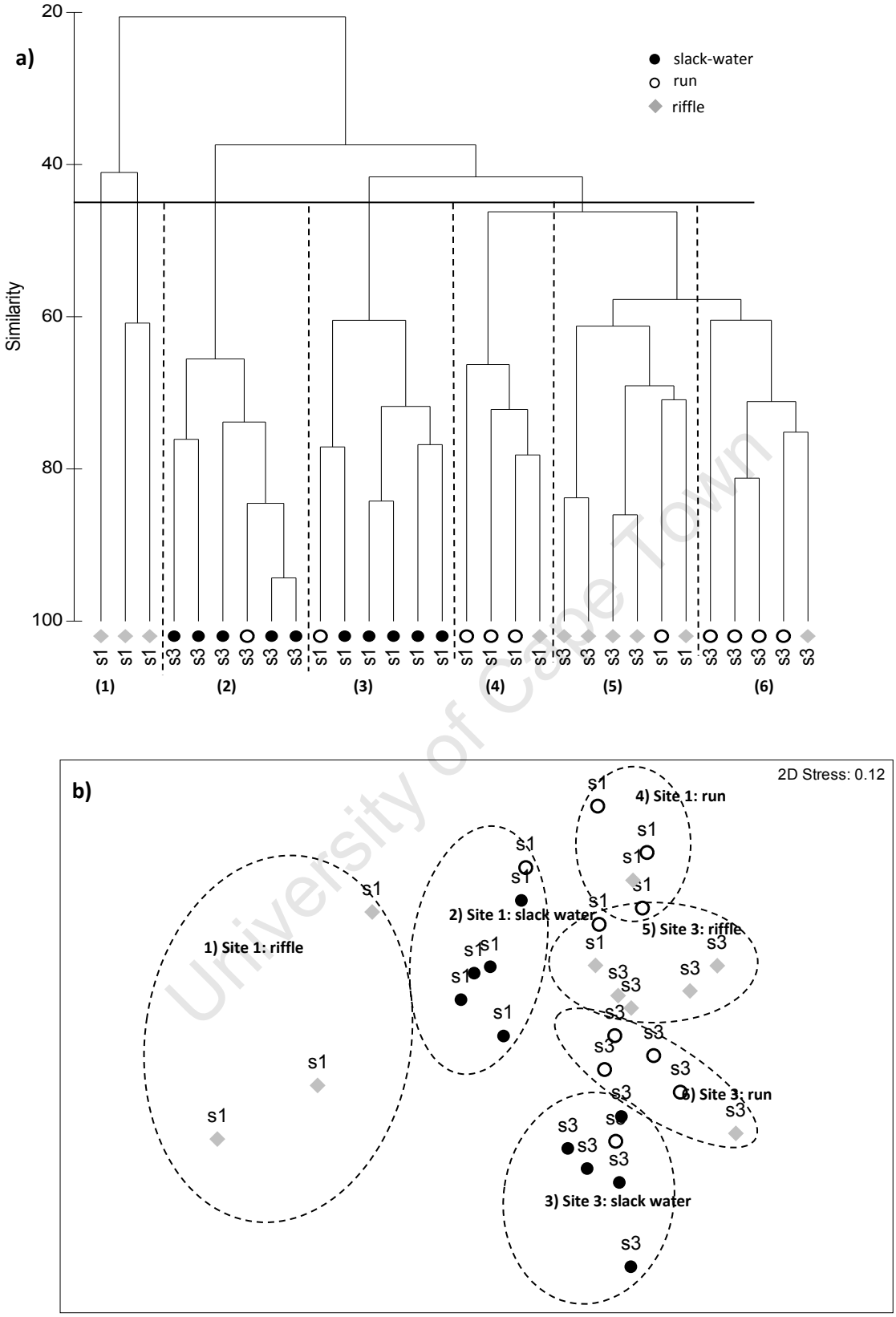


Figure 6.17 **a) hierarchical clustering dendrogram and (b) 2-D MDS ordination of square-root transformed and weighted periphtyon densities based on Bray-Curtis similarities between sites and biotopes during October 2009.**

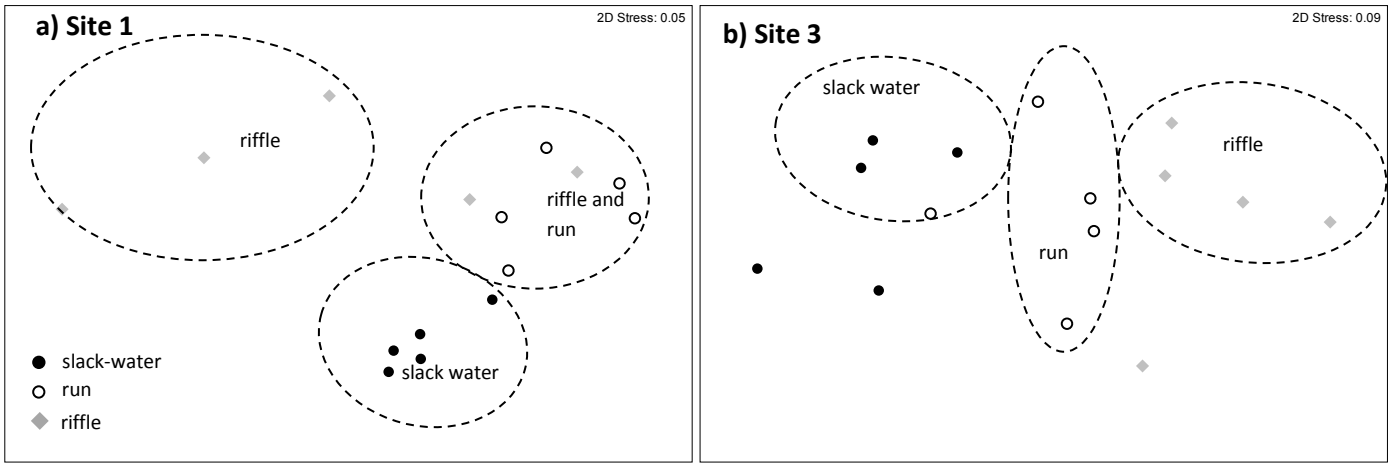


Figure 6.18 **2-D MDS plots of square-root transformed and weighted periphyton densities per biotope based on Bray-Curtis similarities at a) Site 1 and b) Site 3 during October 2009. The ellipsoids indicate similarity among groups at 45% Bray Curtis similarity.**

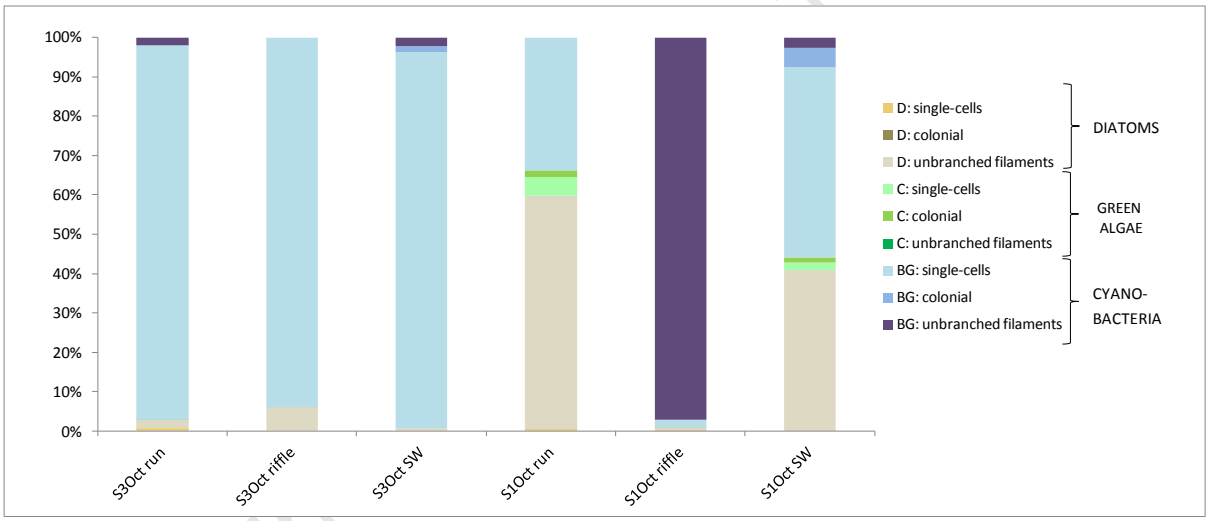


Figure 6.19 **Proportion of taxa by division and form (i.e. structural types) between biotopes and sites during October 2009. D=diatoms; C=green algae; BG=cyanobacteria.**

The taxa responsible for the distinction between biotopes differed between Sites 1 and 3 (Figure 6.20). SIMPER analysis showed that the distinction between biotopes Site 1 was due largely to the high abundance of the filamentous cyanobacteria, *Lyngbya* sp.2 in the riffles and its absence in both runs and slack-waters. High abundances of the single-celled cyanobacterium, *Chamaesiphon* spp. and the presence of *Homeothrix* spp. in both runs and slack-waters at Site 3 were the key determinants of the distinction between riffles and the other two biotopes in October 2009. *Chamaesiphon* spp. was more abundant in the slack-waters at Site 1, however, and was one of the key taxa responsible for the distinction between these biotopes at both sites (Figure 6.20). Nevertheless, *Aphanocapsa* spp. was only present in the slack-waters at both sites and contributed to the distinction between runs and slack-waters (Figure 6.20). The presence of *Merismopedia* spp.,

Eunotia minor and the high abundance of *E. rhomboidae* in the slack-waters also contributed to the distinction between slack-waters and runs at Site 1 (Figure 6.20a).

DistLM analyses of the biophysical variables that might explaining spatial differences if benthic algal community composition are presented in Table 6.15 for Site 1 and Table 6.16 for Site 3. At both sites, biotope was the single best predictor of periphyton community composition during October 2009 (Tables 6.15 and 6.16: marginal tests). A sequential test showed that deposit feeder densities and depth were co-variates to biotope and together these three variables explained 59% of the observed spatial variability at Site 1 (Table 6.15: sequential tests). No co-variates were identified in the sequential tests at Site 3 and therefore biotope alone explained 48.1% of the variability at this site in October 2009 (Table 6.16: sequential tests). The dbRDA ordination for Site 1 (Figure 6.21) shows that biotope, which correlated most strongly with dbRDA2 (ρ = 0.61), separated slack-waters from runs and riffles. Deposit feeder densities, which correlated strongly with dbRDA1 (ρ= -0.82), separated runs and riffles from each other (Figure 6.21). This suggests that abiotic characteristics used to define hydraulic biotopes were largely responsible for differences between slack-waters and the other two biotopes, whereas deposit feeders, as a biotic feature, separated runs from riffles at Site 1.

Biotope was strongly correlated with dbRDA1 at Site 3 (ρ = 0.933) and clearly separated all three biotopes from each other in the dbRDA ordination given in Figure 6.22. This suggests that spatial variation in benthic algal community composition at Site 3 was driven largely by abiotic factors which define biotopes in October 2009.

Table 6.15 **Relationship between periphyton community composition and environmental variables at Site 1 during October 2009 based on a Bray-Curtis dissimilarity matrix. The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at p ≤ 0.05 is given in *bold italics.***

Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
biotope	-	***6023***	***4.0***	***≤0.01***	***23.7***	-
flow type	-	***5983***	***4.0***	***≤0.01***	***23.5***	-
near-bed velocity	-	***5883***	***3.9***	***≤0.01***	***23.1***	-
deposit feeder density	-	***5597***	***3.7***	***≤0.01***	***22.0***	-
scraper density	-	***5332***	***3.5***	***≤0.01***	***21.0***	-
depth	-	***4501***	***2.8***	***≤0.01***	***17.7***	-
substratum	-	2739	1.6	0.2	10.8	-
brusher density	-	688	0.4	0.9	2.7	-
SEQUENTIAL TESTS [2]						
+biotope	***0.18***	***6023***	***4.0***	***≤0.01***	***23.7***	***23.7***
+deposit feeder density	***0.41***	***6552***	***6.1***	***≤0.01***	***25.8***	***49.5***
+depth	***0.48***	***2426***	***2.6***	***≤0.01***	***9.5***	***59.0***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

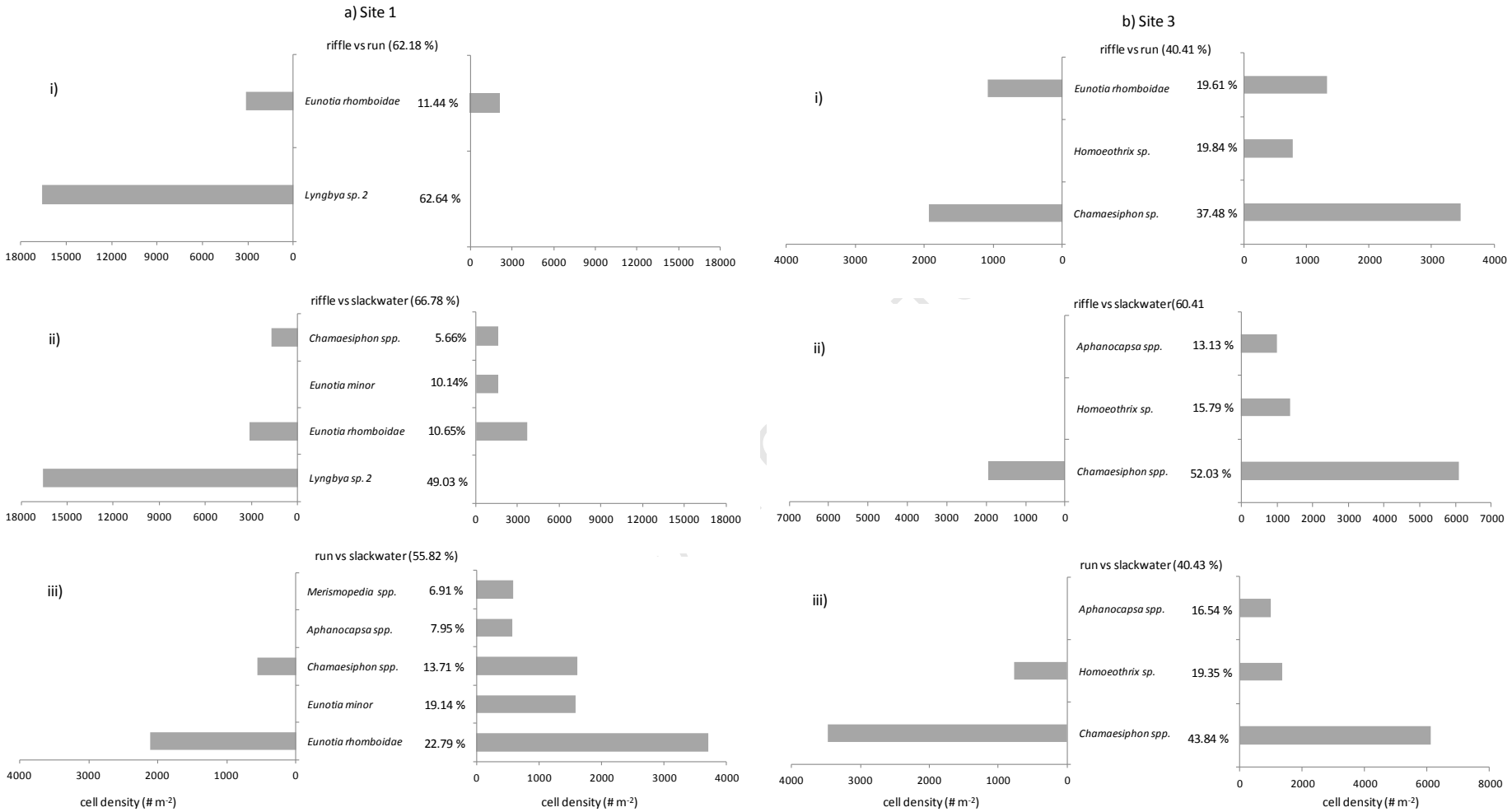


Figure 6.20 **SIMPER results of benthic algal taxa densities for a) Site 1 and b) Site 3 during October 2009 that together contributed at least 70% to the overall differences between i) riffles and runs, ii) riffles and slack waters and iii) runs and slackwaters after square root transformation and Bray-Curtis dissimilarity. The average dissimilary between these two communities as well as the contributions given by each taxon to the average dissimilarities are presented as percentages (%).**

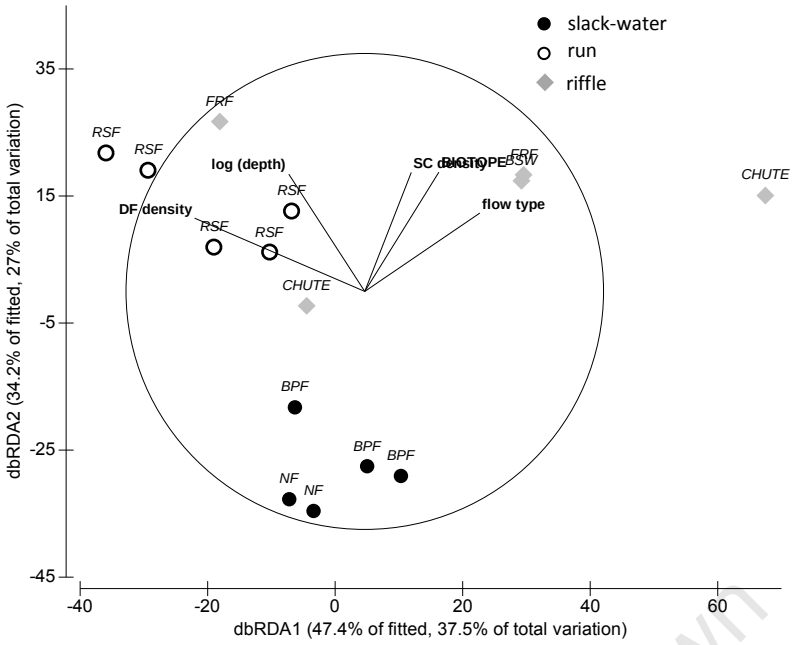


Figure 6.21 **dbRDA ordinations of benthic algal abundances with vectors showing the Spearman correlation between environmental variables and the dbRDA axes at Site 1 during October 2009. Only variables with ρ > 0.5 are shown. Flow types are given as NF = no flow; BPF = barely perceptible flow; RSF = rippled surface flow; FRF=fast riffle flow; BSW=broken standing wave. DF = deposit feeder; SC = scraper.**

Table 6.16 **Relationship between periphyton community composition and environmental variables at Site 3 during October 2009 based on a Bray-Curtis dissimilarity matrix. The 'step-wise' procedure and Adjusted R^2 criteria in DistLM were used. '% var' indicates the percentage variation explained by a variable. 'Cum. % var.' is the cumulative percentage variation explained for each additional co-variate in the sequential tests. Significance at p ≤ 0.05 is given in *bold italics.***

Variable	Ajusted R^2	SS(trace)	*Pseudo-F*	*p*	% var	Cum % var
MARGINAL TESTS [1]						
biotope	-	***6927***	***12.07***	***<0.001***	***48.1***	-
flow type	-	***6260***	***10.01***	***<0.001***	***43.5***	-
near-bed velocity	-	***5071***	***7.07***	***<0.001***	***35.2***	-
substratum	-	1716	1.76	0.15	***11.9***	-
deposit feeder density	-	1463	1.47	0.21	***10.2***	-
brusher density	-	1023	1.00	0.40	***7.1***	-
depth	-	697	0.66	0.59	***4.8***	-
scraper density	-	626	0.59	0.66	***4.4***	-
SEQUENTIAL TESTS [2]						
+biotope	***0.441***	***6927***	***12.07***	***<0.001***	***48.1***	***48.1***

[1] Marginal tests show how much variation each variable explains when considered alone, ignoring other variables.

[2] Sequential tests explain the cumulative variation attributed to each variable fitted to the model in the order specified, taking previous variables into account.

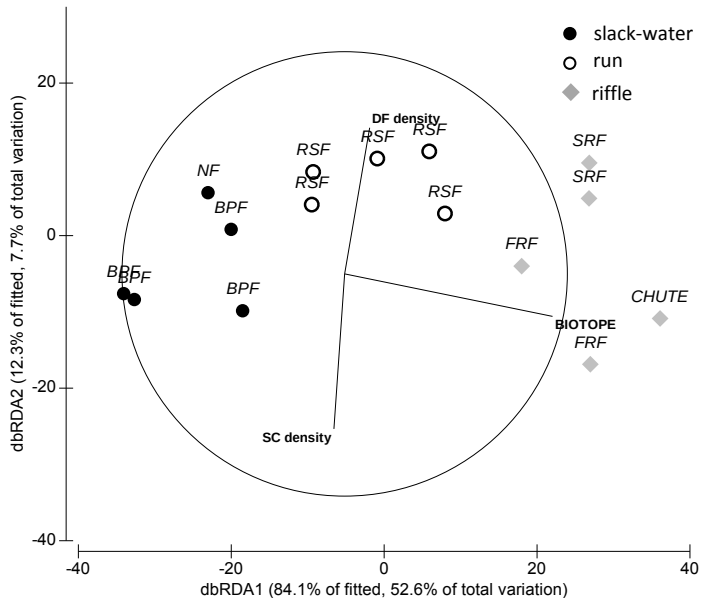


Figure 6.22 **dbRDA ordinations of benthic algal abundances with vectors showing the Spearman correlation between environmental variables and the dbRDA axes at Site 3 during October 2009. Only variables with ρ > 0.5 are shown. Flow types are given as NF = no flow; BPF = barely perceptible flow; RSF = rippled surface flow; FRF=fast riffle flow; BSW=broken standing wave. DF = deposit feeder; SC = scraper.**

6.4 DISCUSSION

Spatial heterogeneity or patchiness in periphyton community composition and biomass is typical of temperate streams and generally reflects heterogeneity of the physical habitat (Stevenson 1997, Cardinale *et al.* 2002). Understanding the factors that govern spatial patterns in periphyton communities is important for predicting how changes in flow or nutrient availability might affect them. Whereas Chapters 3, 4 and 5 focused primarily on temporal changes in communities within a single hydraulic biotope (i.e. runs), the main objective of this study was to develop an understanding of the differences in periphyton communities among biotopes typical of foothill rivers in the south-western Cape, under different nutrient conditions and seasons. Secondly, this study investigated possible links between these spatial patterns in periphyton at a local scale with biophysical features encompassing the range of habitats characteristic of these rivers.

It is well documented (see reviews by Stevenson 1996; Larned 2010) that the relationship between periphyton biomass and near-bed velocity is highly variable in different systems and under different nutrient conditions. Uehlinger (1991) for example, found that periphyton biomass was greater in slow flow relative to fast flow, while others (e.g. Horner *et al.* 1990) found the opposite. Biggs *et al.* (1998) and Biggs *et al.* (2005), among others, however, found a unimodal relationship between periphyton biomass and velocity with peak biomass at intermediate velocities, described as the "subsidy-stress" response. Biggs and Stokseth (1996) showed that the relationship between periphyton biomass across a range of hydraulic habitats differed considerably between communities at different stages of accrual. They found no differences in periphyton biomass among pioneer communities in different habitats but significant differences among climax communities in different habitats during late stages of accrual. By contrast, Villanueva and Modenutti (2004) showed that

grazer effects were less evident at intermediate and late stages of succession than at early stages. This study therefore addressed three hypotheses that are discussed further.

6.4.1 Differences in periphyton biomass among biotopes during late summer

Hypothesis 1 states that, during late summer, periphyton biomass will a) be greatest in the runs but significantly lower is slow-flowing slack-waters and fast-flowing riffles under oligotrophic conditions in late summer; and b) be greatest in slack-waters, and lowest in riffles under enriched conditions (Figure 6.1). As expected, the relationship between periphyton biomass and hydraulic biotopes or near-bed velocity was variable under different nutrient conditions and between seasons representing different periods of stability following disturbance. Surprisingly, no significant differences in periphyton biomass among biotopes were evident during March 2009 under both nutrient-limited and moderately enriched conditions (Table 6.6). This suggests that factors other than hydraulic biotopes influenced local-scale patterns of periphyton biomass during late summer. Somerfield *et al.* (2002) showed that, where gradients of change exist in data sets, linear regression always has greater power than ANOVA to detect differences. Indeed, the DistLM analysis, which is based on the same principles as a multiple linear regression analysis, showed that depth explained nearly 30% and velocity tentatively explained 20% of the observed spatial pattern in biomass during March 2009 at Site 3 (Table 6.8). Similarly, under unenriched conditions, velocity alone explained nearly 40% of the variability in biomass during the summer (Table 6.9). Despite the lack of clear biotope differences, these data suggest that heterogeneity in near-bed velocities during the late summer did contribute somewhat to the patchiness in periphyton biomass at a local spatial scale. Nevertheless, hydraulic biotopes were poor surrogates for explaining biomass-velocity relationships in late summer.

The velocity-biomass relationship in March 2009 was not linear but unimodal at both sites (Figure 6.9). Despite the lack of a difference between biotopes, slack-waters and fast flowing replicates typical of riffles had lower biomass than that at intermediate flows representative of runs. Using regression analysis and not ANOVA therefore provided evidence in favour of the hypothesis that periphyton biomass in the south-western Cape are highest at moderate velocities typical of runs in oligotrophic conditions at the end of summer. Considering the suggested non-linear relationship between near-bed velocity and Chl *a* biomass during March 2009, it is not surprising that the DistLM, which fitted a linear model to the data set, only described a small proportion of the spatial variation across both Sites 1 and 3 (Tables 6.8 and 6.9). It is likely therefore that more complex, non-linear modelling would have provided a better idea of the suite of variables that explained Chl *a* biomass variability across habitats at the end of the growing season. Nevertheless, a far larger more spatially representative data set would be required for such analyses and is recommended for future research.

In a study of the relationship between velocity and periphyton biomass in communities with different growth forms, Biggs *et al.* (1998) found that in highly enriched conditions, nutrient delivery to a community dominated by a mucilaginous mat was already optimal and thus no nutrient subsidy occurred with increases in velocity. Also, they showed that shear stress only became important at

high near-bed velocities. Therefore, over a range of 0 to 0.5 ms^{-1}, which was below the shear stress limit, Biggs *et al.* (1998) found no relationship between near-bed velocity and periphyton biomass. Indeed, Figure 6.9b shows no appreciable increase in periphyton biomass over a similar range at Site 1 during this study, which might explain the less distinct unimodal relationship at Site 1 during March 2009.

Despite moderate enrichment at Site 3, where it was expected that a negative linear relationship would describe a significantly higher biomass in slack-waters relative to riffles, the relationship at this site was also unimodal. This suggests that, like Site 1, increasing velocities at the low end of the spectrum promote the uptake of nutrients at Site 3 by promoting the mass transfer of nutrients to algal cells (see Chapter 1, section 1.3.5.1). This implies that periphyton biomass accrual was nutrient-limited in slower flowing slack-waters where nutrient availability was expected to be optimal for growth. The hypothesis that periphyton biomass decreases linearly with an increase in near-bed velocity under enriched conditions (Hypothesis 1b) must be rejected based on the evidence provided in this study.

During March 2009, Chl *a* biomass peaked at 0.18 - 0.2 ms^{-1} at Site 1, while peak biomass at Site 3 was reached at a higher velocity range (i.e. 0.2 - 0.3 ms^{-1}). Although these figures fall within the range of flows supporting peak biomass reported in the literature, velocity optima vary considerably among different studies. For example, Biggs and Stokseth (1996) reported velocity optima of 0.5-0.7 ms^{-1} in their study while Poff *et al.* (1990) found maximum biomass at velocities <0.17 ms^{-1}. Biggs *et al.* (1998) found that velocity optima for maximum biomass varied considerably depending on the nutrient regime and community growth form. They found that a short filamentous community in an unenriched outdoor flume reached peak biomass at 0.184 ms^{-1} whereas a community with a similar structure in a moderately enriched stream reached peak biomass at 0.23 ms^{-1}, although the biomass peak was greater. Surprisingly, peak biomass was not significantly greater at the moderately enriched Site 3, relative to Site 1, as expected, in late summer. Although, the velocity optimum was greater at Site 3, relative to Site 1, saturating current velocities are usually higher in high-density than in low-density algal communities which are usually supported by greater nutrient availability (Stevenson 1996). Thus the differences in velocity optima for periphyton biomass between Sites 1 and 3 during March 2009 may be influenced by more complex interactions than simply the nutrient subsidy provided by increasing velocity and the drag forces which result in sloughing beyond a certain threshold. Indeed there is evidence to suggest that the velocity optimum at Site 1 under nutrient limited conditions may be somewhat influenced by grazing pressure characteristic of the riffles.

Indeed, scraper densities at Site 1 explained 38.2 % of the observed variation in Chl *a* biomass during March 2009. Deposit feeders too explained 37.1 % of the variation in Chl a biomass at Site 1 during March 2009. Scrapers and deposit feeders were clearly greater in the riffles (≥ 1000 individuals m^{-2}) and lowest in slack-waters and runs. The negative relationship between periphyton biomass and these two groups of grazing invertebrates suggests that during the late summer, grazing pressure in the riffles may suppress periphyton biomass accrual in these habitats under oligotrophic conditions.

In other words, spatial variability in grazing activity may contribute to local-scale patchiness in periphyton biomass observed at Site 1. The controlled grazer experiments in Chapter 8 showed that grazer densities (particularly baetid mayflies) above 1000 individuals m^{-2} were able to suppress periphyton accrual during the spring 2010 and therefore it is possible that grazer densities in the riffles were high enough to effectively suppress algal growth during March 2008. Grazer densities in the runs during March 2008 were similarly low during the grazer experiments in March/April 2011 when no grazer effects were apparent thus suggesting that the ability of grazers to suppress periphyton biomass is somewhat dependant on their densities which differ substantially between biotopes. Although it was predicted that increased drag forces and sloughing during high velocities would result in lower periphyton biomass in riffles, relative to runs, these results suggest that grazing activity at higher velocities and not necessarily increased shear stress at these higher near-bed velocities *per se*, may be an important driver of local-scale patchiness in periphyton biomass under oligotrophic conditions. Similarly, it has been shown that localised high densities of grazing invertebrates are able to influence spatial differences in periphyton biomass at this scale (Steinman *et al.* 1989, Rosemond *et al.* 1993, Peterson *et al.* 2001, Villanueva and Modenutti 2004). Where periphyton biomass is low, algal feeders are able to track food resources, which can therefore result in localised increases in the density and growth rates of these invertebrates and an associated decline in periphyton accrual (Lamberti *et al.* 1989, Hill *et al.* 1992, Peterson *et al.* 2001, Peters *et al.* 2007). On the contrary, it has been shown that grazing activity does not effectively suppress biomass accrual under enriched conditions, where algal biomass is rapid, because the rate of algal production under these conditions exceeds the consumption capacity of consumers (Feminella and Resh 1991, Hillebrand 2002, Hillebrand *et al.* 2002). Indeed, no significant relationship was evident between periphyton biomass and grazing invertebrates under enriched conditions at Site 3 in late summer. This suggests that local scale variability in periphyton biomass at Site 3 may be independent of grazing activity because nutrient availability promotes rapid algal growth that exceeds the requirements for algal feeders. In other words, grazing pressure may suppress biomass accrual in riffles under nutrient poor conditions but not under slightly enriched conditions where algal growth exceeds consumption by grazers. Nevertheless, the results of this study are correlative and therefore tentative. It would be interesting to test whether, in the absence of grazing activity, a linear positive relationship develops between periphyton biomass and near-bed velocities like that observed during October 2009 at Site 1.

6.4.2 Differences in periphyton biomass among biotopes during Spring

Disturbance events in streams create small-scale ($<1m^2$) patches that reflect the differential effects of scouring, deposition or unchanged (stable) conditions associated with flood conditions (Matthaei *et al.* 1999). These effects therefore represent the local disturbance history of a stream. Matthaei *et al.* (2003) demonstrated that the local disturbance history affects benthic algal patchiness that could not be attributed to differences in habitat parameters that define hydraulic biotopes, such as substratum size, local near-bed current velocity or water depth during periods of stable flow. Given therefore that early spring generally follows a period of high disturbance caused by winter flooding,

it was hypothesised that no clear biotope differences would be evident at Site 1 during October 2009 (Figure 6.2). Also, it was hypothesised that, like the late summer period, slack-waters at Site 3 would support the greatest biomass with lowest biomass in riffles at Site 3 but the patterns would be less distinct relative to summer (Figure 6.2). Nutrient enrichment at Site 3 should promote the rapid recovery of the periphyton community (Steinman and McIntire 1990), relative to conditions at Site 1. The temporal extent of the effects based on the local disturbance history would therefore be minimal and local hydraulic conditions would structure the communities according to biotopes.

Unexpectedly, clear distinctions in periphyton biomass among biotopes were evident at *both* Sites 1 and 3 during October 2009. At Site 1, highest biomass occurred in riffles with lowest biomass in slack-waters. This suggests that the nutrient subsidy provided by increasing flows act across the full spectrum of near- bed velocities and thus biomass is limited across all biotopes at Site 1 in early spring. By contrast, the greatest biomass was supported by slack-waters and runs with significantly lower biomass in the riffles at Site 3 thus supporting the hypothesis of a negative relationship between periphyton biomass and flow under moderately enriched conditions. Under these conditions, the advantages of increased nutrient uptake are negligible at lower velocities and the effects of increasing drag with increasing velocity promote sloughing. Thus, a strong *positive* correlation between Chl *a* biomass and near-bed velocity at Site 1, and a *negative* biomass-velocity relationship at Site 3 provides further support to the body of literature that highlights nutrient availability differences as the primary cause for the opposite effects of near-bed velocity on periphyton biomass (Horner and Welch 1981; Stevenson 1984; Poff *et al.* 1990; Uehlinger 1991, Stevenson 1996; Biggs *et al.* 1998; Biggs *et al.* 2005; Larned 2010).

Similar to March 2009, flow related parameters were not the only variables to contribute to the observed variability at Site 1. Indeed, scraper densities at Site 1 explained more than half (52.4%) of the variability in periphyton biomass during October 2009. Unlike March 2009, however, the relationship between periphyton biomass and scraper density was *positive* rather than negative (compare Figures 6.8a and 6.11). The reason for this apparent contradiction in the relationship between periphyton biomass and scraper densities is unclear and warrants further investigation.

6.4.3 Differences in periphyton community structure among biotopes

The third hypothesis addressed in this study was that periphyton community composition would differ significantly among biotopes at both Sites 1 and 3 during late summer but that no clear biotope distinctions in periphyton biomass would be evident at either site in spring at the start of the growing season in October 2009. Contrary to these predictions, periphyton communities were only marginally significantly between biotopes at Site 3 in March 2009. Periphyton communities at Site 1 did however differ significantly between biotopes during March 2009 as predicted (Table 6.12). Flow-related predictor variables all contributed significantly to the distinction between biotopes at Site 1 (Table 6.13) therefore providing further evidence for the role of near-bed velocities as a driver of local scale patterns in periphyton communities. Interestingly grazing pressure did not significantly contribute separately to the distinction in community composition between biotopes at Site 1 during March 2009 but grazer density contributed 15.4% to the overall variability

as a covariate to biotope as the single best predictor of local scale variability (Table 6.13). The effects of variability in grazing pressure on local patterns of periphyton biomass proposed earlier were evidently not reflected in the local-scale variability in periphyton community composition at Site 1 during March 2009. Neither flow nor grazing, or any other predictor variable for that matter could explain local-scale variability in periphyton community composition at Site 3 during March 2009. It has been reported that where nutrients are not limiting and are fairly uniform within a given river reach, bottom up processes, particularly nutrient availability, control periphyton community structure if the range of velocities is below the shear stress limit for a given community (e.g. Biggs *et al.* 1998, Hillebrand 2002; Hillebrand *et al.* 2002). Consequently, under this scenario, spatial patchiness in near-bed velocities and grazing invertebrates are irrelevant. The MDS plot in Figure 6.12 shows far greater variability between replicates at Site 1, relative to Site 3. This suggests that local-scale variability in the community composition at Site 3 was far smaller than that at Site 1 and supports the idea that despite heterogeneity in hydraulic and biotic variables measured in this study, the pattern of periphyton community structure is more uniform and possibly driven by nutrients. Unfortunately measurements of nutrients during this study were not replicated sufficiently to determine the role of nutrients in local scale patterns of periphyton community structure but a comparison of single measurements between biotopes (Table 6.16) suggests that nutrient concentrations were far greater and invariant between biotopes, relative to Site 1. Also, although Site 3 was considered moderately enriched in this study, the SRP concentrations during March 2009 were comparable with the highly enriched conditions in which Biggs *et al.* (1998) found no relationship between velocity and periphyton biomass.

Distinct biotope and community composition differences at Sites 1 and 3 during October 2009 were unexpected because local disturbance history, rather than local heterogeneity was expected to have been the primary driver of local scale variability at the end of the winter high flow period. Despite these winter conditions, approximately 1.5 months had lapsed since the last disturbance flood (Class 3) and the October 2009 sampling event, which may have been sufficient time for recovery in this system. In a European river, Matthaei *et al.* (2003) found that the influence of disturbance history on periphyton communities, rather than local velocity was strongest three months after a flood disturbance. It is possible, however, that higher light and temperatures in the Berg River promote rapid algal growth during spring, compared to the relatively cool, turbid, more shaded conditions represented by the River Isar in which Matthaei *et al.* (2003) undertook their study. In such circumstances, the influence of disturbance history would be outweighed by habitat conditions typical of low flows far sooner than that reported by Matthaei *et al.* (2003). Also, the River Isar is dominated by gravel, unlike the Berg and Molenaars that are dominated by less mobile cobbles. Furthermore, the disturbance event experienced 1.5 months prior to sampling in October 2009 had a return period of about 3-4 per years unlike the River Isar where the disturbance event was much larger (1 in one year event). In streams with large cobbles and smaller disturbance events, like that experienced 1.5 months prior to sampling in the Berg and Molenaars, it is likely that hydraulic biotopes are differentially affected by disturbance with the greatest effect in the riffles where stone movement, sheer stress and abrasion by finer sediments would be the greatest and least along the

channel margins characterising the slack-waters. Indeed, the class 3 flood on the Molenaars and Berg Rivers prior to October 2009 may well have *enhanced* the distinction between riffles, runs and slack-waters through differential effects of bed movement among biotopes. It is possible therefore that the timing of the spring sampling in October 2009 did not adequately represent communities in early stages of accrual during spring conditions. The hypotheses that periphyton biomass and community composition would not differ substantially among biotopes following disturbance therefore was not adequately tested in this study and therefore cannot be accepted or rejected.

In conclusion, this study has shown that periphyton community composition and biomass are influenced by local scale heterogeneity in near-bed velocities, particularly in spring when grazers have not recovered sufficiently following winter flooding. Under these conditions, hydraulic biotopes defined in this study as slack-waters, runs and riffles provide a good surrogate for spatial differences in near-bed velocities that drive periphyton patterns at this scale. Whether disturbance history enhances the biotope effect during the early spring or whether the community had recovered sufficiently to negate the effects of disturbance history on local scale patterns could not be established from this data set. Frequent sampling following the last large disturbance event of the wet season would provide further insight but was beyond the scope of this study. Besides the role of near-bed velocities, there is evidence to suggest that grazing pressure affects local scale difference in periphyton community structure but only under nutrient limited conditions.

The stratified random selection of replicates may not be the best sampling approach for understanding micro-scale heterogeneity in periphyton communities. Rather, a stratified design with the collection of samples along set transects, irrespective of the 'visual' identification of hydraulic biotopes may have been a better approach for understanding the full extent of variability across the stream bed. Nevertheless, the observed variability during this study suggests that biotopes, as a practical means of identifying spatial heterogeneity at a local scale are good predictors of periphyton community differences and thus, from a management perspective, provide a useful approach to understanding within-reach heterogeneity in periphyton communities.

Table 6.16 Nutrient concentrations (mg l^{-1}) measured in each biotope at Sites 1 and 3 during March 2009 and October 2009

	Oct-09						Mar-09					
	Site 1			*Site 3*			*Site 1*			*Site 3*		
	slack-water	run	riffle	slack-water	run	riffle	slack-water	run	riffle	slack-water	run	riffle
TP	0.290	0.135	0.165	0.441	0.250	0.413	0.101	0.122	0.527	0.614	0.621	0.614
PO_4-P	0.062	0.035	0.035	0.230	0.212	0.221	0.006	0.078	0.015	0.417	0.440	0.422
SiO_4	0.845	0.958	0.994	2.015	1.673	2.558	1.099	1.249	1.369	5.188	8.839	6.667
NH_4^{3+}-N	0.068	0.068	0.079	0.255	0.243	0.266	0.058	0.099	0.040	0.018	0.063	0.004
NO_2^--N	0.022	0.023	0.026	0.016	0.023	0.021	0.014	0.016	0.019	0.026	0.030	0.032
NO_3^--N	0.143	0.162	0.166	0.397	0.381	0.380	0.370	0.418	0.395	1.222	1.422	1.393

CHAPTER 7 THE ROLE OF FLOOD EVENTS IN STRUCTURING PERIPHYTON COMMUNTIES

7.1 INTRODUCTION

The temporal pattern of periphyton composition and biomass demonstrated in Chapter 4 and the strong links with flood characteristics (Chapter 5) in the Berg and Molenaars Rivers provide evidence to support the large body of literature which suggests that floods are instrumental in shaping periphyton communities in rivers. Floods, as disturbance events, have been widely debated in the literature but it is generally accepted that flood events which remove benthic organisms at rates faster than recolonisation or accrual rates are considered disturbance events (Peterson 1996, Biggs *et al.* 1999a, Larned 2010; Stanley *et al.* 2010). Thus, disturbance events should be quantified by physical measures of the event itself, as well as the biotic response (Stanley *et al.* 2010) within a specified spatial and temporal scale (Poff 1992).

