Patterns in periphyton biomass and community structure in foothill rivers: A comparison between winter and summer rainfall regions

by
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December 2017

Thesis presented infulfilment of the requirementsfor the degree of Master of Science in the Faculty of AgriSciences at Stellenbosch University

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List of Abbreviations

Abbreviation	Description
EC	Electrical conductivity
NO_2-N	Nitrogen as Nitrite
NO_3-N	Nitrogen as Nitrate
TIN	Total inorganic nitrogen (NO_2 + NO_3 + $NH_{4)}$
PO_4-P	Phosphorous as orthophosphate
Rs_{MIN}	Daily minimum solar irradiation averaged over the inter-sampling period
Rs_{CUM}	Cumulative hourly solar irradiation over the inter-sampling period
WT_{CV}	Daily coefficient of variation in water temperature averaged over the inter-sampling period
WT_{MIN}	Minimum daily water temperature averaged over the inter-sampling period
WT_{MAX}	Maximum daily water temperature averaged over the inter-sampling period
WT_{CUM}	Cumulative average daily water temperature over the inter-sampling period
Q_{CV}	Coefficient of variation in average daily discharge over the inter-sampling period
$\#Flds_{\geq 2}$	Number of floods equal to or greater than a DRIFT class 2 flood over the inter-sampling period
$\#Days_{\geq 1}$	Number of days in flood equal to or greater than a DRIFT class 1 flood over the intersampling period
$\#Days_{\geq 2}$	Number of days in flood equal to or greater than a DRIFT class 2 flood over the intersampling period
$Since_{\geq 2}$	Number of days since a DRIFT class 2 or greater flood was experienced
$Since_{\geq 1:2}$	Number of days since a DRIFT class 1:2 year flood or greater flood was experienced
GR_{DENS}	Density of grazers (scrapers + brushers + deposit feeders)
SCR_{DENS}	Density of scrapers
BR_{DENS}	Density of brushers
DP_{DENS}	Density of deposit feeders
GR_{BMASS}	Biomass of grazers (scrapers + brushers + deposit feeders)
SCR_{BMASS}	Biomass of scrapers
BR_{BMASS}	Biomass of brushers
DP_{BMASS}	Biomass of deposit feeders

Abstract

South Africa has a semi-arid climate with seasonal rainfall whose runoff is jeopardised by high rates of evapotranspiration. These conditions decrease the ability of rivers to dilute point and non-point sources of pollution, which leads to enrichment and the subsequent eutrophication of water bodies. Eutrophication occurs when periphyton communities proliferate through a shift in community structure, accompanied by greater biomass accrual that deteriorates water quality and impairs aquatic ecosystem functioning. The National Eutrophication Monitoring Programme (NEMP) has set water quality guidelines for concentrations of phosphorous and Benthic chlorophyll *a*, but periphyton are understudied in South African waters, which explains their exclusion from the River Eco-Status Monitoring programme (REMP). To underpin the causes of eutrophication, this study aimed to broaden the knowledge on periphyton-environmental relationships in terms of patterns in peripyton biomass and community structure on a regional scale across seasons in a winter (Western Cape) and summer (KwaZulu-Natal) rainfall region. Periphyton biomass and community structure were observed across flow and enrichment categories and a suite of environmental metrics comprising flow, nutrients, water temperature, sunlight and macroinvertebrates. An *in situ* tool known as the Benthotorch® was validated to potentially be used in future rapid assessments of trophic status. Periphyton samples from sites representing a range in environmental conditions were collected in autumn and spring which mark the beginning and end of the periphyton growth seasons. Periphyton biomass in the Western Cape was found to be influenced predominantly by the availability of TIN in autumn and WT_{MAX} in spring. In KwaZulu-Natal, periphyton biomass was influenced mostly by flow metrics and WT_{CV}. Periphyton community structure in the Western Cape was influenced mostly by TIN and the length of the growing season in autumn and by EC and the duration of class 1 floods in spring. In KwaZulu-Natal, periphyton community structure was influenced mostly by water temperature and flow metrics in autumn and by the length of the growing season ($Since_{\geq 2}$), the duration of class 2 floods and PO_4-P in spring. The flow regime is regarded as the primary regulator of flood prone rivers, which was not the case in this study, and calls for future research. Nutrients accounted minimally towards spatial variation in periphyton communities in KwaZulu-Natal possibly due to sites with similar nutrient ranges, or because nutrients are not a key driver of periphyton communities here. The importance of water temperature metrics in both regions stresses the need for water temperature monitoring programmes, that are currently lacking in South Africa. The Benthotorch® estimated periphyton biomass and community structure more accurately at sites that were dominated by diatoms, overestimated cyanobacteria and did not consistently recognize green algae.