Bed movement as a function of discharge is frequently used as a physical measurement of flood disturbance (e.g. Biggs *et al.* 1999a; Ractliffe 2009). Although bed movement as a threshold for disturbance may be applicable to the study of flood effects on invertebrates (Ractliffe 2009; Death and Winterbourn 1994; Townsend *et al.* 1997; Downes *et al.* 1998), significant loss of periphyton biomass can occur at velocities well below these thresholds. Although flood events that induce bed-load transport are usually associated with the most dramatic change in periphyton biomass and community composition (Biggs *et al.* 1999a; Holomuzki and Biggs 2000), increases in shear stress and abrasion by sediments, associated with smaller flood events, are also responsible for the loss of periphyton biomass (see Section 1.4, Chapter 1). Biggs *et al.* (1999a), for example, showed that small flood events cause loss primarily through increased shear stress alone. With greater velocity and shear stress associated with larger events, fine sediments are mobilized which provide an additional impact on periphyton through abrasion (Uehlinger 1991; Webb *et al.* 2006; Francoeur and Biggs 2006; Fuller *et al.* 2011). However, both these mechanisms operate at velocities much lower than that defining bed movement.

Both Biggs and Thomsen (1995) and Francoeur and Biggs (2006) have shown that flood intensity, rather than duration is more effective at removing periphyton biomass. In both these studies, most periphyton loss occurred immediately after a specific threshold of intensity was reached, but the disturbance intensity could be increased by increasing either water velocities (Biggs and Thomsen 1995) or sediment load (Francoeur and Biggs 2006). Therefore, floods of similar size with similar velocity within the same river reach may have widely varying effects on periphyton removal, depending on the level of suspended sediments because of differences in the scour potential under different suspended sediment loads.

Two major processes characterize the biotic response to disturbance. Resistance is a measure of the ability of a community to withstand disturbance, whereas resilience is a measure of a community's ability to recover from disturbance. Grimm and Fisher (1989) stated that the resistance of

periphyton communities might be directly linked to the magnitude of a flood event but the frequency and timing of disturbances dictate the time available for recovery and the resources available for growth and therefore affect the resilience of periphyton communities in rivers.

In terms of resistance, communities with different growth forms may be more or less susceptible to removal than others under similar discharges. That is, filamentous algae with firm attachment mechanisms, or prostrate taxa which occur in the boundary layer of substrata, will be more resistant to floods than those with weak attachments that extend beyond the boundary layer (Francoeur and Biggs 2006). Also, more mature communities that become resource limited start to degenerate and senesce and are therefore less resistant than early successional communities that form thin films that are less limited by both light and nutrients at their base (Peterson and Stevenson 1992).

Resilience, or the recovery of the community following disturbance is dictated by the initial conditions following the disturbance event (Peterson 1996). Following catastrophic events where most of the periphyton are scoured, resilience will depend on the rate of immigration of algal cells from upstream, whereas following smaller events, resilience may rely more on regrowth of basal cells that were able to withstand scour (Peterson 1996).

The primary aim of this study was to investigate periphyton response to individual flood events of different magnitude and to compare the resistance of communities with different pre-flood growth forms. Assuming a similar load of fine sediments in the Berg and Molenaars Rivers in their foothill reaches, it is expected that the amount of biomass lost will vary greatly depending on the magnitude of a flood event and how resistant the community is to disturbance events of different sizes. Enrichment often results in periphyton communities that are characterised by filamentous taxa that are more susceptible to scour during floods than those characteristic of oligotrophic conditions (Steinman and McIntire 1987; Steinman and McIntire 1990). Also, the susceptibly of periphyton communities to flood disturbance generally increases with time since the last disturbance event (Larned 2010). It is therefore hypothesized that:

- The decrease in periphyton biomass and the shift in community composition due to a disturbance event will be greater in the more nutrient-rich Molenaars River than the Berg River if the community has recovered since the last disturbance event;
- Communities at the end of the growing season[8] will be more susceptible to disturbance than those at the start of the growing season. i.e. the decrease in biomass will be greater after the first wet season floods (in autumn) relative to those at the end of the wet season (in spring) when the community should be more resistant to flooding.

A secondary aim was to investigate the recovery of a periphyton community following a disturbance event by monitoring community and biomass changes over the short-term. After a disturbance event, a succession in algal community structure often occurs, beginning with the development of an

[8] Defined at the low flow summer period when flood events are absent (or rare) and resources such as light are not limiting.

organic matrix and bacterial flora followed by a transition from small adnate diatoms to apically attached colonial diatoms and finally to filamentous green algae (Peterson and Stevenson 1990). It is predicted that biomass immediately prior to and after a disturbance event will be significantly different and that the recovering community will show a distinct pattern of succession as suggested in the literature.

7.2 STUDY APPROACH AND METHODS

7.2.1 Approach to this study

In terms of the first part of this study, i.e. to assess periphyton response to floods of different magnitude, samples were collected immediately prior to an event and again immediately following an event. In all cases, sampling took place within one day of any given flood event to minimize the effects of other factors that might influence periphyton.

Site 1 on the Berg River and Site 4 on the Molenaars River were selected for the investigation of periphyton response to floods because these two sites represent nutrient poor and enriched conditions respectively (see a description of sites in Section 2.2), and daily discharge data were reliable for these two sites. Furthermore, samples were collected before and after the first flood event in autumn (May 2010), which marked the onset of the winter high-flow period after an extended period of low disturbance over the dry season (Figure 7.1a and 7.2a). Samples were collected again in the spring (October 2010) around the start of the more stable, low-flow period, following on from the winter high disturbance period (Figure 7.1b and Figure 7.2b). During routine monthly sampling of periphyton as part of the temporal study (Chapter 4), periphyton samples were collected immediately before and after a small flood at Site 3 on the Molenaars River in May 2008 and these data were added to the dataset for this study (Figure 7.3).

In order to assess post-flood recovery of a periphyton community, samples were collected at Site 2 before and after a planned experimental flood with an instantaneous peak volume of 200 m^3 s^{-1} released on the 11th and 12th June 2008 from the Berg Dam. Samples for periphyton biomass and species composition determination were collected on the 10th June 2008 (immediately prior to the flood) and again on the 13th June, the 17th June and the 24th June 2008 (Figure 7.4).

Table 7.1 provides a summary of the sampling dates, as well as the magnitude, duration and DRIFT class of each of the flood events in this study.

Table 7.1 Summary of flood events sampled on the Berg and Molenaars rivers

Site	Date	Flood peak (Q_{max} in m^3 S^{-1})	DRIFT flood class	Duration (days)	Season
Site 3	May-08	14.50	Class I (upper end)	3	late autumn
Site 2	Jun-08	21.15	Class III	2	winter
Site 1	May-10	43.18	Class IV	12	late autumn
Site 4	May-10	69.52	Class IV	13	late autumn
Site 1	Oct-10	18.42	Class III	3	Spring
Site 4	Oct-10	49.56	Class III	4	Spring

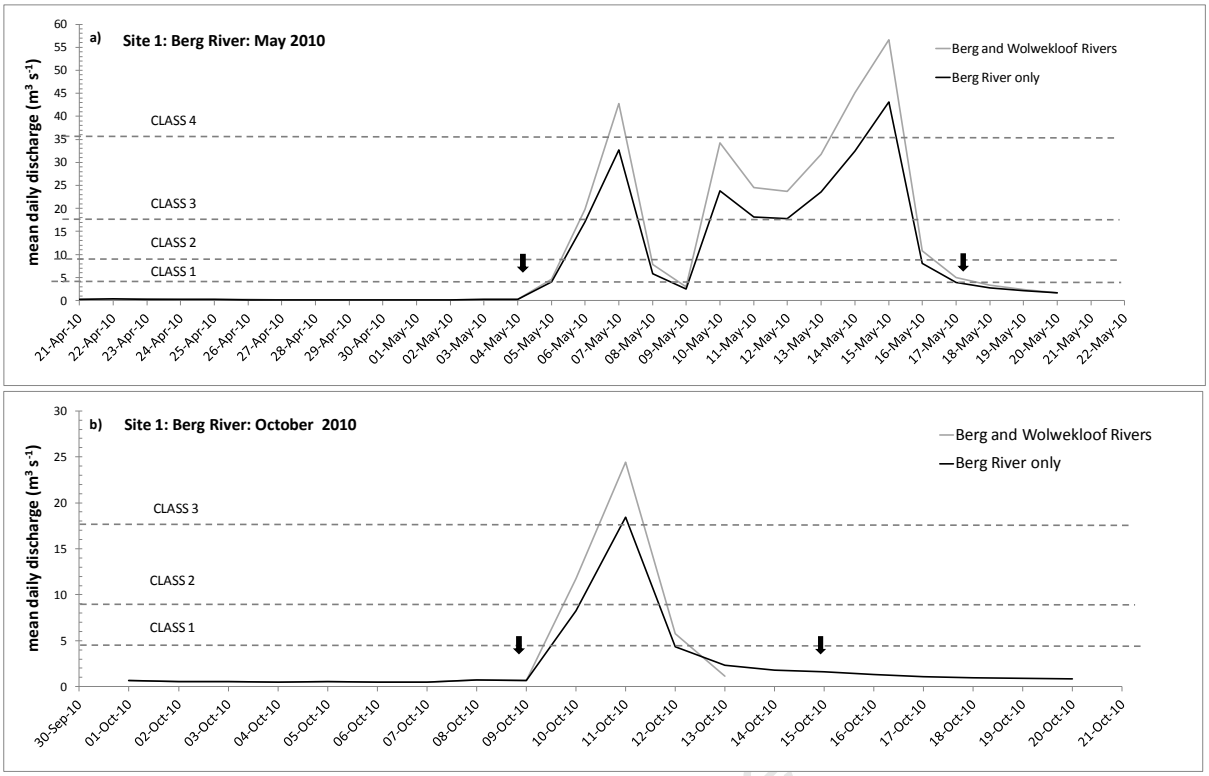


Figure 7.1 **Flood events during (a) May 2010 and (b) October 2010 on the Berg River at Site 1. Sampling dates are marked with arrows. Daily flow data for the Berg and Wolwekloof Rivers together are also shown because the DRIFT flood classes were based on flow data downstream of the confluence of the Wolwekloof and Berg Rivers.**

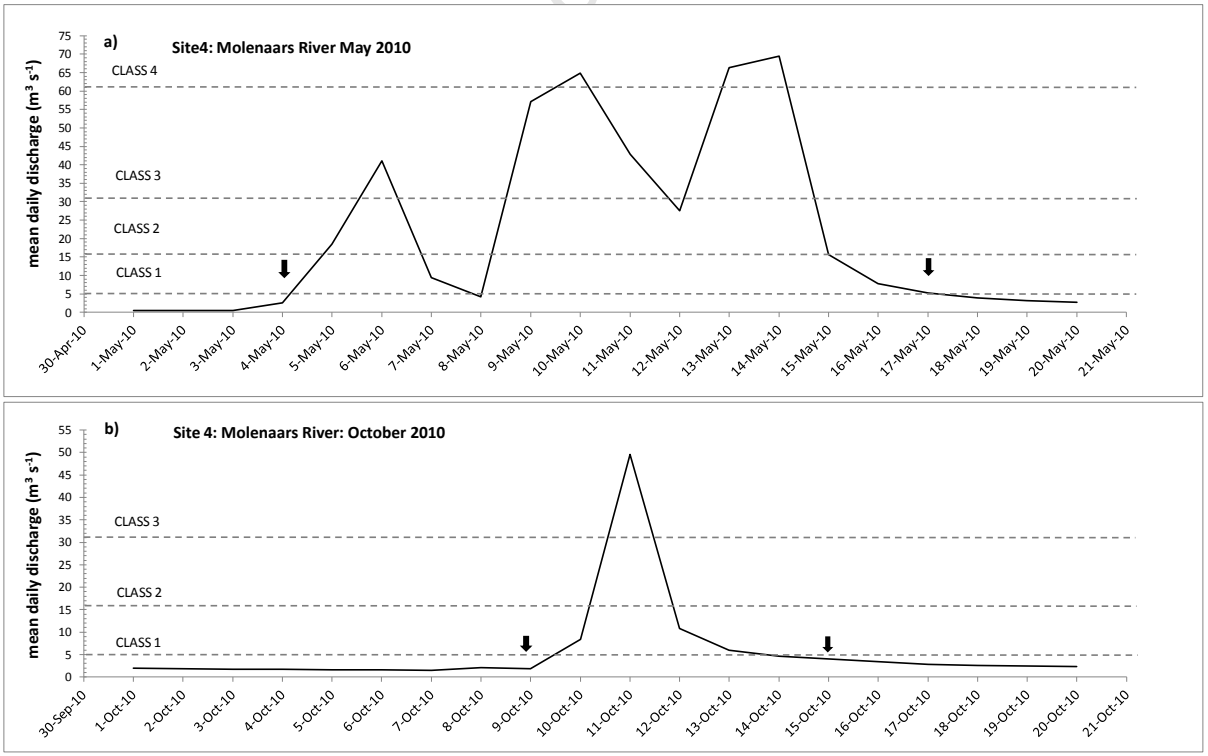


Figure 7.2 **Flood events during (a) May 2010 and (b) October 2010 on the Molenaars River at Site 4 showing the DRIFT classes. Sampling dates are marked with arrows.**

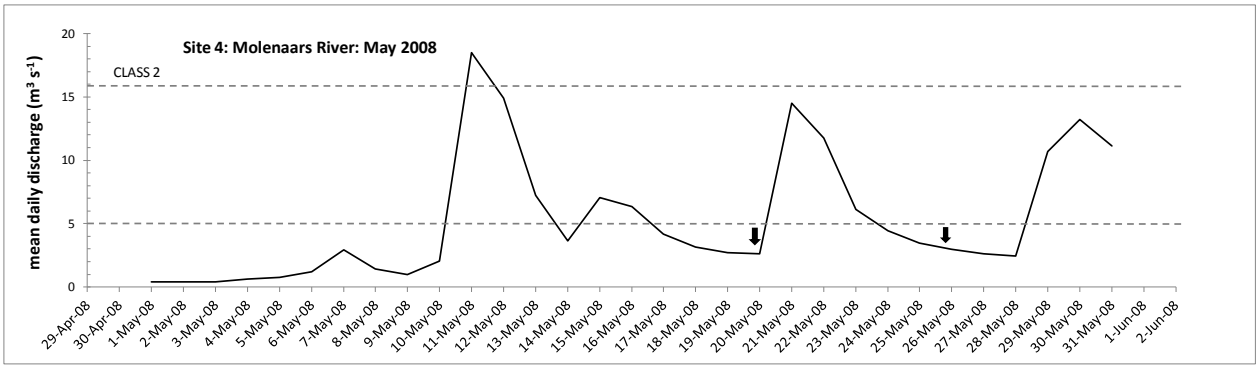


Figure 7.3 Flood event during May 2008 at Site 3 on the Molenaars River showing the relevant DRIFT classes. Sampling dates are marked with arrows.

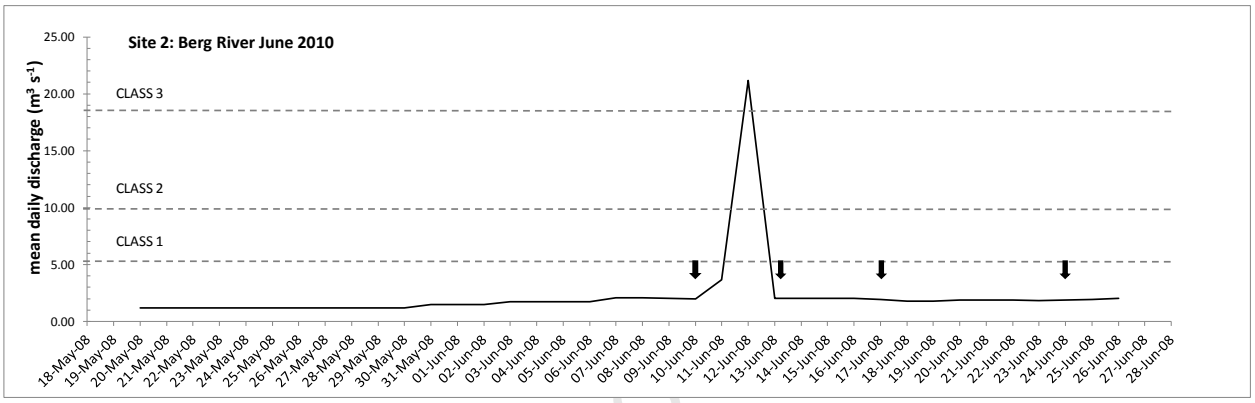


Figure 7.4 Experimental flood release from the Berg Dam during June 2008 showing the relevant DRIFT classes. Sampling dates are marked with arrows.

7.2.2 Sample collection

To reduce within-stream variability associated with different hydraulic biotopes, all samples were collected from the run biotope, where variability is the lowest among biotopes (see Section 2.3.2) and all replicate stones were of similar size. A total of six replicates were collected on each sampling occasion from Sites 1 and 4 for the assessment of periphyton response to floods. In the case of the data collected from Site 3 in May 2008, a total of eight replicates were collected immediately prior to and after the flood event. Also, eight replicate samples were collected on each sampling occasion from Site 2 as part of the post-flood recovery study. Details of the collection and processing of samples are provided in Chapter 2, Section 2.3.

7.2.3 Flood magnitude based on discharge as a measure of flood intensity

The use of discharge *per se* as a measure of flood intensity has been criticized because discharge acts at a scale that is irrelevant to biota at the bed surface (Stanzer *et al.* 1988; Nikora *et al.* 1998; Hart and Finelli 1999). Instead, periphyton are affected by hydraulic conditions at a much finer scale (Larned 2010) and periphyton response to flood events should ideally be linked to physical forces acting at the bed. Most of the criticism of using discharge to measure flood effects has been aimed at studies that report periphyton biomass change in response to flood events as a multiple of base flow conditions where no link was made between flood discharge and the physical forces acting on

the biota (e.g. Biggs and Close 1989). Essentially, similar sized floods relative to mean base flow may be associated with very different hydraulic conditions acting on biota at the river bed because of differences in channel and bed properties.

In this study, flood magnitude was categorized using the DRIFT methodology based on long-term flow data as described in Section 3.2. . Briefly, Class 1 to 4 floods are the intra-annual flood events and the flood size for each class is defined by the Class 5 floods (i.e. those with a return period of 2 years). The magnitude of this event is halved to define the upper threshold of the Class 4 flood (King and Brown 2006). This flood size is then halved to define the upper threshold of the Class 3 flood and so on until the upper threshold of the Class 1 flood is defined. The halving of flood magnitude is thought to represent a shift in the bed-load characteristics of the channel and was determined specifically for Western Cape River systems. For example, Class 1 floods are thought to flush out poor-quality water, whereas Class 2 floods also flush out poor-quality water but entrain some fine sediment (King and Brown 2006). Class 3 floods are those that sort sediments by size, maintain physical heterogeneity, flush riffles and scour cobbles. Class 4 floods also sort sediments and maintain heterogeneity of the river bed but are believed to also maintain the active channel by flushing riparian seedlings from the channel margins (King and Brown 2006). Discharge ranges for each DRIFT flood class are summarized in Section 3.2, Table 3.2 of this thesis. Unlike the measurement of flood magnitude as a multiple of base flows, which may have widely variable biotic responses, DRIFT therefore provided a method of classifying the flow regime and thus flood size into categories that are ecologically relevant. It is however acknowledged that these categories may be associated with variable hydraulic conditions that affect the removal of periphyton at the bed surface. Fortuitously, a study by Ractliffe (2009) on invertebrate responses to floods in the Molenaars and Berg Rivers linked flood magnitude to flow forces acting on individual river stones and stone movement during a series of flood events at Sites 1 and 3 of this study. These data therefore provided a means of linking periphyton response to floods of different magnitude in this study to average physical forces acting at the bed as measured previously.

7.2.4 Statistical analysis

Both univariate and multivariate statistical methods were used for the analysis. Both 'site' and 'treatment' (i.e. pre- and post flood sampling dates) effects were tested as fixed factors in the analyses using both univariate and multivariate statistical methods. Parametric ANOVA analyses in Statistica v.10 were performed on all biomass data after log (x+1) transformation to meet the assumptions of normality and homoscedasticity (Quinn and Keough 2003).

Both the MDS and cluster ordination techniques in PRIMER 6.1 (Clarke and Gorley 2001) were used to visualize potential shifts in periphyton community structure and recovery thereafter in response to flood events. Significance of changes in community structure were then analysed using a two-way PERMANOVA (Anderson *et al*. 2008) between sites and treatments. In the case were data were collected from one site only (i.e. Site 3 during May 2008), a one-way PERMANOVA of differences between treatments was performed. The PERMDISP routine in PRIMER 6.1 was used to test for the

homogeneity of multivariate dispersions among samples to assess the validity of significant differences determined by PERMANOVA.

The SIMPER routine in PRIMER 6.1 was used to identify the taxa most responsible for the differences between communities. All data were square root transformed prior to analysis to reduce variability in skewness in the data set, although this is not a requirement for these analyses.

Detailed descriptions of these analytical techniques are provided in Sections 4.2.2 and 5.2.2 of Chapters 4 and 5, respectively.

7.3 PERIPHYTON RESPONSE TO FLOODS

7.3.1 The effects of a Class I flood on the periphyton community in autumn 2008

A spate on the Molenaars River in May 2008 with a peak discharge of 14.5 m^3 s^{-1} did not result in a significant change in the Chl *a* biomass at Site 3 (F=1.82; df=1, p=0.198), although the mean Chl *a* biomass declined from 2.2 mg m^{-2} prior to the spate to 1.4 mg m^{-2} after the event (Figure 7.5a). Nevertheless, the event resulted in a significant reduction in AFDM (F=8.05; df=1; p=0.013) from a pre-flood biomass of 0.68 g m^{-2} to a post-flood biomass of 0.41 g m^{-2} (Figure 7.5b). This suggests that a Class 1 flood with a magnitude at the upper end of the Class 1 flood range (Table 3.2) was able to effectively remove organic matter derived either from bacteria, fungi, protozoa, dead algal cells or detritus. By contrast, biomass of live benthic algae as measured by Chl *a*, did not decline significantly in response to the event, although this may have been a consequence of the high pre-flood variability indicated by the error bars in Figure 7.5a.

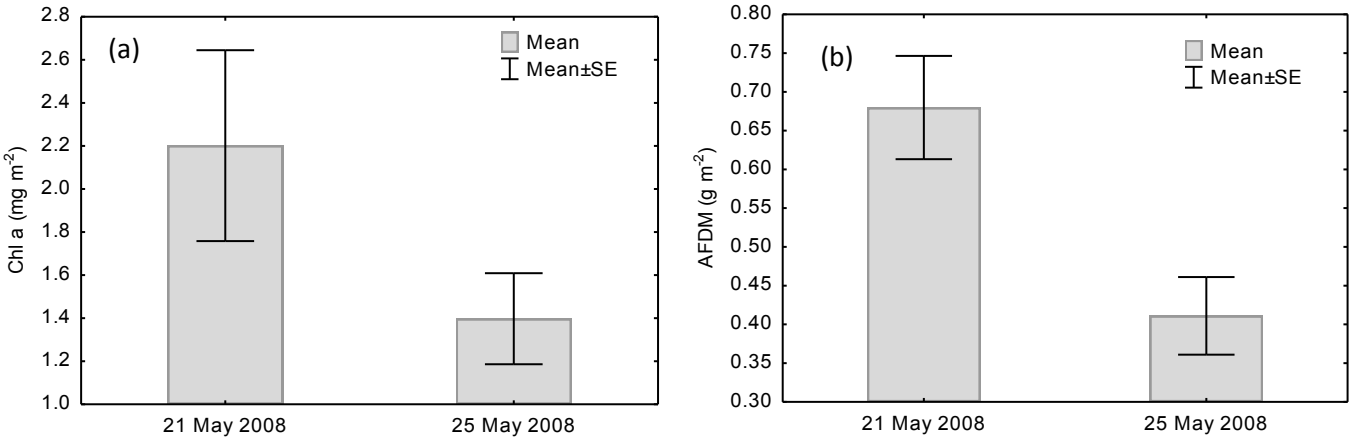


Figure 7.5 Mean a) Chl *a* biomass in mg m^{-2} and b) Ash Free Dry Mass (AFDM) in g m^{-2} measured immediately before and after a Class 1 flood event at Site 3 on the Molenaars River in May 2008.

Similarly, no clear distinction between pre- and post-flood benthic algal communities was evident following the spate in May 2008 at Site 3 on the Molenaars River (Figure 7.6), and this was confirmed by the PERMANOVA analysis (pseudo F= 1.1, df=1, p=0.34).

Figure 7.7 provides an indication of the contribution of different taxa to the overall algal density, as well as the relative contribution of each taxon given as a percentage of the total both before and after the spate. Prior to the spate, more than 70% of the community was comprised of the single

celled cyanophyte, *Chamaesiphon* spp. which maintained its dominance following the small flood with an increase in its relative proportion to 85% of the community (Figure 7.7b). Despite the lack of a significant change in the community composition following the spate, both *Gomphonema lagenula* and *Frustulia vulgaris*, two single-celled diatoms, were present in small numbers prior to flooding but were absent from the community following the event. Densities of the diatoms *Eunotia minor* and *E. rhomboidae* and the colonial chlorophyte, *Chlorococcum* spp. also declined following flooding. Nevertheless, the most notable difference between the pre-and post-flood communities was a decline in the colonial chlorophyte, *Desmococcus* spp. following flooding (Figure 7.7).

These data suggest that despite subtle changes in the community composition following a Class 1 flood in the Molenaars River, a flood of this magnitude did not result in a significant shift in community structure immediately following the event.

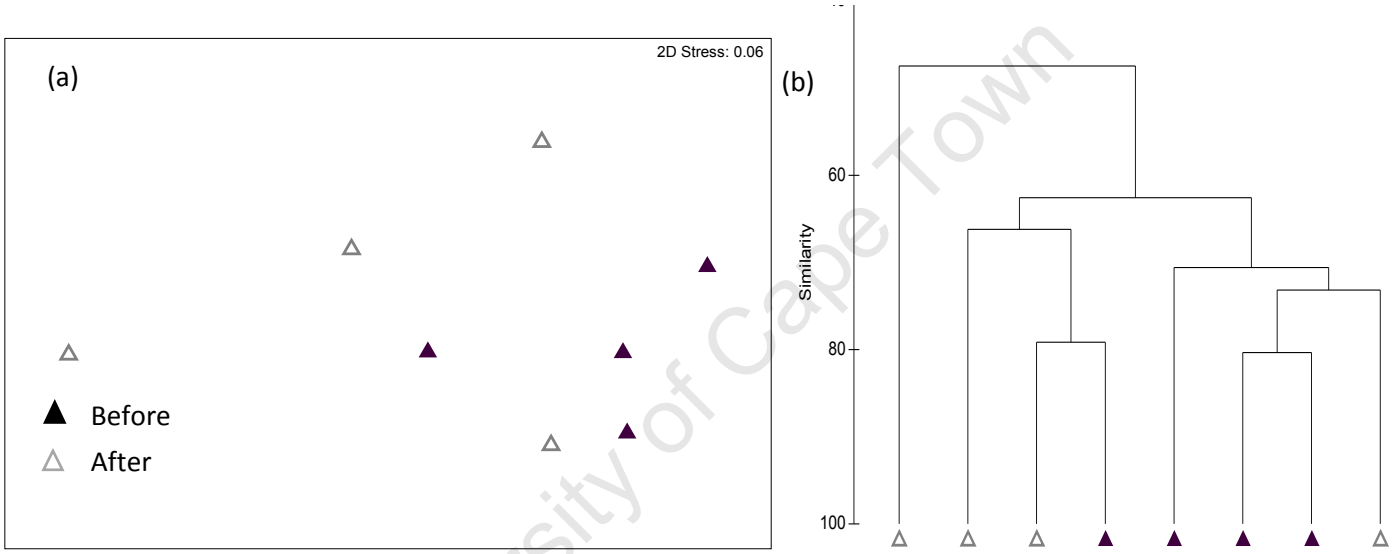


Figure 7.7 **a) 2-D MDS plot and b) Hierarchical clustering dendrogram of Bray-Curtis similarities based on square-root transformed benthic algal taxa at Site 3 on the Molenaars River before and after a Class 1 flood event in May 2008**

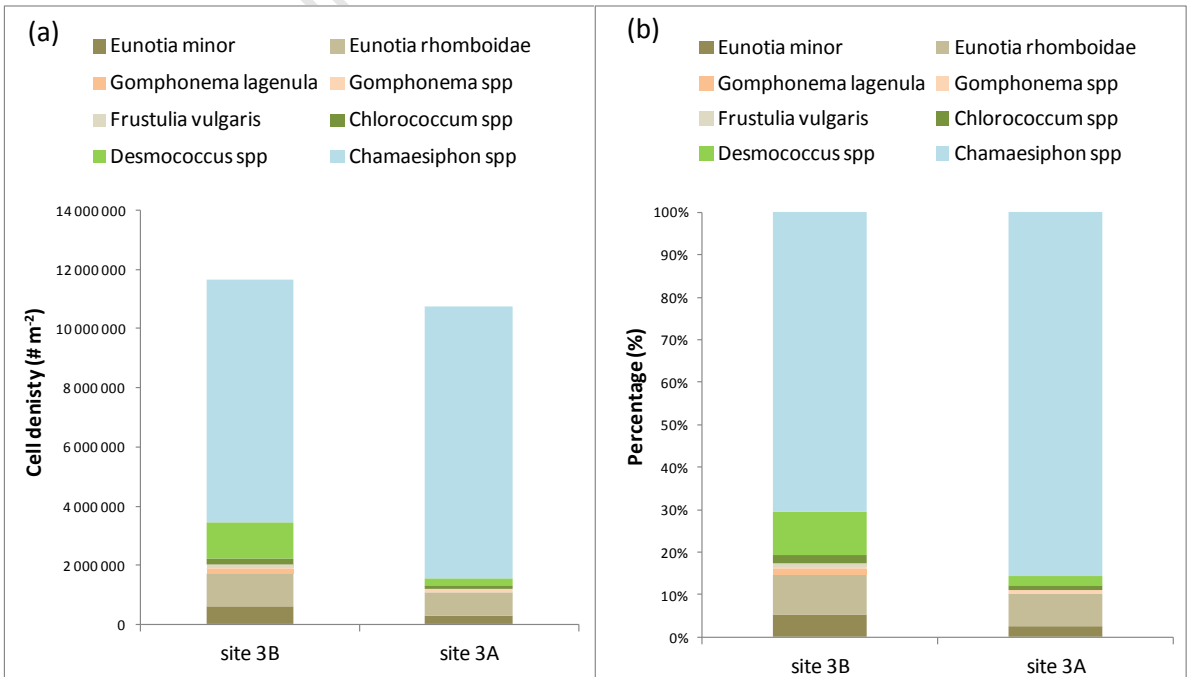


Figure 7.6 **a) Density and b) Relative percentage contribution of benthic algal taxa at site 3 on the Molenaars River before and after a Class I flood event In May 2008**

7.3.2 The effects of a Class 4 flood on periphyton communities in autumn 2010

Despite differences in the trophic status between the Berg and Molenaars Rivers, periphyton biomass, measured as both Chl *a* biomass and AFDM, was not significantly different between Site 1 and Site 4 prior to flooding (Tables 7.2 and 7.3). A Class 4 flood event on both rivers, which marked the first disturbance event of the wet season (Figure 7.1a and 7.2a), however, resulted is a significant decline in Chl *a* biomass at both sites (Table 7.2). A 92 % decline in Chl *a* biomass was observed at Site 1 whereas Chl *a* biomass declined by 73.4 % at Site 4 (Figure 7.8). This suggests that the flood event on the Berg River was more effective at benthic algal removal than that on the Molenaars River (Figure 7.8).

Table 7.2 **a) A two-way ANOVA of Chl *a* biomass between sites and between treatments (i.e. pre- and post-flood) in May 2010. b) P-values for the post-hoc comparisons of sites and treatments based on Tukey tests. Significant values at p≤0.05 are given in *bold italics***

a)	SS	Df	MS	F	p
Site	0.10	1	0.10	1.96	0.177
Treatment	2.87	1	2.87	57.22	***<0.001***
Site x Treatment	0.29	1	0.29	5.70	***0.027***
Error	1.00	20	0.05		

b)	S1: Before	S1: After	S4: Before
S1: Before			
S1: After	***<0.001***		
S4: Before	0.897	***<0.001***	
S4: After	***0.002***	0.064	***0.008***

Interestingly, the 24 % decline in AFDM at Site 4 (Figure 7.9b) was not significant as indicated by the *post-hoc* comparisons in Table 7.3. By contrast, AFDM decreased by 69% on the Berg River at Site 1 in response to the flood (Figure 7.9a) and this decline was significant (Table 7.3).

Table 7.3 **a) A two-way ANOVA of AFDM between sites and between treatments (i.e. pre- and post-flood) in May 2010. b) P-values for the post-hoc comparisons of sites and treatments based on Tukey tests. Significant values at p≤0.05 are given in *bold italics* and at p≤0.1 in *italics only***

a)	SS	Df	MS	F	p
Site	0.00	1	0.00	0.11	0.743
Treatment	0.21	1	0.21	11.87	***0.003***
Site x Treatment	0.07	1	0.07	4.13	*0.056*
Error	0.36	20	0.02		

b)	S1: Before	S1: After	S4: Before
S1: Before			
S1: After	***0.005***		
S4: Before	0.364	0.157	
S4: After	0.065	0.633	0.752

Prior to the first wet season floods in May 2010, benthic algal communities on the Berg (Site 1) and Molenaars (Site 4) Rivers were clearly distinct from each other (Figure 7.10). Following a Class 4 flood on both rivers, the benthic algal community shifted considerably (Figures 7.11 and 7.12), particularly at Site 4 on the Molenaars (Figure 7.12).

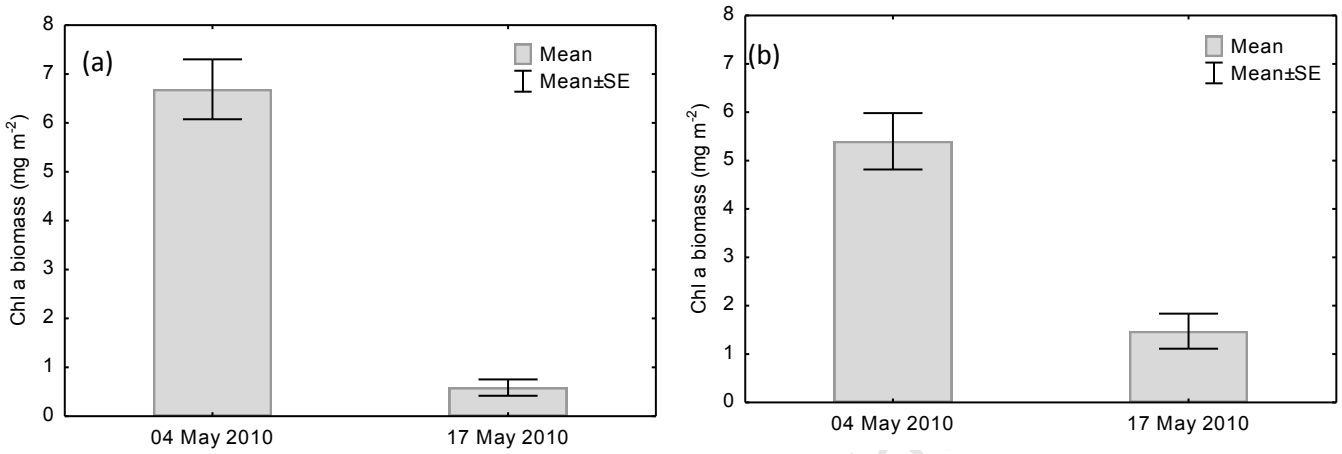


Figure 7.8 **Mean chlorophyll *a* biomass in mg m^{-2} at a) Site 1 on the Berg River and b) Site 4 on the Molenaars River measured immediately before and after a Class 4 flood event in May 2010.**

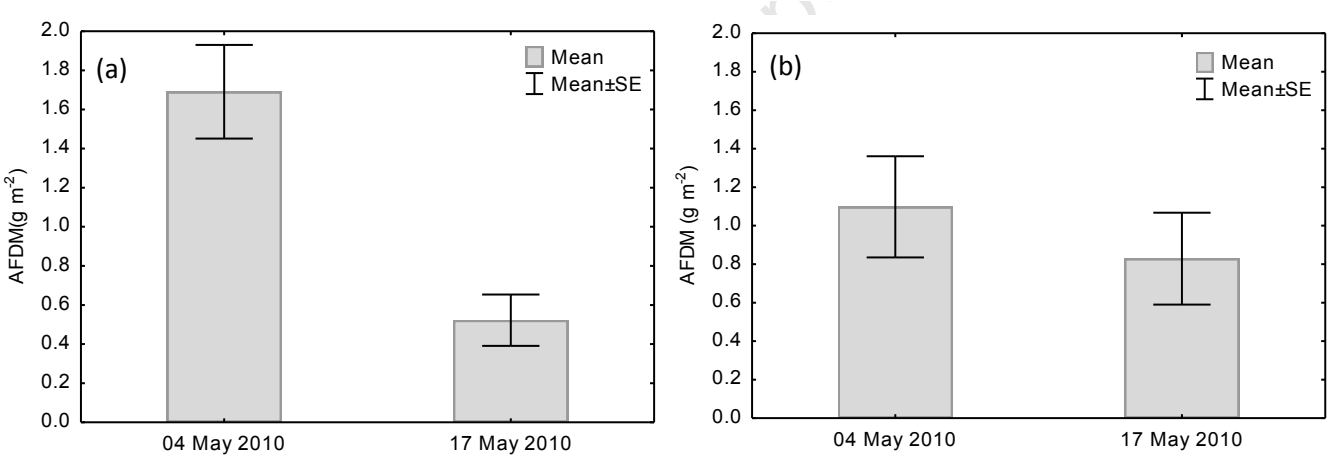


Figure 7.9 **Mean Ash Free Dry Mass (AFDM) in g m^{-2} at a) Site 1 on the Berg River and b) Site 4 on the Molenaars River measured immediately before and after a Class 4 flood in May 2010**

The MDS and hierarchical cluster diagram in Figure 7.11 show that the post-flood community at Site 1 was highly variable with no clear grouping of replicate samples. By contrast, the post-flood community at Site 4 was less variable and 5 of the 6 replicate samples grouped together at 60% similarity with the remaining replicate forming part of the pre-flood community (Figure 7.12).

A two-way PERMANOVA of differences in algal community structure showed significantly different communities both between sites and between treatments (pre- and post-flood) (Table 7.4). Despite the indication of a dispersion effect among replicates in Figure 7.10, a test of the homogeneity of multivariate dispersions using PERMDISP, showed that there were no significant differences in the variability between groups (Table 7.5: pair-wise comparisons), and thus the significance given by the PERMANOVA is most likely valid (Anderson *et al.* 2008). Indeed, the distinction between benthic

algal communities before and after the flood event is evident from the MDS ordinations and hierarchical cluster diagrams given for each site independently (Figure 7.11 and 7.12). Nevertheless, the average distance to centroid, as an indicator of patchiness within the run biotope, shows the greatest dispersion among replicates for Site 1 after flooding (Table 7.5: average distance to centroid), as indicated in the MDS ordination and hierarchical cluster diagram in Figure 7.11 and Figure 7.12.