Opsomming

Suid-Afrika het ‘n half-woestyn klimaat met seisoenale reënval. Die dreinering van hierdie reënval word in gevaar gestel deur hoë evapotranspirasie. Hierdie omstandighede verminder die kapasiteit van riviere om punt- en nie-punt bronbesoedeling te verdun, wat lei tot geweldige eutrofikasie van warm waterbronne. Eutrofikasie kom voor waneer periphyton vermenigvuldig deur verhoogde biomassa en verskuiwings in gemeenskapstruktuur wat waterkwaliteit verswak. Gevolglik word die funksionering van akwatiese ekosisteme benadeel. Die Nasionale Eutrofikasie Monitorprogram (NEMP) het waterkwaliteit riglyne opgestel vir die konsentrasie fosfor en chlorofil *a*. Daar word egter min klem gelê op periphyton in Suid-Afrikaanse waterbronne, wat die uitsluiting daarvan in die Rivier Ekostatus Monitorprogram (REMP) verduidelik. Dit is nodig om kennis op te doen oor die verhoudings van periphyton in die omgewing om besluite te kan maak wat eutrofikasie geheel en al kan verhoed. Die kern van hierdie studie is om die kennis van periphyton biomassa en gemeenskapstrukture te verbreed op ‘n plaaslike skaal oor seisoene, nl. ‘n winterreënvalseisoen (Weskaap) en ‘n somerreënvalseisoen (Kwazulu Natal). Die relatiewe belang van verryking- en vloeikategorieë is getoets, sowel as ‘n hoeveelheid omgewingsfaktore (vloei, voedingstowwe, water temperatuur, sonlig en algeëtende insekte). Die Benthotorch®, ‘n *in situ* gereedskapsmodel is beskikbaar gemaak vir toekomstige gebruik vir vinnige assesserings van die trofiese statusse van waterbronne. Periphyton monsters is geneem gedurende herfs- en lenteseisoene, want dit val saam met die groeiseisoene van die periphyton. ‘n Verskeidenheid van areas is gekies vir hierdie studie om ruimtelike variasie en omgewingsfaktore in ag te neem. Daar is gevind dat periphyton biomassas in die Weskaap meestal deur die beskikbaarheid van TIN in die herfsmaande en WT_{MAX} in die lentemaande beïnvloed word. In Kwazulu Natal is dit hoofsaaklik deur vloeifaktore en WT_{CV} beïnvloed. In die Weskaap is periphyton gemeenskapstrukture meestal deur TIN asook die lengte van die groeiseisoen in die herfs beïnvloed, waar dit deur EC en die lengte van klas 1 vloede in die lente beïnvloed is. In Kwazulu Natal is periphyton gemeenskapstrukture in herfsmaande meestal deur watertemperature en vloeifaktore beïnvloed en gedurende lentemaande deur die lengte van die groeiseisoen ($sedert_{\geq 2}$), die lengte van klas 2 vloede en PO_4-P. Daar is ‘n behoefte aan toekomstige navorsing wanneer daar na die klein bydrae van vloeiings relatief tot voedingstofkonsentrasies en tot ruimtelike variasie in periphyton gemeenskappe gekyk word. Die vloei regime word as die primêre reguleerder, in periphyton gemeenskappe in riviere wat maklik vloed, gesien. Die klein bydrae van voedingstowwe tot die ruimtelike variasie in periphyton gemeenskappe in Kwazulu Natal is waarskynlik die gevolg van die onvoldoende omvang van voedingstowwe in die bestudeerde areas. Die behoefte aan watertemperatuur monitorprogramme word duidelik wanneer daar

na die belangrikheid van die watertemperatuurfaktore in albei areas gekyk word. Huidiglik is daar ‘n leemte aan hierdie tipe program in Suid-Afrika. Die Benthotorch® het het goed vergelyk ten opsigte van biomassa skattings, veral in die areas met diatome in groot maat. Dit het egter, in terme van gemeenskapstruktuur-skattings die cyanobacteria oorskat by sekere geleenthede en nie groen alge herken nie.

Acknowledgements

I would firstly like to thank my parents, Graham and Jenny Grainger for always believing in my capabilities and for supporting me during this financially stressful period. Next I would like to thank the project leader and my external supervisor, Dr Justine Ewart-Smith for teaching me not only so much about periphyton, statistics and report writing, but also about myself. I have grown leaps and bounds under your mentorship, thank you for always challenging me! Thank you to my academic supervisors Dr Shayne Jacobs and Dr John Simaika for all your support, guidance and aid towards helping me submit this thesis. Thank you Dr Vere Ross-Gillespie for assisting on this project, I appreciated your enthusiasm and enjoyed working with you. Thank you Dr Mark Graham for your valuable inputs at the Water Research Commission steering meetings.

An extended thanks goes out to Mannie Sewcharran from Umgeni Water in KwaZulu-Natal for analysis of Benthic chlorophyll *a* and nutrients. Similarly, thanks to Liesl Phigeland from the University of Cape Town for analysis of Benthic chlorophyll *a* and Ash Free Dry Weights. Thanks to Raïssa Philibert and Mhlanga Mdutyana from the University of Cape Town for nutrient analyses. Howard Waldron, thank you for allowing me to do the nutrient analyses myself and for having a radio in the lab. Thank you Pashni Pillay for your specialist input regarding periphyton identification.

I would like to thank my friends, Colin Tucker and Lorain Den Boogert as well as my sister, Stacey for assisting me with field work and scrubbing periphyton off cobbles with a smile.

Thank you to Jody-lee Reizenberg for assisting me with statistics and for being so critical about my writing, your teachings will always stick with me. Thank you to Irene Joubert from the Agricultural Research Council for providing me with solar irradiance and air temperature data. Thank you Helen Dallas from the Freshwater Research Centre for providing metadata on study sites and water temperature data. Thanks to Tricia Theunissen for helping me to translate my abstract into Afrikaans.

Thank you Helen Dallas, Bruce Paxton and Heather Malan for providing valuable presentation tips in preparation for the SASAQS conference. Thank you Belinda Day for validating my invertebrate identification work and Helen Barber-James for assisting me with invertebrate identification.

Finally, thank you to the Water Research Commission (WRC) for the provision of funding that allowed me to undertake this project and ultimately contribute to the knowledge of periphyton in South African Rivers.

Chapter 1. General Introduction and thesis overview

1.1 South Africa's water shortage

South Africa is a water stressed country owing to a mean annual precipitation of 500 mm, which is highly seasonal and unevenly distributed, and equates to 60% of the world average (Dallas & Rivers-Moore 2014). Evaporative rates often exceed those of precipitation (Schulze et al. 2001; Ashton 2010), and are expected to be exacerbated by increasing air temperatures (Hewitson & Crane 2006) across the country and decreasing precipitation (Thieme et al. 2010) in provinces such as the Western Cape and North West, according to predictive climate change models of South Africa (Schulze et al. 2001; Roux & Nel 2013). Alien woody vegetation consumes more water compared to native plants, and decreases the amount of runoff in rivers (Schulze et al. 2001). The majority of the country's river lengths are dammed, diverted, connected and abstracted in order to satisfy consumptive requirements (Davies & Day 1998, Dallas & Rivers-Moore 2014). These activities alter the flow and thermal regime of rivers (Rader et al. 2008), especially in the Western Cape that has highly seasonal and unpredictable rainfall (Giorgi & Lionello 2008).

1.2 River eutrophication

Low flow conditions, especially in the rivers in the summer of the Western Cape are susceptible to eutrophication, due to the decreased ability of these rivers to dilute point (e.g. urban sewerage) and diffuse (e.g. agricultural runoff) nutrient loading, which is exacerbated when rivers are regulated through water abstraction and damming (McDowell & Omernik 1977; Robinson et al. 2004; Ryder 2004). River regulation decreases water volumes and stabilises flows, which increases nutrient concentrations and retention times respectively (Behrendt & Opitz 1999). This encourages shifts in periphyton biomass and community structure, which can be harmful to the environment (Hart et al. 2013). This is especially the case in Mediterranean-type ecosystems that experience periods of simultaneous low flows, warm temperatures and high nutrient loading (Ohte et al. 2007; Ponsat et al. 2016). Such conditions enable dormant periphyton propagules to bloom (Villanueva et al. 2000 Schneider & Lindstrøm 2011) which results in a proliferation of filamentous green algae and cyanobacteria (Power 1996; Stevenson et al. 2006; Stewart & Lowe 2008; Villeneuve et al. 2009).

Periphyton blooms are associated with an increase in biomass due to community structure shifts from single celled diatoms to elaborate forms of filamentous green algae and cyanobacteria (Power 1996; Stewart & Lowe 2008). These shifts usually result in water quality deterioration and hinder the flow of energy through the food web (Cashman et al. 2013). It is therefore important to understand the

environmental conditions that lead to enrichment and the proliferation of undesirable periphyton taxa to effectively manage river systems through the maintenance of their ecosystem integrity for protection of their resource value.

Enrichment is assessed by measuring nutrient (nitrates and phosphates) concentrations in the water column (Dodds & Smith 2016). However, the spatio-temporal variation of nutrient movements is complex, involving the quantification of nutrient inputs from natural processes as well as those of anthropogenic origin, of which the major portion could be harbored within periphyton (Stevenson et al. 2006), which responds to slight changes in nutrient concentrations (Biggs & Thompson 1995 and Gaiser et al. 2005). This provides an opportunity to investigate patterns in periphyton biomass and community structure as a proxy for enrichment (Dodds 2006; Li et al. 2010).

1.3 Introduction to periphyton

Periphyton collectively consist of autotrophic organisms (benthic algae), heterotrophic organisms (bacteria, fungi and protists) that colonise substrates such as macrophytes, silt, sand, gravel and cobbles (Burns & Ryder 2001). This thesis only considers the cobble inhabiting (epilithic) benthic algae (diatoms, green algae and cyanobacteria). Periphyton are in direct contact with the abiotic environment, as they assimilate light and nutrients in order to photosynthesize. In open-canopied foothill rivers, they contribute substantially to the energetic demand of higher trophic levels (Uehlinger 1979; Hill et al. 2011), and are crucial to nutrient cycling (Minshall 1978; Mulholland et al. 2009). Periphyton mats also provide refugia for aquatic biota such as chironomids and help to maintain biodiversity (Chester & Norris 2006). However, proliferations of filamentous green algae and cyanobacteria are often unpalatable and non-nutritious, unlike carbohydrate-rich single-celled diatoms, which are typically found during early succession (Chester & Norris 2006; Guo et al. 2016). Such shifts lead to trophic decoupling, by shifting consumer feeding behaviours from herbivory to omnivory and carnivory (Cashman et al. 2013; Guo et al. 2016). The level of succession therefore gives a sense of the energy available to higher trophic guilds (McCormick et al. 1997; Dodds 2006 ; Li et al. 2010 ; Davie et al. 2012).

In addition, filamentous green algae such as *Oedogonium* spp. clog waterways and spoil recreational areas while blooms of cyanobacteria such as *Lyngba* spp. and *Nostoc* spp. release harmful cyanotoxins which lead to water odour and taste problems that are responsible for fish kills (Biggs 2000). It is thus important to understand which environmental variables keep periphyton at early succession to keep

water quality at acceptable standards and maximize the flow of energy through the trophic web (Davie et al. 2012).

1.4 Periphyton variability across space

Spatial patterns in periphyton range from micro-habitats to regions. At the finest scale (millimetres), periphyton are governed by the hydraulic biotope, due to variability in near-bed flow velocities associated with substrate distribution and type (Biggs 2000). At the medium scale (metres), periphyton are governed by the river reach (Sabater and Sabater 1992), which from source to mouth changes in geomorphology, extent of canopy cover and the availability of nutrients (Biggs et al. 2000). At the broad scale (kilometres), and scale of this study, periphyton reflect regional differences in climate, geology and surrounding land use, which together contribute to the variability in hydrological and nutrient characteristics (Biggs 1996).

1.5 Effect of environmental variables on periphyton

Section 1.5 considers the separate effects of environmental variables (flow, grazers, nutrients, temperature and sunlight) on periphyton communities. These variables however also act in concert and their more complex effects on periphyton biomass and community structure are explained in Chapters 3 and 4 respectively.

Periphyton communities are shaped by the balance between resources (light, temperature and nutrients) and disturbances (flow and grazers) (Uehlinger 1979; Francoeur et al. 1999; Stevenson 2014; Dalu et al. 2015). In flood prone rivers, the flow regime which is described by the timing, frequency and magnitude of elevated base flows and flood events is widely considered to primary regulate periphyton communities.(Biggs & Close 1989; Boulêtreau et al. 2006; Davie et al. 2012; Dalu et al. 2015). Periphyton growth during stable or sub-critical flow periods is then determined by the rate of grazing (Feminella & Hawkins 1995) versus the rate of growth based on the availabilities of light, temperature and nutrients (Stevenson et al. 2006; Allan & Castillo 2007; Stevenson 2014). What follows is a widely accepted conceptualization of these variables.