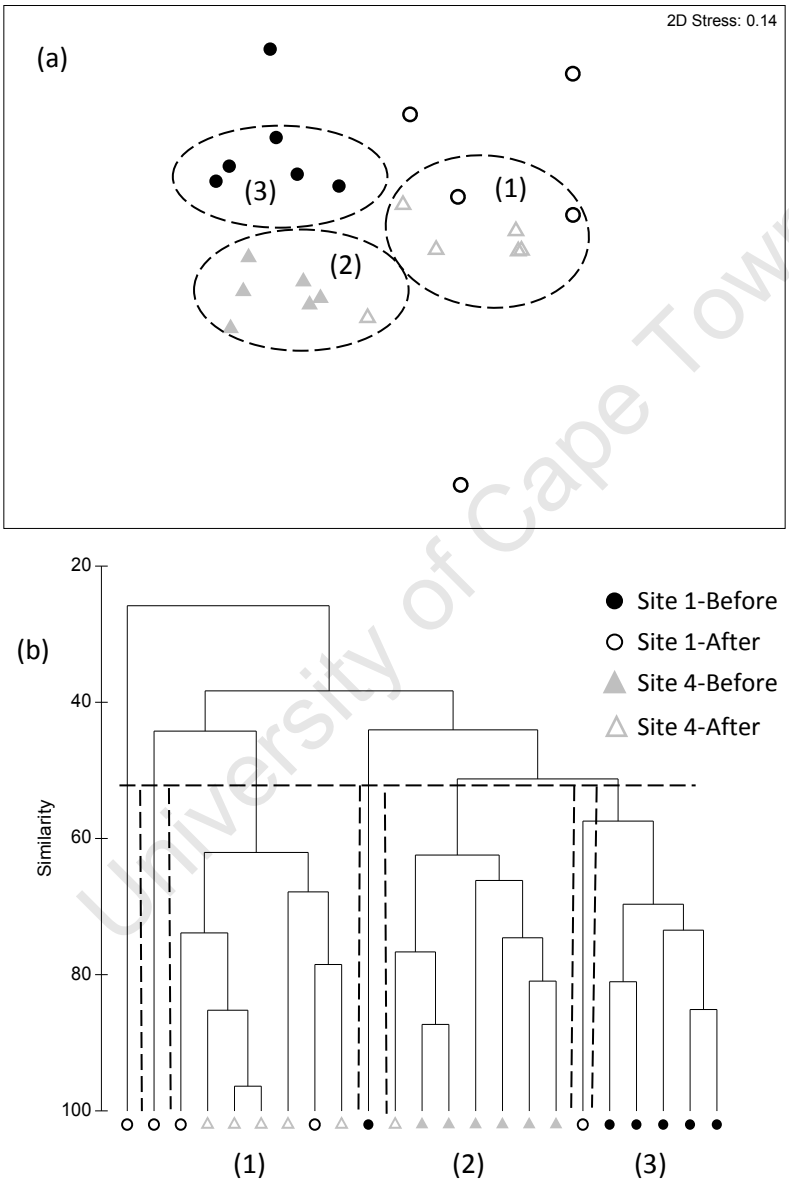


Figure 7.10 **a) 2-D MDS plot and b) Hierarchical clustering dendrogram of Bray-Curtis similarities based on square-root transformed benthic algal taxa for each site and treatment (before and after the flood) in May 2010.**

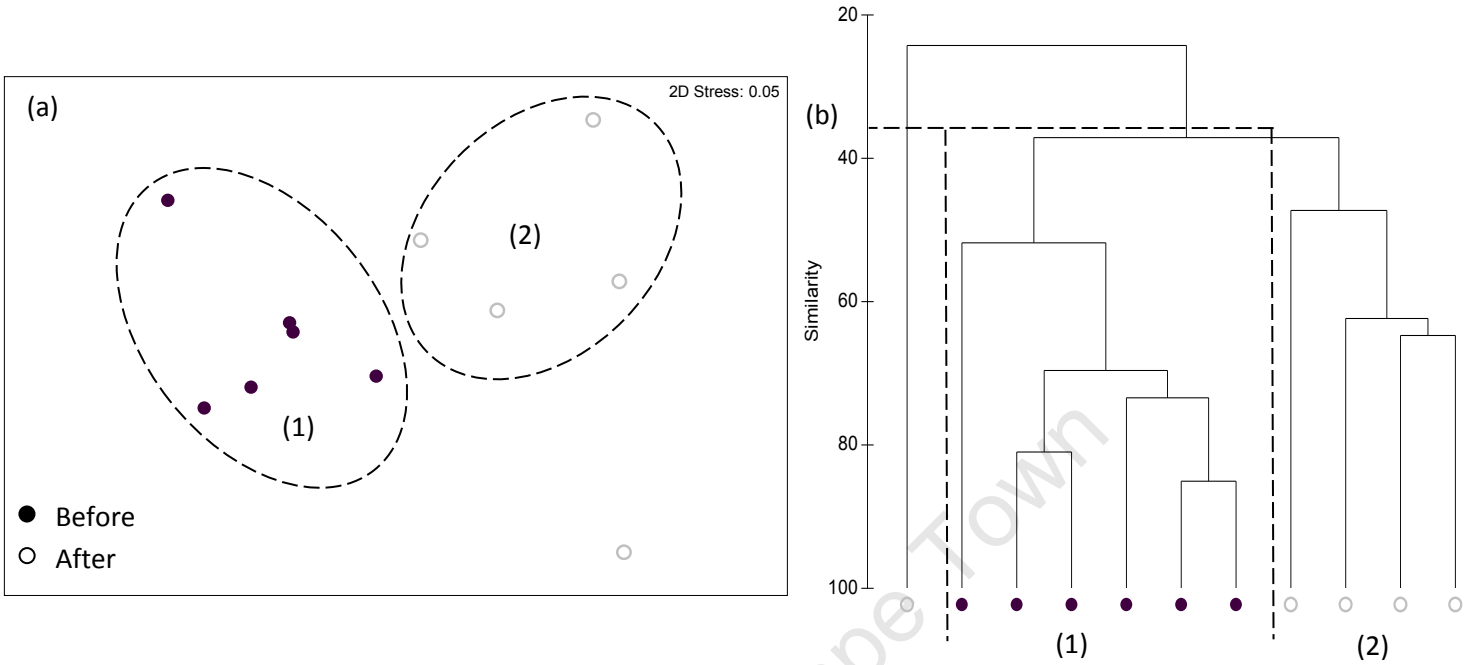


Figure 7.11 **a) MDS plot and b) Hierarchical clustering dendrogram of Bray-curtis similarities of benthic algal community structure at Site 1 before and after a Class 4 flood event in May 2010.**

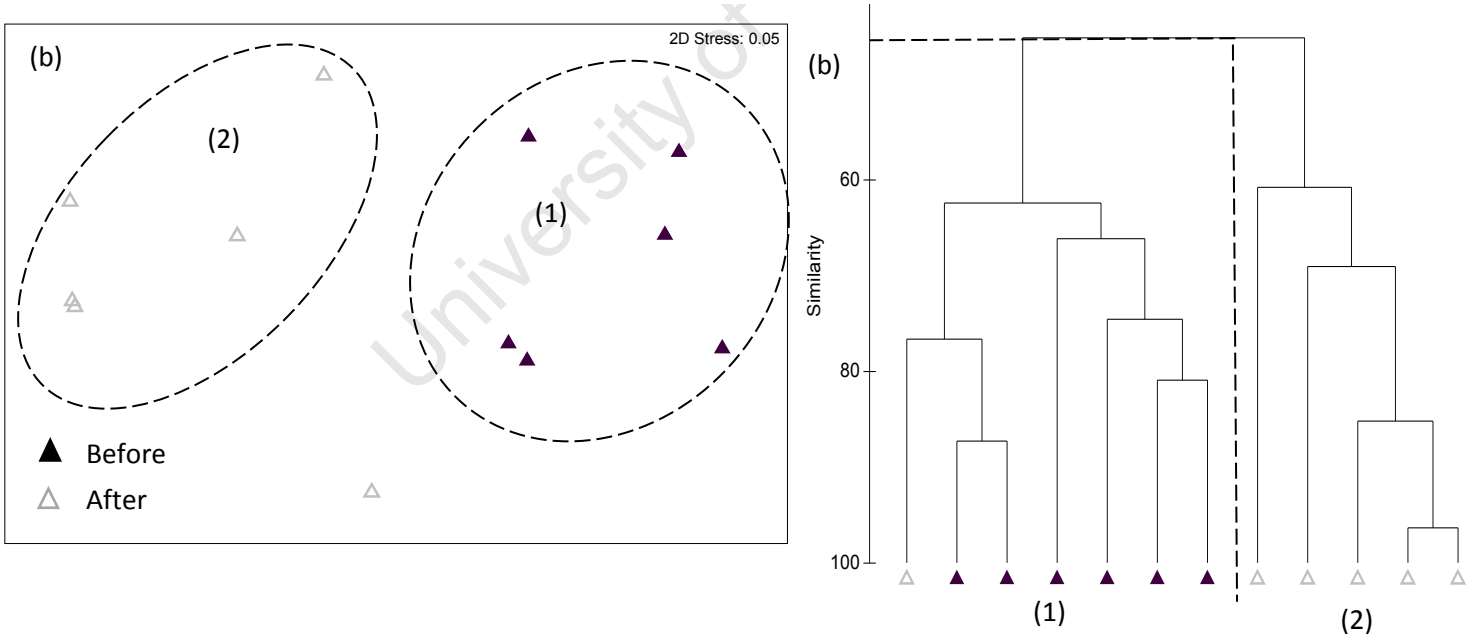


Figure 7.12 **a) MDS plot and b) Hierarchical clustering dendrogram of Bray-curtis similarities of benthic algal community structure at Site 4 before and after a Class 4 flood event in May 2010**

Table 7.4 **a) A two-way PERMANOVA of differences in community composition between Sites 1 and 4 and before and after the May flood event in 2010 and b) results of the pair wise tests before and after the flood event for each site separately. Significant values at p≤0.05 are given in *bold italics*.**

Main test

Groups	***df***	***SS***	***MS***	***Pseudo-F***	***p***
Site	1	5446	5446	6.742	***0.0004***
Treatment	1	11677	11677	14.456	***0.0001***
Site x Treatment	1	2451	2451	3.035	***0.0231***
Residuals	19	15347	808		
Total	22	34863			

Pairwise tests	*t*	*p*
Site 1: before and after	2.663	***0.0021***
Site 4: before and after	3.3777	***0.0024***

Table 7.5 **a) P-values for pair-wise comparisons of differences in variability between sites and treatments based on the Bray-Curtis similarity matrix of square-root transformed benthic algal taxa using PERMDISP and b) the average distance to centroid based on Euclidean distances where the greater the distance, the greater the dispersion (or variability) among samples.**

Pairwise comparisons

Groups	t	P
Site 1B vs Site 4B	0.616	0.671
Site 1B vs Site 1A	1.821	0.135
Site 4B vs Site 4A	1.121	0.393
Site 1A vs Site 4A	1.818	0.205

Average distance to centroid

Site	Before	After
Site 1	21.6	34.1
Site 4	19.0	22.7

A comparison between the relative contribution of different taxa to the benthic algal communities at Sites 1 and 4 prior to the flood (Figure 7.13) shows why these two communities were distinct as expected under different levels of enrichment. The Berg River community prior to flooding (Site 1B) was dominated by the single-celled cyanophyte, *Chamaesiphon* spp. with an equal but much smaller portion of the diatom *E. rhomboidae* and the colonial chlorophyte, *Desmococcus* spp. Other taxa present in low abundance included the unbranched filamentous cyanophyte, *Homeothrix* spp., the colonial cyanophyte, *Aphanocapsa* spp., the Euglenoid, *Trachelomonas* spp., and the colonial chlorophytes, *Cosmarium* spp., *Actinotaenium* spp. and Chl*orococcum* spp. *Hapalosiphon* spp. was the only branched chlorophyte present but was present in very low numbers, comprising only 0.2%

of the community prior to flooding (Figure 7.13). The community therefore was dominated by single-celled diatoms and cyanophytes, with some colonial cyanophytes and chlorophytes and very low densities of filamentous taxa (Figure 7.13).

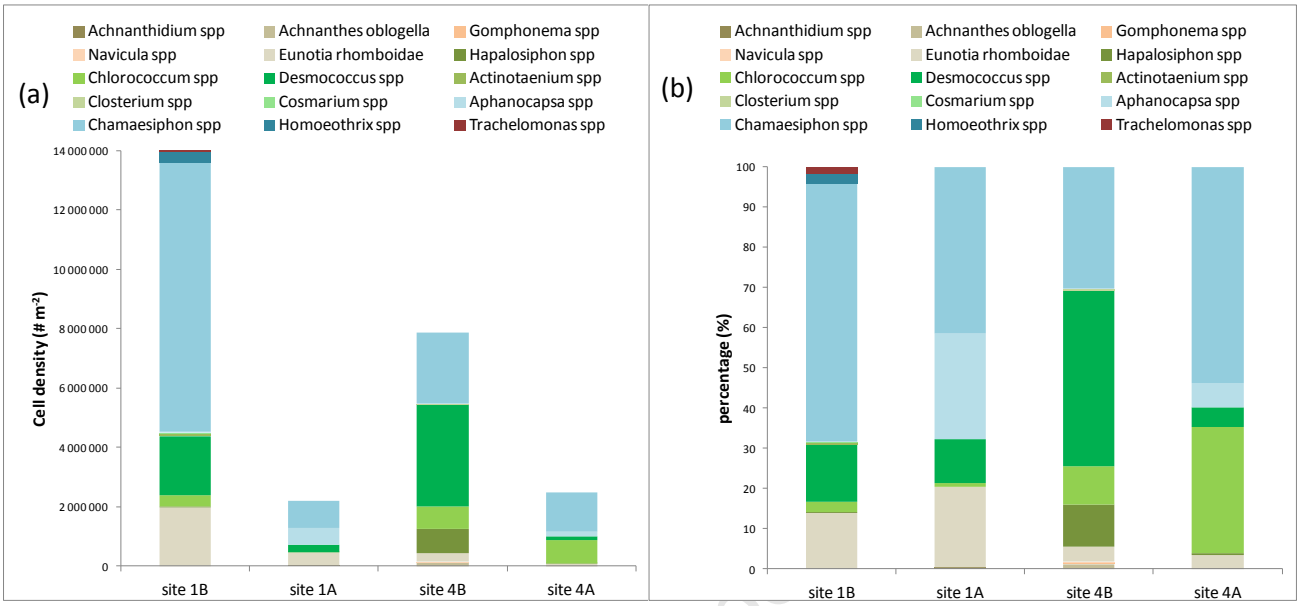


Figure 7.13 **a) Density and b) relative percentage contribution of benthic algal taxa at Sites 1 and 4 on the Berg and Molenaars Rivers respectively before and after a Class 4 flood event in May 2010.**

By contrast, the benthic algal community in the Molenaars River (Site 4B) was dominated by chlorophytes, mainly colonies of *Desmococcus* spp., but also branched-filaments of *Hapalosiphon* spp. and colonies of Chl*orococcum* spp. prior to the flood (Figure 7.13). *Chamaesiphon* spp. was however present in relatively high abundance (Figure 7.13). Besides *E. rhomboidae*, other diatoms that occurred in low densities included *Achnanthes oblogella*, *Gomphonema* spp., and *Navicula* spp. The community structure on the Molenaars River was therefore comprised of a greater abundance of taxa with larger cells and far more complex physiognomies relative to the Berg River, which was dominated by a high abundance of small single-celled cyanophytes prior to flooding at the end of the growing season in 2010.

Nevertheless, a SIMPER analysis of differences between Sites 1 and 4 prior to flooding shows that these sites were only 48.12% dissimilar (Figure 7.14). A higher abundance of *Chamaesiphon* spp. and *E. rhomboidae* and a lower abundance of the two chlorophytes, *Desmococcus* spp. and *Hapalosiphon* spp. at Site 1 were the key taxa responsible for the significant difference in the pre-flood communities a these two sites (Figure 7.14).

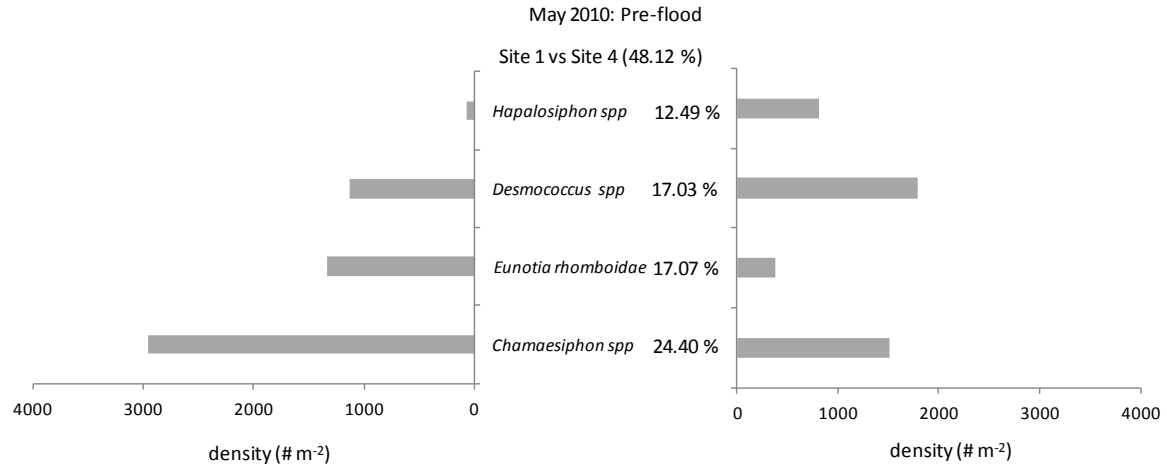


Figure 7.14 SIMPER analysis of benthic algal density data representing the community before the flood disturbance event at Site 1 and Site 4 in May 2010. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.

The greatest change in the proportion of taxa pre- and post-flooding on the Berg River was a reduction in the abundance of *Chamaesiphon* spp. and an increase in the relative contribution of *Aphanocapsa* spp. Taxa that disappeared with the flood included the large-celled desmids, *Actinotaenium* spp. and *Cosmarium* spp., the filamentous cyanophyte, *Homeothrix* spp. and *Trachelomonas* spp., a Euglenophyte that is usually planktonic rather than attached to the substrate (Figure 7.13). Thus the total number of taxa halved (i.e. 10 pre-flood to 5 post-flood) in response to the flood event. Pre- and post-flood communities on the Berg River were 66.21% dissimilar according to a SIMPER analysis (Figure 7.15). Despite the elimination of 5 taxa following the flood event, the significant difference between these two communities was driven largely by a reduction in the relative abundances of the dominant taxa, *Chamaesiphon* spp., *E. rhomboidae* and *Desmococcus* spp., in response to the flood event (Figure 7.15).

The greatest difference between pre-and post-flood communities on the Molenaars River was a large decrease in the abundance of *Desmococcus* spp., with an increase in the relative proportion of *Chamaesiphon* spp., and Chl*orococcum* spp. The branched chlorophyte, *Hapalosiphon* spp. and the diatoms, *Gomphonema* spp., and *Navicula* spp. disappeared completely, while most of the other taxa declined in abundance. The pre- and post-flood communities on the Molenaars River were 53.84% dissimilar and this difference was driven largely by a significant decline in the abundance of *Desmococcus* spp. following the flood event (Figure 7.16). A reduction in the abundance of *Hapalosiphon* spp., Chl*orococcum* spp. and *Chamaesiphon* spp., also contributed to the distinction between these two communities (Figure 7.16).

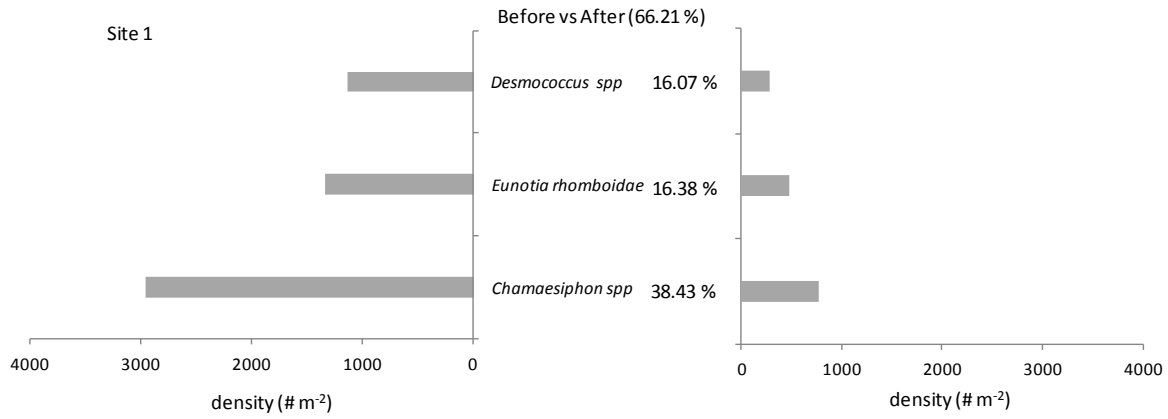


Figure 7.15 **SIMPER analysis of benthic algal density data before and after the Class 4 flood event in May 2010 at Site 1 on the Berg River. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.**

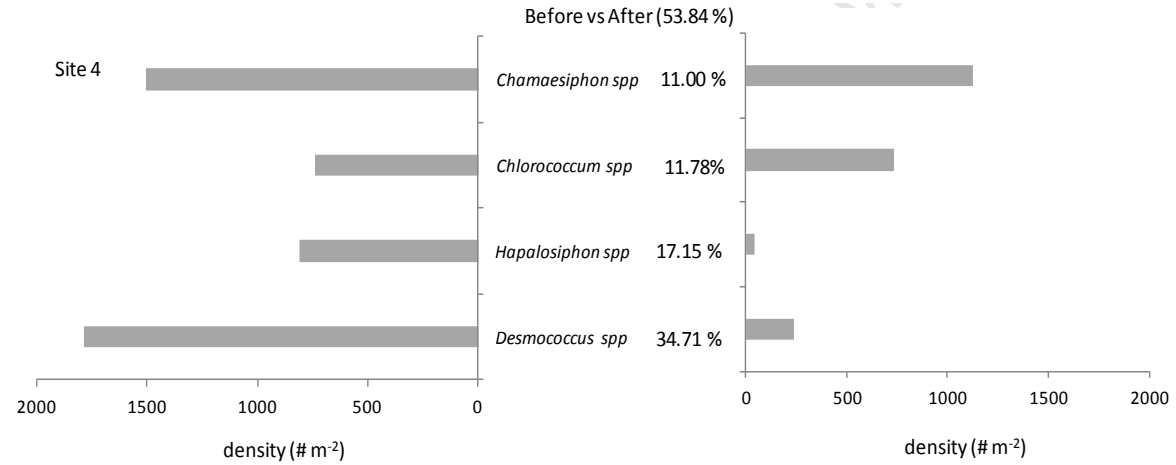


Figure 7.16 **SIMPER analysis of benthic algal density data before and after the Class 4 flood event in May 2010 at Site 4 on the Molenaars River. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.**

7.3.3 The effects of a Class 3 flood on periphyton communities in spring 2010

Despite greater nutrient enrichment at Site 4 on the Molenaars River, the pre-flood Chl *a* biomass at Site 1 in October 2010 was greater than that recorded at Site 4 (Figure 7.17), although the difference was not significant (Table 7.6). A significant decrease of 56 % in Chl *a* biomass was observed at Site 1 in response to a Class 3 flood event whereas Chl *a* biomass only declined by 9% at Site 4 and this slight decline was not significant (Table 7.6; Figure 7.17).

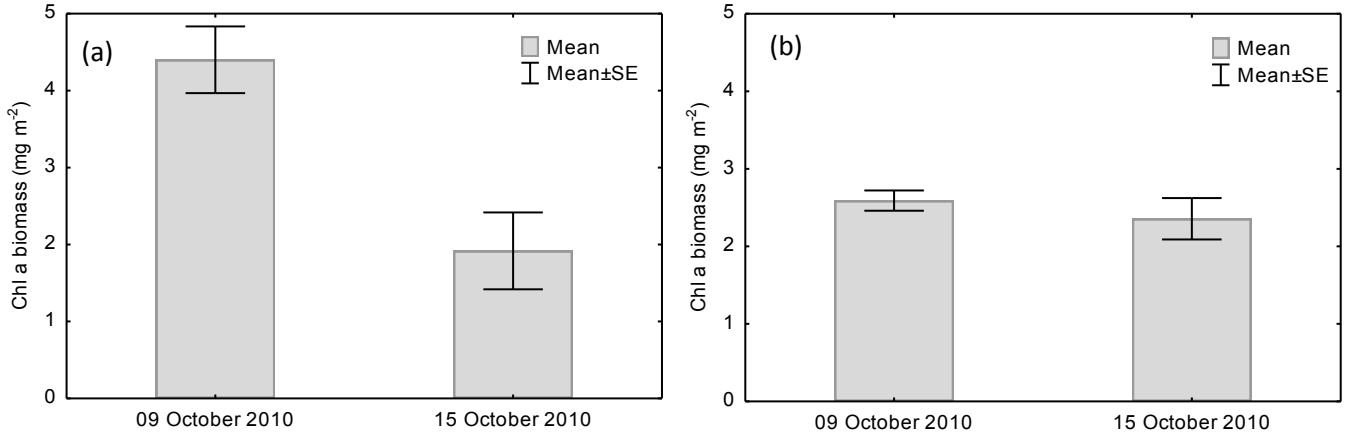


Figure 7.17 **Mean Chl *a* biomass in mg m^{-2} at a) Site 1 on the Berg River and b) Site 4 on the Molenaars River measured immediately before and after a Class 3 flood event in October 2010.**

Table 7.6 **a) A two-way ANOVA of Chl *a* biomass between sites and between treatments (i.e. pre- and post-flood) in October 2010 and b) P-values for the post-hoc comparisons of sites and treatments based on Tukey tests. Significant values at p≤0.05 are given in *bold italics.***

	SS	Df	MS	F	p
Site	0.007	1	0.007	0.471	0.504
Treatment	0.103	1	0.103	7.096	***0.019***
Site x Treatment	0.066	1	0.066	4.571	*0.051*
Error	0.203	14	0.014		

	S1: Before	S1: After	S4: Before
S1: Before			
S1: After	***0.005***		
S4: Before	0.235	0.521	
S4: After	0.129	0.737	0.988

AFDM was marginally greater at Site 4 relative to Site 1 prior to the onset of the Class 3 flood in October 2010 (Figure 7.18, Table 7.7). Nevertheless, AFDM did not change significantly at either site

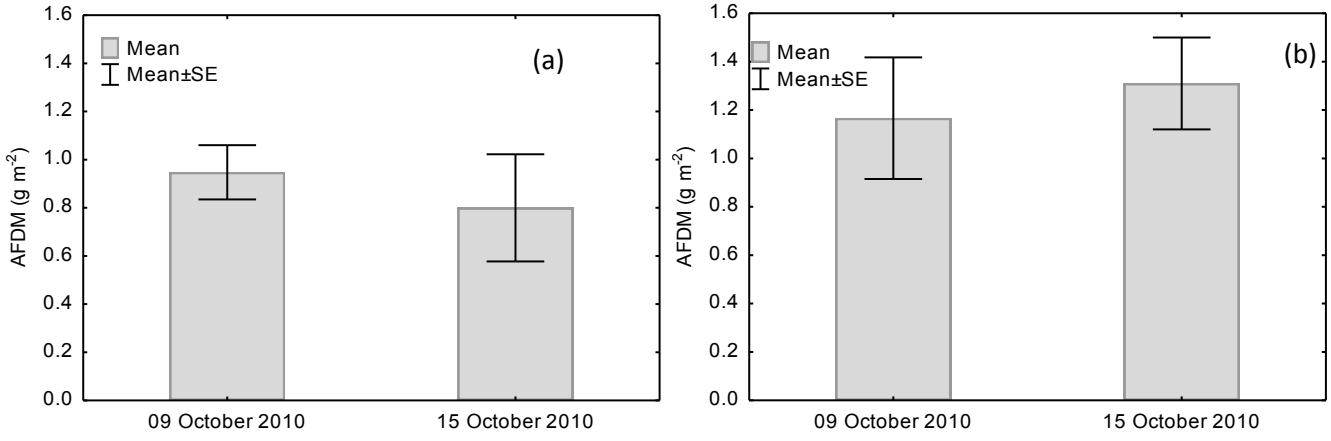


Figure 7.18 **Mean Chl *a* biomass in mg m^{-2} at a) Site 1 on the Berg River and b) Site 4 on the Molenaars River measured immediately before and after a Class 3 flood event in October.**

in response to the Class 3 spring flood (Figure 7.18, Table 7.7).

Table 7.7 **A two-way ANOVA of AFDM between sites and between treatments (i.e. pre- and post-flood) in October 2010. There were no significant differences at p≤ 0.05, but significance at p≤0.1 is given in *italics*.**

	SS	Df	MS	F	p
Site	0.134	1	0.134	3.439	*0.085*
Treatment	0.006	1	0.006	0.142	0.712
Site x Treatment	0.038	1	0.038	0.981	0.339
Error	0.544	14	0.039		

Despite the marginal site and treatment differences in periphyton based on measures of biomass, the MDS plot and hierarchical cluster diagram in Figure 7.19 shows that periphyton communities at Sites 1 and 4 were distinctly different from each other both before and after the Class 3 flood in October 2010. These differences were confirmed by the PERMANOVA (Table 7.8) and most likely reflect differences in nutrient availability between these sites, as expected.

Besides these site differences, the PERMANOVA results indicate that the pre- and post-flood communities differed significantly at both sites (Table 7.8). Unlike May 2010 when the pre- and post-flood communities were more distinct than the site differences (Table 7.4), the flood effects were less distinct than the site differences during October 2010 (Table 7.8, Figure 7.19). The Molenaars River community at Site 4 did, however, separate distinctly into two groups representing pre- and post-flood communities, with the exception of one replicate sample after flooding which grouped with the pre-flood community (Figure 7.20). Differences in the community structure at Site 1 on the Berg River were less distinct with two of the post-flood replicates grouping with the pre-flood community (Figure 7.21).

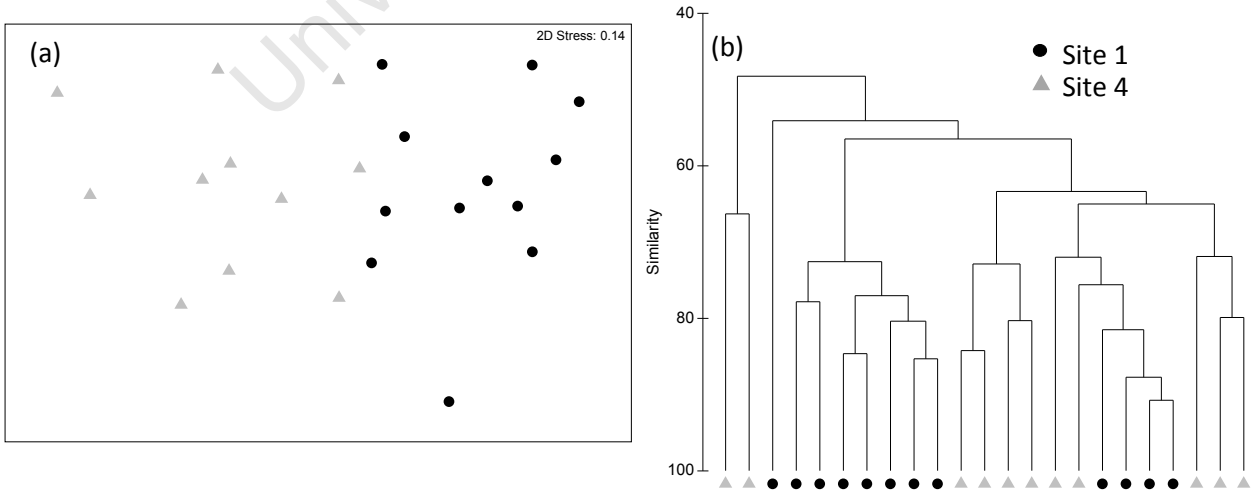


Figure 7.19 **a) MDS plot and b) Hierarchical clustering dendrogram of Bray-curtis similarities of benthic algal community composition based on square-root transformed benthic algal taxa for each site and treatment (pre- and post-flood) in October 2010.**

Table 7.8 **Two-way PERMANOVA results for differences in community structure between sites 1 and 4 and before and after the spring flood event.**

Main test

Groups	***df***	***SS***	***MS***	***Pseudo-F***	***p***
Site	1	6831	6831	14	***0.0001***
Treatment	1	1688	1688	3	***0.0192***
Site x Treatment	1	1492	1492	3	***0.0298***
Residuals	19	9572	504		
Total	22	19340			

Pairwise tests	*t*	*p*
Site 1: before and after	1.665	*0.053*
Site 4: before and after	1.871	***0.002***

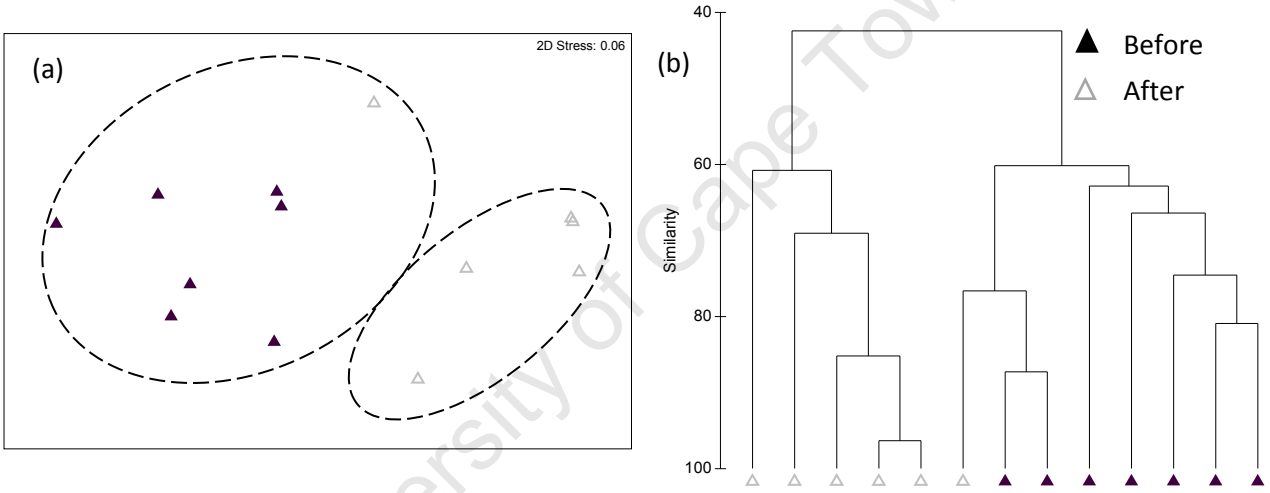


Figure 7.20 **a) MDS plot and b) Hierarchical clustering dendrogram of Bray-curtis similarities of benthic algal community composition based on square-root transformed benthic algal taxa at Site 4 before and after a Class 4 flood event in May October 2010.**

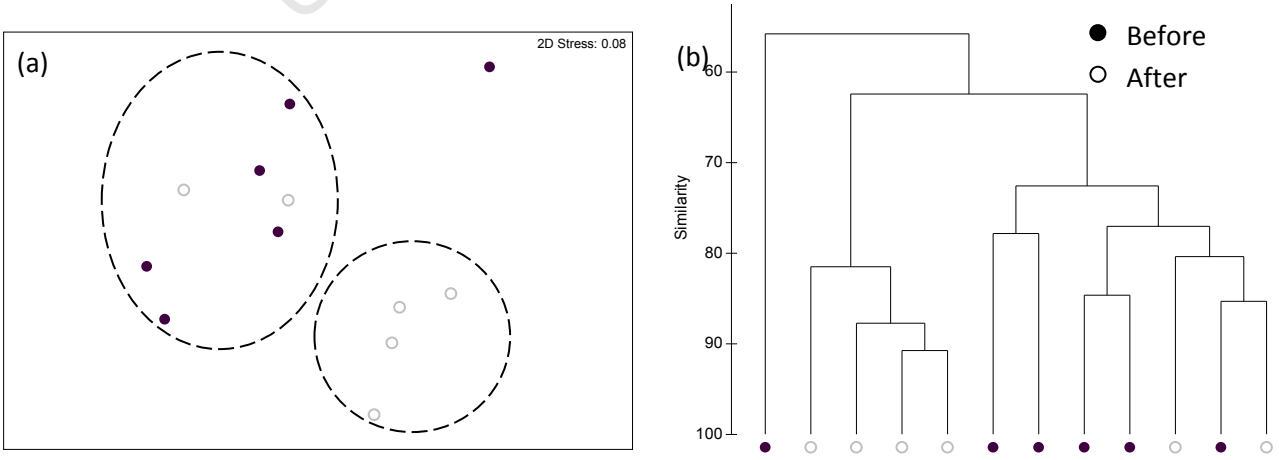


Figure 7.21 **a) MDS plot and b) Hierarchical clustering dendrogram of Bray-curtis similarities of benthic algal community composition based on square-root transformed benthic algal taxa at Site 1 before and after a Class 4 flood event in October 2010.**

Benthic algal densities in October 2010 reflected the patterns in Chl *a* biomass between sites and treatments (Figures 7.18a and 7.22a) with the greatest overall density at Site 1 prior to flooding (Figure 7.22a). Figure 7.22 suggests that the colonial chlorophyte, *Desmococcus* spp., and the single-celled diatom, *Eunotia rhomboidae* were far more abundant at Site 1 compared to Site 4, regardless of the flood event. The communities in both rivers were however dominated by *Desmococcus* spp., both before and after the flood event (Figure 7.22). Nevertheless, two filamentous chlorophytes, *Hapalosiphon* spp., and *Mougeotia* sp.1, were prevalent at Site 4 on the Molenaars River prior to flooding but were absent at Site 1. The presence of taxa with more complex, branched structures at Site 4 compared to Site 1 where single-celled diatoms were more prevalent, most likely reflects differences in the nutrient availability at these two sites as expected. Indeed, SIMPER analysis shows that the distinction between Sites 1 and 4 prior to flooding was driven largely by the greater abundance of *Desmococcus* spp., *Eunotia rhomboidae* and the single celled cyanophyte, *Chamaesiphon* spp., and the absence of *Hapalosiphon* spp. at Site 1 (Figure 7.23).

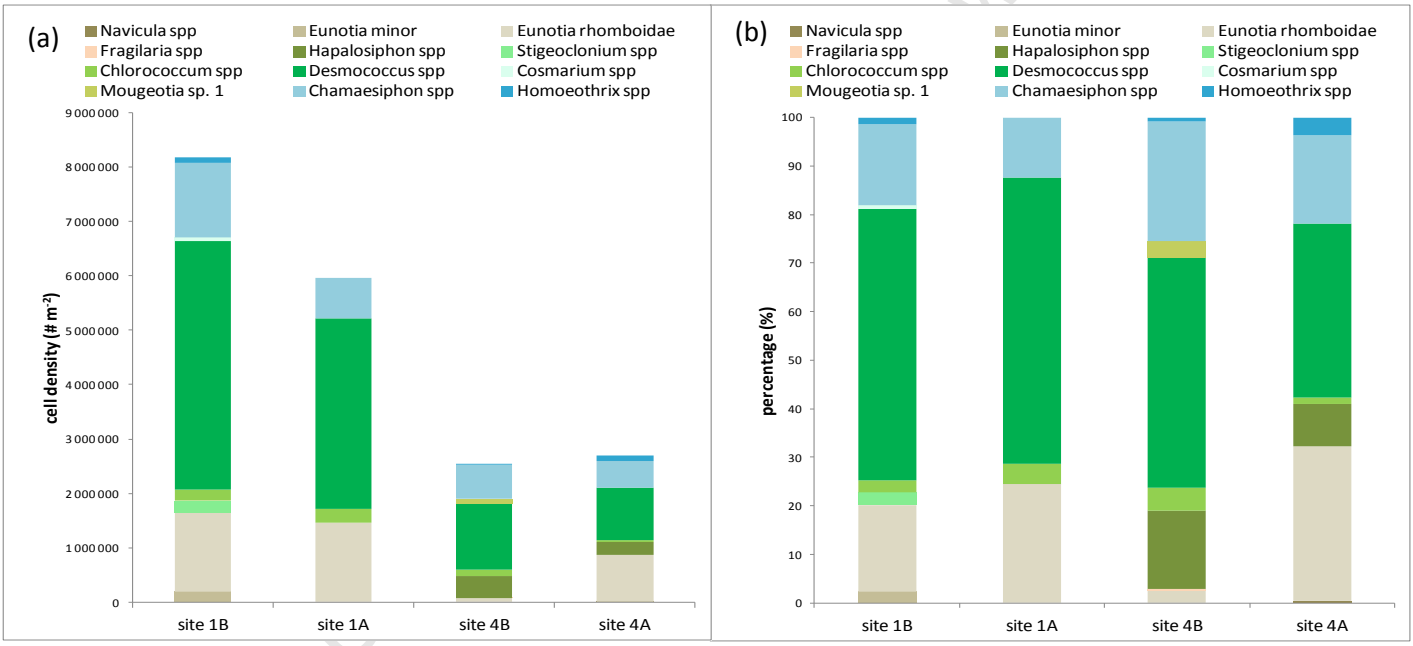


Figure 7.22 **a) Density and b) relative percentage contribution of benthic algal taxa at Sites 1 and 4 before and after flooding in October 2010**

A comparison between the pre- and post-flood community structure at Site 1 shows a loss of the diatom, *Eunotia minor*, the large-celled desmid, *Cosmarium* spp., as well as two branched taxa, *Stigeoclonium* spp. and *Homeothrix* spp., which were all present in low densities prior to flooding (Figure 7.22). Nevertheless, the communities before and after flooding were only 34.72% dissimilar and the distinction between these communities was driven largely by a decline in the dominant taxa and not the loss of those that were rare prior to flooding (Figure 7.24).

The spring flood at Site 4 resulted in a loss of *Mougeotia* sp. 1, and a large decline in *Hapalosiphon* spp., both filamentous chlorophytes (Figure 7.22). Nevertheless, an increase in the single-celled diatom, *E. rhomboidae* after flooding was the main driver for the distinction between pre- and post-flood communities in the SIMPER analysis (Figure 7.25).