1.5.1 Flow

The flow regime regulates periphyton communities in three manners: Firstly by shear force due to currents exerting a drag force on periphyton filaments. Secondly by abrasion by fine sediments, which in addition to shear stress scours periphyton, and thirdly by substrate toppling, which may embed periphyton communities under substrata (Biggs & Close 1989; Uehlinger et al. 1996; Hoyle et al. 2016). Many studies however agree that abrasion has a significant impact on periphyton communities, irrespective of the level of shear force (Power & Stewart 1987; Webb et al. 2006; Yang et al. 2009; Hoyle et al. 2016).

1.5.2 Grazers

Lamberti (1993), Feminella and Hawkins (1995) and Taylor et al. (2002) showed that grazers regulate periphyton abundance, which according to Poff and Ward (2002) and Stevenson et al. (2006) are particularly effective under stable current conditions. However, grazers can also benefit periphyton communities. Dudley (1992) found that *Baetis*, a deposit feeder, had negative effects on periphyton during early succession, but positive effects once established. Murdock et al. (2011) explains that grazing stimulates periphyton through the removal of dead cells, which remineralises nutrients, and is particularly beneficial to thick mats that are nutrient deprived. Townsend et al. (2012) found that the preferential feeding on diatoms by many grazers allows for colonization of late succession groups such as filamentous green algae. However, preferential grazing temporarily benefits late successional taxa as they colonise on top of diatoms, which when removed, weakens the mat foundation and causes dislodgement (Graba et al. 2014). Periphyton in turn are able to differentially influence grazers. Guo et al. (2016) found that increases in shading and nutrients increased the highly unsaturated fatty acid content in periphyton, which increased the growth of stream grazers. By contrast, Cashman et al. (2013) found that elevated light and nutrients decreased the availability of highly unsaturated fatty acids, which may lead to shifts in the assemblage of aquatic consumers or encourage herbivores to become omnivores or carnivores.

1.5.3 Nutrients

Nitrogen (nitrates and ammonia) and phosphorous (orthophosphate) compounds are the most important nutrients regulating plant growth (Stevenson 2014; Dodds & Smith 2016). However, it is their

stoichiometric proportions that are relevant to periphyton, not their absolute concentrations (Dodds 2007; Dodds & Cole 2007; Schade et al. 2011).The ideal nitrogen: phosphorus ratio for plant growth (by mass), known as the Redfield ratio is 16:1. A ratio (< 10) is considered nitrogen limited, while a ratio (> 17) is considered phosphorous limited (Dodds 2003). When flow conditions permit periphyton growth, the availability and ratio of nutrients determines the rate of succession and biomass accrued (Stevenson et al. 2006). A sudden discharge of herbicides for example elevates nutrient concentrations which promote shifts from low nutrient demanding single celled diatom taxa to high nutrient demanding filamentous diatom and green algae (Piggott et al. 2015). Nutrient availability is not only determined by water column concentrations, but also by current velocities, which when increasing, act as nutrient pulses, especially in oligotrophic streams Dodds et al. (1998). Faster current velocities increase nutrient transport, but also thin out the boundary layer of periphyton mats, thereby increasing diffusion rates (Biggs et al. 1998). There however is a limit to the advantages of increased current velocities, as was noted by Ponsat et al. (2016) that observed stronger responses to nutrients during base flow conditions than during floods.

1.5.4 Sunlight

Sunlight is critical for autotrophic production and is influenced by riparian shading, water depth and turbidity of the water column (Larned 2010). High sunlight intensities can decrease periphyton growth (Bothwell et al 1993; Kiffney et al 1997) and depending on the degree of sunlight can determine the composition of periphyton communities. Passy (2007) and Villeneuve et al. (2009) found that shade tolerant diatoms could be found in heavily shaded streams while filementous green algae, that require adequate sunlight, were limited to open canopied rivers. However these trends may not always hold true, as according to (Hill 1996 & Tuchman 1996), periphyton can adjust to various levels of sunlight by regulating photosynthetic efficiency in order to maintain maximum photosynthetic rates in shaded environments, a process known as photoacclimation.

1.5.5 Water temperature

Optimal temperatures for periphyton range from 10-30 °C, with higher temperatures causing heat stress and reduced growth (DeNicola 1996). Diatoms prefer cooler temperatures (< 20°C) relative to green algae, which prefer cooler temperatures compared to cyanobacteria, that thrive above 30°C (Wilde & Tilly 1981; Lamberti & Resh 1985, in DeNicola 1996) .Water temperature is important for enzyme

catalyzed reactions for all aquatic biota (DeNicola 1996; Larras et al. 2013; Teittinen et al. 2015) which determines the rate of photosynthesis (Morin et al. 1999). Periphyton are able to overcome limitations by temperature by means of thermal acclimation. Thermal acclimation however is nutrient demanding, due to increased enzyme synthesis, and may lead to nutrient limitation (Larned 2010). River temperatures oscillate more downstream than upstream of impoundments, but are usually warmer, which may affect periphyton assemblages (Biggs 2000).

1.5.6 pH

Periphyton taxa have pH specific physiologies at which carbon uptake and proton influxes are maximised (Gross 2000), which determines growth and community composition (Ledger & Hildrew 1998; Sabater et al. 2003). Activities such as mining leads to river acidification, which induces osmotic and cell division stress on periphyton, favouring communities of depauperate chlorophytes (Visviki & Santikul 2000). Furthermore, acidification of rivers promotes the dissolution of metals which changes membrane permeability, inhibits photosynthetic electron transport and forms precipitations of metal phosphates that decrease the availability of phosphorous in the water (Kinross et al. 2000). Schneider et al. (2013) mention that pH must be understood within the context of nutrient concentrations, and noted that acid-tolerant taxa are associated with nutrient poor rivers, with diatom taxon richness increasing with nutrients, and non-diatom taxa decreasing in acidic rivers. Ledger and Hildrew (1998) found that *Eunotia* spp , small coccoid green algae and filamentous green algae were common in the acidic rivers of the UK. Acidic rivers are expected to have a lower periphyton species diversity compared to more neutral rivers (Winterbourn et al. 1992; Ledger and Hildrew 1998) which has yet to be compared between the Western Cape and KwaZulu-Natal, that are naturally acidic and alkaline respectively (Davies & Day 1998).