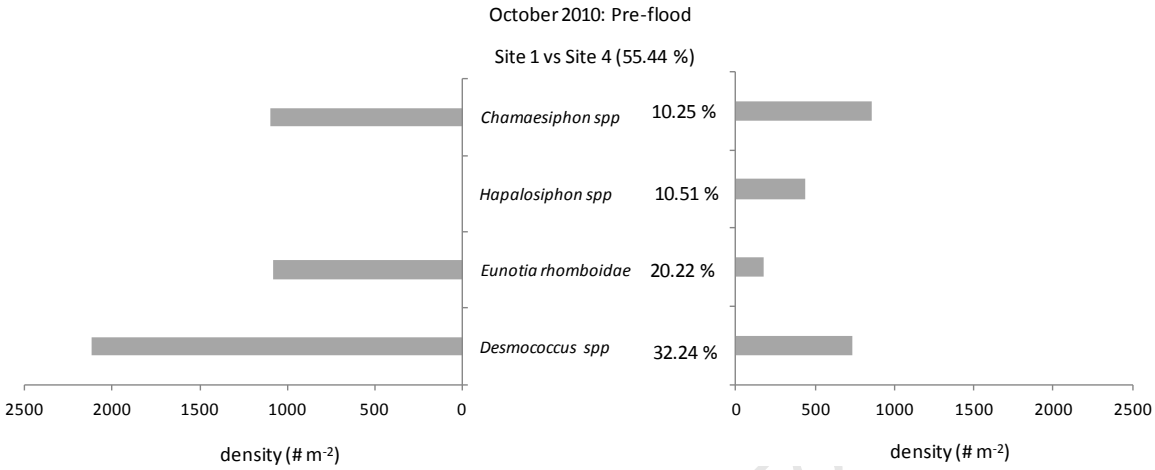


Figure 7.23 **SIMPER analysis of benthic algal density data representing the community before the flood disturbance event at Site 1 and Site 4 in October 2010. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.**

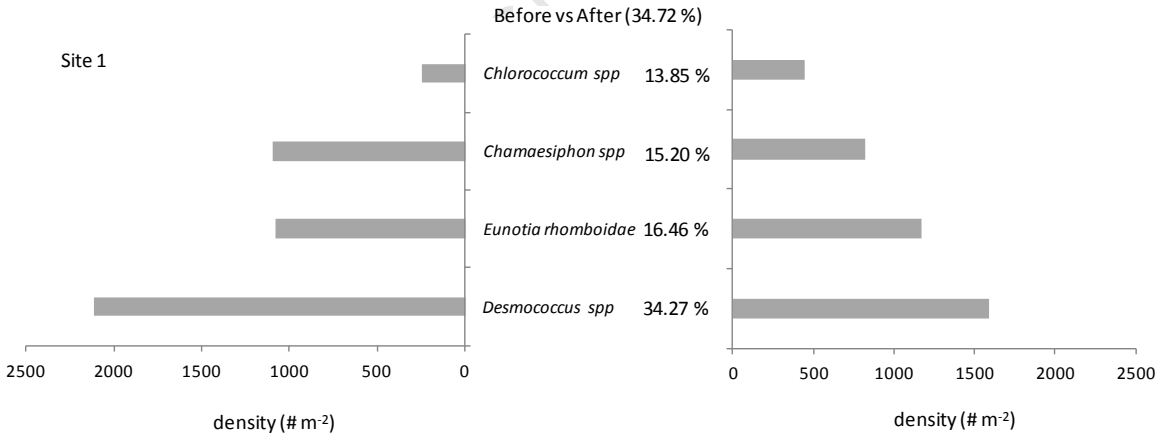


Figure 7.24 **SIMPER analysis of benthic algal density data before and after the Class 3 flood event in October 2010 at Site 4 on the Molenaars River. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.**

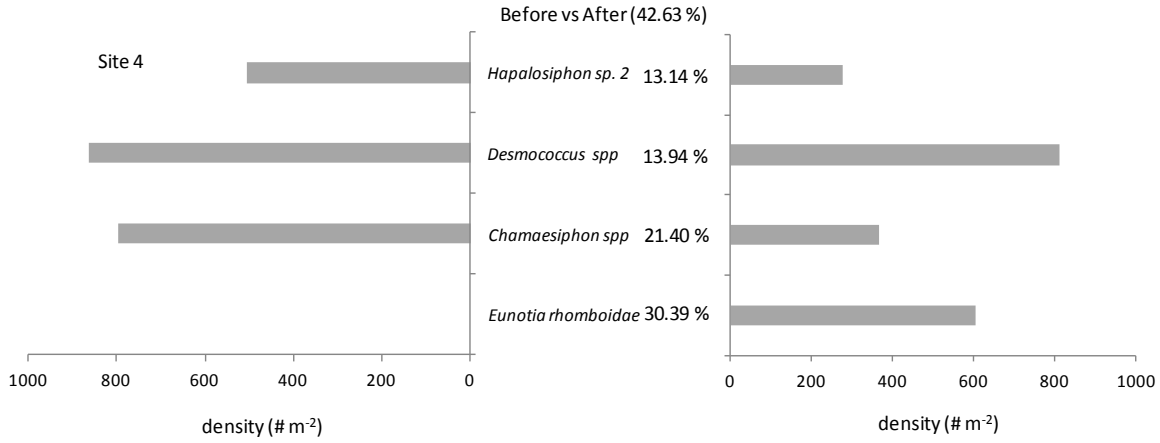


Figure 7.25 **SIMPER analysis of benthic algal density data before and after the Class 3 flood event in October 2010 at Site 1 on the Berg River. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxa is shown for each bar in the graph respectively.**

7.4 POST-FLOOD RECOVERY OF PERIPHYTON

7.4.1 Short- term changes in periphyton biomass following a flood event

A one-way ANOVA of Chl *a* biomass before a Class 3 flood and that on three subsequent sampling occasions over a two week period showed no significant change in Chl *a* biomass (F=1.57; df=3; p=0.22; Figure 7.26). Nevertheless, Figure 7.26 suggests that two weeks after the flood event, average Chl *a* biomass was double that prior to flooding. Although the increase was not statistically significant in the ANOVA analysis, a t-test between Chl *a* biomass prior to flooding (i.e. 10 June 2008) with that two weeks later (i.e. 24 June 2008) shows a weakly significant increase in Chl *a* biomass (F=4.05; df=1; p=0.065). Figure 7.26 shows an increase in the standard error around mean Chl *a* biomass after flooding suggesting an increase in local-scale patchiness of periphyton following the flood. It is not surprising therefore that the increase in Chl *a* biomass following the event was not statistically significant in the ANOVA analysis.

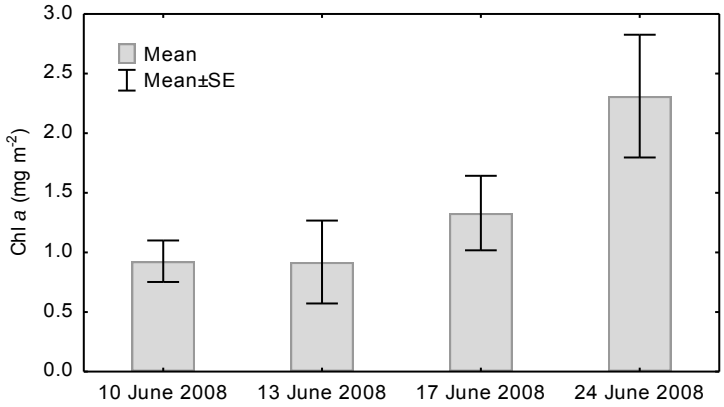


Figure 7.26 **Mean Chl *a* biomass measured prior to a Class 3 flood event (10 June 2008) and on three subsequent occasions following the event at Site 2 on the Berg River.**

By contrast, a significant reduction in AFDM with little variability around the mean was measured immediately after the event (F=19.27; df=3; p=0.000; Figure 7.27). Like Chl *a* biomass, however, AFDM increased rapidly thereafter (Figure 7.27). After one week, AFDM was slightly lower than that prior to the flood event and after two weeks, pre- and post-flood AFDM was similar (Figure 7.27). A *post-hoc* Tukey test of differences between individual sampling occasions shows that AFDM was only significantly lower immediately following the flood event and recovered rapidly thereafter (Table 7.9).

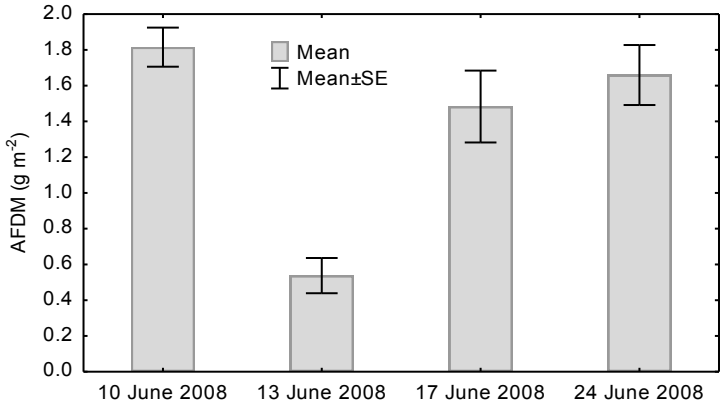


Figure 7.27 **Mean Ashfree Dry Mass (AFDM) measured prior to a Class 3 flood event (10 June 2008) and on three subsequent occasions following the event at Site 2 on the Berg River.**

Table 7.9 **Tukey post-hoc tests showing differences in AFDM biomass (g m^{-2}) between different sampling occasions over the two-week recovery period at Site 2 on the Berg River.**

	10 June 2008	13 June 2008	17 June 2008	24 June 2008
10 June 2008		***0.000***	0.489	0.916
13 June 2008	***0.000***		***0.000***	***0.000***
17 June 2008	0.489	***0.000***		0.848
24 June 2008	0.916	***0.000***	0.848	

The autotrophic index (AI), as a measure of the balance between autotrophic and heterotrophic components of the periphyton (Figure 7.28), declined considerably after the flood event. The high pre-flood AI measure, relative to that after two weeks suggests that the community moved from one that was almost completely dominated by heterotrophs and/or organic detritus to one where viable algae became more prolific. Nevertheless, the AI measure remained over 400 suggesting that the heterotrophs and/or detritus community remained dominant, even after flooding (Biggs and Close 1989; Fayolle *et al*. 1998).

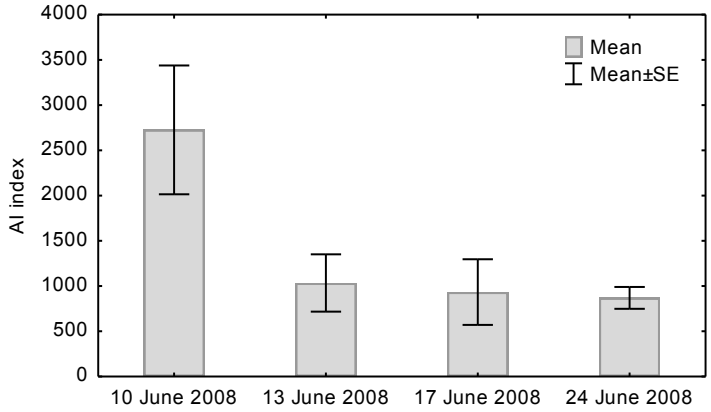


Figure 7.28 **Autotrophic Index (AI) of the periphyton communities before and after a Class 3 flood event at Site 2 on the Berg River in June 2008.**

7.4.2 Short term changes in periphyton community structure following a flood event

The MDS ordination and cluster diagram in Figure 7.29 suggest that there was no distinct shift in the benthic algal community immediately following the Class 3 flood. Nevertheless, the arrows in Figure 7.29a suggest that there may be some change in community structure with time, with the greatest difference a week after the flood event (A2). Interestingly, some of the replicates from before the flood grouped with replicates from the community two weeks after the event. This suggests that the community may have shifted somewhat over the recovery period but there is evidence to suggest that after two weeks, the community may have recovered somewhat to the pre-flood community structure. A PERMANOVA analysis of differences in algal community structure showed that there were indeed significant differences in these communities over the short term (Pseudo-F=1.99; df=3; p=0.026). As suggested by the MDS ordination and cluster diagram (Figure 7.29), the pair-wise tests

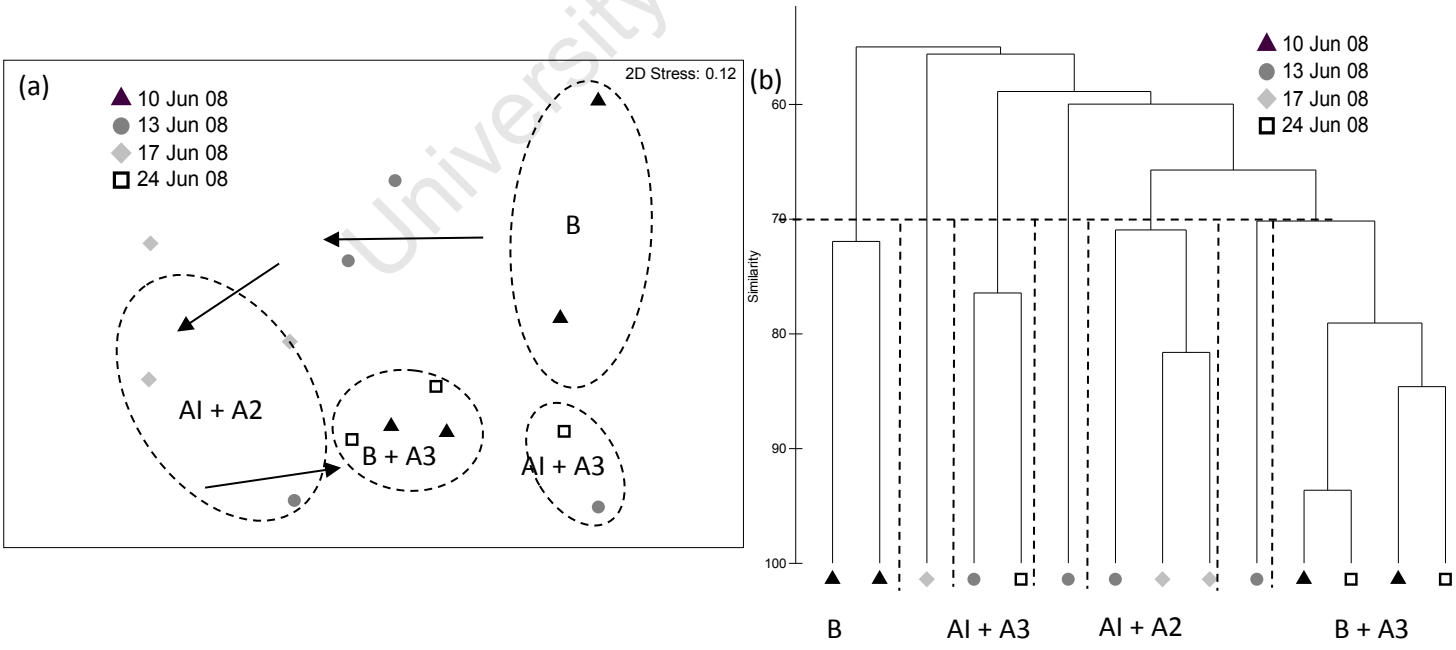


Figure 7.29 **a) MDS ordination and b) Hierarchical cluster diagram of square root transformed algal densities at Site 2 based on replicate samples collected prior to flooding (10 June 2008: B), immediately after flooding (13 June 2008: A1), a week later ((17 June 2008: A2) and two weeks later (24 June 2008: A3). The ellipses indicate the groupings of replicates at 70 % Bray Curtis similarity given in the cluster diagram.**

show that the community one week after the event (A2) was significantly different from that before the flood (B) (Table 7.10). Interestingly, the algal community before and immediately after the event (i.e. A1) were not significantly different from each other (Table 7.10) and two weeks after the event (A3), the community structure was similar to that prior to the flood event with nearly 70% similarity between these two communities (Table 7.11).

Table 7.10 Pair-wise comparisons of PERMANOVA results for differences between the four sampling occasions before the flood event, immediately after the event (A1), a week after the event (A2) and two weeks after the event (A3). Significant differences at p ≤ 0.5 are shown in bold italics and those at p≤0.1 are given in *italics only*.

	Before	A1	A2
A1	0.34		
A2	***0.03***	0.15	
A3	0.40	0.57	0.09

Table 7.11 Similarity comparisons between treatment pairs based on Bray-Curtis similarities

	B	A1	A2	A3
B	66.4			
A1	59.3	57.1		
A2	***53.7***	60.0	73.5	
A3	68.7	64.1	58.6	69.4

Chamaesiphon spp. comprised 54 % of the community composition prior to flooding and maintained its dominance after the flood and over the two week recovery period (Figure 7.30). Despite the persistently high dominance by *Chamaesiphon* spp. over the recovery period, changes in the densities (Figure 7.30a) and relative proportions (Figure 7.30b) of taxa other than *Chamaesiphon* spp. provided an indication of the resistance and resilience of various taxa to flood disturbance (Figure 7.30).

The colonial chlorophyte, *Desmococcus* spp., the desmid, *Gongrosira* spp. and the diatom *E. rhomboidae* each contributed around 10 % to the total density of the community prior to flooding. Other taxa included the branched chlorophyte, *Hapalosiphon* spp., and the diatom, *Navicula* spp. Most of these taxa disappeared with the flood event and the relative proportion of *E. rhomboidae* declined. By contrast, the relative proportion of *Desmococcus* spp. and *Chamaesiphon* spp. both increased with the flood event. The relative density of both *Desmococcus* spp. and *Chamaesiphon* spp. increased further and contributed 27% and 59% respectively to the community structure a week after the flood (A2). A SIMPER analysis between the pre-flood community and that after a week showed that these two taxa were the most influential driving the significant differences between these communities (Figure 7.31). The absence of both the desmid, *Gongrosira* spp. and the diatom, *E. rhomboidae* a week after the flood also contributed to the distinction between these two communities (Figure 7.31).

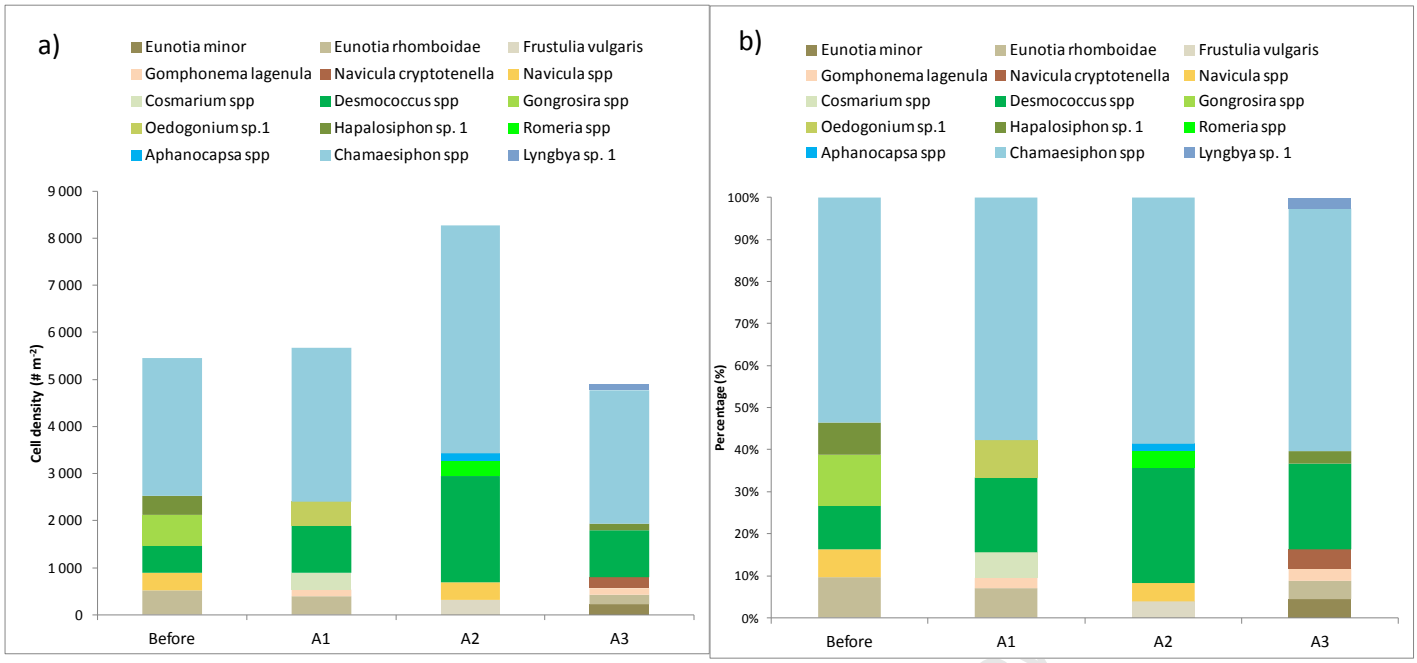


Figure 7.30 **a) Density and b) relative percentage contribution of benthic algal taxa prior to flooding (10 June 2008: Before), immediately after flooding (13 June 2008: A1), a week later (13 June 2008: A2) and two weeks later (24 June 2008: A3).**

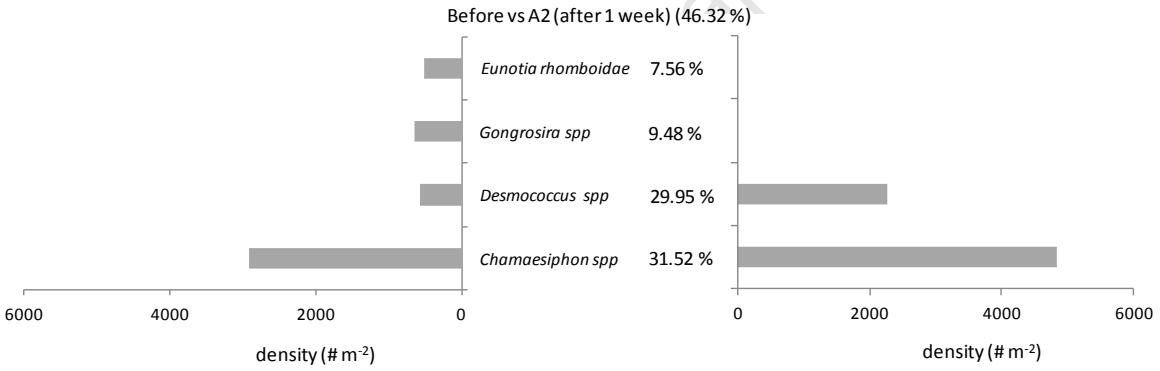


Figure 7.31 **SIMPER analysis of benthic algal density data before and one week after the Class III flood event in June 2008 at Site 2 below the dam on the Berg River. Only those taxa which together contribute 70% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.**

Nevertheless, two weeks after the flood event (A3), the number of taxa present had increased slightly due to the presence of more diatom species (i.e. *Eunotia minor, G. lagenula and Navicula cryptotenella*) that were not present in the community prior to flooding. Also, the branched chlorophyte, *Hapalosiphon* spp. returned to the community after two weeks and the filamentous cyanophyte, *Lyngbya* sp.1 was also present in this sample. The relative contribution of *Chamaesiphon* spp. and *Desmococcus* spp. also declined slightly between the first week (A2) and the second week (A3) after the flood.

7.5 DISCUSSION

7.5.1 Response to floods of different sizes

Flood events may not always result in the loss of periphyton biomass or a shift in the community structure of benthic algae in rivers because shear stress associated with small spates may not be great enough to dislodge the benthic mat. Grimm and Fisher (1989), for example, found a significant correlation between the removal of Chl *a* biomass and flood magnitude but showed that this relationship is not linear such that removal will only occur above a specific threshold disturbance magnitude. Evidently, a spate at the upper limit of a Class 1 flood event on the Molenaars River during May 2008 did not significantly change the algal community at Site 3, although a significant decrease in AFDM was observed. AFDM, as a measure of the overall biomass of the periphyton matrix, includes all organic material whereas Chl *a* biomass is a measure of the actively living benthic algae only. A number of studies have shown that where the non-algal component of the periphyton is composed largely of unattached dead or decaying organic matter, only a small flood event is required to significantly change the AFDM whereas Chl *a* biomass is far more resistant to scour (Biggs and Close, 1989; Fayolle *et al*. 1998). Biggs and Close (1989), for example, found that floods greater than 6 times the preceding base flow lead to scour of AFDM whereas far larger events were necessary to significantly shift Chl *a* biomass. Despite a non-significant change in the Chl *a* biomass at Site 3 following the spate in May 2008, the decrease in both measures of biomass and the absence or decline in the abundance of specific taxa suggest that the threshold for disturbance of periphyton communities may be slightly larger than the Class 1 event measured in this study. Indeed, correlations between Chl *a* biomass and the recovery period following each of the four DRIFT flood classes in Chapter 5 (Table 5.1), suggested that floods within the range of a Class 2 were the best predictors of intra-annual patterns of Chl *a* biomass. Although there was little difference between the strength of the correlation for Class 1 and 2 floods at Site 3, the Distance Based Linear Model (DistLM) in Chapter 5 showed that, of the floods measured in the Berg and Molenaars Rivers over 21 months, those within the range of a Class 2 or larger were the best predictors of temporal change in both Chl *a* biomass and benthic algal community composition.

In a study of macro-invertebrate response to flood disturbance in the Berg and Molenaars Rivers, Ractliffe (2009) found that the disturbance threshold for macro-invertebrates was defined by bed movement in these rivers, which translate into DRIFT Class 3 floods. As discussed in Chapter 5, disturbance of the periphyton mat probably occurs at a lower discharge than that defining bed movement because shear stress and sediment scour can remove considerable biomass, prior to bed movement (Biggs and Thomsen 1995; Jowett and Biggs 1997; Francoeur and Biggs 2006; Webb *et al*. 2006). Class 4 floods in the Berg and Molenaars Rivers are able to move between 25 and 43% of the bed, ranging in size from small cobbles through to boulders (Cullis *et al.* 2008). It is therefore not surprising that a large intra-annual flood (DRIFT Class 4 flood) resulted in a 92 % decrease in Chl *a* biomass at Site 1 and a 73 % decrease in Chl *a* biomass at Site 4 during May 2010. The flood had similar effects to those previously described as catastrophic events for periphyton communities in both arid (Grimm and Fisher 1989; Lytle 2000) and pre-alpine (Uehlinger *et al*. 1996; Robinson *et al.*

2004) rivers. Less Chl *a* biomass loss was evident following a Class 3 flood with magnitudes in both rivers above the bed movement discharges described by Ractliffe (2009) for these rivers. The effect of a flood expected to impose similar shear stresses near the bed, was however significantly different between Site 1 with a 56 % decrease in Chl *a* biomass and Site 4, with only a 9% change in biomass in October 2010. Flood magnitude and associated increases in near-bed velocity and shear stress are not the only factors affecting the response of periphyton communities to flood disturbance, however.

7.5.2 Resistance to flood disturbance

A number of studies on the resistance of periphyton to physical disturbance indicate that susceptibility to disturbance is influenced not only by flood magnitude but also by the growth form and age of the community (Tett *et al.* 1978; Biggs and Close 1989; Uehlinger 1991; Peterson and Stevenson 1992; Biggs and Thomsen 1995; Biggs *et al.* 1999; Francoeur and Biggs 2006; Fuller 2011). Consequently, this study hypothesized that, given a flood with similar shear forces acting at the bed, benthic algal communities in the Molenaars River would be more susceptible to change compared with the Berg River community because enrichment of the Molenaars River would promote the growth of a periphyton mat with a higher biomass and more complex structure. On the contrary, a greater loss of Chl *a* biomass was evident at Site 1 on the Berg River compared with Site 4 on the Molenaars River, following both the Class 4 flood in May 2010 and the Class 3 flood in October 2010. In their study of the relative effects of flows and nutrients on periphyton dynamics in New Zealand rivers, Biggs and Close (1989) found that the efficiency of scour was driven more by periphyton biomass immediately prior to flooding, than the actual magnitude of the flood itself. Similarly, Uehlinger *et al*. (1996) found that the flow required for detachment of periphyton decreases with increasing biomass. Chl *a* biomass was indeed greater prior to flooding at Site 1 compared with Site 4 during October 2010 which might explain the significant decrease in biomass observed at Site 1 with no significant change in biomass at Site 4. Nevertheless, no significant differences in pre-flood Chl *a* biomass were evident during May 2010 between these sites, yet the community at Site 1 appears less resistant than that at Site 4. Francoeur and Biggs (2006) also found a large difference in resistance between communities with similar initial biomass.

Differences in resistance under conditions of similar physical disturbance can however be linked to differences in composition and physiognomy that may not be detected by differences in pre-flood Chl *a* biomass *per se*. Tett *et al.* (1978) found that small increases in flow removed loosely adhering cyanobacteria and diatoms leaving communities dominated by encrusting chlorophytes and those cyanobacteria that are more resistant to scour. In their study of desert streams, Grimm and Fisher (1989) found that diatoms were most resistant, followed by filamentous chlorophytes and then cyanobacteria, although their post-flood sample was collected a week following the disturbance event. Generally, susceptible growth forms include chain-forming diatoms, uniserate filaments and loosely attached cyanobacterial mats, whereas resistant growth forms include prostrate diatoms and basal cells of chlorophytes (Larned 2010). The communities present at both sites in this study were dominated by either single-celled taxa, mainly the cyanobacteria *Chamaesiphon* spp. and the diatom

E. rhomboidae, or the colonial chlorophyte, *Desmococcus* spp. The community at Site 4 was, however, more structurally complex with a greater proportion of both unbranched and branched filamentous taxa. Whereas filamentous taxa present in low abundance at Site 1 disappeared with the floods in both May 2010 and October 2010, branched filamentous taxa such as the chlorophyte *Hapalosiphon* spp. and the cyanophyte *Homeothrix* spp. did not decrease significantly in October 2010 at Site 4 when no significant change in Chl *a* biomass was measured. In other words, taxa with more complex growth forms were more resistant to flooding at Site 4, below the trout farm compared with Site 1. Similarly, a flood at Site 2 below the Berg Dam with a similar magnitude (Class 3) to that in October 2010 on the upper Berg River did not significantly affect either Chl *a* biomass or the community composition.

It is possible that the physical structure (e.g. the presence of mucilage) of the periphyton mat contributed to differential resistance. Fuller *et al.* (2011) for example, found that communities with *Nostoc* spp. were more resistant to flood disturbance because of a high proportion of mucus in the periphyton mat associated with *Nostoc* colonies. Similarly, Peterson *et al.* (1994) found that benthic mats dominated by an interwoven matrix of filamentous cyanobacteria with mucilage producing diatom taxa were more resistant to flooding than those without mucilage. *Navicula* spp., which was abundant at Site 2 prior to the flood, produces mucilage and may have contributed to creating a more cohesive community matrix that increased its resistance to flooding. Nevertheless, Peterson et al. (1994) indicate that the mucilage produced by motile diatoms does not significantly affect cohesiveness of the community. Also, mucilage producing diatoms were uncommon at Site 4 on the Molenaars River. It is however possible that at both Sites 2 and 4 bacteria and/or fungi that can produce mucilage helped to bind the periphyton mat and thus created a community that was structurally, rather than taxonomically resistant to flood disturbance. These heterotrophic components, although not dominant, may well consist of mucilage-producing bacteria and fungi as indicated by the slimy texture of the substrata at Site 4 (pers. obs.). Also, contrary to the study of Biggs and Close (1989) who found that Chl *a* biomass was significantly more resistant to flooding than AFDM, AFDM at Site 4 was particularly resistant to flooding during May 2010. Whereas Biggs and Close (1989) attributed the greater susceptibility of AFDM to the presence of decaying or dead organic matter that was unattached, it is possible that the non-algal component of the periphyton downstream of the trout farm on the Molenaars River was comprised largely of mucilage producing bacteria and/or fungi which could render the periphyton community as a whole more resistant than the live algal component alone. Without identification of the non-algal components of the periphyton during this study, this explanation is speculative and requires further investigation. It is noteworthy, however, that heterotrophs generally did not dominate the community under enriched conditions at Sites 3 and 4 on the Molenaars River, as suggested by the temporal patterns in the autotrophic index (AI) in Chapter 4.

Differences in resource supply can also affect resistance to flood disturbance (Biggs *et al.* 1999) because thicker mats with more complex forms supported by higher nutrient availability, extend beyond the boundary layer and are therefore more susceptible to scour. Under conditions of high nutrient stress, Biggs *et al.* (1999) found that communities with the same taxonomic structure were

less resistant to scour than those where nutrients were not limiting. Considering that nutrients are more limiting in the Berg River, it may be that nutrient stress accounted for the difference in resistance between these two rivers. Rather than greater nutrient availability in the Molenaars River promoting growth forms that are more susceptible to floods as predicted, this suggests that nutrient availability maintains a community that is more resistant to flood disturbance. The first hypothesis addressed in this study, which stated that periphyton biomass loss would be greater in the Molenaars River compared to the Berg River must be rejected. The importance of resource limitation to community resistance is not unequivocal. Mulholland *et al.* (1991), for example, found that nutrient levels did not affect the resistance of periphyton communities to flood disturbance.

Age or maturity of the periphyton mat is another factor which influences susceptibility to flood disturbance (Biggs and Close 1989; Peterson 1989; Peterson 1996). Consequently, it was hypothesized that communities at the end of the growing season (i.e. May) would be more susceptible to the first floods of the wet season compared with those at the end of the wet season in spring (i.e. October) because mature, senescent mats typical of autumn are less resistant to flooding than communities dominated by early successional taxa at the start of the growing season (Peterson and Stevenson 1992, Larned 2010). Indeed, Chl *a* biomass decreased by 92 % and 73 % at Sites 1 and 4 respectively following the first wet season flood event in May 2010, whereas the spring flood in October 2010 resulted in a far smaller decline of 56% and 9% at Sites 1 and 4 respectively. Periphyton removal was therefore far greater at the end of the growing season in autumn relative to spring at both sites, thus confirming this prediction. Besides the loss of filamentous taxa at Site 1 in both May and October 2010, reflecting their susceptibly to scour, differences between the pre- and post-flood communities during both May and October 2010, were driven largely by a reduction in the abundant taxa at these sites i.e. the single-celled diatom, *Eunotia rhomboidae*, the single-celled cyanobacterium, *Chamaesiphon* spp., and the colonial chlorophyte, *Desmococcus* spp. Similarly, a decrease in the abundance of *Chamaesiphon* spp., *Desmococcus* spp. and *Hapalosiphon* spp. distinguished pre-and post-flood communities in both May and October 2010 at Site 4. Pre-flood differences in community composition between May 2010 and October 2010 were therefore not driven by significant differences in their taxon composition but by differences in the abundance of their dominant taxa.

Temporal differences in resistance to flooding depend to some extent on the physiological condition of cells within algal mats (Peterson and Grimm 1992; Peterson 1996). As the mat thickens, access to light and nutrients becomes limiting for cells at the base of the mat (Dodds 1989; Biggs *et al*. 1999; Peterson and Stevenson 1992). If this condition persists, the mat may slough autogenically even in the absence of high flows (Bouletreau *et al.* 2006). Autogenic sloughing was evident on both the Berg and Molenaars Rivers towards the end of the growing season in 2008 when a significant decrease in periphyton biomass was observed in autumn in the absence of flood disturbance (see Chapter 4, Section 4.3.1). This suggests that the periphyton communities in both rivers experience resource stress towards the end of the growing season following peak biomass. Under such conditions, the periphyton communities at both Sites 1 and 4 during May 2010 may have been more susceptible to even slight increases in velocity, thus causing a greater distinction between pre- and

post-flood communities in autumn compared with spring. Nevertheless, both flood magnitude and duration were greater in May 2010 compared with October 2010. Although this study endeavoured to compare community resistance to floods of similar size, this was unfortunately not possible due to the natural flood regime experience in 2010. The differential effect of near-bed velocities, shear stress and bed movement associated with the larger flood event during May 2010, relative to October 2010 therefore cannot be discounted as a potential factor accounting for the greater change in biomass and community composition evident at both sites during May 2010.

7.5.3 Post-flood recovery of periphyton communities

Despite evidence to suggest that a DRIFT Class 3 flood can significantly change Chl *a* biomass and shift benthic algal community structure in the Berg and Molenaars Rivers, the Class 3 event in June 2008 downstream of the Berg Dam failed to significantly change either Chl *a* biomass or community composition. As discussed in Section 7.5.2, this may be a consequence of mucilage production that creates a more cohesive and thus resistant community. Nevertheless, the AFDM changed considerably following the event. The Autotrophic Index also declined significantly following the flood event, suggesting the removal of organic detritus and/or bacteria and fungi present in the periphyton mat. The post-flood community was therefore far less dominated by heterotrophs with a greater contribution by autotrophic algae compared with the pre-flood community. Fayolle *et al.* (1998) found that in the absence of hydrological disturbance, AI values were higher than in natural systems because increases in AFDM were not accompanied by an increase in Chl *a* biomass. Rather, an accumulation of allochthonous detritus accounted for the high AFDM and restricted the growth of algae, as measured by Chl *a* biomass. Similarly, the flood at Site 2 in June 2008 marked the first flood of the wet season after an extended period of stability, relative to the natural condition upstream of the dam. Nevertheless, a week after the flood event, Chl *a* biomass was marginally greater than prior to flooding, but after two weeks Chl *a* biomass was significantly greater than the pre-flood biomass. Similarly, Biggs and Close (1989) found that Chl *a* biomass increased by 500% after just 19 days following a flood event. Following disturbance which differentially removes detritus and/or heterotrophs but not the live benthic algae, renewed access to resources such as light and nutrients may promote the rapid regrowth of persistent cells as demonstrated previously (Power and Stewart 1987; Humphrey and Stevenson 1992; Peterson and Stevenson 1992; Fuller 2011). This suggests that the Class 3 flood may have promoted the growth of benthic algae in the short-term through release from growth-limiting conditions created by the predominance of either organic matter or heterotrophs prior to flooding. Also persistently lower AI values after the flood event indicate that the community was able to prevent the particularly high dominance of either detritus or heterotrophs over the short term.

Shifts in the abundance and taxon composition over the short-term suggest that both regrowth of persistent cells and, to a lesser extent, colonization by new immigrates contributed to the rapid recovery of the community following the flood event at Site 2. Two dominant taxa in the pre-flood community, *Chamaesiphon* spp. and *Desmococcus* spp. which withstood the flood, responded positively to the flood event with a rapid increase in abundance in excess of pre-flood abundances

after only one week. Interestingly, *E. rhomboidae* was able to withstand the flood event but seemed to disappear from the community with the rapid increase in *Chamaesiphon* spp. and *Desmococcus* spp. The presence of new taxa after 2 weeks however, suggests that recolonisation also played a part in short term recovery following the flood event and indeed contributed to the similarity between the pre-flood community and that after 2 weeks. In particular, *Hapalosiphon* spp. returned to the community while new immigrants present in low numbers included a number of diatoms as well as the filamentous cyanobacterium, *Lyngbya* sp. 1. This suggests that the single-celled cyanobacterium, *Chamaesiphon* spp. and the colonial chlorophyte *Desmococcus* spp. are particularly resilient to disturbance, while diatoms and taxa with structurally more complex growth forms showed slightly slower recovery. Both Fisher *et al.* (1982) and Peterson *et al.* (1990) found that unattached diatoms such as *Navicula* spp. resettle rapidly following disturbance. Nevertheless, the flood event encountered by Fisher *et al*. (1982) considerably scoured the bed, unlike that in the Berg River downstream of the dam and thus recovery was driven by immigration of new cells, rather than regrowth of persistent cells. The conditions from which recovery begins can be highly variable and therefore result in a multiple of different recovery trajectories (Peterson 1996). In cases were severe storms completely scour substrata, or pre-flood communities are dominated by susceptible taxa, colonization processes may be more influential than regrowth of persistent taxa. The recovery of periphyton communities in this study was based on the effects of a single flood event downstream of a dam and therefore interpretation of the mechanisms of recovery are particularly limited by the lack of both spatial and temporal replication. An understanding of these mechanisms is however fundamental for predicting potential consequences of altered flow regimes and thus requires further attention.

In conclusion,

- This study used flood magnitude, standardize into ecologically relevant flood categories, for comparison between systems with different channel morphology to show that floods of different magnitude in the Berg and Molenaars Rivers differentially affected both periphyton biomass and community composition.
- Although tentative, there is some evidence to suggest that flood timing and the physical structure (i.e. mucilage content), and not necessarily the complexity of the community growth form may affect the resistance to flood events as indicated by the different response to flooding between May and October 2010 and sites on the Berg and Molenaars rivers.
- Antecedent conditions prior to flooding, particularly elevated summer base-flows and the absence of autumn floods may enhance the resistance of periphyton communities and thus affect the response to flood events as suggested by the difference in response to disturbance below the Berg Dam relative to that above the dam.
- A limited assessment of post-flood recovery in this study suggests that floods may promote algal growth in some instances, rather than reduce algal biomass, particularly if the pre-flood community is resistant to flooding.

- This study recognizes that metrics that describe near-bed flow at spatial and temporal scales relevant to periphyton such as shear stress and near-bed turbulence intensity are far more accurate descriptors of the hydraulic conditions experienced by benthic organisms. These hydraulic parameters are therefore better indicators of disturbance than flood magnitude. Although the use of ecologically-relevant flood categories in this study may be less accurate than hydraulic measures at the bed surface, its application for water resource management is far more appropriate.
- This study focused specifically on flood disturbance within the run biotope of the foothill reaches of south-western Cape Rivers. The resistance and resilience of periphyton communities to floods may therefore differ between habitats and river reaches within and between catchments. The relevance of these findings to a broader spatial scale are therefore limited and further investigation of the role of floods to other biotopes and other rivers that encompass variability at a broader scale is warranted.
- This study provides useful information on the responses of periphyton communities to ecologically significant flood events and the factors which affect resistance and resilience to floods of different magnitude, albeit at a limited spatial scale.

CHAPTER 8 THE EFFECTS OF INVERTEBRATE GRAZERS ON STREAM PERIPHYON: RESULTS OF AN *IN SITU* EXCLUSION EXPERIMENT

8.1 INTRODUCTION

Grazing by aquatic invertebrates can strongly affect periphyton biomass and community structure in rivers (Rosemond *et al.* 1993; Feminella and Hawkins 1995; Steinmann 1996; Holomuzki *et al.* 2006; Holomuzki *et al.* 2010). In their review of grazer studies, Feminella and Hawkins (1995) found that periphyton biomass declined in response to grazers in 70% of studies and that grazers altered the composition and structure of benthic algae in 81% of these studies. Their study contradicted the widely held notion that grazers are unimportant regulators of periphyton in river ecosystems (Holomuzki *et al.* 2010). There are studies, however, which show that algal biomass remains unchanged (Jacoby 1987; Feminella *et al.* 1989) or increases (McCormick and Stevenson 1991) in response to grazers. Essentially, the effects of grazing in streams can vary considerably due to differences in the densities, mobility and feeding modality of grazers and the growth form and composition of periphyton communities upon which they feed (Steinman *et al.* 1987; Hill and Knight 1988; Dudley and D'Antonio 1991; Peterson *et al.* 2001) as described in Chapter 1, Section 1.4.5.

Resources such as light and nutrients may also determine whether or not grazers are able to reduce periphyton biomass (Holomuzki *et al.* 2010; Law 2011). Both DeNicola and McIntire (1991) and Wellnitz *et al.* (1996), for example, found that grazing had little effect on periphyton communities at low light levels but they were significantly altered at intermediate and high light intensities. Similarly, when nutrients are not limiting to the growth of benthic algae, grazers may be unable to reduce periphyton biomass (Feminella and Resh 1991; Dube *et al.* 1997) because algal growth and accrual outweigh the negative effect of grazing under such conditions (Biggs *et al.* 1998; Hillebrand 2002). Although uncommon, some studies have shown that grazers can stimulate algal growth at low densities because dead cells are consumed or dislodged thus promoting light and nutrient availability to remaining cells (Gelwick and Matthews 1992; Liess and Hillebrand 2004); grazers remove epiphytes, which promotes the growth of the filamentous host (Dudley 1992); grazer excretion fertilizes the algal community and therefore promotes growth in nutrient-limited conditions (McCormick and Stevenson 1991); and communities shift to those which are more photosynthetically active or resistant to grazing (Lamberti *et al*. 1989).

The dominant grazers identified in the foothill reaches of south-western Cape rivers are baetid mayflies, which typically consume algae and detritus and belong to the deposit feeder functional feeding group (FFG) defined in Chapter 2, Table 2.1. Chironomids, and elmid beetles, which are scrapers feeding mainly on benthic algae, can also occur in relatively large numbers, particularly in the spring and autumn (Chapter 3, Figure 3.3.1). Heptageniid mayflies, which were classified as brushers in this study (Chapter 2, Table 2.1), are also relatively dominant during autumn (Chapter 3, Figure 3.3.1). Baetids generally reduce erect and filamentous algae, whereas heptageniids reduce

stalked growth forms but neither group affected prostrate diatoms in the study by Wellnitz and Ward (1998). Chironomids preferentially feed on diatoms and the basal cells of filamentous green algal and cyanobacteria (Tall *et al.* 2006; Maasri *et al.* 2010). Although relatively small grazers, like those in south-western Cape rivers are considered less effective herbivores than either snails or caddisflies (Jacoby 1985; Lamberti *et al.* 1987; Hill 1992; Biggs and Lowe 1994), Lamberti *et al.* (1995) and, more recently, Maasri *et al.* (2010) found that small grazers such as mayflies and chironomids can effectively reduce periphyton biomass if they are present in high enough densities.

In their habitat matrix conceptual model of stream periphyton, Biggs *et al.* (1998) pointed out that grazing activity, as a potential top-down control on periphyton biomass is generally limited to stable periods between floods because flood disturbance is the key driver of invertebrate and periphyton community structure and biomass during the wet season when flooding is frequent (see Chapter 6). Indeed, correlative evidence between temporal patterns in periphyton communities and densities of grazers provided in Chapters 5 and 6 suggest that grazing may be a significant top-down control on periphyton communities in late spring when periphyton communities are in their early stages of succession in foothill reaches of the south-western Cape. Conversely, it was suggested that bottom-up factors such as nutrient availability may be more important controls of periphyton biomass towards the end of the stable, dry season when the community is dominated by less palatable taxa and grazer densities are lower (Chapter 5). It was therefore proposed that the effects of grazing activity on periphyton community structure and biomass will change temporally due to shifts in the abiotic and biotic factors that determine the outcome of the periphyton-grazer interaction.

The objective of the study reported in this chapter was to determine whether invertebrate grazers limit periphyton biomass and change benthic algal community structure during spring and again during early autumn, using controlled *in situ* experiments. It was expected that periphyton biomass would increase in the absence of grazers and that the community structure would differ significantly between grazed and ungrazed treatments during the spring. Conversely, no difference in either periphyton biomass or community structure was expected during early autumn when resource availability is a more important bottom-up control of periphyton accrual and community composition, compared with the top-down control by grazing invertebrates.

It is therefore hypothesized that:

1 Periphyton biomass during late spring will be greater in enclosures without invertebrate grazers compared with enclosures that include them.

2 Benthic algal community structure will be significantly different between enclosures with and without grazers during the late spring.

3 There will be no difference in periphyton biomass or community structure between enclosures with and without grazers during the early autumn.

8.2 METHODS

8.2.1 General methods

A 100 m section of the foothill reaches of the Berg River at Site 1 was selected for undertaking the *in situ* experiments conducted during late spring (November/December 2010) and again during early autumn (March/April 2011). A detailed description of the study area at Site 1 is provided in Section 2.2.

A number of transparent rectangular plastic bins (each 0.35m x 0.2m x 0.2m; 0.014m^3) were modified to exclude invertebrate grazers for the experiment (Figure 8.1). A 0.15m x 0.15m opening was cut in the short sides of each bin and replaced with 80 µm mesh using silicone to seal the enclosure. Together with a sealed Perspex lid, this prevented the movement of invertebrates either in or out of the enclosure. The meshed sides were orientated upstream and downstream to allow water movement through the enclosure. Three large cobbles were used as the substratum for algal growth and these were placed on a bed of sand, gravel and pebbles to imitate the natural stream bed. Whereas the cobbles were already colonized with benthic algae thus representing the community at the time of the experiment, the smaller bed materials were taken from the dry channel margins and were therefore devoid of both live benthic algae and invertebrates. Cobbles were selected randomly from run biotopes for placement within the enclosures. For the exclusion of invertebrate grazers, cobbles were carefully removed, ensuring that no periphyton was lost and all invertebrates were removed with forceps. For enclosures with grazers, cobbles were lifted and placed within an 80 µm mesh net situated immediately downstream to ensure that all invertebrates associated with the stone were captured. These stones, together with the invertebrate grazers were then placed within the enclosures used as the inclusion treatment. Although cobbles were taken from the run biotopes, enclosures were placed in riffles to maximize the movement of water through each enclosure and thereby minimize the enclosure effect. Despite attempts to maintain conditions within the enclosures as near as possible to those in the open stream, there were differences in physico-chemical conditions between the treatment enclosures and the open stream (Table 8.1). In particular, near-bed velocities were much lower in the enclosures compared to the open stream.

8.2.2 Experimental design

Two field experiments were conducted: the first took place from the 25th November to the 8th December 2010 (experiment 1), and the second from the 20th March 2011 to the 2nd April 2011 (experiment 2). These two sampling periods were selected to represent conditions typical of late spring (i.e. early in the dry season: experiment 1) and early autumn (i.e. late in the dry season: experiment 2). The algal community in each enclosure therefore represented the ambient composition and biomass at the time. The inclusion enclosures were not stocked with known densities of invertebrate grazers but, as far as possible, included natural invertebrate densities typical of either late spring (experiment 1) or early autumn (experiment 2).

In the first experiment, 10 enclosures were placed randomly in five pairs along the 100 m section of the river channel. Each pair consisted of an enclosure with and without grazers. Together with these two treatments, a randomly selected cobble (Figure 8.2), as a third experimental unit was used to control for the effects of the box in each pair of enclosures (i.e. the box control). Each set of three experimental units (i.e. the exclusion treatment, inclusion control and box control) therefore comprised five 'blocks' in a randomized complete block (RCB) design (Quinn and Keough 2003). After 13 days, a single cobble from each enclosure, as well as one from the run biotope (representing the three experimental units) was removed and sampled for Chl *a* analysis, algal taxon composition and invertebrate grazer densities and biomass. The field and laboratory methods used for processing samples and weighting algal cell densities to compensate for the broad size range of cells in the community are described in detail in Section 2.3.2, Chapter 2.

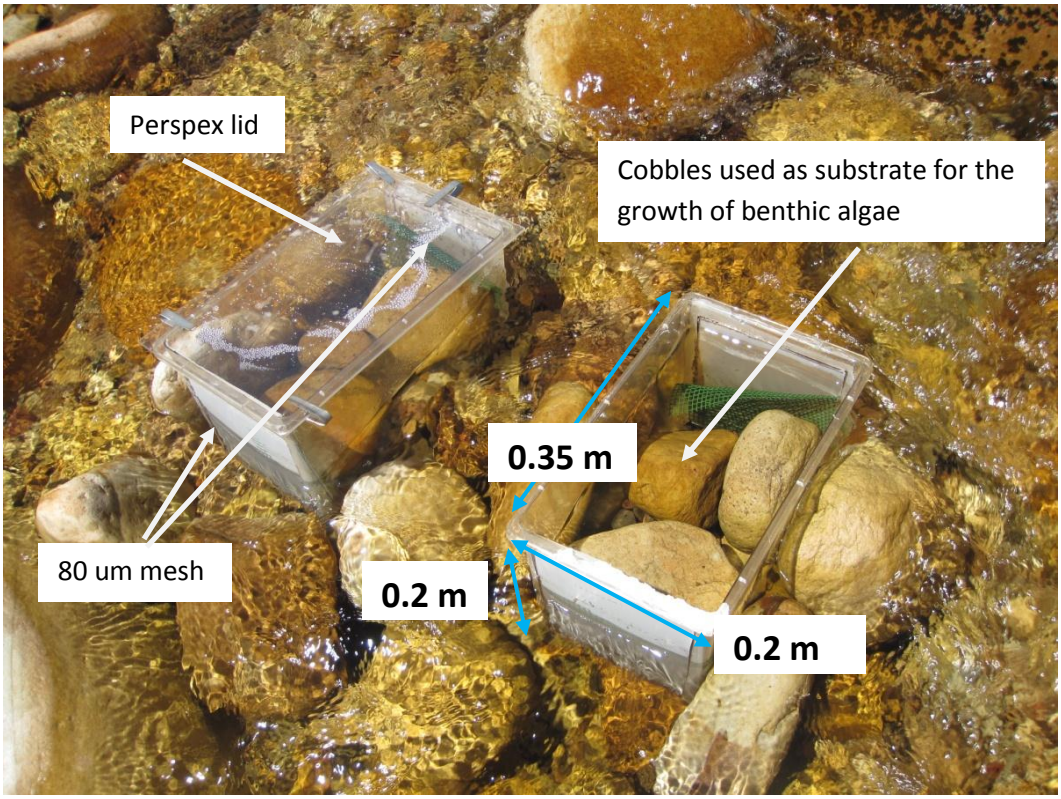


Figure 8.1 **Enclosures used for the exclusion of invertebrate algivores during the exclusion experiments. The blue arrows show the dimensions of the bins and the mesh at the upstream and downstream ends are indicated.**

Thirteen days after the set-up of experiment 1, unattached filaments of green algae were clearly visible in the enclosures without grazers, suggesting that within only two weeks, the periphyton mat had already sloughed in the absence of grazing (Figure 8.3). Therefore a sample of the surrounding water with algae in each enclosure was extracted for the estimation of Chl *a* concentrations, which were quantified by estimating the volume of water in each enclosure. General laboratory procedures for the processing of these samples are described in Section 2.4, Chapter 2.

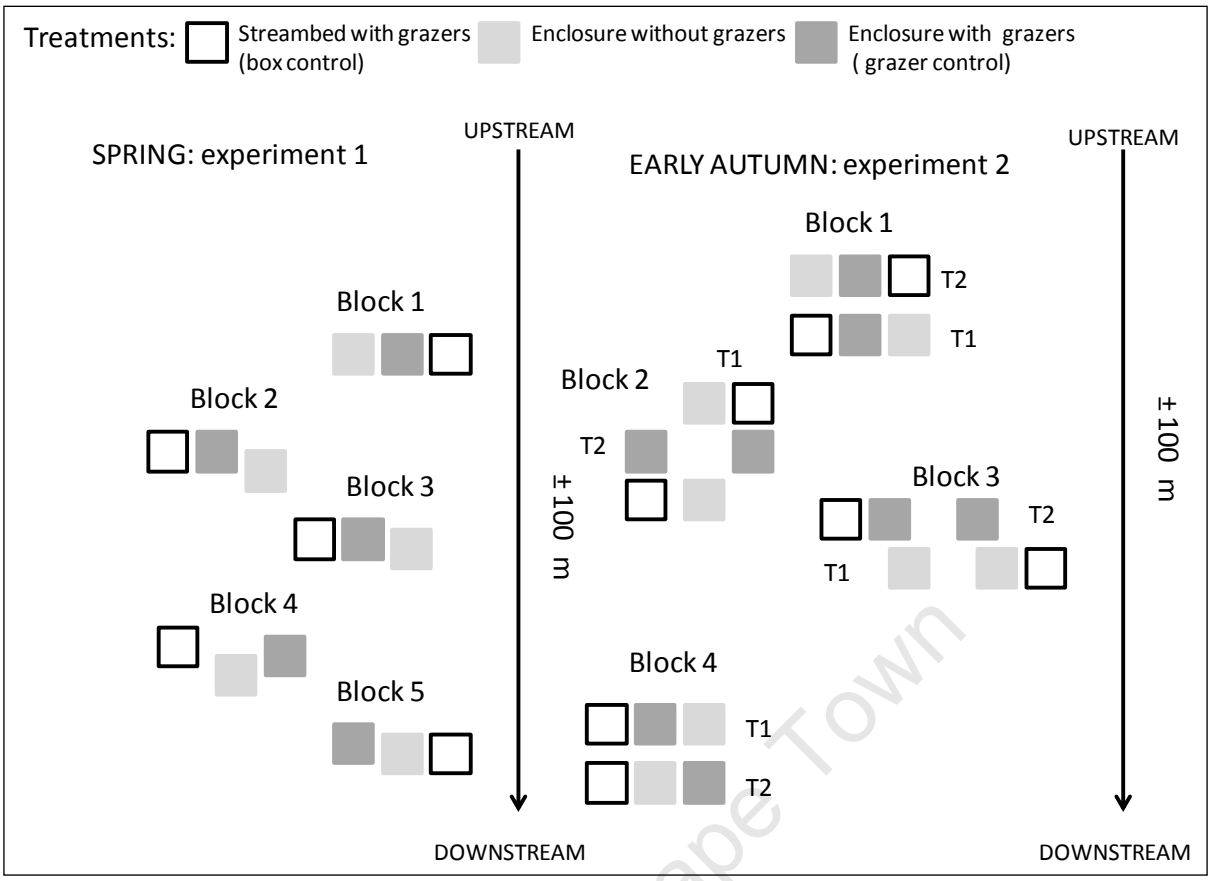


Figure 8.2 **Spatial layout of the experiments in spring and early autumn demonstating the different treatments and blocks used in a ramdonised complete block design.**

During the second experiment, each treatment was sampled after 5 days (T1) in an attempt to sample the algal community before it reached peak biomass and became detached. A second, independent set of treatments was sampled again after 13 days (T2) and each set of treatments were replicated in four 'blocks' in a randomized complete block design (Quinn and Keough 2003)(Figure 8.3).

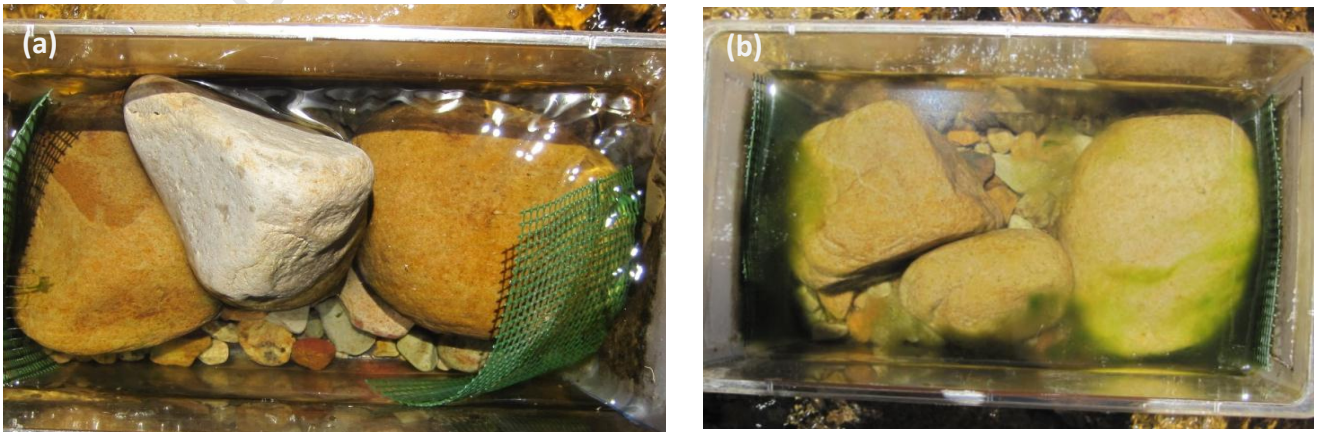


Figure 8.3 **Enclosures from Block 2 after 13 days during experiment 1 during December 2010 showing a) the enclosure with grazers and b) the enclosure without grazers. The unattached filaments of green algae are clearly visible in the enclosure with no grazers.**

Table 8.1 Physico-chemical data averaged for the inclusion and exclusion treatments and the control during late spring 2010, early autumn 2011 at T1 and T2.

	Experiment 1 late spring 2010			Experiment 2: T1 early autumn 2011			Experiment 2: T2 early autumn 2011		
	open stream	*exclusion*	*inclusion*	*open stream*	*exclusion*	*inclusion*	*open stream*	*exclusion*	*inclusion*
near bed velocity ($m\ s^{-1}$)	0.20	0.02	0.03	0.02	0.02	0.07	0.00	0.00	0.10
water depth (m)	0.21	0.14	0.14	0.14	0.15	0.18	0.10	0.10	0.20
pH	5.08	5.72	5.42	5.18	5.19	5.07	5.00	5.00	5.00
conductivity ($\mu S\ cm^{-2}$)	10.40	10.31	10.42	21.98	22.03	21.90	18.70	18.70	18.70
O_2 (%)	98.04	102.12	80.44	97.88	96.45	99.90	103.60	103.30	106.30
water temperature (^0C)	19.82	20.20	19.66	17.00	16.88	16.90	17.50	17.50	17.50

8.2.3 Statistical analysis

Mixed-model ANOVAs in STATISTICA, version 10 (2011), were used to test for differences in periphyton biomass measured as Chl *a* and AFDM and length-weighted algal cell densities (see Chapter 2, Section 2.4.2) between treatments (i.e. grazed and ungrazed enclosures and the open stream with grazers) and treatment blocks (Figure 8.2) during both experiments. For these analyses, treatments were considered fixed factors while blocks were random factors accounting for spatial variability in periphyton biomass within the river reach (Quinn and Keough 2003). Tukey HSD tests were used for post-hoc pair-wise comparisons between treatments. All data were log (x+1) transformed to meet the assumptions of equal variance and normality (Quinn and Keough 2003). Simple linear regressions between periphyton biomass and grazer densities were used to further investigate the relationship between periphyton biomass and grazers.

Benthic community structure in ungrazed and grazed communities during the late spring and early autumn was compared using a combination of MDS and cluster ordination techniques in PRIMER 6.1 (Clarke and Gorley 2001) and PERMANOVA (Anderson *et al.* 2008). Similar to the ANOVA analysis, the PERMANOVA design tested for differences between treatments as fixed factors and blocks as random factors. The PERMDISP function in the PERMANOVA add-on in PRIMER 6.1 was used to test for dispersion effects among treatments because significant dispersion affects can affect the significance of treatment effects (Anderson *et al*. 2008). SIMPER analyses were undertaken to determine which periphyton taxa were most responsible for differences between treatments. All data were either square root or 4^{th} root transformed where necessary. Detailed descriptions of these analytical tools are given in Chapter 4, Section 4.2.2 and Chapter 5, Section 5.2.2.

8.3 RESULTS

8.3.1 Experiment 1: Spring 2010

The experimental design for this study was aimed at excluding all potential grazers from the exclusion treatment and including densities of grazers typical of the natural stream in the inclusion treatments (Section 8.2.2). Although every attempt was made to remove all grazers during the experimental set up, invertebrate grazers were present in the exclusion enclosures, particularly in block 4 (i.e. 4E) where chironomids were present in relatively large numbers (Figure 8.4). It is likely that first instar hatchlings, which are highly active and have head capsules and body widths smaller

than 80 µm, moved into the these enclosures during the experiment (Prof. Cranston, Department of Entomology, University of California, Davis, pers. comm.). Grazer densities in the exclusion enclosures therefore ranged from 265 to 916 individuals m^{-2} and provided a treatment of reduced grazing intensity, rather than one with no grazers. Also, some mortality and emergence of invertebrate grazers was evident in the inclusion boxes, particularly in block 3 (i.e. 3I) where grazer densities were similar to those in the exclusion boxes and grazer densities were generally lower than in the open stream (Figure 8.4). Densities ranged from 730 to 1970 individuals m^{-2} and from 2100 to 5780 individuals m^{-2} in the inclusion boxes and the open stream respectively. Nevertheless, baetids were the dominant grazers in both the inclusion control and the open stream, whereas chironomids dominated the exclusion treatment (Figure 8.4).

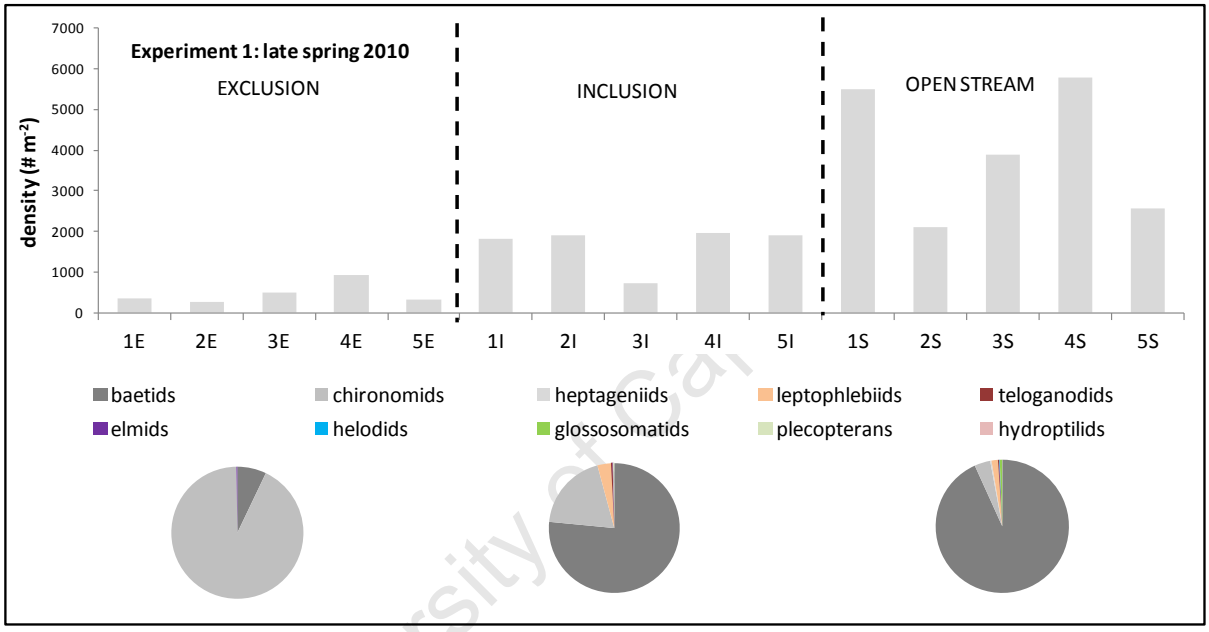


Figure 8.4 **Density of invertebrate grazers (# m^{-2}) present in each sample representing the exclusion and inclusion treatments, relative to the control during late spring 2010 (experiment 1). S= open stream; I = inclusion enclosure; E=exclusion enclosure. The pie charts represent the proportion of each grazer taxon averaged for each treatment and the control.**

Table 8.2 **A mixed-model ANOVA of Chl *a* biomass (mg m^{-2}) and algal cell density (# m^{-2}) between treatments and blocks, together with the post-hoc results showing differences between treatments. Significant values at p≤0.05 are given in *bold italics* and at p≤0.1** in *italics only*

Chl a biomass

Main tests	SS	Df	MS	F	p
Treatment (fixed)	0.087	2	0.043	3.63	*0.08*
Block (random)	0.066	4	0.016	1.37	0.32
Error	0.095	8	0.012		

algal cell density

	SS	Df	MS	F	p
Treatment (fixed)	0.31	2	0.15	10.72	***0.01***
Block (random)	0.12	4	0.03	2.00	0.19
Error	0.12	8	0.01		

Tukey post-hoc

	exclusion	inclusion
inclusion	0.984	
open stream	0.123	*0.096*

	exclusion	inclusion
inclusion	***0.01***	
open stream	***0.01***	0.93

Unexpectedly, Chl *a* biomass was not significantly greater in the exclusion treatment, relative to the inclusion treatment according to the ANOVA results (Table 8.2; Figure 8.5a) However, Chl *a* biomass in the inclusion treatment was significantly greater than that for the open channel, suggesting a 'box effect' on the accrual of Chl *a* biomass during the 13-day experiment.

By contrast, a significant treatment effect between inclusion and exclusion enclosures was observed for both algal cell densities (Table 8.2; Figure 8.5a) and AFDM (Table 8.3; Figure 8.5b), suggesting a significant grazer effect on periphyton biomass during the late spring. Relative to the start of the experiment, AFDM not only increased in the exclusion treatment, but decreased in the presence of grazing in the inclusion treatment (Figure 8.5b).

Despite similar Chl *a* biomass on the stones between the inclusion and exclusion treatments, unattached algal filaments (mainly *Mougeotia* sp. 1) within the water column were abundant in the exclusion enclosures. Indeed, Chl *a* and AFDM concentrations were significantly greater in the exclusion enclosures relative to the inclusion enclosures after 13 days (Chl *a*: F=1.15; p=*0.005* and AFDM: F=8.22; p=*0.05*) (Figure 8.6).

Table 8.3 **A mixed model ANOVA of AFDM between treatments and blocks, together with the *post-hoc* results showing differences between treatments. Significant values at p≤0.05 are given in *bold italics* and at p≤0.1 in *italics only***

Main tests

	SS	Df	MS	F	p
Treatment (fixed)	872712	2	436356	5.662	***0.029***
Block (random)	235800	4	58950	0.765	0.577
Error	616524	8	77066		

Tukey post-hoc

	exclusion	inclusion
inclusion	***0.025***	
open stream	0.158	0.454

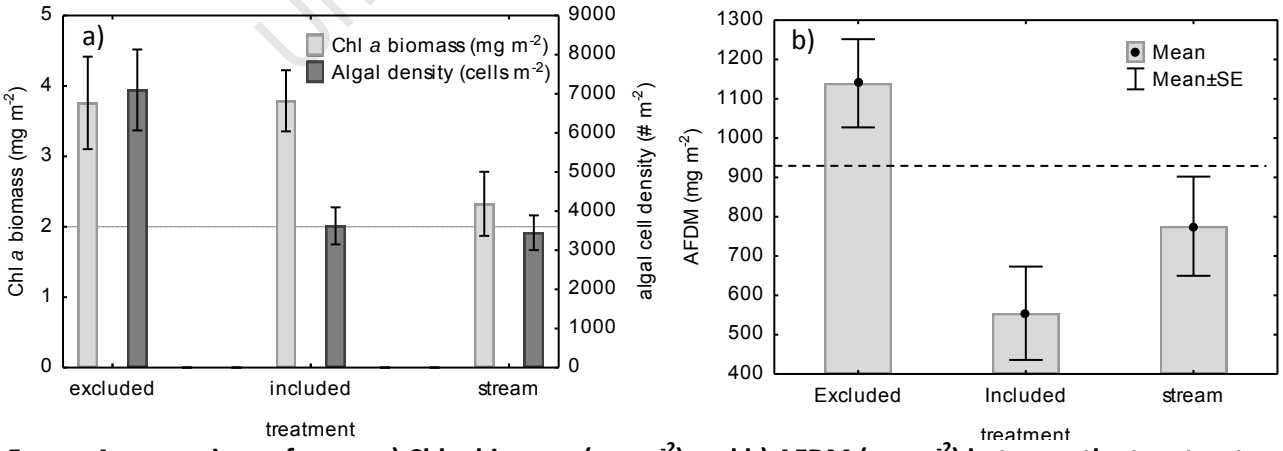


Figure 8.5 **A comparison of mean a) Chl *a* biomass (mg m^{-2}) and b) AFDM (mg m^{-2}) between the two treatments (excluded and included grazers) and the control for experiment 1. The dotted reference lines indicate the average Chl *a* and AFDM values at the start of the experiment.**

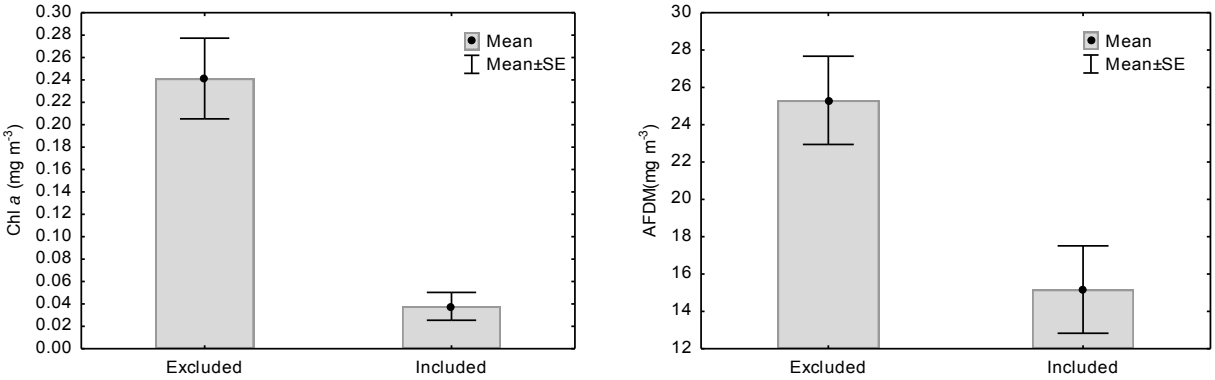


Figure 8.6 A comparison of mean concentration of a) Chl *a* biomass (mg m^{-3}) and b) AFDM (g m^{-3}) in the water column of the enclosures between the two treatments for experiment 1.

An inverse relationship (r = -0.63) between baetid density (as the dominant grazer) and Chl *a* biomass was observed in spring 2010 (Figure 8.7). Three of the five exclusion enclosures fell well below the regression line, indicating a low Chl *a* biomass associated with low grazer densities (Figure 8.7). Considering the high biomass of unattached algae within the exclusion enclosures, it is possible that periphyton mats dominated by the green filamentous alga, *Mougeotia* sp. 1 had grown rapidly in the absence of grazing, and then detached during the 13-day grazing period. By removing these three values from the regression analysis, the inverse relationship between grazer densities and Chl *a* biomass is particularly strong (r = -0.86; p≤0.001; r=0.74). Despite its categorization as an inclusion treatment, the inclusion enclosure with grazer densities below 1000 individuals m^{-2} had a higher biomass than the other four inclusion treatments (Figure 8.7).

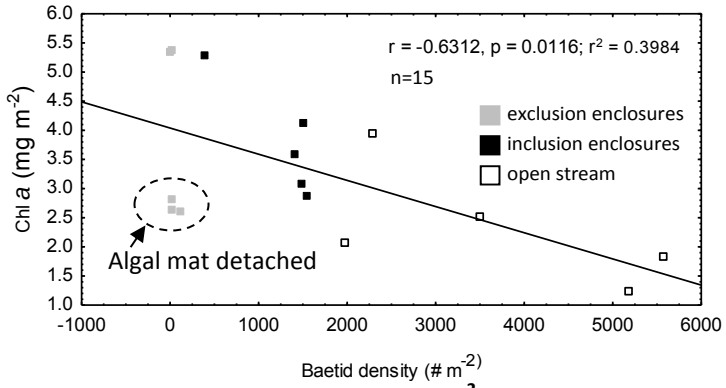


Figure 8.7 Relationship between baetid density (# m^{-2}) and Chl *a* biomass (mg m^{-2}) after 13 days of grazing in late spring 2010. Each point represents a single block and treatment.

Nevertheless, the significant difference in AFDM between the inclusion and exclusion enclosures (Table 8.2, Figure 8.5b), the significant difference in both Chl *a* biomass and AFDM floating within the enclosures between treatments (Figure 8.6) and the significant inverse relationship between grazer densities and Chl *a* biomass (Figure 8.7) suggest that grazing activity suppressed periphyton accrual during the spring experiment.

Interestingly, a significant positive relationship between Chl *a* biomass and chironomid densities was observed (Figure 8.8), suggesting that chironomids are able to proliferate when algal biomass is

relatively high, but do not effectively suppress algal accrual at the densities recorded in this experiment.

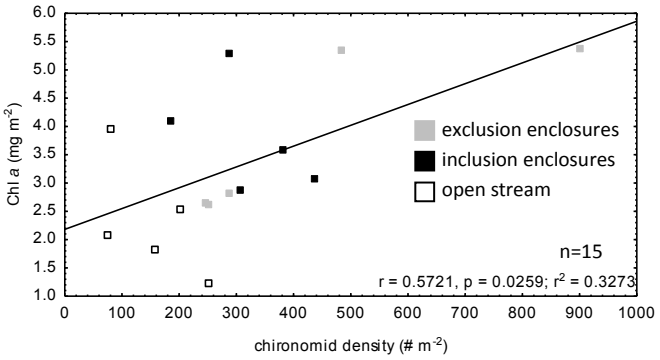


Figure 8.8 Relationship between chironomid density (# m^{-2}) and Chl *a* biomass (mg m^{-2}) after 13 days of grazing in late spring 2010. Each point represents a single block and treatment.

With the exception of the exclusion enclosure in block 4 (i.e. 4E), the benthic algal communities were distinct between treatments with no clear separation between communities in the inclusion control and the open steam as indicated by the MDS plot and hierarchical cluster diagram in Figure 8.9. A bubble plot of grazer densities over the MDS plot shows that 4E had a higher density of grazers relative to the other exclusion boxes (Figure 8.9b).

These differences were confirmed by the PERMANOVA results, which showed that the algal community in both the inclusion control and the open stream were significantly different from the community with low grazer densities in the exclusion boxes (Table 8.4). Despite the higher density of grazers in the open stream relative to the inclusion control, their algal communities were not different indicating no box affect on the structure of communities during the experiment (Table 8.4, Figure 8.9).

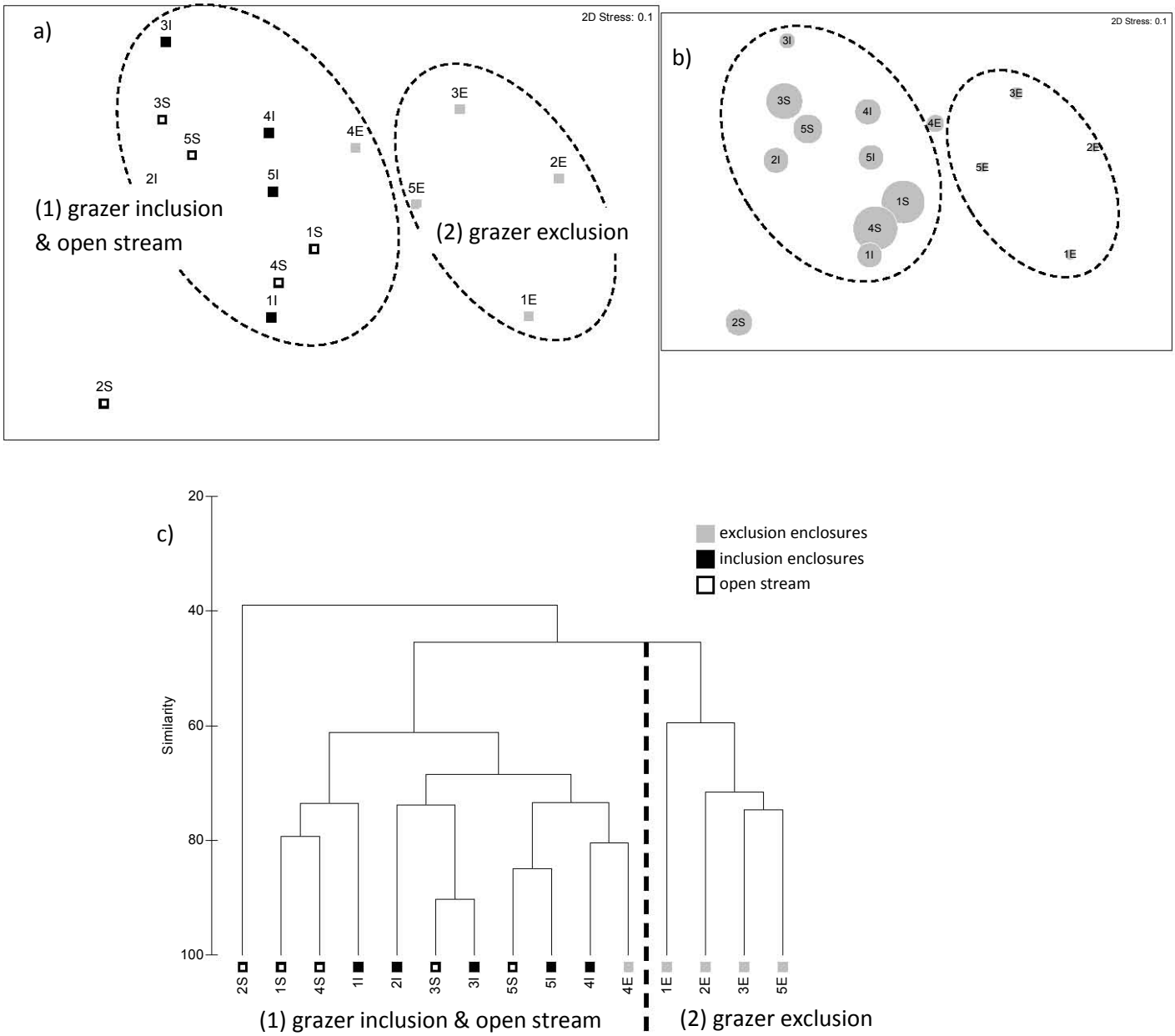


Figure 8.9 **a) 2-D MDS plot and b) overlaid with a bubble plot of baetid densities together with c) a hierarchical clustering dendrogram of Bray-Curtis similarities based on square-root transformed benthic algal taxa for each treatment and the control in each of the 5 blocks during experiment 1 in late spring 2010. S= open stream; I = inclusion enclosure; E=exclusion enclosure.**

Table 8.4 **PERMANOVA results of differences in community composition between treatments and blocks after the 13-day grazer exclusion experiment in spring 2010. Significant values at p≤0.05 are given in *bold italics***

Main test

Groups	***df***	***SS***	***MS***	***Pseudo-F***	***p***
treatment	2	7268.5	3634	6.789	***0.0001***
block	4	4604.5	1151	2.150	0.0519
residuals	8	4282.8	535		
Total	14	16156			

Pairwise tests

	t	p
exclusion vs inclusion	2.67	***0.014***
open stream vs inclusion	1.05	0.384
open stream vs exclusion	3.32	***0.001***

A comparison between the relative contribution of benthic algal taxa by division and growth form shows a clear difference in the composition of periphyton communities among treatments (Figure 8.10). The most obvious difference is the high proportion of the green filamentous algae, *Mougeotia* spp., in the exclusion enclosures relative to both the inclusion control and the open stream where *Mougeotia* spp. was mostly absent or present in low abundance (Figure 8.10). Despite the community at 4E grouping with the inclusion community in the MDS and hierarchical clustering dendrogram (Figure 8.9), it too included *Mougeotia* spp., but the proportion of this taxon was lower in 4E, compared with the communities typical of the other exclusion boxes. Diatoms, particularly *Eunotia rhomboidae*, were also more abundant in the exclusion enclosures relative to the inclusion

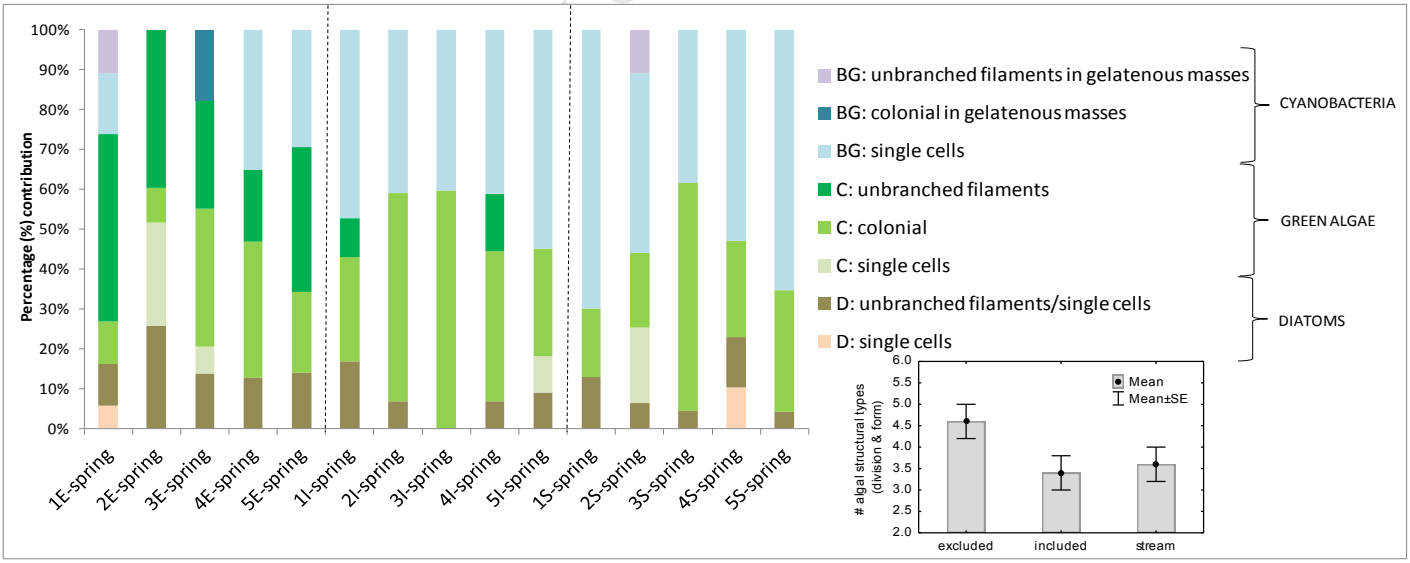


Figure 8.10 **Proportion of taxa by division and form (i.e. structural types) in each treatment during late spring 2010. BG=cyanophytes; C=chlorophytes; D=diatoms. S= open stream; I = inclusion enclosure; E=exclusion enclosure. The dotted lines separate the samples from each block representing the treatment and control. The inserted graph shows the average number of structual types per treatment.**

boxes and the open stream. By contrast, the single-celled cyanobacterium, *Chamaesiphon* spp., was conspicuously more abundant in the grazer control and the open stream (Figure 8.10). The inserted graph in Figure 8.10 shows that the community was generally more complex, with a greater number of algal forms in the absence of grazers.