1.5.7 Electrical Conductivity

Electrical conductivity (EC) indicates the dissolved salt content in the water medium and how well it conducts electricity (Davies & Day 1998). EC reflects geology (Biggs & Gerbeaux (2010), biogeochemistry, and land use and is frequently reported as a key variable for diatom distributions (Teittinen et al. 2015). EC is also considered a broad proxy for nutrient content as it has been correlated with nutrient concentrations, but unlike nutrients, does not decrease with increasing periphyton biomass (Biggs & Close 1989).

1.6 Periphyton as bioindicators

Historically, periphyton were overlooked as aquatic indicators due to a lack of taxonomic skills and standardised methods with which to measure them. Periphyton are currently used as bioindicators in New Zealand, North America, Europe and Australia (Chessman et al. 1999; Hill et al. 2000). Examples include diatom use in pollution assessments (Kelly et al. 1998), periphyton indices of biotic integrity (Hill et al. 2000) and trophic status (Dodds 2006; Schneider & Lindstrøm 2011). The latest index by Kelly et al. (2016), known as the Rapid Assessment of Periphyton Ecology in Rivers (RAPPER), uses periphyton genera to inform ecological condition based on stress tolerant and competitive taxa under nutrient pressures. Periphyton make for ideal bioindicators because they are stable, sessile, regenerate rapidly, are cosmopolitan in distribution, mirror environmental change, are relevant to ecosystem functioning and provide a wealth of rich ecological information (McCormick & Cairns 1994). Abroad, workers have shown diatoms to be sensitive to flow changes (e.g. Van Dam et al. 1994; Kelly & Whitton 1995) and diatom indices are already in use (e.g. Prygiel & Coste 1993; Kelly & Whitton 1995). Diatoms are easier to identify and preserve compared to filamentous green algae and cyanobacteria (Blinn & Herbst 2003; Taylor et al. 2004; Taylor et al. 2007) and form the basis of preliminary periphyton research in South Africa.

In South Africa, Taylor et al. (2004) showed that diatoms responded better to alterations in water quality compared to macroinvertebrates. Bate et al. (2004) developed a water quality index for South Africa using known attributes of diatoms to compare water quality between catchments. Taylor et al. (2007) tested a European diatom index called the Specific Pollution Sensitivity Index to support and motivate for the inclusion of diatoms in biomonitoring procedures in South Africa. Azim et al (2005) and Ewart-Smith (2012) showed that periphyton respond to gradients of enrichment and flow alterations in rivers. Vos (2015) tested periphyton responses to water quality. Oberholster et al. (2016) linked environmental conditions to the proliferation of green algae under various agricultural land uses

1.7 The South African water monitoring trajectory

The focus of monitoring in South African rivers is water quality based, and currently does not consider periphyton monitoring. Water quality is inferred through measurement of pollutants against acceptable concentration standards (Taylor et al. 2004; Taylor et al. 2007). There are however many pollutants that need to be measured, which is costly, time consuming and subject to analytical error (Abdel-Hamid et al. 2014) In addition, these measurements are but a combined snapshot of natural and anthropogenic

inputs in time, and considering their spatio-temporal variation, are difficult to relate to ecosystem health (Aubin et al. 2011; Abdel-Hamid et al. 2014). Stevenson et al. (1999) suggested that water quality instead be inferred using fauna and flora, provided the challenges associated with chemical monitoring can be overcome. Indeed, two South African monitoring programmes, the National Biomonitoring Programme for Aquatic Ecosystems, (NBPAE) and the River Eco- Status monitoring programme, (REMP) employ fauna and flora in the following programmes: the Index of Biotic Integrity (IBI) incorporates fish, the Riparian Vegetation Index (RVI) incorporates macrophytes and the South African Scoring System (SASS) uses macroinvertebrates.

These indices are limited to water quality monitoring and even so, only infer their general causes, such as accidental spills and habitat destruction (Bate et al. 2004). As such, they cannot provide information about flow or nutrient changes over time and space, which are of interest to this study. Currently, the effects of altered flows are assessed according to changes in downstream communities in a South African programme known as the Downstream Response to Imposed Flow Alteration (DRIFT) (Brown et al. 2005). DRIFT and its subcomponent, the Ecological Reserve do not currently incorporate periphyton community changes. Similarly, eutrophication in South Africa is measured by the National Eutrophication Monitoring Programme (NEMP), which also does not make use of periphyton indicators. Periphyton provide early warning signs of environmental change, especially when they are flow and nutrient related, and could potentially be used as proxies for flow and nutrient changes in South African rivers, considering that periphyton have been successfully developed in monitoring programmes abroad.

1.8 Gaps in the South African periphyton literature and advances in field techniques

Most studies involving periphyton in South African rivers have focused only on the diatom group as a means of testing and comparing to water quality and understanding seasonal changes in their community structure (e.g. Slinger 2015) Oberholster (2011) showed that filamentous green algae respond to changes in water quality during high and medium flow periods. Ewart-Smith (2012) piloted research on several periphyton groups (diatoms, green algae and cyanobacteria) whose intention was to understand temporal patterns in periphyton community structure across levels of flow alteration and enrichment. The findings of this research were however not valid for the whole of South Africa, considering that the study area was limited to the south-Western Cape. A follow up study by Singh

(2016) then aimed to explore temporal variability of periphyton in KwaZulu-Natal, to form a conceptual basis for periphyton communities each month. These studies did not build in spatial variability, as each study only contained four sites, motivating for a spatial understanding of periphyton biomass and community structure to complement it's temporal component, and build sufficient literature for inclusion in South African monitoring programmes.

As mentioned, analysis of periphyton biomass and community structure is time consuming, expensive and subject to handling error. Periphyton monitoring would be encouraged if such challenges could be overcome. Indeed, a German *in situ* tool called the Benthotorch® (bbe Moldaenke GmbH) was developed in order to provide instant and comparable results in the field that can be operated by the non-specialist. Demonstrating that this tool accurately estimates periphyton biomass and community structure in South African rivers would further motivate for the inclusion of periphyton in South African monitoring programmes.