A SIMPER analysis of differences in community composition between treatments confirmed these findings (Figure 8.11). Indeed, the high abundance of *Mougeotia* sp. 1 and *Eunotia rhomboidae* in the exclusion treatment relative to inclusion treatment together accounted for 60% of the difference between these communities (Figure 8.11).

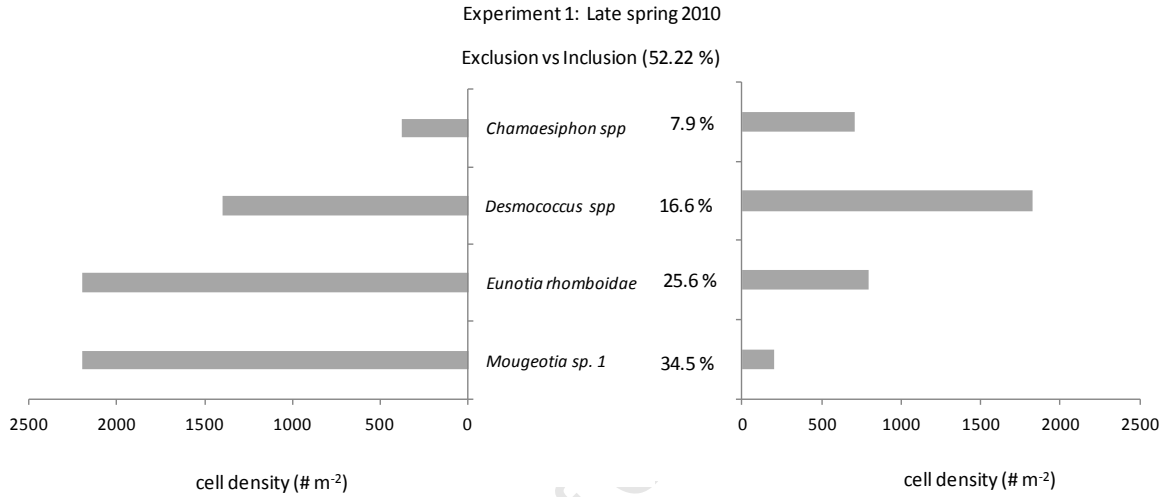


Figure 8.11 **SIMPER analysis of benthic algal density data representing the community in the exclusion and inclusion treatments after 13 days of grazing in late spring 2010. Only those taxa which together contribute 75% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets, while the contribution given by each taxon is shown for each bar in the graph respectively.**

8.3.2 Experiment 2: Early autumn 2011

Grazer abundance was much lower during the autumn experiment in March/April 2011 (Figure 8.12), compared with the levels recorded in experiment 1. Densities ranged from 15 to 160 individuals m^{-2} in the exclusion boxes, 65 to 1100 individuals m^{-2} in the inclusion boxes and 920 to 1010 individuals m^{-2} in the open stream for the first set of treatments sampled after 5 days (T1) (Figure 8.12a). For the second set of treatments sampled after 13 days, grazer densities ranged from zero to 100 individuals m^{-2} in the exclusion boxes, 300 to 1600 individuals m^{-2} in the inclusion boxes and 920 to 2200 individuals m^{-2} in the open stream (Figure 8.12b). The composition of grazers was similar between the open stream and the inclusion treatment at both T1 and T2. Baetids were the dominant grazers at T1, with relatively high densities of chironomids in the inclusion treatment compared to the open stream (Figure 8.12a). Although grazer densities were relatively low in the exclusion treatment at this time, those that were present were dominated by chironomids, probably because early hatchlings could move through the mesh.

The grazer community at T2 was again similar between the inclusion treatment and the open stream but the composition differed substantially from that recorded at T1 (Figure 8.12b). In particular, elmid larvae and leptophlebiids were abundant at this time, although baetids and chironomids were still present in relatively high numbers. Also, heptageniids contributed to the grazer community sampled after the 13-day grazing period. Very few grazers were recorded in the exclusion treatment at T2 but those that were present included chironomids and leptophlebiids in equal proportions.

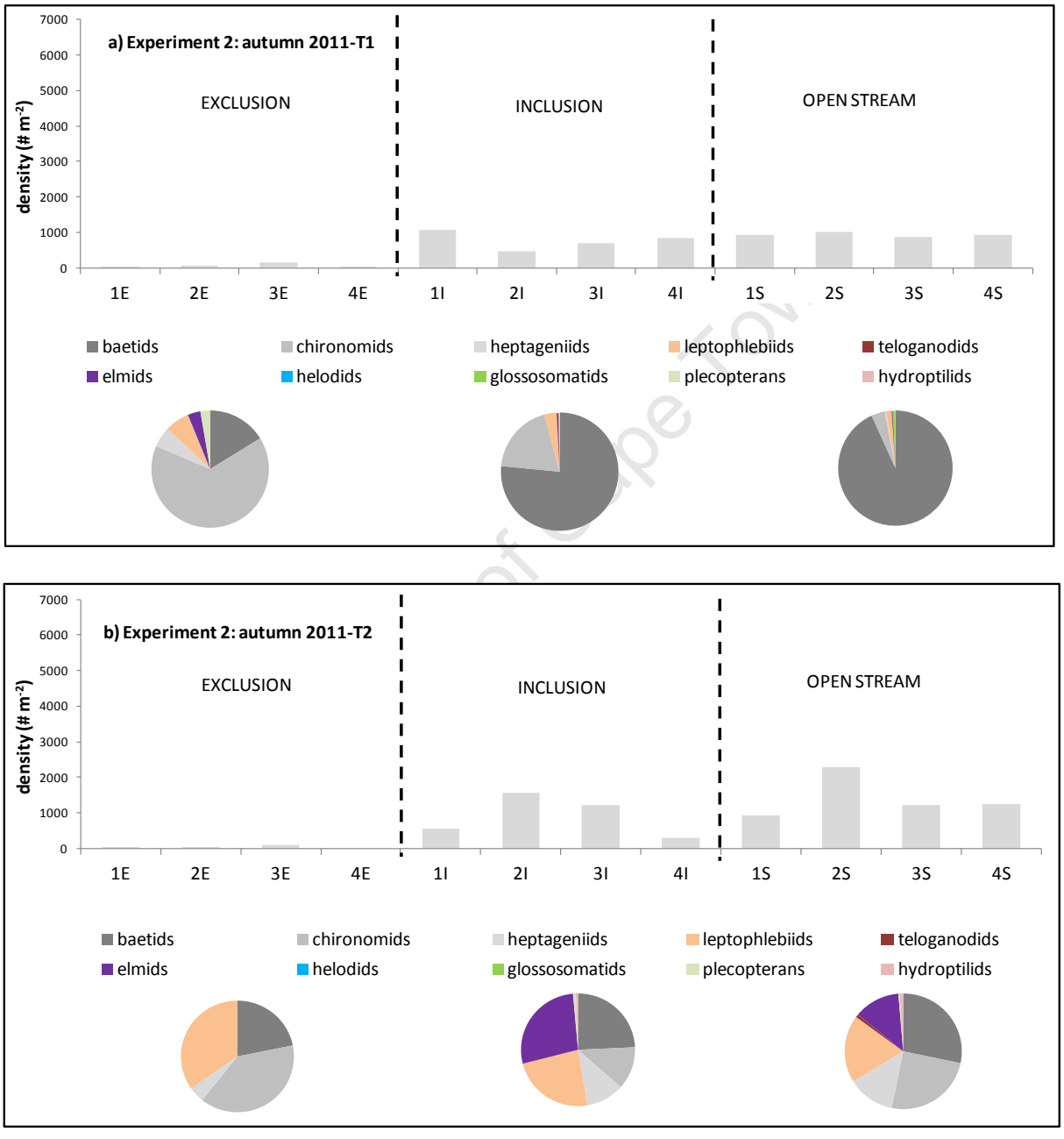


Figure 8.12 **Density of invertebrate grazers (# m^{-2}) present in each sample representing enclosures in each treatment, relative to that in the open stream (control) during autumn 2011 sampled a) after a 5-day grazing period and b) after a 13-day grazing period. S= open stream; I = inclusion enclosure; E=exclusion enclosure. The pie charts represent the proportion of each grazer taxon averaged for each treatment and the box control.**

No significant grazer effect or box effect on either Chl *a* biomass (F=0.286; p=0.76) or AFDM (F=0.34; p=0.73) was observed after the 5-day grazing period in autumn 2011 (Figure 8.13). Similarly, no differences in Chl *a* biomass (F=0.29; p=0.76) or AFDM (F=1.27; p=0.35) were evident at T2 (Figure 8.14). Unlike the spring experiment when unattached green filamentous algae were found in large quantities in the exclusion treatment, there was no difference in either Chl a biomass (F=0.3, p=0.6) or AFDM (F=0.03, p=0.89) of the water column between the inclusion and exclusion chambers after the 13-day period in experiment 2.

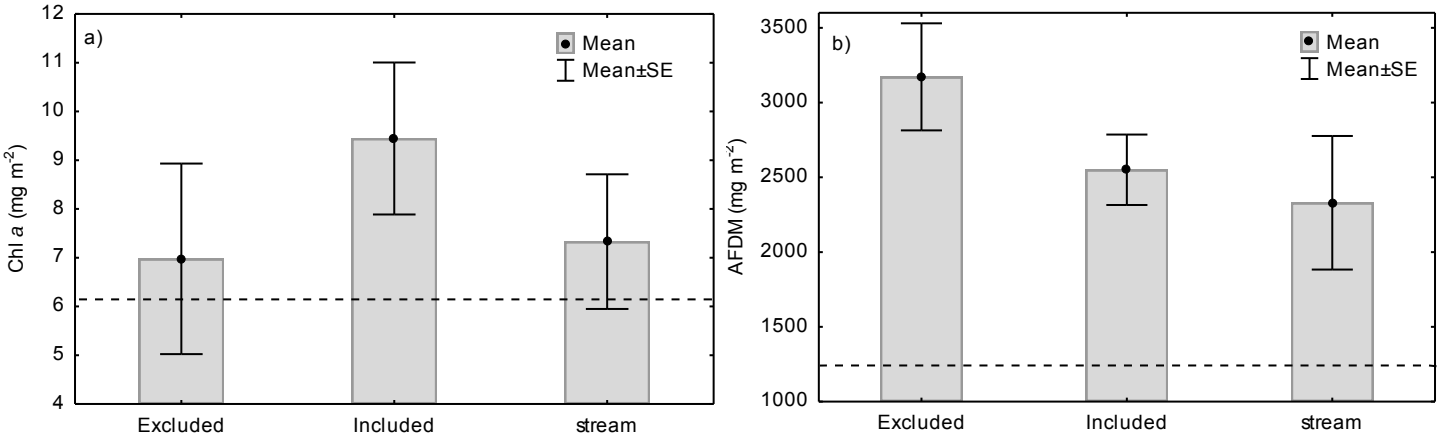


Figure 8.13 **A comparison of mean a) Chl *a* biomass and b) AFDM between the three different treatments for experiment 2 after 5 days in autumn 2010 (T1). The dotted reference lines indicate the average Chl *a* and AFDM values at the start of the experiment.**

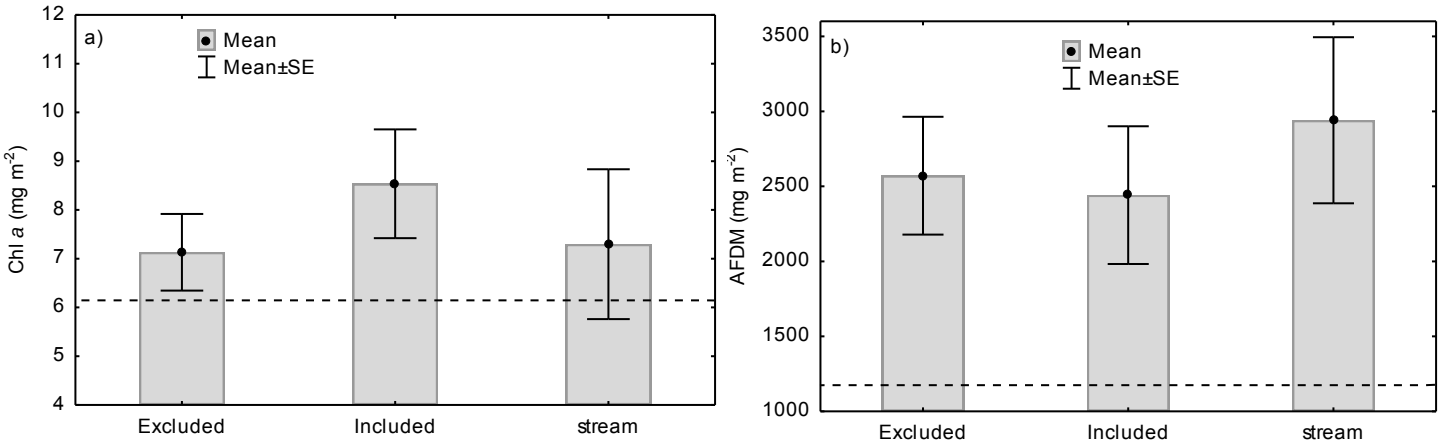


Figure 8.14 **A comparison of mean a) Chl *a* biomass and b) AFDM between the three different treatments for experiment 2 after 13 days in autumn 2010 (T2). The dotted reference lines indicate the average Chl *a* and AFDM values at the start of the experiment.**

Despite no grazer effects on the periphyton biomass during autumn 2011, the benthic algal community separated according to treatments with no distinction between T1 and T2 (Figure 8.15).

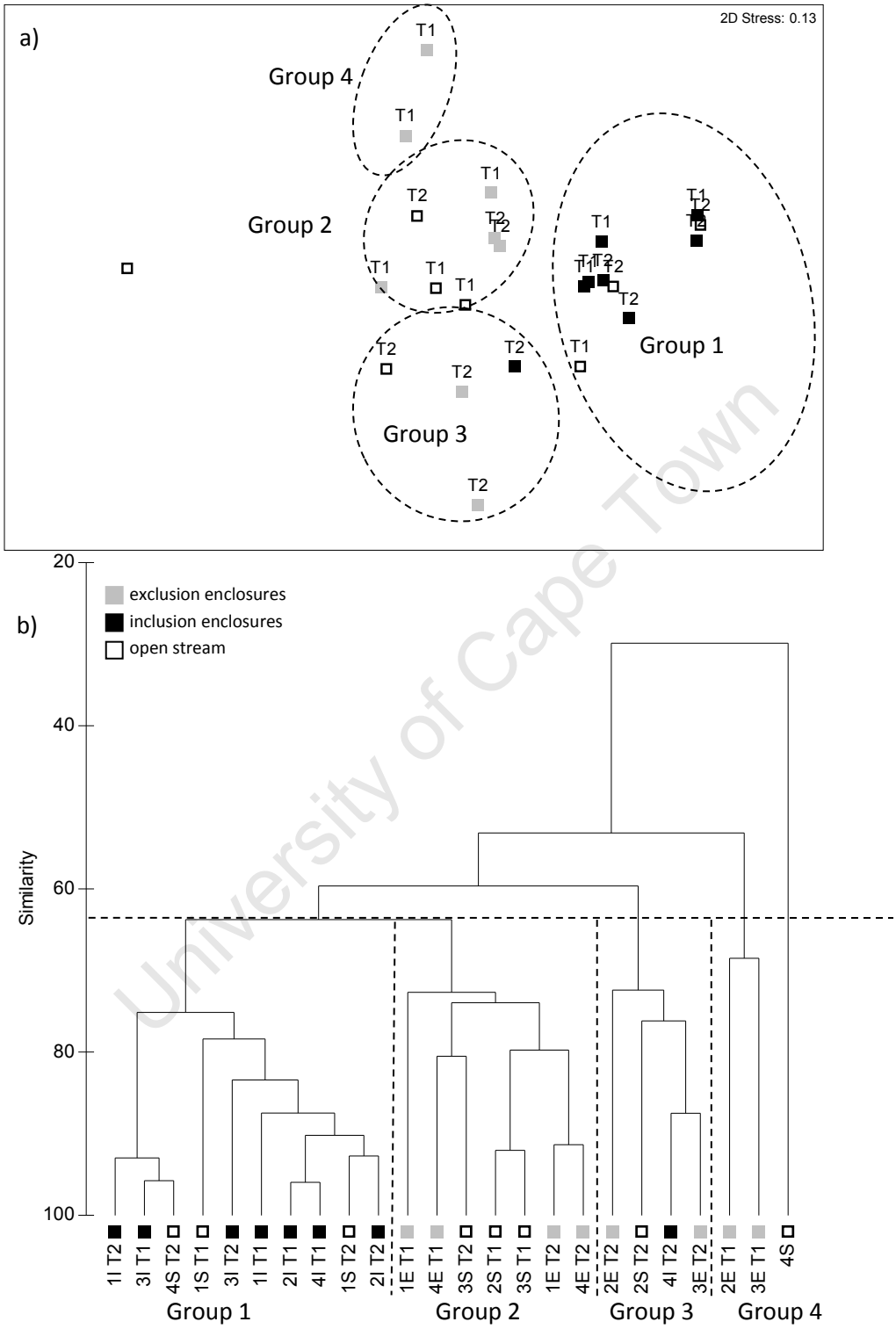


Figure 8.15 **2-D MDS plot and b) hierarchical clustering dendrogram of Bray-Curtis similarities based on 4th-root transformed benthic algal taxa for each treatment and the control in each of the 5 blocks during experiment 2 after a 5-day and 13-day grazing period in early autumn 2011. S= open stream; I = inclusion enclosure; E=exclusion enclosure.**

Experimental unit 4I was an exception to this pattern and was more similar to the exclusion samples in group 3 (Figure 8.15). Interestingly, a single large gomphid was found in 4I at T2. As a predator, it is likely that this individual consumed other invertebrate grazers in the enclosure representing 4I because grazer densities in this enclosure were much lower than that recorded at the other three samples representing the inclusion treatment (Figure 8.12a). This sample effectively represented an exclusion sample therefore and it is not surprising that it grouped more closely with the other exclusion samples. Also, some control samples grouped with the inclusion enclosures while others grouped with the exclusion enclosures, showing no clear pattern. Within the exclusion treatment, three separate groups were evident and the variability among replicates was far greater than that for the group representing the inclusion treatment (group 1) (Figure 8.15a).

Considering that there was no distinction between T1 and T2 among all treatments and the control (Pseudo-F 0.77; p=0.57), samples from both time periods were grouped for an assessment of treatment effects on benthic algal community composition. Sample 4I at T2 was categorized as part of the exclusion treatments for the reasons described above. The PERMANOVA results for differences between treatments show that the exclusion and inclusion treatments were significantly different from each other, suggesting a grazing effect on benthic algal community composition during autumn 2011 (Table 8.5). However, there was a slight difference between the inclusion treatment and the open stream (Table 8.4), suggesting that there may have been a box effect on benthic algal community structure during this experiment. An analysis of dispersion (PERMDISP), however showed a significant dispersion effect (F=7.49; p=0.003) between treatments, confirming a greater variability among samples from the exclusion treatment, relative to the inclusion treatment indicated by the spread of samples in the MDS plot (Figure 8.15a). Considering that both the PERMDISP tests were significant, the implied grazer effects on community composition from the PERMANOVA results in Table 8.5 are questionable, suggesting that there is scope for further work with greater replication on the effects of grazing during autumn.

Table 8.5 **PERMANOVA results of differences in community composition between treatments and blocks after the 5-day grazer exclusion experiment in autumn 2010. Significant values at p≤0.05 are given in *bold italics.***

Main test

Groups	***df***	***SS***	***MS***	***Pseudo-F***	***p***
treatment	2	3766	1883	3.30	***0.05***
block	3	1900	633	1.17	0.34
residuals	11	5958	542		
Total	22	15129			

Pairwise tests

	t	p
exclusion vs inclusion	2.71	***0.001***
open stream vs inclusion	2.05	***0.040***
open stream vs exclusion	1.32	0.156

Despite the tentative grazer effects on benthic algal community composition during autumn 2011, there is some indication that benthic algal communities differ between treatments and therefore differences in the community composition were further explored. The periphyton community throughout experiment 2 was dominated by the single-celled cyanobacterium, *Chamaesiphon* spp. (Figures 8.16 and 8.17). However, green algae, particularly the branched filaments of *Stigeoclonium* spp. at T1 and the unbranched filaments of *Mougeotiopsis* spp. at T2 were present in relatively high abundance in the exclusion enclosures at blocks 2 and 3 (Figures 8.16 and 8.17). The number of structural algal types based on their division and growth form at both T1 and T2, shows that the community was far more structurally complex in the absence of grazers. With the exception of 4I after 13 days, the only two algal taxa recorded in the inclusion treatment of all four blocks were *Chamaesiphon* spp. and the colonial green alga, *Desmococcus* spp. As described above, the presence of a large predator and associated low grazer density in 4I may have accounted for its distinction from the other inclusion enclosures at T2. Like the exclusion treatments at blocks 2 and 3, *Mougeotiopsis* spp. was relatively abundant in the inclusion enclosure at block 4 (i.e. 4I), which explains why it grouped with the exclusion samples in the MDS analysis (Figure 8.15).

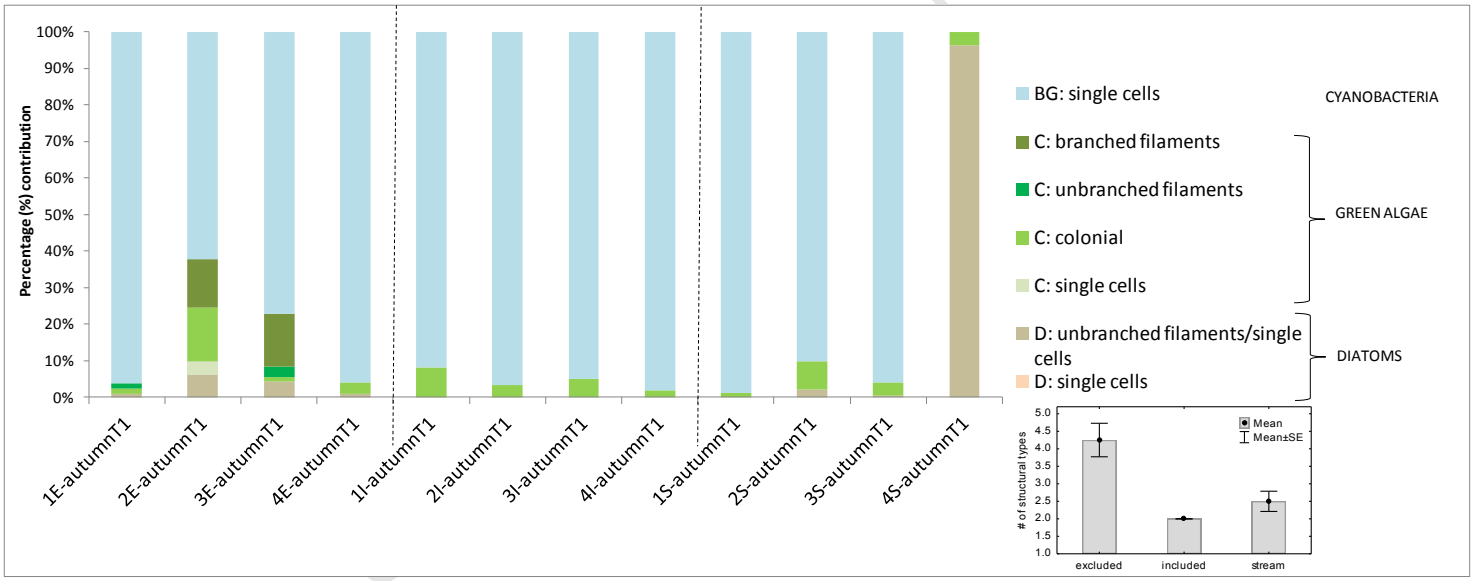


Figure 8.16 **Proportion of taxa by division and form (structural types) in each treatment at T1 during early autumn 2011. BG=cyanophytes; C=chlorophytes; D=diatoms. S= open stream; I = inclusion enclosure; E=exclusion enclosure. The dotted lines separate the samples from each block representing the treatment and control. The inserted graph shows the average number of different strutural types per treatment.**

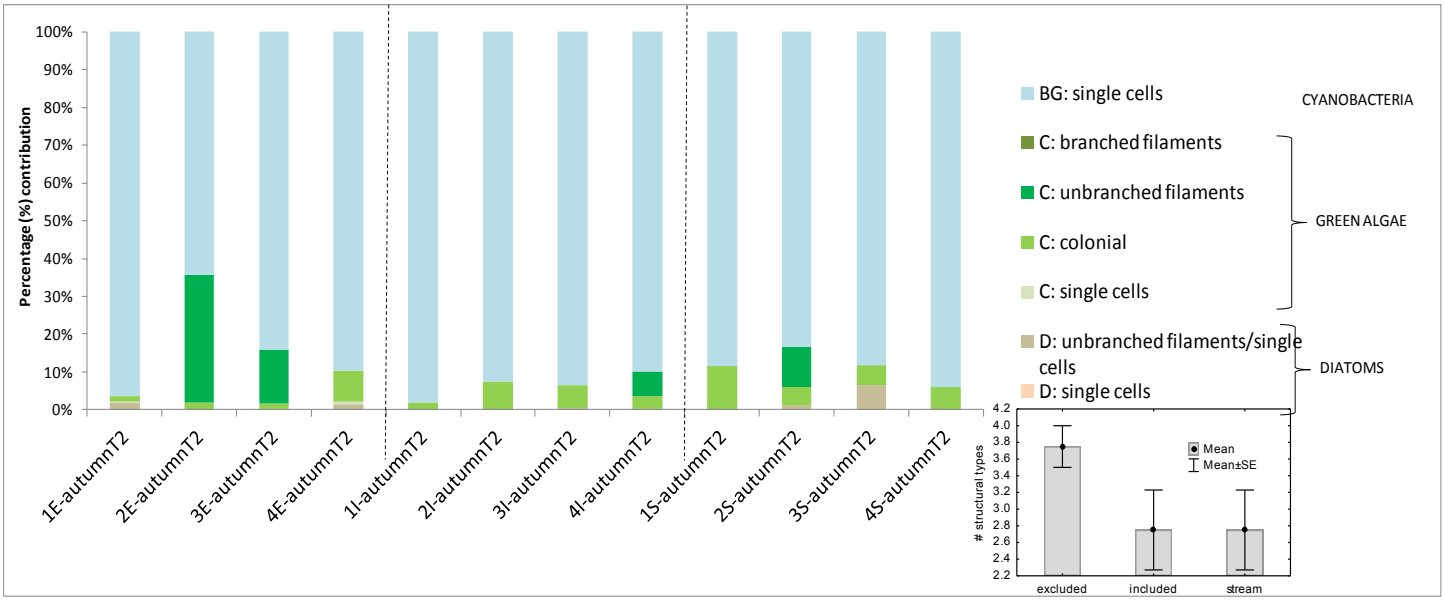


Figure 8.17 **Proportion of taxa by division and form (structural types) in each treatment at T2 during early autumn 2011. BG=cyanophytes; C=chlorophytes; D=diatoms. S= open stream; I = inclusion enclosure; E=exclusion enclosure. The dotted lines separate the samples from each block representing the treatment and control. The inserted graph shows the average number of different strutural types per treatment.**

A similar benthic algal composition was recorded in the open stream at both T1 and T2, with the exception of a single outlier at T1 (i.e. 4S) when the community was dominated by the diatom, *Eunotia rhomboidae* at block 4.

A SIMPER analysis of the taxa responsible for the differences between treatments in autumn 2011 showed that the separation was driven largely by the absence of the upright diatom, *Eunotia rhomboide*, and the filamentous chlorophytae, *Mougeotiopsis* spp. and *Mougeotia* spp. in the inclusion treatment and the slightly greater abundance of the colonial green alga, *Chlorococcum* spp in the inclusion treatment (Figure 8.18).

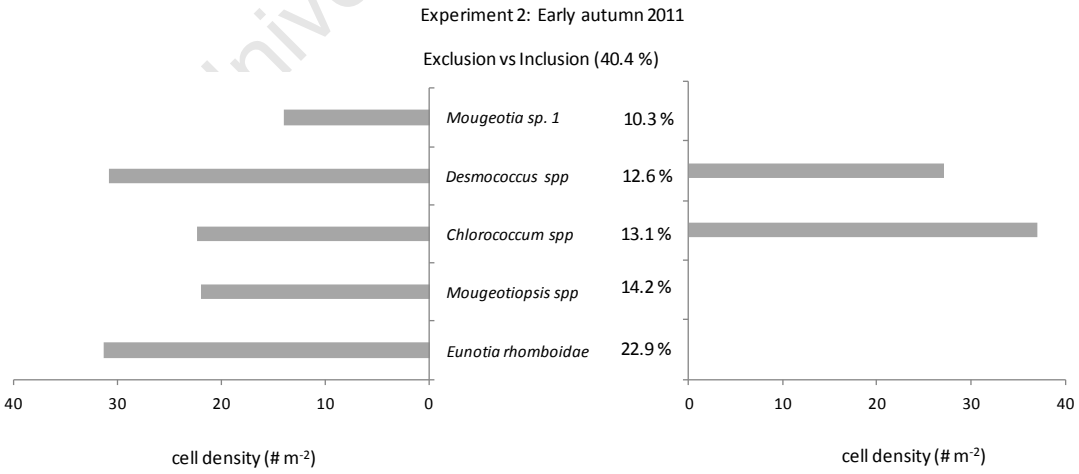


Figure 8.18 **SIMPER analysis of benthic algal density data representing the community in the exclusion and inclusion treatments after 13 days of grazing in early autumn 2011. Only those taxa which together contribute 75% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets.**

Despite the significant difference between the open stream control and grazer control (Table 8.5), there was only 31.1 % dissimilarity between them (Figure 8.19). This marginal difference was explained largely by a higher abundance of *Chlorococcum* spp. and *Chamaesiphon* spp. and an absence of *Eunotia rhomboidae* and lower abundance of *Desmococcus* spp. in the inclusion treatment relative to the open stream.

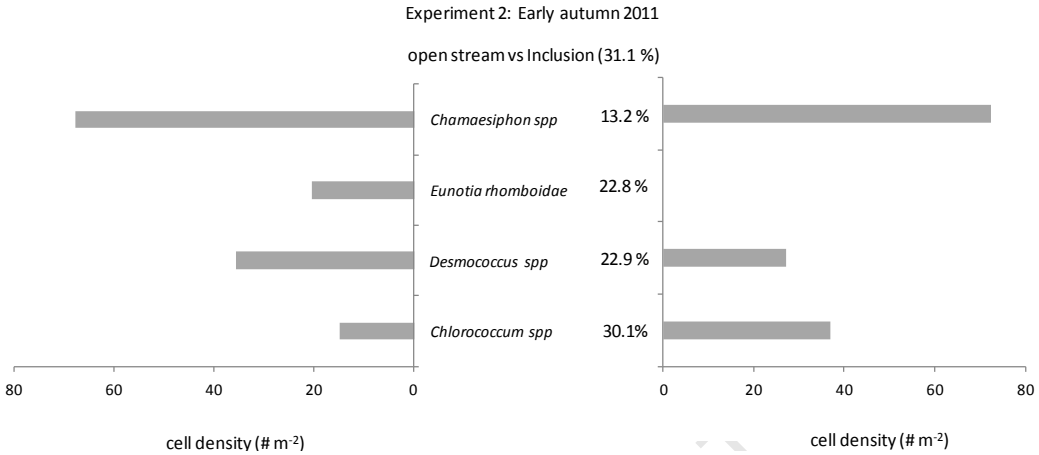


Figure 8.19 **SIMPER analysis of benthic algal density data representing the community in the control and inclusion treatment after 13 days of grazing in early autumn 2011. Only those taxa which together contribute 75% or more to the differences between communities are presented. The overall % dissimilarity is given in brackets.**

8.4 DISCUSSION

Results from the experimental control of grazer densities in the Berg River support the prediction that grazing activity affects both the biomass and composition of periphyton communities at the onset of the stable dry season (Chapters 4 and 5). These findings agree with the large body of literature which shows that periphyton biomass almost always decreases in the presence of grazers (Feminella and Hawkins 1995, Steinman 1996; Hillebrand 2009).

Although AFDM and algal cell densities on the substrata were significantly greater in the exclusion treatments, it was surprising that these grazer effects were not demonstrated by differences in Chl *a* biomass on the substrata. It is likely that mat degeneration and sloughing in the exclusion enclosure was responsible for this result. Within the 13-day experiment in late spring 2010 conspicuous filaments of the green alga, *Mougeotia* spp. developed under low grazing pressure (< 1000 individuals m^{-2}). Compared with the inclusion treatment, Chl *a* biomass was 6-fold higher in the water column of the exclusion treatment. This suggests that the benthic algal community was highly productive during the spring and, in the absence of invertebrate grazers rapidly accrued biomass that created a thick mat dominated by green filamentous algae that reached peak biomass and sloughed within a particularly short period (i.e. 13 days). Similarly, Jacoby (1987) found that peak biomass was reached on day 12 of his grazer exclusion study in a foothill steam in Washington. Thereafter the community sloughed as the basal algae cells died. In nutrient poor streams, the time to peak biomass is often rapid because basal cells become nutrient-limited resulting in mat degradation and detachment from the substratum as cells senesce (Biggs 1996). Considering that

the enclosures during this experiment reduced near-bed velocities considerably, the time to peak biomass may have been even quicker than in the natural stream where higher near-bed velocities enhance nutrient availability in nutrient poor rivers (see Chapter 6). The remaining senescent algal cells following sloughing generally have a lower Chl *a* content than those in mats that are grazed because grazers generally remove dead algal cells, which promotes the availability of resources thus enhancing the biomass specific productivity of the periphyton mat (Gelwick and Matthews 1992; Liess and Hillebrand 2004). Under the circumstances observed in this study, algal cell density and AFDM therefore appear to be better measures of periphyton abundance for detecting grazer effects. Nevertheless, the first hypothesis, which stated that periphyton biomass will be greater in the absence of invertebrate grazers compared with enclosures with grazers during late spring, is supported.

The significant negative relationship between baetid densities, as the dominant grazer and the biomass of periphyton during late spring suggests that variation in invertebrate grazing pressure either due to changes in densities or grazer type, is an important factor affecting the dynamics of periphyton communities in south-western Cape rivers. Indeed, chironomids, as the second most dominant grazers in the Berg River did not show this relationship but apparently increased in density with increasing periphyton biomass. Baetids are larger, more mobile and able to consume a wider range of cell sizes compared to chironomids (Tall *et al.* 2006; Maasri *et al.* 2011), and occur in higher densities. These factors therefore make baetids more effective grazers than chironomids in the Berg River. Although some studies have shown that chironomids can impose a top-down control on periphyton biomass accrual in streams (Maasri *et al.* 2011), in a meta-analysis of grazer control on periphyton biomass across aquatic ecosystems, Hillebrand (2009) found that chironomids were the only grazers that did not exhibit a significant negative effect on periphyton biomass.

Most studies show that the effects of grazers on periphyton biomass are a direct function of grazer density (Hill and Knight 1987; Steinman *et al*. 1987; Hill *et al.* 1992; Rosemond *et al.* 1993). In this study, periphyton biomass in enclosures with baetid densities less than 1000 individuals m^{-2} were similar to those without baetids. This suggests that baetids were only effective at removing algae when densities were greater than 1000 individuals m^{-2}. Similarly, Colletti *et al*. (1987) reported that heptageniid mayflies have little or no effect on periphyton biomass at densities lower than 1000 individuals m^{-2}. DeNicola *et al.* (1990) found that baetid mayflies at densities of 500 individuals m^{-2} had little effect on periphyton biomass and generally cleared patches of algae. Taylor *et al.* (2002) however, found that a distinct grazer effect was observed between mesocosms without grazers and those stocked with 319 baetid mayflies m^{-2} and that periphyton biomass continued to decrease in mesocosms with increasingly greater densities. Considering the low replication of enclosures with baetid densities between zero and 1000 individuals m^{-2}, and the fact that baetids occur naturally at densities above 1000 individuals m^{-2} in late spring when periphyton biomass maintained a low steady state (Chapter 5, Figure 5.1), it is uncertain whether or not these grazers would effectively control periphyton biomass at densities below 1000 individuals m^{-2} in foothill reaches of south-western Cape rivers. More focused experimentation with greater replication of grazers at different

densities is therefore warranted to establish the threshold of grazing pressure that is necessary to control periphyton biomass in these rivers.

A second hypothesis tested in this study was that benthic algal community structure would be significantly different between treatments with and without grazers in late spring. Indeed, algal communities stocked with ambient grazer densities grouped together with those in the open stream but were distinctly different from those in the exclusion enclosures. This hypothesis is therefore supported. Despite apparent detachment of the algal mat in the exclusion enclosures, the green filamentous taxon, *Mougeotia* spp. was particularly prevalent under low grazer densities, compared with the inclusion enclosure were it was recorded in low abundance and the open stream, where it was completely absent. Besides *Mougeotia* spp., the diatom *Eunotia rhomboidae* and to a lesser extent *Gomphonema* spp. and *Frustulia* spp. were more abundant in the exclusion enclosures compared to those with higher grazer densities, suggesting that they are particularly palatable to invertebrate grazers. Similarly, Holomuzki and Biggs (2006) found that *Mougeotia* spp. were only found in ungrazed controls in a grazer exclusion study in New Zealand. They also found that erect diatoms were higher in the absence of grazers. Furthermore, Wellnitz ad Ward (1998) found that baetid mayflies were able to effectively reduce erect and filamentous algae. Filamentous green algae such as *Mougeotia* spp., as well as the large upright diatoms like *E. rhomboidae*, *Gomphonema spp.* and *Frustulia* spp. may therefore be more accessible to invertebrate grazers in the Berg River.

By contrast, both the adnate cyanobacterium, *Chamaesiphon* spp. and the colonial green alga, *Desmococcus* spp. increased in the presence of grazers. The cells of both these taxa are particularly small, relative to other dominant taxa found in the periphyton mats of south-western Cape foothill rivers. Jacoby (1987) found that taxa with larger cells were removed more readily than those with small cells by a Heptageniid mayfly. Also, cyanobacteria are considered less palatable than both green algae and diatoms (Hart 1985; Feminella and Resh 1991). Both Rosemond *et al.* (1993) and Wellnitz et al. (1996) also found that *Chamaesiphon* spp. responded positively to grazing activity. The increased representation of these taxa in the inclusion treatment relative to the exclusion treatment in late spring suggests that they are tolerant to grazers in the Berg River and can therefore thrive in the absence of competition from more palatable taxa that are removed by grazers. It therefore follows that a reduction in the representation of *Chamaesiphon* spp. and *Desmococcus* spp. under conditions of low grazing pressure may be a consequence of competitive displacement by green filamentous taxa and diatoms, which were able to proliferate in the absence of grazing.

The third hypothesis investigated in this study stated that no difference in periphyton biomass or community structure would be observed between treatments with and without grazers in early autumn. As predicted, no significant decrease in periphyton biomass was observed between treatments with and without grazers after both 5 days and 13 days during experiment 2. Unlike the late spring community, which included diatoms and single-celled chlorophytes, more than 90% of the algal community during late autumn was naturally dominated by the small, prostrate cyanophyte, *Chamaesiphon* spp. Also, grazer densities were generally below 1000 individuals m^{-2} and were rarely above 1500 individuals m^{-2} in either the inclusion enclosures or the open stream

during early autumn. A combination of low grazing intensity (Colletti *et al*. 1987; DeNicola *et al*. 1990; Holomuzki *et al.* 2006) and low food quality may therefore account for an insignificant grazer effect on periphyton biomass in early autumn.

Nevertheless, a significant difference in the periphyton community composition between treatments was observed in early autumn 2011, and therefore my third hypothesis must partially be rejected. Hill and Harvey (1990) and Rosemond (1993) also reported no change in periphyton biomass in response to grazing but a significant shift in community structure. Hill and Harvey (1990) attributed their results to the removal of overstory algal cells by grazers, which shifted the community to one that was more grazer-resistant taxa but promoted their growth such that biomass remained unchanged in the presence of grazers. Indeed, in the absence of grazers, palatable, more structurally complex taxa, such as the diatom *E. rhomboidae* and the filamentous chlorophyta, *Mougeotiopsis* spp. and *Mougeotia* sp. 1 were found in the exclusion enclosures but were absent in the presence of grazers. Despite the release from grazers, however, these more structurally complex and productive taxa did not proliferate as was the case in late spring. Under limited nutrient conditions like those typical of early autumn in the upper reaches of the Berg River when availability is generally lower (Chapter 5) the rapid growth and accrual of competitively superior taxa may be limited, despite their release from grazing. These results supports the idea that periphyton growth is controlled from the top-down at the beginning of the dry season but bottom-up factors (i.e. nutrient availability) become more important towards the end of the growing season. Naturally greater periphyton biomass in early autumn (Chl *a* biomass: 6.1 mg m^{-2}; AFDM: 1.3 g m^{-2}) compared to late spring (Chl *a* biomass: 1.8 mg m^{-2}; AFDM: 0.86 g m^{-2}) suggests that under bottom-up control by nutrients in late autumn, the community can attain a higher biomass than that limited from the top-down by grazing in the late spring in foothill rivers of the south-western Cape.

In conclusion, this study shows that grazing may be an important top-down control of periphyton biomass during the late spring. However, the effects of grazing on periphyton communities are dependent on both grazer type as well as grazer density. In early autumn, grazers may shift the community towards one that is dominated by prostrate cyanophytes, thereby influencing the trajectory of succession towards a more complex community. Nevertheless, grazers do not seem to affect periphyton biomass at this time, possibly due to a limited source of good quality food and low grazer densities. Besides the direct effects of climate change and anthropogenic impacts on periphyton communities, shifts in invertebrate community structure could significantly change the spatiotemporal dynamics of periphyton communities in foothill rivers of the south-western Cape, particularly during the dry, stable summer period when the balance between top-down and bottom-up forces control periphyton community structure and biomass.

CHAPTER 9 GENERAL DISCUSSION

9.1 DISTURBANCE, SUCCESSION AND THE INTERPLAY BETWEEN BOTTOM-UP AND TOP-DOWN CONTROLS

In Mediterranean climates, such as the south-western Cape, that have a defined period of flooding over the wet season, flood disturbance is generally the overriding driver of biotic communities in perennial rivers. Classical definitions of disturbance stipulate that disturbance includes only unpredictable events (Resh *et al.* 1988). It could therefore be argued that flood events in the south-western Cape should *not* be considered disturbances because they are generally confined to the wet season to which algal communities are adapted as a predictable component of the natural environment. However, flood events in rivers of the south-western Cape are 'predictably unpredictable' as postulated by Davies *et al*. (1995) and demonstrated by Ractliffe (2009). In other words, floods predictably occur over the wet season but the occurrence of any one event of a particular magnitude within this period is unpredictable. Larned (2010) argued that whether flood events are predictable or not is irrelevant to immobile periphyton communities because, unlike motile organisms, which might be adapted to predictable flood events, the effects of flooding on periphyton communities are independent of their predictability. Physical disturbance was therefore defined as 'episodic events that remove organisms at rates faster than rates of accrual or recruitment' (Larned 2010), regardless of their predictability.

Considering that the flow regime in south-western Cape rivers is defined by a distinct wet season with frequent floods, followed by a period of flow stability when communities follow a sequence of change, or succession, both flood disturbance and succession were two central themes that formed the basis of this thesis. Chapters 4 and 5 in particular demonstrated firstly that flood disturbance (particularly flood frequency over the wet season) is the primary factor shaping periphyton communities in south-western Cape rivers and secondly, that periphyton community succession in systems with similar flow regimes is affected by nutrient enrichment. Also, seasonal cues such as light and water temperature may affect successional changes in community structure over the dry season (Chapter 5).

The role of grazers as a top-down control on periphyton communities was implied by the temporal patterns observed in Chapter 4 and the relationships established in Chapter 5. The relative importance of grazing pressure in south-western Cape rivers, particularly during the spring and early summer, was confirmed by the outcome of the controlled experiments detailed in Chapter 8. These results suggest a shift from a top-down abiotic control of periphyton communities over the wet season driven by flood disturbance (Figure 9.1: 1) to a bottom-up control driven by the availability of nutrients during the dry season (Figure 9.1: 2). During the late spring and early summer, this bottom-up control of periphyton may work antagonistically to the top-down biotic control imposed by grazing pressure (Figure 9.1: 3). In other words, community succession is mediated by various environmental variables that operate from the bottom-up (nutrients, light, and water temperature)

or top-down (grazers) and shifts in the timing or strength of any one of these factors or the frequency of disturbance can result in a shift in periphyton community structure.

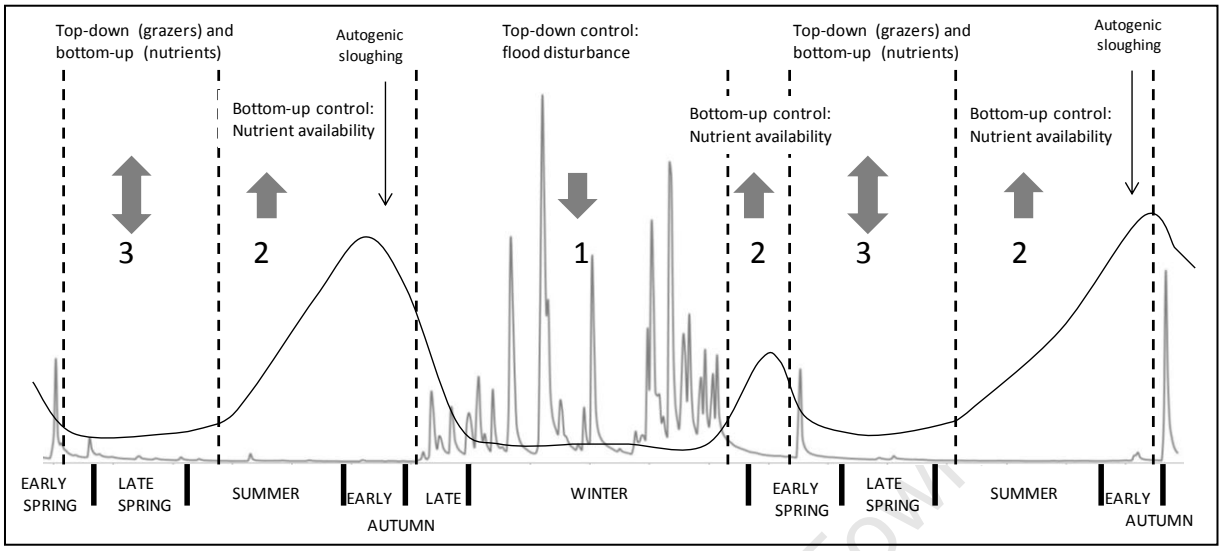


Figure 9.1 **A typical cycle of natural periphyton biomass accrual and loss under oligotrophic (—) conditions, superimposed on a hydrograph typical of south-western Cape foothill rivers (—). Proposed temporal shifts in top-down and bottom-up control of periphyton communities are also shown.**

In a broad sense, these results support the habitat matrix conceptual model for stream periphyton proposed by Biggs *et al.* (1998), which is based on the general habitat templet concept of Southwood (1988). Their model predicts that flood disturbance interacts with nutrient supply to determine the community structure, productivity and trajectory of succession of periphyton communities in unshaded temperate streams. Essentially, they suggest that gradients in disturbance frequency and resource supply result in a predictable community that can be described by three main life-history "strategies". These include the "competitive strategy" (C-selection), which is typical of productive, undisturbed habitats; the "stress-tolerant strategy" (S-selection), which is typical of nutrient-limited, undisturbed habitats; and the "ruderal strategy" (R-selection), which involves adaptation to physically disturbed, moderately enriched habitats (Figure 9.2). Indeed, under natural flow conditions, the moderately enriched Molenaars River generally supported C-S selected taxa at the end of the growing season and R-selected taxa following flood disturbance. The oligotrophic Berg River typically supported taxa that are S-selected in late summer and a community dominated by R-selected taxa in summer (Figure 9.2). Grazers operate within this framework by mediating shifts between communities with different life history adaptations (Figure 9.2). Based on the findings in this thesis, this model may be applicable to the evaluation of periphyton communities that are driven primarily by flood disturbance, nutrient supply and invertebrate grazing. The ability to predict periphyton biomass and community structure under different nutrient and flow regimes, and the role of grazers within this framework, is fundamental to the application of periphyton communities as indicators of human-induced ecosystems changes in rivers.

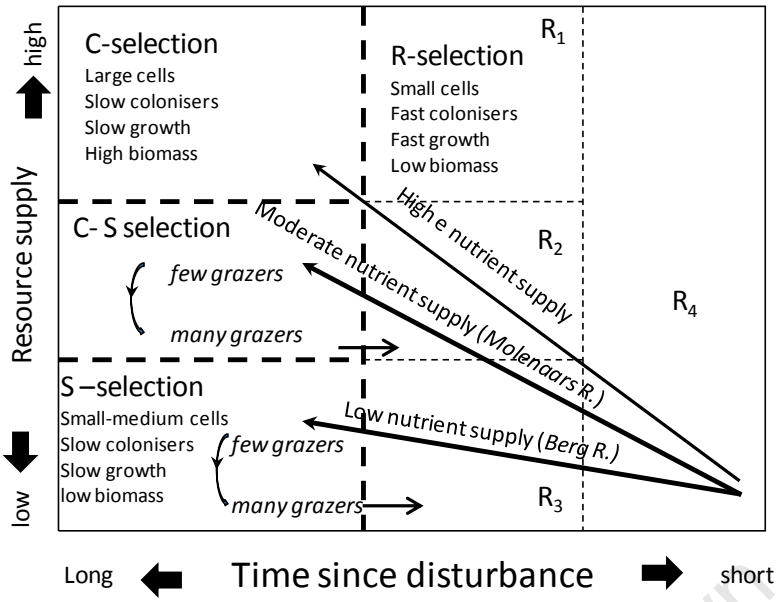


Figure 9.2 **Hypothetical succesional trajectories of periphyton communities following flood disturbance in habitats with high nutrient supply, moderate nutrient supply, and low nutrient supply. If inter-flood periods are suffienciently long, then invertebrate grazer densities can increase to levesl that cause periphtyon community structure to change back toward that characteristic of moderately disturbed, mesotrophic habitats or moderately disturbed, oligotrophic habitats. The sub-groups in the R-selected strategists define specific taxa with variable growth and cell size (after Biggs *et al.* 1998).**

9.2 KEY FINDINGS AND THEIR IMPLICATIONS FOR RIVER MANAGEMENT IN SOUTH AFRICA

In the absence of any previous ecological research on periphyton communities in South African rivers, an understanding of the key factors that drive the spatio-temporal patterns in periphyton communities under natural conditions provided a basis for understanding how changes in these factors might affect community structure in south-western Cape rivers.

This thesis demonstrated that the frequency and timing of flood events and flood size (or disturbance threshold), as well as dry season base-flow conditions, affect periphyton communities. Furthermore, nutrient availability affects the biotic response to different flow conditions.

9.2.1 Frequency of flood disturbance

A key finding in this thesis was that periphyton communities shift frequently as indicated by the distinct monthly changes in biomass and community structure, particularly over the wet season (Chapter 4). Less frequent shifts, often coinciding with seasonal changes were evident during the dry season. This suggests that periphyton communities are highly dynamic both *within* and *between* seasons and the frequency of change differs between the wet season when the frequency of flooding is high and the dry season, which rarely experiences floods and has stable low flows. Under natural conditions, frequent flood disturbances over the wet season maintain the periphyton community in an early stage of accrual. Considering that flood frequency is the primary factor driving temporal patterns in periphyton communities (Chapter 4 and 5), a reduction in the frequency of

disturbance floods over the wet season may alter natural cycles of biomass loss and accrual typical of the wet season in south-western Cape rivers. Unnaturally extended period of flow stability of over this time could allow the accumulation of biomass and the community may proceed through a succession from diatoms towards one with more complex algal forms such as large green filamentous algae or cyanobacteria that may be less palatable to invertebrate grazers. Indeed, flow regulation downstream of the Berg Dam showed that an unnaturally extended period of stability in the absence of floods during the first half of winter resulted in an aseasonal, mid-winter peak in periphyton biomass, despite low light availability and cold conditions (Chapter 4). Unlike Site 1 where frequent floods maintained a periphyton community in early stages of succession dominated by diatoms, the community at Site 2 was dominated by cyanobacteria during mid-winter. Consequently, aseasonal winter proliferations of periphyton may reduce the food quality of the periphyton community by allowing the community to proceed through a succession, uninterrupted by floods. In terms of managing altered flow regimes, this suggests that frequent, small floods over the wet season (above the disturbance threshold: see Section 9.3) rather than a few larger events would maintain periphyton communities in the early stages of succession typical of the wet season.

An important finding of this thesis was that accrual cycles during the inter-flood period are affected by the availability of nutrients (Chapter 4). Given sufficient time for biomass accrual following flooding, a comparison of temporal patterns in periphyton development between Site 1, 3 and 4, which represent sites that range in their availability of nutrients from oligotrophic, to slightly enriched to moderately enriched respectively, showed that both the rate of accrual and the size of peak biomass were greater with increasing levels of nutrient availability (Figure 9.3) The effects of nutrient availability during the inter-flood period therefore has particular implications for making decisions about the actual frequency of disturbance floods necessary to maintain the integrity of periphyton communities in regulated rivers.

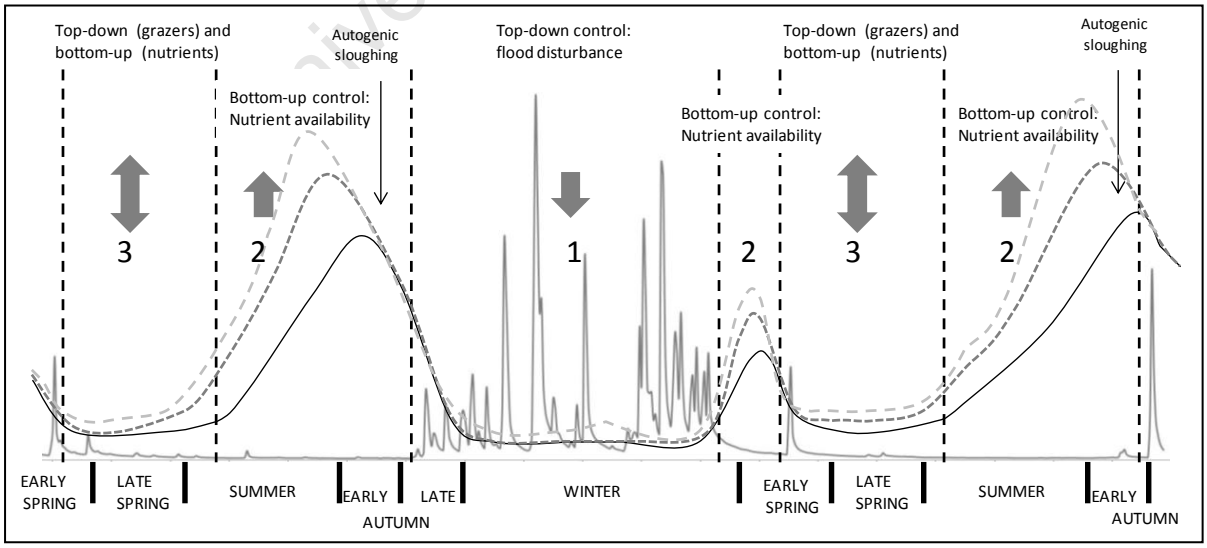


Figure 9.3 **Schematic diagram showing the proposed effects of periphyton biomass accrual and loss under oligotrophic (—), slightly enriched (---) and moderately enriched (- - -) conditions over an annual cycle, superimposed on a hydrograph typical of south-western Cape foothill rivers (—). Proposed temporal shifts in top-down and bottom-up control of periphyton communities are also shown.**

Figure 9.4 provides a hypothetical model of the effects of reduction in flood frequency under nutrient poor and enriched conditions. Under a natural flow regime, frequent flooding maintains a low biomass community characterised by pioneer taxa. Under an altered flow scenario, a reduction in the frequency of flooding will shift the community towards one that reaches a higher maturity and has a higher biomass. These effects will be more severe under enriched conditions than under unenriched conditions because the availability of nutrients promotes rapid periphyton growth during inter-flood periods. Essentially, over a given inter-flood period, periphyton growth and accrual will be greater under enriched conditions than under unenriched conditions and therefore the negative effects on periphyton communities of reducing flood frequency will be greater under enriched conditions. Simply stated, under enriched conditions, flood releases will need to be more frequent over the wet season to maintain the community in its pioneer state typical of the early stages of accrual compared with that under nutrient poor conditions, where periphyton accrual takes longer and therefore fewer floods are needed to maintain the community in its pioneer state.

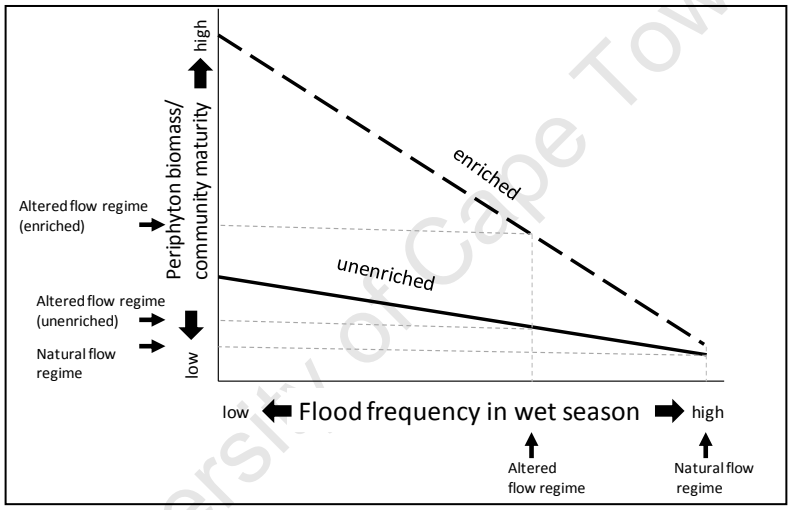


Figure 9.4 Conceptual relationship between flood frequency over the wet season and its influence on periphyton biomass and community structure.

9.2.2 Timing of the last wet season disturbance flood event

This study showed that the last disturbance flood event of the wet season, which generally occurs in spring, marks the start of the accrual cycle for periphyton biomass over the dry season. During the dry season, bottom-up factors such as nutrients, light and water temperature control how much periphyton accrues and what taxa dominate the community (Chapter 4). The periphyton community provides an important source of food for aquatic invertebrates over this period. In particular, different invertebrate functional feeding groups seem to depend on the availability of algae for food at different stages during the growing season: scrapers during early spring; deposit feeders during late spring and early summer; and brushers during late summer and autumn (Chapter 5). If the spring flood is lost, then the accrual cycle would start earlier and the spring and early summer periphyton community would be higher in biomass and possibly more complex in structure. This mismatch between invertebrates and periphyton could therefore result in a reduction in the abundance of invertebrates, or at least a shift in species composition. In turn, this could reduce the

flow of energy to higher levels of the food chain and thereby compromise the ecological integrity of the system.

9.2.3 The role of dry season base flows

The autotrophic/heterotrophic balance in the periphyton community changes under conditions of altered base flows and enrichment (Chapter 4). Under elevated base flow conditions downstream of the Berg Dam, the heterotrophic component (i.e. bacteria/fungi/protozoa/organic matter) was much higher than upstream of the dam. This was particularly the case during the summer when base flows were much higher at Site 2 than at Site 1 due to artificial releases of water for irrigation purposes. Although near-bed velocities at Site 2 were generally lower than at Site 1 under these conditions, the run biotope was generally deeper than 0.5 m, whereas the runs at Site 1 upstream of the dam were typically shallower than 0.2 m over the dry season (Figure 3.27). During these periods, mucilaginous diatoms or heterotrophs that are more resistant to these altered base flow conditions may render the community more resistant to floods. However, this study failed to provide conclusive evidence to this effect and further investigation of the mucilaginous content of the periphyton community as a factor affecting resistance to floods is warranted.

9.2.4 The threshold of disturbance

In their review of disturbance in stream ecology, Stanley *et al.* (2010) emphasised that disturbance events should be quantified by physical measures of the event itself (e.g. size of a flood), as well as by the biotic response, which indicates the resistance and resilience of a community. An important finding in this study (Chapter 5) was that the DRIFT Class 2 flood most likely defines the disturbance threshold for periphyton communities in the south-western Cape under natural flow conditions. Nevertheless, Chapter 7 demonstrated that quantifying the disturbance threshold in terms of flood size is complicated by the resistance of the community to disturbance. Under some conditions, a Class 3 flood event did not constitute a disturbance to the periphyton community downstream of the Berg Dam (i.e. Site 2), possibly because the community had developed a resistance to flooding following an extended dry season with elevated constant base flows. These conditions seem to have resulted in a community with high mucilage content, and therefore a higher resistance to scour.

Periphyton biomass downstream of the Berg Dam was significantly *greater* two weeks after the Class 3 flood event than prior to flooding in June 2008 (Chapter 7). This suggests that, under certain circumstances, a flood event may *promote* the growth of benthic algae in the short-term through release from growth-limiting conditions created by the predominance of either organic matter or heterotrophs prior to flooding. A similar situation in the Mitta Mitta River, Australia, showed that flood releases under an altered flow regime removed the long, filamentous branches of the dominant algal taxa but that floods were not large enough to scour the algal holdfasts. The removal of dead matter and silt promoted regrowth from the holdfasts and resulted in an algal bloom that was larger than that prior to flooding (Watts *et al.* 2009). This scenario emphasises the need to understand the links between the biotic community and its response to flood flows to make informed decisions about the size of flood events necessary to achieve specific ecological goals.

Even in the absence of elevated summer base flows, nutrient enrichment (i.e. Site 4) seems to have promoted the development of periphyton communities with high mucilage content that may have increased their resistance to floods. This suggests that larger disturbances may be needed to reset periphyton communities where altered flow conditions (Figure 9.5a) or enrichment (Figure 9.5b) have increased the resistance of the pre-flood periphyton community.

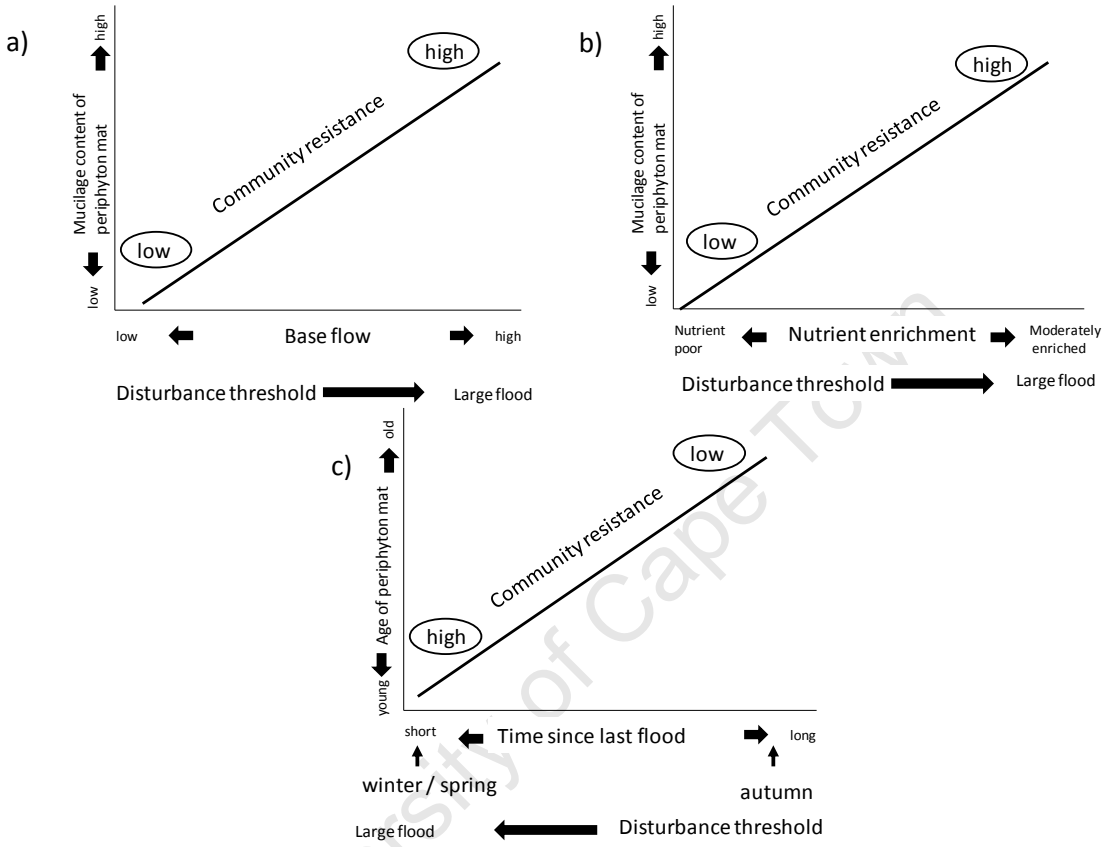


Figure 9.4 **The conceptual relationship between a) summer base flows, and the mucilagenous content of the periphyton mat; b) moderate enrichment and the mucilagenous content of the periphyton mat; and c) the length of the stable, dry season following the last flood and the age of the periphyton mat.**

Differences in the resistance to flood disturbance were also affected by the age of the community, which in turn is determined by the length of the accrual cycle (Figure 9.5c) because algal cells at the base of the mat eventually die and the mat is easily detached by even small events at the end of the accrual cycle. Conversely, pioneer taxa typical of the early stages of the accrual cycle are usually more resistant to flood disturbance than mature communities (Grimm and Fisher (1989). The size of the flood needed to reset the community to its early stages of accrual, or threshold of disturbance, may be smaller during autumn compared with spring as long as base flow conditions and nutrient availability over the dry season remain natural (Section 9.2.3).

Understanding the flow and nutrient factors that affect the resistance and resilience of periphyton communities, and the disturbance necessary to reset periphyton accrual cycles, has major implications for managing modified flow regimes. Firstly, the spring flood needed to reset the accrual cycle at the start of the growing season may need to be larger than the flood in autumn

when the community is mature and less resistant to disturbance. Secondly, when deciding on the magnitude of flood releases under altered flow conditions, water managers would need to take cognisance of antecedent base flow conditions and levels of nutrient impairment that may enhance resistance to flooding.

9.2.5 Heterogeneity in periphyton communities between hydraulic habitats – the implications of changes in near-bed velocity and nutrients

Although this thesis focused largely on periphyton community structure and biomass within a temporal realm, reach-scale spatial patterns were also addressed, with the main objective of understanding the interactive effects of near-bed velocity and nutrient availability on periphyton communities among different habitats at a local scale. Chapter 6 demonstrated that differences in near-bed velocities were a key driver of within-reach variability in periphyton communities. In particular, periphyton communities differed between hydraulic biotopes that represent a range of near-bed velocities.

An important finding of this study was that the functional significance of near-bed velocity appears to vary depending on the level of enrichment and the age of the periphyton mat. In spring, periphyton mats are young, while in autumn they are mature as they reach the end of the accrual cycle. In particular, under both moderately enriched and unenriched conditions during autumn, periphyton biomass peaked in the run biotope with lowest biomass in the slack-waters and riffles (Figure 9.6). During October 2010 (spring) however, low flow conditions had opposing effects on the local periphyton community under different enrichment scenarios (Figure 9.6). Under nutrient poor conditions in spring, lowest biomass occurred in the slow flowing slack-waters and highest biomass in the riffles where near-bed velocities were the greatest. By contrast, highest biomass occurred in the slack-waters and lowest biomass in the riffles under enriched conditions. Essentially, where nutrients are limiting, the higher flows in riffles promote the transfer of nutrients to the algal mat and therefore promote algal growth at higher velocities compared with growth in runs and slack-waters (Chapter 6). Where nutrients are in ample supply, however, large algal mats can accumulate in the slack-waters.

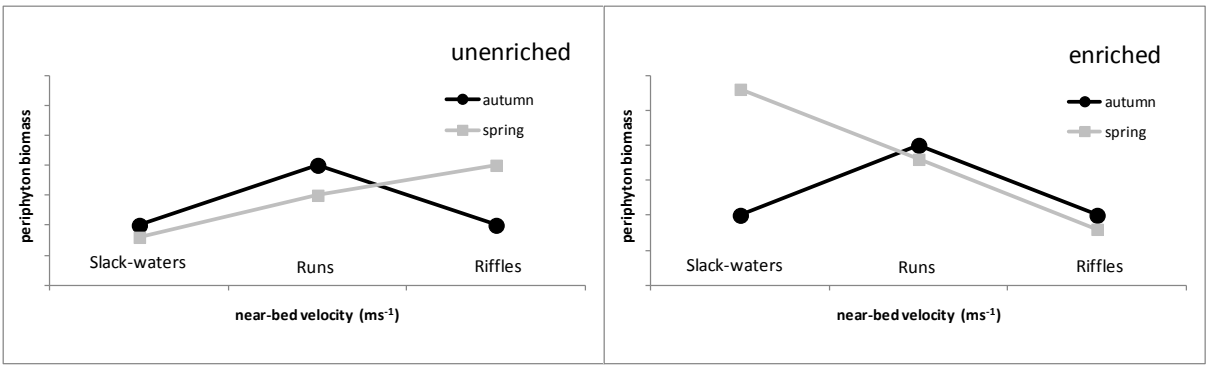


Figure 9.5 Conceptual relationship between periphyton biomass and near-bed velocities in different seasons and under different enrichment conditions based on findings in Chapter 6.

Depending on the level of enrichment therefore, different low flow conditions can result in different periphyton communities within a river reach. For instance, a reduction in dry season low flows can significantly change biotope characteristics such that slack-waters and runs dominate and riffles become scarce. These changes could lead to an overall decrease in periphyton biomass in oligotrophic rivers but an overall increase in biomass in enriched rivers (Figure 9.5). Maintaining local-scale heterogeneity in both the physical habitat and the biota that depend on them is critical for the maintenance of biodiversity in streams.

Proposed changes in periphyton community structure associated with altered flow and nutrient conditions in streams are summarised in Figure 9.6. This conceptual model shows how 1) increases or 2) decreases in summer base flows might affect the dry season community and 3) a decrease in flood frequency or 4) loss of the spring flood could affect the late spring and summer periphyton community structure. Seven different alternative communities that could replace natural periphyton communities under altered flow and nutrient conditions are predicted (shaded blocks). Many of these proposed outcomes are tentative, largely because this research was limited to only two foothill rivers in the south-western Cape. However, the model provides a framework for developing specific research projects for addressing specific components of altered flow regimes under different nutrient conditions.

9.2.6 Periphyton biomass as a measure of trophic status

By virtue of the sampling design of this study, trophic status of sites on the Berg and Molenaars Rivers were inferred by the presence or absence of trout farms upstream of each site and not by ambient nutrient concentrations in the water column. Chapter 4 showed that peak biomass (P_B) was greater under enriched conditions than under nutrient poor conditions and that benthic algal communities at P_B were particularly distinct between sites with different levels of enrichment. These differences in trophic status were not always reflected by differences in dissolved nutrient concentrations measured at the time of sampling. Essentially, concentrations of dissolved nutrients in the water column reflect the residual concentration after removal by periphyton. In other words, ambient nutrient concentrations do not reflect the total nutrient load available for uptake and thus the trophic potential of the system, but instead reflect what is left after the periphyton has removed what is needed for growth (Biggs 2000a). This suggests that P_B in open-canopied rivers provides a far better indicator of trophic status in rivers than do N and P concentrations in the water column.

The effects of nutrient enrichment were limited to mesotrophic conditions typical of the Molenaars River and therefore the response of periphyton to higher levels of enrichment could not be established in this study. Incorporation of Chl *a* biomass determination into routine monitoring initiatives will provide the information necessary for setting thresholds of oligotrophic, mesotrophic and eutrophic conditions. Trophic status thresholds have been established successfully in other countries, particularly the United States and New Zealand where they are currently used for monitoring and resource evaluation (e.g. Biggs 2000b).

9.3 PERIPHYTON AS INDICATORS IN RESERVE DETERMINATIONS

Considerable effort has been invested in developing appropriate methods for setting environmental flow requirements for water resources in South Africa. DRIFT, for example, is a holistic methodology that has received wide acceptance both in South Africa and elsewhere because relationships between DRIFT flow categories and a number of biophysical ecosystem components based on a number of biotic indictors are used to produce predictions of ecosystem response to flow alteration (King and Brown 2006). Biotic ecosystem components currently include invertebrates, riparian vegetation and fish (King and Brown 2006). The findings of this study, however, suggest that periphyton could contribute substantially to the suite of biotic indicators used for predicting ecosystem response to flow alteration in South Africa.

Useful biotic indicators obviously need to be sensitive to flow and nutrient alterations but they also need to have well-understood unidirectional responses to specific alterations in the flow regime. From a practical perspective, indicators need to be easy to measure and cost-effective (Burns and Ryder 2001). The development of such indicators is far less advanced for periphyton than for invertebrates and fish. Those periphyton indicators that have been established often require detailed assessments of community structure. This requires specialised taxonomic skills and the process is costly and time-consuming (see Section 1.6 of the literature review, Chapter 1). Nevertheless, there is some indication from the findings in this study that indicators based on a combination of algal division and growth form could be established. The three main taxonomic groups include green algae, diatoms and cyanobacteria and three broad growth forms include single-celled, colonial and filamentous and branched filamentous types. Chl *a* biomass and the Autotrophic Index, which measures the balance between autotrophs and heterotrophs, are two indicators that may prove useful for predicting and monitoring the biotic response to flow alteration. Identifying which, if any, of the proposed periphyton indicators are robust predictors for monitoring ecological change requires extensive testing and should be included in future research initiatives.

Considering the limited extent of this study, the relevance of key findings to a broader geographic area is limited. Insights into spatial and temporal variability in periphyton communities and the factors which drive these patterns in other rivers of South Africa will improve our understanding of their role in maintaining ecosystem integrity and therefore their value as biotic indicators of altered flow and nutrient regimes. Considering that rivers of the south-western Cape are particularly different from those throughout the rest of South Africa, there is a clear need to expand the geographic range of this research.

9.4 CONCLUDING REMARKS

This thesis constitutes the first detailed assessment of periphyton community structure and biomass in South African rivers. It provides information on temporal and reach-scale spatial patterns in periphyton communities and the environmental factors that drive them. Key findings support the widely accepted notion that flood disturbance is the overriding factor structuring periphyton communities in open-canopied temperate rivers that have distinct seasonality in environmental

conditions. Furthermore, this thesis demonstrated that nutrients and grazers are important factors that affect the structure and biomass of periphyton communities over the stable dry season. In his review of temporal patterns of periphyton communities, Biggs (1996) describes this pattern as typical of Mediterranean climates. Larned (2010) pointed out that a large volume of the existing literature on periphyton describes spatial and temporal patterns in these communities but rarely provides evidence for the cause of these patterns because of insufficient statistical power and lack of experimental control.

This thesis demonstrated that flood events and their interactions with multiple other environmental variables operate over shorter time scales than those defining seasons. Establishing causal links between environmental drivers and periphyton communities is often limited by the frequency of sampling, because once-off seasonal sampling limits the ability to invoke any causal links between changes in periphyton and potential environmental drivers. Monthly sampling, rather than seasonal sampling in this study therefore provided a valuable opportunity for teasing apart the causal effects among variables that change over multiple time scales. Furthermore, this thesis showed that floods are not confined to the winter but straddle the early spring and late autumn such that there is a mis-match between the period of flooding over the wet season, and seasonal shifts in factors such as light and temperature in the south-western Cape. However, seasonal conditions during the spring, such as temperature and light, are favourable for the rapid growth of periphyton, particularly if invertebrate grazer densities have not yet recovered after flooding. Although monthly sampling during this study provided some understanding of the relative importance of specific environmental drivers of periphyton, sampling at a frequency greater than monthly would significantly improve our ability to determine the exact cause of changes in periphyton communities, particularly during the spring when multiple interactions between environmental factors are particularly complex.

Nevertheless, two components of this thesis managed to establish the causal relationship between periphyton communities and environmental drivers. Firstly, the flood study in Chapter 7 isolated specific flood events and measured their effects on periphyton communities in the absence of other factors. This study provided valuable insights into the size of floods defining the disturbance threshold for periphyton communities and the resistance and reliance of these communities. Secondly, the controlled grazer experiment in Chapter 8 provided clear evidence of the importance of invertebrate grazers as a factor regulating periphyton communities during the spring. This thesis therefore contributes to our understanding the causal links between periphyton communities and the key environmental factors that shape them. Increasing sampling intensity, together with further controlled experiments that include some measure of nutrient availability should therefore be the focus of any future research that endeavours to tease apart the causal relationship between environmental drivers and periphyton communities.

Finally, this thesis contributes significantly to the body of literature which aims at predicting ecological responses to environmental drivers. In turn, this thesis provides a foundation for understanding periphyton communities in south-western Cape rivers and therefore complements the body of knowledge that should be used in managing and monitoring aquatic ecosystem integrity.