1.9 Research aims

This study aimed to broaden the baseline knowledge on spatial patterns associated with periphyton biomass and community structure. This would potentially serve as complimentary information to research already done in South Africa, and assist to provide a comprehensive basis on periphyton communities in the country. The scales of spatial variability consider regional differences due to summer and winter rainfall climatic areas and at a finer scale, site differences due to various levels of flow alteration (none, abstraction, impoundment and abstraction and impoundment) and enrichment (oligotrophic, oligotrophic-mesotrophic, mesotrophic, mesotrophic-eutrophic and eutrophic).

The research aims were to:

1) Broaden the knowledge on periphyton taxa in South African rivers
2) Improve on the understanding of the relative strength of nutrients and flow alteration towards regulating periphyton biomass and shaping periphyton communities
3) Assess whether periphyton biomass and community structure mirrors the level of enrichment and identify environmental variables that act as a proxy for enrichment
4) Improve on the understanding of which environmental variables explain shifts in periphyton biomass and community structure across regions and seasons

5) Assess whether there is a correlation between Benthotorch® and spectrophotometric biomass estimations and whether the Benthotorch® can accurately identify periphyton groups (diatoms, green algae and cyanobacteria).

1.10 Chapter outline

Chapter 1: General introduction and thesis overview

This chapter starts with an introduction to the water shortage in South Africa and how it promotes eutrophication, and the subsequent blooming of periphyton., which results in blooms of periphyton. A basic conceptualization is given on the periphyton–environment relationship to provide an understanding on how key environmental variables such as (flows, nutrients, grazers, sunlight and water temperature) shape periphyton communities. A worldly view is then provided on the use of periphyton as indicators to shed light on how periphyton could benefit biomonitoring programmes in South Africa, along with the recent efforts made by researchers to minimise the periphyton knowledge gap.

Chapter 2: Site selection and general methods

Chapter 2 explains the rationale for site selection and what criteria were used to categorise them according to levels of flow alteration and enrichment. The experimental design explains when sampling was done, whose procedures in terms of field and lab analysis are then detailed. The calculation steps of environmental metrics and sample units are given and the choice of statistical tests explained.

Chapter 3: Spatial patterns in periphyton biomass

Chapter 3 aims to explain spatial variation in periphyton biomass in relation to differences in characteristics of environmental variables. The separate effects of nutrient enrichment and flow alteration are tested in order to ascertain which variable has a stronger influence on periphyton biomass. Periphyton biomass is then scrutinised against a subset of environmental variables consisting of flow, sunlight, water temperature, grazer metrics and measurements of nutrients, pH and electrical conductivity. The first objective was to understand which individual or subset of variables best explain periphyton biomass. These variables were further tested for consideration as potential proxies of enrichment.

Chapter 4: Spatial patterns in periphyton community structure

Chapter 4 introduces the reader to the periphyton taxa identified in this study, where they were found and how their abundances in terms of their attributes (periphyton group and growth form) change across regions, seasons and levels of enrichment. This chapter also aims to explain spatial variation in periphyton community structure (taxa) in terms of differences in characteristics of environmental variables. The separate effects of nutrient enrichment and flow alteration are tested in order to ascertain which variable has a stronger influence on periphyton community structure. Periphyton community structure is then analysed in relation to a subset of environmental variables consisting of flow, sunlight, water temperature, grazer metrics and measurements of nutrients, pH and electrical conductivity. The first objective was to understand which individual or subset of variables best explain periphyton community structure. These variables were further tested for consideration as potential proxies of enrichment.

Chapter 5: Validation of an *in situ* tool (Benthotorch®)

Chapter 5 highlights the need for a rapid in situ tool with which to inform water resource management. The Benthotorch® is introduced as a solution and a short literature review on it's application to date given. The Benthotorch® is validated as a tool to estimate periphyton biomass and community structure (diatoms, green algae and cyanobacteria) by comparing against conventional techniques. The sampling approach is explained, as well as calculations of Benthotorch® biomass. A conclusion is then drawn based on it's efficacy at measuring these parametres.

Chapter6: General synthesis

Chapter 6 summarises the main findings of Chapters 3 and 4 in terms of contrasting on what aspects of the environment are most influential in each region with efforts to explain these findings. Potential proxies of enrichment are mentioned, for future consideration in eutrophication monitoring monitoring programmes. Chapter 5 explains whether the Benthotorch® is a suitable tool in South African rivers, and provides a set of recommendations for future use.

Chapter 2. Site selection and general methods

2.1 Site selection

To ensure that adequate spatial variability was built into this study, sites were selected from two distinct regions of South Africa, namely the Western Cape and KwaZulu-Natal. Freshwaters in the Western Cape flow over Table Mountain Sandstone which gives rise to clear waters and are typically acidic, due to the leeching of tannins from the surrounding fynbos (Allanson et al. 1990). Rivers of KwaZulu-Natal are turbid and slightly alkaline as they flow over young, weathering igneous rocks such as tillite (Allanson et al. 1990). A GIS desktop assessment was performed to shortlist sites from these regions that were located in open canopied foothill river reaches. Periphyton provide a significant proportion of energy in this longitudinal zone because an open canopy and shallow water maximizes autotrophic production (McCormick et al. 1997). The availability of time series data of flow and water temperature were treated as prerequisites for the selection of sites, as they explain regional variability in periphyton (Larned 2010). Nutrient data was not considered in the desktop assessment, as records were only available in two month intervals, which did not always coincide with autumn and spring, when sampling took place. Thirteen sites from twelve rivers in the Western Cape and nine sites from six rivers in KwaZulu-Natal were selected (Figure 2.1).

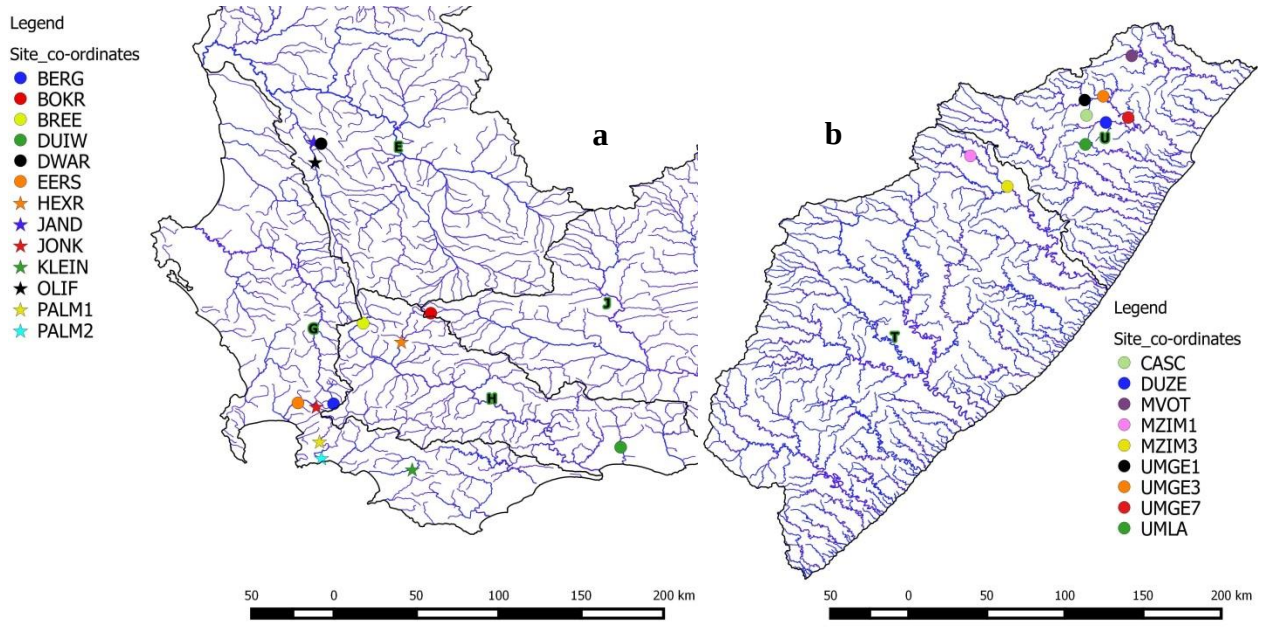


Figure 2.1: Maps showing 1:500000 rivers for the primary drainage regions in a) The Western Cape and b) KwaZulu-Natal. E = Olifants, G = Berg, H = Breë, J = Gourits, U = uMgeni and T =Mzimvubu

2.2 Site categorisation

Sites were categorised in terms of their level of flow alteration and enrichment in order to assess for their separate effects on peripyton biomass and community structure. Flows were categorised based on hydrographs that were drawn from 5 years of average daily discharge time series data obtainable from the Department of Water Affairs and Sanitation's (DWS) website. The hydrographs were investigated for natural flow patterns in the dry and wet seasons, with any deviations informing the level of alteration. Four categories of flow alteration (in order of the level of alteration) were identified:

natnat: rivers that flow naturally with no flow alteration in either the dry or wet season;

natdec: rivers with natural wet season flows but decreased dry season flows due to abstraction;

decinc: rivers with decreased wet season flows (i.e. loss of floods due to damming) and increased dry season flows, most likely due to agricultural return flows and

decdec: rivers with decreased wet and dry season flows.

Enrichment was categorised based on the Present Ecological State (PES) wetland model by Malan and Day (2012), which was modified for application on rivers. The model treats the relative percentage of surrounding land use types as surrogates for estimating the level of nutrient enrichment. The 2013-2014 National Landcover Dataset was used to calculate these percentages to derive an impact score for each site. The approach is described in detail in Ewart-Smith et al. (2016). Five categories of enrichment (in order of the level of enrichment) were identified in Table 2.1 below. Table 2.2 summarises the sites in this study, when they were sampled, as well as their enrichment and flow class attributes.

Table 2.1: Enrichment categories derived from Malan and Day (2012)

Altered Impact Score	Trophic Status
0	Oligotrophic
0-0.15	Oligo-Mesotrophic
0.16-0.45	Mesotrophic
0.46-1.0	Meso-Eutrotrophic
1.1-2	Eutrophic
>.2.1	Hypertrophic

Table 2.2: Sites in the Western Cape and KwaZulu-Natal sampled in 2015, and their assignment of enrichment and flow alteration categories.