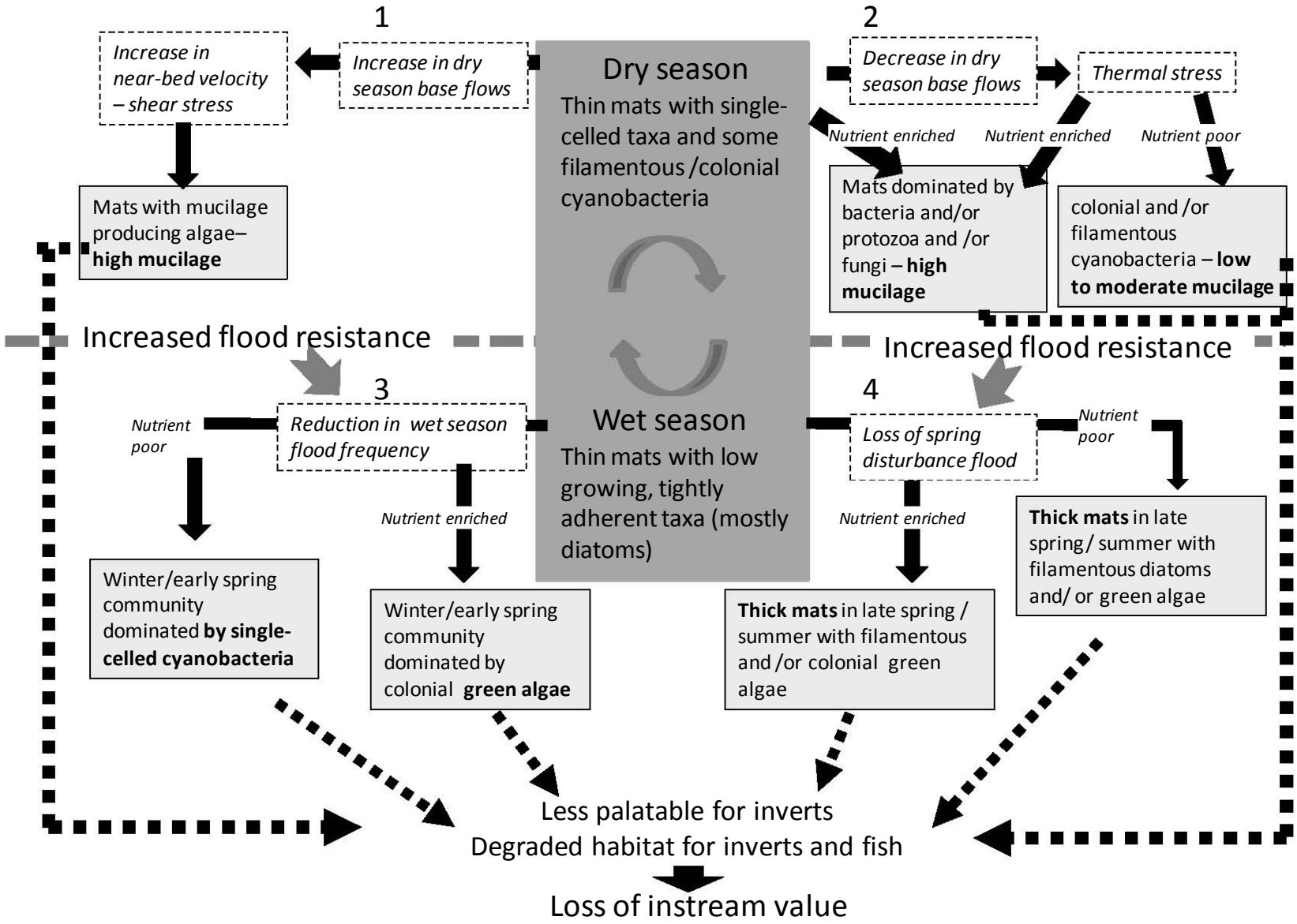


Figure 9.6 **Conceptual model of the proposed changes in periphyton community structure associated with altered flow and nutrient conditions in streams. The darker grey block represents periphtyon communities under natural flow and nutrient conditions in the Berg River. The ultimate effect of altered periphyton community structure on ecosystem integrity of streams (dotted line) is also shown..**

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APPENDIX 1a

List of benthic algal taxa by taxonomic division and growth form given as a mean number per m^2 x10^3 for each month sampled at Site 1 on the Berg River between September 2007 and May 2009

SITE 1: Berg River

			late spring			summer			early autumn	late autumn	winter				early spring		late spring	summer			early autumn	late autumn
Division	**Growth form**	**Taxon**	*Oct-07*	*Nov-07*	*Dec-07*	*Jan-08*	*Feb-08*	*Mar-08*	*Apr-08*	*May-08*	*Jun-08*	*Jul-08*	*Aug-08*	*Sep-08*	*Oct-08*	*Nov-08*	*Dec-08*	*Jan-09*	*Feb-09*	*Mar-09*	*Apr-09*	*May-09*
Bacillariophyta	***single cells***	*Achnanthes oblogella*	0	38	7	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Asterionella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Encyonema spp	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema lagenula	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema laticollum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0	0	0	0	279	0	0	0	0	0	0
		Gomphonema pseudoaug	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema spp	0	22	12	0	53	0	0	0	0	0	0	59	131	164	0	0	0	0	0	0
		Gomphonema truncatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula cryptotenella	0	0	0	0	0	242	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula spp	0	0	0	0	1072	484	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Netrium spp	0	0	0	0	573	1368	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia irremisa	0	0	0	0	38	351	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia palea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia spp	0	0	0	0	0	157	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Pinnularia subcapitata	0	0	0	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	0
		Surirella angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments/single cells	*Eunotia bilunaris*	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Eunotia exigua	645	235	812	483	412	161	0	143	0	0	0	158	155	0	0	0	0	0	0	0
		Eunotia minor	568	583	773	0	0	0	0	120	64	27	0	0	61	148	0	0	0	0	0	0
		Eunotia rhomboidae	41741	2845	4939	3499	10370	5361	2834	1422	2081	27	3250	8255	14575	2979	5136	26120	1613	7924	7316	1238
		Fragilaria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Fragilaria capucina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia crassinervia	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Tabellaria flocculosa	0	0	0	0	647	0	0	0	32	0	0	0	48	0	0	0	0	0	0	0
	colonial	*Achnanthidium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Aulocosceria* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyta	***single cells***	*Actinotaenium* spp	0	0	0	0	376	936	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Closterium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Cosmarium spp	0	0	218	0	0	577	0	0	0	0	0	0	376	0	751	0	0	0	0	20
		Gongrosira spp	0	0	0	0	2099	0	0	0	0	0	0	0	0	0	0	0	0	6023	1082	1011
		Penium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Staurastrum spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	colonial	*Chlorococcum* spp	764	8178	62	240	0	0	169	0	0	11	87	871	157	22	230	343	496	1517	362	24
		Coelosphaerium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Desmococcus spp	4965	416	2115	2672	3369	1528	7086	13848	4228	77	234	41	11706	1912	3244	3183	1042	1192	9393	2488
		Micractinium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Scenedesmus spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Mougeotia* sp 1	0	0	28	35	6243	6152	0	0	0	0	0	0	54	0	0	0	0	0	0	0
		Mougeotia sp 2	0	0	0	0	18049	0	0	0	0	0	0	0	444	0	0	0	0	0	0	0
		Oedogonium sp 1	0	1254	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Oedogonium sp 2	0	0	0	0	3745	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Palmella spp	0	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Spirogyra spp	0	0	0	0	0	32355	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Ulothrix spp	0	0	0	0	313	0	0	0	0	0	0	0	362	0	0	0	0	0	0	0
	branched filaments	*Stigeoclonium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanophyta	***single cells***	*Chamaesiphon* spp	4444	2524	701	3458	17116	21951	14179	9792	5482	15	2557	2712	1265	3863	2366	65697	40051	48922	2400	470
	colonial	*Aphanocapsa* spp	0	50	26	0	0	0	0	0	0	0	0	0	5323	521	860	0	0	11220	22895	1591
		Merismopedia spp	0	0	0	123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Hapalosiphon* spp	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0
		Homoeothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	121	0	0	0	0	0	0	0
		Lyngbya sp 1	0	0	0	0	1114	0	0	0	0	0	0	0	0	0	0	1371	168	0	0	0
		Lyngbya sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Oscillatoria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Phormidium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Romeria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	filaments in gelatenous masses	*Nostoc* spp	0	0	0	124	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Rivularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysophyta	***colonial***	*Dinobryon* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglenophyta	***single cells***	*Euglena* spp	0	0	0	0	0	0	0	0	0	0	0	0	10117	0	0	691	0	0	0	0

1.1 APPENDIX 1b

List of benthic algal taxa by taxonomic division and growth form given as a mean number per m^2 x10^3 for each month sampled at Site 2 on the Berg River between September 2007 and May 2009

SITE 2: Berg River

			late spring			summer			early autumn	late autumn	winter			early spring		late spring	summer			early autum	late autumn
Division	**Growth form**	**Taxon**	*Oct-07*	*Nov-07*	*Dec-07*	*Jan-08*	*Feb-08*	*Mar-08*	*Apr-08*	*######*	*Jun-08*	*Jul-08*	*Aug-08*	*Oct-08*	*Nov-08*	*######*	*Jan-09*	*Feb-09*	*Mar-09*	*######*	*May-09*
Bacillariophyta	***single cells***	*Achnanthes oblogella*	6434	99	474	0	163	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Asterionella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	459	0	0
		Encyonema spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema lagenula	0	416	0	232	1667	360	285	0	79	0	0	0	0	0	0	0	0	0	0
		Gomphonema laticollum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema pseudoaug	0	690	0	568	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema spp	0	0	0	400	0	0	0	0	0	0	0	341	0	0	0	0	0	280	1302
		Gomphonema truncatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula angusta	0	1961	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula cryptotenella	29649	9469	4788	2462	8783	7635	1018	0	0	0	0	0	0	0	0	0	13727	0	3375
		Navicula spp	11755	3234	0	0	0	1287	0	0	0	0	0	0	0	0	0	4976	0	2076	1537
		Netrium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1035	0	0
		Nitzschia irremisa	0	735	724	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia palea	0	46	0	811	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia spp	0	0	0	608	159	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Pinnularia subcapitata	0	374	0	0	0	0	0	0	0	0	0	0	0	193	0	0	0	0	0
		Surirella angusta	0	1223	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments/single cells	*Eunotia bilunaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Eunotia exigua	9099	3581	1092	605	4138	0	0	0	0	0	0	405	168	0	896	0	565	0	0
		Eunotia minor	0	232	1841	785	0	0	0	477	0	0	0	0	0	0	0	0	0	0	0
		Eunotia rhomboidae	55321	3172	6564	5501	16003	2262	855	2373	323	2357	406	812	168	191	2562	1660	2849	1985	2506
		Fragilaria spp	2083	72802	0	0	13665	59000	3767	0	0	0	0	0	0	0	0	9386	1523	1002	4961
		Fragilaria capucina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia spp	239	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia crassinervia	0	2803	1086	1108	993	0	0	0	0	0	0	0	0	0	0	0	0	248	0
		Frustulia vulgaris	5940	93	2967	406	198	0	0	0	0	0	0	0	0	0	0	0	895	201	1181
		Tabellaria flocculosa	8237	60353	2071	110	476	541	0	0	0	0	0	893	0	309	34326	7639	4936	451	843
	colonial	*Achnanthidium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Aulocosceria* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyta	***single cells***	*Actinotaenium* spp	0	0	0	0	0	415	0	0	0	0	0	0	0	0	0	292	280	129	133
		Closterium spp	0	1210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	226	201	189
		Cosmarium spp	7677	2871	10347	6082	1314	743	388	0	321	0	0	0	0	0	0	4429	3301	682	0
		Gongrosira spp	0	0	0	0	0	0	2696	0	0	0	0	0	0	0	0	0	0	0	0
		Penium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	602	637	404
		Staurastrum spp	0	0	0	0	0	33817	4441	0	0	0	0	0	0	0	0	0	1300	1929	3937
	colonial	*Chlorococcum* spp	0	1624	769	138	0	405	0	0	0	0	24	0	0	0	517	0	115	0	0
		Coelosphaerium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Desmococcus spp	2391	1373	868	6095	2109	772	22891	1058	1880	0	100	0	519	1259	0	470	382	91	903
		Micractinium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Scenedesmus spp	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	58
	unbranched filaments	*Mougeotia* sp 1	11508	28404	0	0	0	1931	305	144	0	0	0	223	0	0	0	0	0	0	0
		Mougeotia sp 2	35390	18260	0	0	0	0	0	0	0	0	0	0	0	0	590443	42863	5686	0	0
		Oedogonium sp 1	0	110387	3480	8591	0	0	0	0	1885	0	0	0	0	0	0	0	0	0	0
		Oedogonium sp 2	0	22383	4997	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Palmella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Spirogyra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Ulothrix spp	0	0	0	0	1631	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	branched filaments	*Stigeoclonium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	174
Cyanophyta	***single cells***	*Chamaesiphon* spp	0	894	18826	18346	18655	27466	24572	24163	11477	60192	4176	71626	33702	42272	109265	92154	23628	7865	13900
	colonial	*Aphanocapsa* spp	0	0	0	0	0	0	0	0	0	0	0	406	0	0	0	0	0	1008	969
		Merismopedia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Hapalosiphon* spp	0	2210	0	0	0	0	361	301	0	755	0	215	0	191	0	0	0	24	0
		Homoeothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7035	0
		Lyngbya sp 1	18475	4625	1290	1869	4393	1066	611	0	0	2303	0	0	0	0	0	423	0	703	0
		Lyngbya sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1058	452	1927	1976
		Oscillatoria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Phormidium spp	0	523	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Romeria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	filaments in gelatenous masses	*Nostoc* spp	0	0	0	0	993	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Rivularia spp	0	1585	3688	959	0	0	0	0	0	0	0	0	0	0	0	0	0	570	1384
Chrysophyta	***colonial***	*Dinobryon* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3075	0	856	16	0
Euglenophyta	***single cells***	*Euglena* spp	0	0	0	0	0	0	0	0	0	0	0	19811	0	0	0	0	0	0	0

1.2 APPENDIX 1c

List of benthic algal taxa by taxonomic division and growth form given as a mean number per m^2 x10^3 for each month sampled at Site 3 on the Molenaars River between September 2007 and May 2009

SITE 3: Molenaars River

			late spring		summer				early autumn	late autumn	winter				early spring		late spring	summer			early autumn	late autumn
Division	**Growth form**	**Taxon**	*Oct-07*	*Nov-07*	*Dec-07*	*Jan-08*	*Feb-08*	*Mar-08*	*Apr-08*	*May-08*	*Jun-08*	*Jul-08*	*Aug-08*	*Sep-08*	*Oct-08*	*Nov-08*	*Dec-08*	*Jan-09*	*Feb-09*	*Mar-09*	*Apr-09*	*May-09*
Bacillariophyta	***single cells***	*Achnanthes oblogella*	0	115	0	0	0	0	0	0	0	47	9	0	0	0	0	0	0	0	0	0
		Asterionella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Encyonema spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema lagenula	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0
		Gomphonema laticollum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0
		Gomphonema pseudoaug	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema spp	5542	0	59	0	0	115	778	99	0	0	0	0	342	0	178	0	378	1619	0	60
		Gomphonema truncatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula cryptotenella	0	112	0	0	910	0	360	0	33	0	0	0	0	0	0	0	0	0	0	0
		Navicula spp	0	224	105	0	0	194	0	0	0	0	0	0	0	0	0	0	461	0	813	0
		Netrium spp	0	0	0	0	0	0	0	0	0	195	0	0	0	0	0	0	0	0	0	0
		Nitzschia irremisa	0	0	0	0	126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia palea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia spp	0	337	0	0	252	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0
		Pinnularia subcapitata	1348	0	0	0	0	0	0	0	0	0	0	0	97	0	0	0	0	0	0	0
		Surirella angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments/single cells	*Eunotia bilunaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Eunotia exigua	1631	0	0	0	0	0	0	0	0	0	26	0	17	0	0	0	0	0	0	0
		Eunotia minor	0	0	0	0	0	434	469	276	0	76	26	132	0	0	0	0	0	0	0	0
		Eunotia rhomboidae	10358	1278	604	953	2227	0	752	817	265	719	193	420	165	324	615	464	209	1885	1463	251
		Fragilaria spp	4038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Fragilaria capucina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia spp	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia crassinervia	0	0	168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia vulgaris	0	0	0	0	135	0	0	0	0	0	14	0	0	0	0	0	90	0	185	0
		Tabellaria flocculosa	823	0	0	0	485	0	0	0	0	0	0	0	0	0	0	0	0	0	386	0
	colonial	*Achnanthidium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Aulocosceria* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3201	0
Chlorophyta	***single cells***	*Actinotaenium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Closterium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Cosmarium spp	0	0	0	0	0	0	0	0	0	0	0	0	35	0	0	0	328	0	0	0
		Gongrosira spp	0	0	0	1788	0	0	7031	0	0	0	0	0	0	0	0	0	0	0	0	0
		Penium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Staurastrum spp	5640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	colonial	*Chlorococcum* spp	2937	11257	0	87	0	875	1028	117	0	92	3	88	1053	162	254	6754	1876	63645	46453	455
		Coelosphaerium spp	0	0	1955	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Desmococcus spp	941	0	982	485	1012	36930	19281	229	820	25	1794	44	0	0	0	729	4456	1526	1738	32
		Micractinium spp	0	0	0	0	0	0	0	0	0	145	0	0	0	0	0	0	0	0	0	0
		Scenedesmus spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Mougeotia* sp 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	270	0	141	0
		Mougeotia sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5020	0
		Oedogonium sp 1	0	2910	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Oedogonium sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Palmella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Spirogyra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Ulothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	branched filaments	*Stigeoclonium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanophyta	***single cells***	*Chamaesiphon* spp	0	7769	10286	15055	16527	13206	15927	9230	9549	2618	1036	5749	1838	38683	28423	14154	8232	7665	3222	483
	colonial	*Aphanocapsa* spp	1433	537	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29239	5692	2863
		Merismopedia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Hapalosiphon* spp	0	0	0	0	56	382	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Homoeothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	3554	68399	2751	1872	1905	0	3364	0
		Lyngbya sp 1	0	133	0	0	5212	93	0	0	0	0	0	0	5875	0	0	0	68	0	290	88
		Lyngbya sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	579	0
		Oscillatoria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Phormidium spp	15738	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Romeria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	filaments in gelatenous masses	*Nostoc* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Rivularia spp	0	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysophyta	***colonial***	*Dinobryon* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglenophyta	***single cells***	*Euglena* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1.3 APPENDIX 1d

List of benthic algal taxa by taxonomic division and growth form given as a mean number per m^2 x10^3 for each month sampled at Site 4 on the Molenaars River between September 2007 and May 2009

SITE 4: Molenaars River

			late spring			summer			early autumn	late autumn	winter			early spring		late spring	summer			early autumn	late autumn
Division	**Growth form**	**Taxon**	*Oct-07*	*Nov-07*	*Dec-07*	*Jan-08*	*Feb-08*	*Mar-08*	*Apr-08*	*May-08*	*Jun-08*	*Jul-08*	*Aug-08*	*Oct-08*	*Nov-08*	*Dec-08*	*Jan-09*	*Feb-09*	*Mar-09*	*Apr-09*	*May-09*
Bacillariophyta	***single cells***	*Achnanthes oblogella*	819	36	21	0	60	45	0	0	0	96	0	924	0	0	0	0	0	0	0
		Asterionella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Encyonema spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema lagenula	0	0	0	0	2505	957	0	0	0	0	48	0	0	0	0	0	0	0	0
		Gomphonema laticollum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3524	0	0
		Gomphonema parvulum	0	0	0	0	0	0	0	0	0	0	0	387350	0	0	0	0	0	0	0
		Gomphonema pseudoaug	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Gomphonema spp	19010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1800	2032	19619	0
		Gomphonema truncatum	0	0	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula cryptotenella	0	0	0	0	17484	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Navicula spp	0	228	0	0	4602	0	0	0	0	0	0	0	0	0	0	6421	3213	2611	0
		Netrium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia irremisa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia palea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nitzschia spp	0	0	0	0	0	114	0	0	0	0	0	0	0	0	0	0	0	0	0
		Pinnularia subcapitata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Surirella angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments/single cells	*Eunotia bilunaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Eunotia exigua	4668	79	0	0	0	0	0	0	0	0	16	834	0	0	0	0	0	0	0
		Eunotia minor	721	42	82	0	0	0	485	0	0	0	0	0	0	0	0	0	0	0	0
		Eunotia rhomboidae	22050	2042	811	520	3810	0	0	105	59	108	130	461	621	0	0	2591	0	1724	0
		Fragilaria spp	6441	0	0	0	0	0	0	0	0	0	0	1787	0	0	0	0	0	0	0
		Fragilaria capucina	0	0	0	0	0	0	0	0	0	0	0	209170	0	0	0	0	0	0	0
		Frustulia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
		Frustulia crassinervia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Frustulia vulgaris	1330	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Tabellaria flocculosa	4740	0	0	0	0	0	0	0	0	50	0	5811	618	0	0	0	0	0	0
	colonial	*Achnanthidium* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Aulocosceria* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorophyta	***single cells***	*Actinotaenium* spp	0	0	0	0	0	0	0	0	0	0	0	1636	0	0	0	0	0	0	0
		Closterium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Cosmarium spp	0	272	0	0	1019	0	0	0	0	0	0	3668	0	0	0	0	0	240	0
		Gongrosira spp	0	0	0	1072	18316	6154	2031	0	0	0	0	0	0	0	0	0	0	3342	0
		Penium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Staurastrum spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	colonial	*Chlorococcum* spp	0	3319	180	0	3058	2881	0	11	0	0	1430	25229	2024	1133	811	47499	21808	8594	73
		Coelosphaerium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Desmococcus spp	2278	362	1126	9522	41770	35550	36109	3014	1109	234	44	0	559	295	488	0	0	875	0
		Micractinium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Scenedesmus spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Mougeotia* sp 1	0	0	0	0	0	0	0	0	0	0	0	3225	0	0	0	0	0	1060	0
		Mougeotia sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20985	15220	14136	0
		Oedogonium sp 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Oedogonium sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Palmella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Spirogyra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Ulothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	branched filaments	*Stigeoclonium* spp	0	0	0	0	0	0	0	0	0	0	0	26062	0	0	0	0	1279	287	0
Cyanophyta	***single cells***	*Chamaesiphon* spp	3800	7715	6376	38568	36500	13258	7730	6026	4450	4932	1839	20710	62744	20700	29656	65422	43035	4186	564
	colonial	*Aphanocapsa* spp	0	1062	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17305	25145	1657
		Merismopedia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unbranched filaments	*Hapalosiphon* spp	0	0	228	550	928	672	190	145	113	13	0	0	0	0	0	0	0	0	68
		Homoeothrix spp	0	0	0	0	0	0	0	0	0	0	0	25649	67106	0	0	0	0	0	0
		Lyngbya sp 1	0	0	0	768	1901	0	0	0	0	0	0	92160	0	1772	379	2795	773	809	0
		Lyngbya sp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Oscillatoria spp	449	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Phormidium spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Romeria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	filaments in gelatenous masses	*Nostoc* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Rivularia spp	0	684	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysophyta	***colonial***	*Dinobryon* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglenophyta	***single cells***	*Euglena* spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2

PERMANOVA pair-wise comparisons between months over the sampling period at Site 1. Comparisons for both intra- and inter-annual differences are shown, as well as months representing seasonal extremes. Only consecutive months for the intra-annual differences are shown. Significant differences at p≤0.05 are indicated in bold italics and at p≤0.1 in italics only.

Groups	t	p	% similarity	seasons
Intra-annual (months)	*t*	*p*		
October2007, November2007	***2.39***	***0.03***	***33***	***early spring - late spring***
November2007, December2007	***2.09***	***0.03***	***48***	***late spring***
December2007, January2008	***3.05***	***0.03***	***68***	***late spring - summer***
January2008, February2008	***3.16***	***0.03***	***41***	***summer***
February2008, March2008	*1.45*	*0.09*	*47*	*summer*
March2008, April2008	***2.04***	***0.03***	***51***	***summer - early autumn***
April2008, May2008	***1.88***	***0.03***	***77***	***early autumn - late autumn***
May2008, June2008	***1.88***	***0.03***	***70***	***late autumn - winter***
June2008, July2008	***2.45***	***0.03***	***15***	***winter***
July2008, August2008	***2.37***	***0.03***	***22***	***winter***
August2008, September2008	***2.13***	***0.03***	***71***	***winter***
September2008, October2008	***3.10***	***0.03***	***43***	***winter - early spring***
October2008, November2008	***2.01***	***0.03***	***40***	***early spring***
November2008, December2008	0.75	0.77	56	late spring
December2008, January2009	***2.92***	***0.03***	***37***	***late spring - summer***
January2009, February2009	***2.39***	***0.02***	***60***	***summer***
February2009, March2009	1.17	0.26	61	summer
March2009, April2009	***2.26***	***0.03***	***39***	***summer - early autumn***
April2009, May2009	***1.80***	***0.03***	***48***	***early autumn - late autumn***
Seasonal extremes				
March 2008, July 2008	***2.77***	***0.03***	***6***	***summer 2008 vs winter 2008***
July 2008, March 2009	***2.39***	***0.03***	***8***	***winter 2008 vs summer 2009***
Inter-annual				
October2007, October2008	***2.17***	***0.03***	***46***	***early spring***
November2007, November2008	***1.88***	***0.03***	***36***	***late spring***
December2007, December2008	***2.26***	***0.03***	***49***	***late spring***
January2008, January2009	***5.33***	***0.03***	***40***	***summer***
February2008, February2009	***2.94***	***0.01***	***38***	***summer***
March2008, March2009	*1.53*	*0.08*	***44***	***summer***
April2008, April2009	***2.47***	***0.03***	***53***	***early autumn***
May2008, May2009	***3.16***	***0.03***	***38***	***late autumn***

APPENDIX 3

PERMDISP pair-wise comparisons between months over the sampling period at Site 1. Comparisons for both intra- and inter-annual differences are shown. Only consecutive months for the intra-annual differences are shown. Significant differences at p≤0.05 are indicated in *bold italics.*

Intra-annual (months)	t	p
October2007, November2007	1.42	0.20
November2007, December2007	***4.25***	***0.03***
December2007, January2008	1.80	0.11
January2008, February2008	***3.14***	***0.03***
February2008, March2008	1.08	0.46
March2008, April2008	***5.63***	***0.03***
April2008, May2008	1.01	0.57
May2008, June2008	***2.47***	***0.03***
June2008, July2008	2.17	0.14
July2008, August2008	***2.99***	***0.03***
August2008, September2008	0.38	0.68
September2008, October2008	***4.52***	***0.03***
October2008, November2008	1.59	0.14
November2008, December2008	0.50	0.72
December2008, January2009	1.99	0.14
January2009, February2009	0.86	0.48
February2009, March2009	1.27	0.31
March2009, April2009	0.52	0.65
April2009, May2009	1.30	0.24
Inter-annual		
October2007, October2008	0.16	0.78
November2007, November2008	0.01	1.00
December2007, December2008	*2.55*	*0.05*
January2008, January2009	***3.77***	***0.03***
February2008, February2009	1.06	0.43
March2008, March2009	0.60	0.77
April2008, April2009	***3.88***	***0.03***
May2008, May2009	***6.75***	***0.03***

APPENDIX 4

SIMPER results of key discriminator taxa contributing to the differences between significantly different months (p≤ 0.1, Appendix 2) at Site 1. Densities are in number of cells (x 10 000) m^{-2} . Diss/SD indicates the consistency with which taxa discriminate between months across all samples.

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
October2007 vs November2007 (67%)	***October 2007***	***November2007***				
Eunotia rhomboidae	3814.7	227.7	25.1	2.1	37.7	37.7
Chlorococcum spp	19.1	645.6	11.2	1.8	16.7	54.5
November2007 vs December2007 (53%)	***November2007***	***December2007***				
Chlorococcum spp	645.6	4.1	14.4	2.2	27.5	27.5
Eunotia rhomboidae	227.7	487.1	6.8	1.3	13.0	40.5
Chamaesiphon spp	99.3	67.4	6.2	1.4	11.7	52.3
December2007 vs January2008	***December2007***	***January2008***				
Chamaesiphon spp	67.4	343.9	7.3	4.4	22.6	22.6
Eunotia minor	69.9	0.0	5.8	3.2	18.1	40.8
Eunotia rhomboidae	487.1	342.1	3.2	1.5	10.0	50.7
January2008 vs February2008	***January2008***	***February2008***				
Mougeotia spp2	0.0	1322.4	13.7	1.6	23.2	23.2
Chamaesiphon spp	343.9	1673.7	8.5	2.6	14.4	37.6
Mougeotia spp1	0.9	496.0	7.9	1.9	13.3	50.9
February2008 vs March2008 (53%)	***February2008***	***March2008***				
Mougeotia spp2	1322.4	0.0	10.5	1.5	19.7	19.7
Spriogira spp	0.0	1609.9	9.2	1.0	17.3	37.0
Mougeotia spp1	496.0	344.8	5.0	1.3	9.4	46.4
Desmococcus spp	322.1	58.0	3.7	2.2	7.0	53.5
March2008 vs April2008 (49%)	***March2008***	***April2008***				
Spriogira spp	1609.9	0.0	12.6	1.0	25.5	25.5
Desmococcus spp	58.0	676.3	8.7	1.7	17.7	43.2
Mougeotia spp1	344.8	0.0	6.2	1.2	12.5	55.7
April2008 vs May2008 (23%)	***April2008***	***May2008***				
Desmococcus spp	676.3	1363.6	7.0	1.7	30.8	30.8
Chamaesiphon spp	1328.2	973.2	6.5	2.4	28.5	59.3
May2008 vs June2008 (30%)	***May2008***	***June2008***				
Desmococcus spp	1363.6	301.9	15.4	1.3	51.8	51.8
June2008 vs July2008 (85%)	***June2008***	***July2008***				
Chamaesiphon spp	537.0	1.3	38.0	2.8	44.8	44.8
July2008 vs August2008 (78%)	***July2008***	***August2008***				
Eunotia rhomboidae	2.3	305.5	33.9	5.7	43.4	43.4
Chamaesiphon spp	1.3	247.9	31.7	5.3	40.6	84.0
August2008 vs September2008 (29%)	***August2008***	***September2008***				
Eunotia rhomboidae	305.5	783.4	11.2	1.5	38.5	38.5
Chlorococcum spp	6.1	79.3	6.7	1.9	23.0	61.5
September2008 vs October2008	***September2008***	***October2008***				
Euglena spp	0.0	708.1	13.0	1.4	22.8	22.8
Desmococcus spp	1.0	680.0	11.1	1.3	19.4	42.1
Aphanocapsa spp	0.0	500.8	10.9	5.4	19.0	61.1
October2008 vs November2008 (60%)	***October2008***	***November2008***				
Euglena spp	708.1	0.0	13.2	1.4	21.9	21.9
Eunotia rhomboidae	1419.6	276.2	10.4	2.7	17.3	39.2
Desmococcus spp	680.0	158.2	8.7	1.2	14.5	53.8
December2008 vs January2009 (63%)	***December2008***	***January2009***				
Chamaesiphon spp	228.9	6410.0	30.2	6.4	47.5	47.5
Eunotia rhomboidae	367.1	2471.3	14.7	1.8	23.1	70.7
January2009 vs February2009 (40%)	***January2009***	***February2009***				
Eunotia rhomboidae	2471.3	32.3	19.1	2.6	47.3	47.3
Chamaesiphon spp	6410.0	3824.6	9.5	1.6	23.5	70.8
March2009 vs April2009 (62%)	***March2009***	***April2009***				
Chamaesiphon spp	4408.3	156.6	21.8	2.0	35.4	35.4
Aphanocapsa spp	280.5	1751.0	15.6	1.8	25.4	60.8
April2009 vs May2009 (52%)	***April2009***	***May2009***				
Aphanocapsa spp	1751.0	129.9	17.1	1.4	32.7	32.7
Eunotia rhomboidae	569.1	58.4	10.7	1.6	20.5	53.3

APPENDIX 5

SIMPER results of key discriminator taxa contributing to the inter-annual differences between months (p≤ 0.1, Appendix 2) at Site 1. Densities are in number of cells (x 10 000) m^{-2} . Diss/SD indicates the consistency with which taxa discriminate between months across all samples.

Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
October 2007 vs October 2008 (54.46%)	***October 2007***	***October 2008***				
Euglena sp	0	2661	10.32	1.42	18.96	18.96
Eunotia rhomboidae	6176.31	3767.76	9.84	1.28	18.06	37.02
Aphanocapsa sp	0	2237.82	8.64	5.19	15.87	52.89
Desmococcus spp	1789.73	2607.62	7.96	1.53	14.62	67.51
Chamaesiphon sp	1770.53	1116.25	4.88	1.87	8.96	76.47
November 2007 vs November 2008 (64.39%)	***November 2007***	***November 2008***				
Chlorococcum spp	2540.92	74.34	17.19	2.42	26.7	26.7
Chamaesiphon sp	996.71	1438.53	10.12	1.31	15.71	42.41
Eunotia rhomboidae	1508.89	1662.05	5.96	1.64	9.25	51.66
Desmococcus spp	586.66	1257.92	5.9	1.39	9.16	60.82
Oedogonium sp 1	771.92	0	5.34	0.94	8.29	69.11
Eunotia minor	649.35	192.23	4.72	1.23	7.34	76.45
December 2007 vs December 2008 (50.65%)	***December 2007***	***December 2008***				
Eunotia rhomboidae	2206.98	1916.05	8.71	2.02	17.21	17.21
Desmococcus spp	1440.45	1306.54	8.18	1.89	16.15	33.36
Eunotia exigua	881.96	0	6.64	4.28	13.12	46.47
Eunotia minor	836.33	0	6.27	3.01	12.38	58.86
Aphanocapsa sp	80.18	800.82	5.96	1.56	11.76	70.62
January 2008 vs January 2009 (59.78%)	***January 2008***	***January 2009***				
Chamaesiphon sp	1854.51	8006.27	27.26	6.83	45.59	45.59
Eunotia rhomboidae	1849.57	4971.24	13.93	2.82	23.31	68.9
Desmococcus spp	1602.52	1109.55	6.4	2.14	10.71	79.61
February 2008 vs February 2009 (61.69%)	***February 2008***	***February 2009***				
Mougeotia sp 2	3636.44	0	13.19	1.59	21.38	21.38
Eunotia rhomboidae	3000.17	567.91	9.11	2.24	14.76	36.15
Mougeotia sp 1	2227.13	0	7.91	2.05	12.82	48.97
Chamaesiphon sp	4091.08	6184.37	7.29	1.52	11.81	60.78
Desmococcus spp	1794.84	778.45	3.57	1.48	5.79	66.57
Oedogonium sp 2	967.55	0	3.25	0.56	5.27	71.84
March 2008 vs March 2009 (55.59%)	***March 2008***	***March 2009***				
Spyrogira spp	4012.33	0	10.99	0.95	19.78	19.78
Chamaesiphon sp	4674.03	6639.51	9.55	1.36	17.19	36.96
Mougeotia sp 1	1856.99	0	5.35	1.11	9.63	46.59
Eunotia rhomboidae	2202.36	2392.43	5.11	1.36	9.2	55.79
Aphanocapsa sp	0	1674.82	4.36	0.54	7.84	63.62
Chlorococcum spp	0	1063.35	3.59	1.37	6.46	70.09
April 2008 vs April 2009 (47.02%)	***April 2008***	***April 2009***				
Aphanocapsa sp	0	4184.55	19.75	2.24	42.01	42.01
Chamaesiphon sp	3644.46	1251.23	12.19	1.8	25.93	67.94
Eunotia rhomboidae	1631.45	2385.67	5.67	1.49	12.07	80
May 2008 vs May 2009 (61.56%)	***May 2008***	***May 2009***				
Chamaesiphon sp	3119.63	559.73	20.84	3.19	33.85	33.85
Desmococcus spp	3692.74	1533.65	17.43	2.99	28.31	62.15
Aphanocapsa sp	0	1139.6	8.94	2.01	14.52	76.68

APPENDIX 6

PERMANOVA pair-wise comparisons between months over the sampling period at Site 2. Comparisons for both intra- and inter-annual differences are shown, as well as months representing seasonal extremes. Only consecutive months for the intra-annual differences are shown. Significant differences at p≤0.05 are indicated in *bold italics* and at p≤0.1 in *italics only.*

Groups	*t*	*p*	*% similarity*	*seasons derived a priori*
Intra-annual (months)				
October2007, November2007	***2.63***	***0.03***	***30***	***early spring - late spring***
November2007, December2007	***3.07***	***0.03***	***30***	***late spring***
December2007, January2008	1.29	0.17	54	late spring - summer
January2008, February2008	***2.01***	***0.03***	***44***	***summer***
February2008, March2008	***1.74***	***0.03***	***50***	***summer***
March2008, April2008	*1.68*	*0.08*	*48*	*summer - early autumn*
April2008, May2008	***1.80***	***0.03***	***51***	***early autumn - late autumn***
May2008, June2008	*1.60*	*0.06*	*57*	*late autumn - winter*
June2008, July2008	*1.48*	*0.09*	*45*	*winter*
July2008, August2008	***2.34***	***0.03***	***35***	***winter***
August2008, October2008	***2.60***	***0.03***	***28***	***winter - early spring***
October2008, November2008	***2.17***	***0.03***	***41***	***early spring***
November2008, December2008	0.48	0.97	68	late spring
December2008, January2009	***2.28***	***0.03***	***34***	***late spring - summer***
January2009, February2009	1.29	0.14	60	summer
February2009, March2009	***2.61***	***0.03***	***45***	***summer***
March2009, April2009	***1.64***	***0.03***	***31***	***summer - early autumn***
April2009, May2009	0.96	0.60	41	early autumn - late autumn
Seasonal extremes				
March2008, July2008	***3.06***	***0.03***		***Summer 08 vs Winter 08***
July2008, March2009	***3.25***	***0.03***		***Winter 09 vs Summer 09***
Inter-annual (years)				
October2007, October2008	***2.95***	***0.03***	***7***	***early spring***
November2007, November2008	***5.85***	***0.03***	***4***	***late spring***
December2007, December2008	***2.61***	***0.03***	***29***	***late spring***
January2008, January2009	***3.05***	***0.03***	***20***	***summer***
February2008, February2009	***3.21***	***0.03***	***33***	***summer***
March2008, March2009	***2.15***	***0.03***	***44***	***summer***
April2008, April2009	***1.56***	***0.03***	***30***	***early autumn***
May2008, May2009	***2.57***	***0.03***	***38***	***late autumn***

APPENDIX 7

Selection of invertebrate functional feeding groups potentially affecting temporal patterns in periphyton communities for inclusion in the DISLM analysis

Chapter 3 presented temporal shifts in the abundance of the dominant invertebrate taxa that were grouped according to their feeding mode into three functional feeding groups (Figure 3.3.1), namely 'brushers' (dominated by heptogeniids), 'deposit feeders' (dominated by baetids) and 'scrapers' (dominated by chironomids) These groups are described in more detail in Chapter 2, Section 2.3.4. Identification of the variables that represent grazing pressure requires some understanding of which of thesw three functional feeding groups potentially imposes a top-down effect on Chl *a* biomass at any given time. In Chapter 4 it was suggested that the Chl *a* biomass peak in late winter under natural flow regimes represented a period when Chl *a* biomass could accrue in the absence of invertebrate algal feeders because their recovery lagged that of Chl *a* biomass. It was also suggested that the sudden increase in algal feeding invertebrates, mainly deposit feeders dominated by baetids, may have accounted for the maintenance of Chl *a* biomass in a stable, low state over the late spring and early summer.

Figure A1 shows that Chl *a* biomass peaked in late summer in both 2008 and 2009. Seasonal increases in both scraper and brusher abundance (Figure A1a-d) tended to lag slightly behind Chl *a* biomass increases over the sampling period at Site 1. By contrast, deposit feeder abundance peaked before Chl *a* biomass during both annual cycles (Figure A1e-f). Also, of the three functional feeding groups, deposit feeders were most abundant, particularly over the spring and early summer. This suggests that deposit feeders such as baetids may affect the pattern of Chl *a* biomass as a top-down control at certain times, which does not seem to be the case for collector gatherers and scrapers because of their relatively low abundance and lagged response to Chl *a* biomass accrual.

Where grazing pressure exerts a top-down effect on Chl *a* biomass a negative relationship would be expected because Chl *a* biomass should decline as grazers become more abundant and losses via consumption outweigh production of periphyton. Interestingly, a simple linear regression between Chl *a* biomass and deposit feeder densities (Figure A2) showed a relatively strong positive relationship between these two variables. This supports the suggestion that brushers do not impose a top-down control on Chl *a* biomass. In fact, a positive relationship suggests that greater Chl *a* biomass supported a greater grazer density (Figure A2). In other words, Chl *a* biomass affected brusher densities, rather than the other way round. Although these results are tentative and require further testing, the inclusion of brusher and scraper densities might obscure the significance of other environmental factors important to periphyton dynamics. Brusher and scraper densities were therefore excluded from the DistLM analyses for assessing temporal patterns.

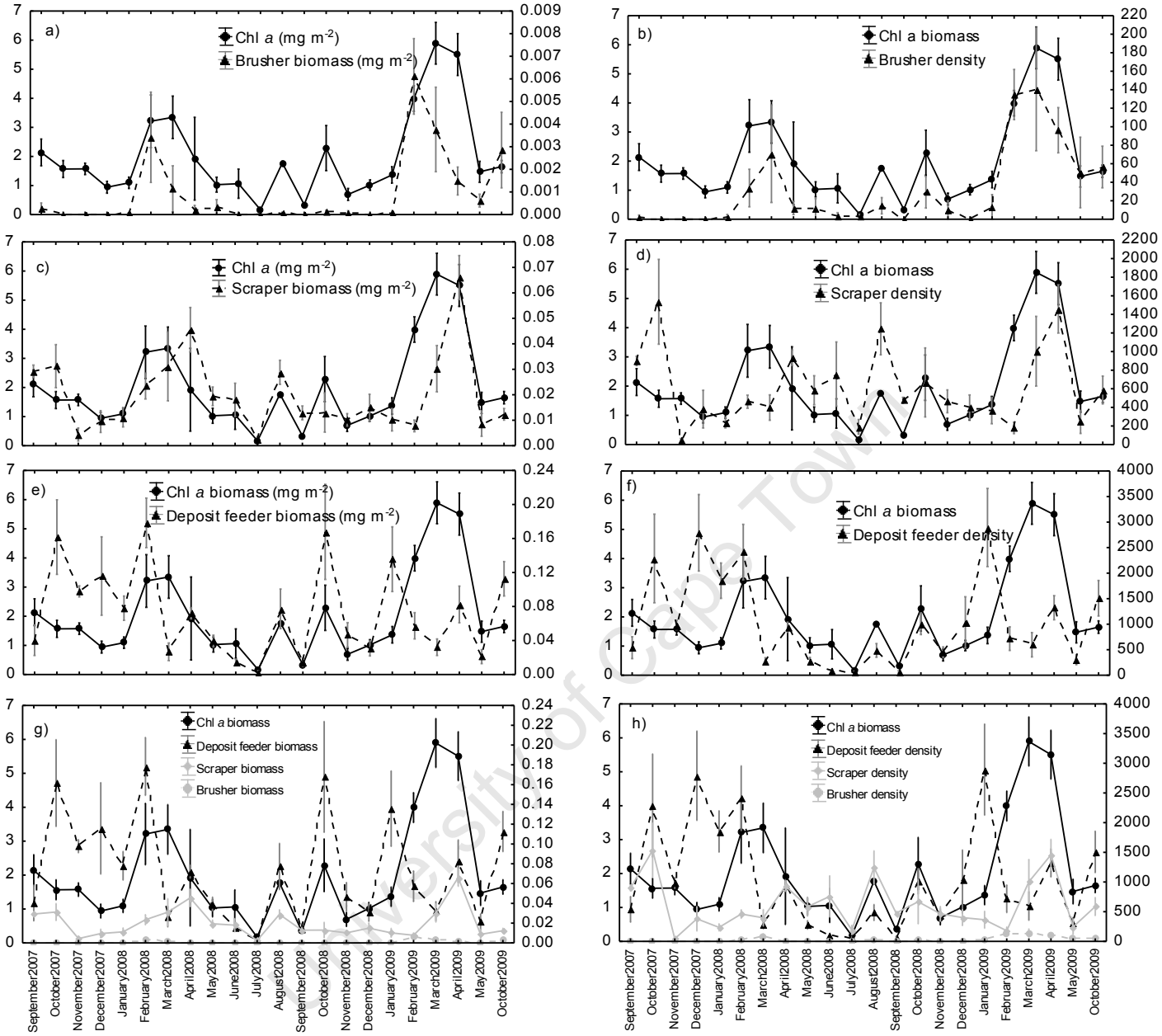

Figure A1 **Mean monthly Chl *a* biomass and a) brusher biomass and b) brusher density (dominated by heptageniids), c) scraper biomass and d) scraper density (dominanted by baetids), e) deposit feeder biomass and f) deposit feeder density (dominated by chironomidae) at Site 1. A comparision of abundance for each invertebrate functional feeding group is indicated in g) biomass and h) density.**

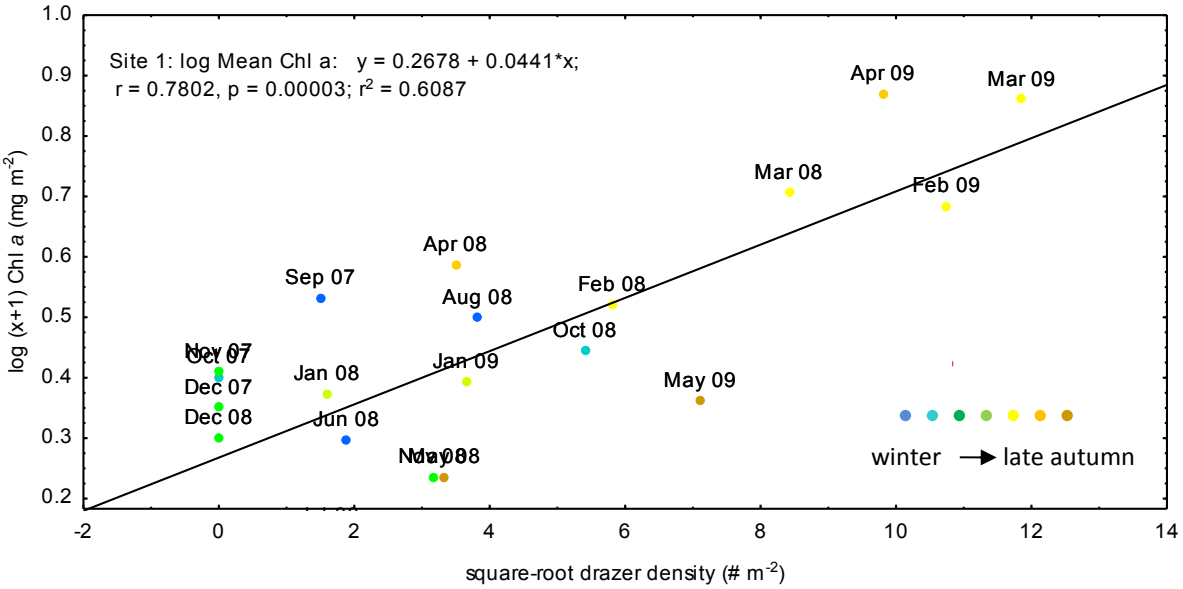

Figure A2 **Relationship between monthly Chl *a* biomass and brusher density at Site 1.**