SITE	RIVER	SITE DESCRIPTION	LEVEL OF FLOW ALTERATION	LEVEL OF ENRICHMENT	SAMPLING DATES Autumn	Spring
		Western Cape				
BERG	Berg	Catchment natural with wide open canopy.	natnat	oligotrophic	17.04.15	13.10.15
BOKR	Bok	Catchment natural with narrow open canopy.	natnat	oligotrophic	07.05.15	15.10.15
JONK	Jonkershoek	Catchment natural with wide open canopy.	natnat	oligotrophic	not sampled	15.10.15
DWAR	Dwars	Catchment natural. Stream with patches of open and closed canopy.	natnat	oligo-mesotrophic	16.04.15	17.10.15
JAND	Jan Dissels	Catchment natural and u/s Clanwilliam town. Partial riparian shading.	natnat	oligo-mesotrophic	not sampled	16.10.15
EERS	Eerste	D/s of WWTW and in feed of the polluted Plankenbrug river. Riparian fringe dominated by patches of alien trees.	natnat	eutrophic	06.05.15	23.10.15
PALM2	Palmiet	Situated 30km downstream of PALM1, with minor abstraction in the summer months.	natdec	oligotrophic	not sampled	14.10.15
BREE	Breede	Upper catchment natural, but situated downstream a WWTW plant.	natnat	oligo-mesotrophic	15.04.15	22.10.15
KLEIN	Klein	Catchment subject to dairy farming with abstraction drying up summer baseflows.	natdec	meso-eutrophic	not sampled	14.10.15
DUIW	Duiwenhoks	Upper catchment subject to agricultural farming with abstraction in summer months.	natdec	meso-eutrophic	20.04.15	17.10.15
OLIF	Olifants	Citrusdal town upstream along with extensive agriculture that requires water in summer. Stream subjected to riparian shading.	decinc	mesotrophic	16.04.15	not sampled
HEXR	Hex	Abstraction taking place for Viticulture upstream.	decdec	meso-eutrophic	15.04.15	22.10.15
PALM1	Palmiet	Immediately d/s the Kogelberg Dam with upper catchment subject to agriculture.	decdec	meso-eutrophic	20.04.15	17.10.15
		KwaZulu-Natal				
CASC	Cascades	Passes through residential and commercial infrastructure. Flows are natural.	natnat	eutrophic	08.06.15	08.10.15
MZIM1	Mzimkhulu	Upper catchment subject to agriculture and plantations but flows natural.	natnat	meso-eutrophic	03.06.15	13.10.15
MZIM3	Mzimkhulu	Upper catchment comprises agriculture, plantations,rural communities and grasslands. Low flows are altered.	natdec	eutrophic	02.06.15	13.10.15
DUZI	Mzunduzi	D/s of WWTW and receives sewage from several u/s tributaries.	decinc	eutrophic	27.05.15	14.10.15
UMGE1	uMngeni	Upper catchment dominated by agriculture with abstraction taking place.	decinc	oligo-mesotrophic	09.06.15	08.10.15
UMGE3	uMngeni	D/s of the Albert Falls Dam and heavily flow regulated.	decinc	mesotrophic	09.06.15	07.10.15
UMGE7	uMngeni	Upper catchment subject to farming activities and rural settlements. Flows are regulated.	decinc	oligo-mesotrophic	11.06.15	07.10.15
UMLA	uMlazi	Situated d/s of Baynedfield dam. Catchment dominated by agriculture and plantations.	decdec	eutrophic	11.06.15	13.10.15
MVOT	Mvoti	Upper catchment subject to agriculture and plantations.	decdec	meso-eutrophic	12.06.15	08.10.15

* WWTW = Waste Water Treatment Works *u/s = upstream *d/s = downstream

2.3 Experimental design

Periphyton biomass and community structure were sampled in the Western Cape and KwaZulu-Natal in 2015 at the end of summer/beginning of autumn (April/May) and the beginning of spring (October). These periods represent the end and beginning of the periphyton growing seasons respectively (Ewart-Smith 2012). For each site, six cobbles were sampled for Benthic chlorophyll *a*, of which three subsamples were drawn for taxonomic analysis. From Table 2.2 it can be derived that a total of 39 site visits were made (21 in the Western Cape and 18 in KwaZulu-Natal), giving 234 biomass and 117 community structure samples. These samples were factored for region, season and levels of enrichment and flow alteration in statistical analysis.

2.4 Sampling procedures

This section applies to Chapters 4-6. Six individual cobbles (replicates) were randomly selected within a 100 m stretch at the study sites. Periphyton were only sampled from runs to reduce variability associated across biotopes (Biggs 2000; Ewart-Smith 2012). For each cobble, the near-bed flow velocity and water column depth were measured using a Flo - Mate^{TM} 2000 flow meter with a built-in ruler (March and McBirney Inc.). The percentage cover of surrounding substrata such as sand, gravel, boulders and bedrock, defined by Rowntree and Wadeson (1999) was estimated for an area of 1m^2 within which the cobble was identified.

An macroinvertebrate sampling net with an 80 micron mesh and aperture area of 300mm x 300mm was placed immediately downstream of each cobble. The cobble was then dislodged, allowing macroinvertebrates to be washed into the net and then placed into a white plastic tray. Macroinvertebrates that did not wash into the net's collection jar were picked with a forceps from the net and off the cobble and altogether fixed in 96% v/v ethanol, which afterwards was diluted to 50% v/v ethanol. Other non-periphytic matter such as bryophytes was removed from the cobble to ensure that only periphyton were being analysed later on in the lab. Periphyton were removed by systematically brushing all cobble surfaces with a toothbrush until the rinsing water remained clear. The collected slurry was placed in black jars to prohibit sunlight and stop photosysthesis, and on ice to prevent decomposition of Benthic chlorophyll *a*. Of the six Benthic chlorophyll *a* samples per site, three random samples were chosen to produce subsamples for periphyton identification. Before decanting, the slurry was manually homogenised by shaking, after which a 50 ml subsample was dyed with Lugol's iodine.

The cobble was measured in order to obtain a surface area with which to standardise biomass and cell density to $1m^2$. The largest length (x), width (y) and height axes (z) was measured and fed into the regression equation determined by Ewart-Smith (2007) and provided in Ewart-Smith (2012) as follows:

Surface area in $m^2 = (0.014(xy + xz + yz)+ 33.819)/10000$

Spot measurements of pH, EC and water temperature were measured with a SensoDirect 150 multimeter, calibrated at a pH of 4 and 7 and an EC of 1413 µS cm. Water samples were collected in 50ml bottles and placed on ice. In the laboratory, the water samples were analyzed for nitrite (NO_2-N), nitrate (NO_3-N), ammonium (NH_4-N), total inorganic nitrogen (TIN), (NO_2-N + NO_3-N and NH_4-N) and orthophosphate (PO_4-P).

2.5 Laboratory procedures

2.5.1 Biomass (Benthic chlorophyll *a*)

The volume (ranging from 30ml to 300ml) of slurry for spectrophotometric analysis was measured and approximately half this volume filtered through Whatman glass fibre filters (MNGF-5, 47mm). The filters were placed in test tubes containing 30ml 90% v/v ethanol and covered with parafilm. Test tubes were placed in a hot water bath for five minutes which was preheated to 78 °C, the boiling point of ethanol. Thereafter, the test tubes were placed in a fridge overnight to complete Benthic chlorophyll *a* extraction. The test tubes were then vortexed for five seconds using a Heidolph vortexer and centrifuged for 10 minutes at 3000 rpm using a Beckman GS-6 centrifuge. A Pipetman pipette was used to transfer 4 ml of sample into a cuvette for spectrophotometric analysis. A Spectroquant Pharo 100 spectrophotometer was used to measure Benthic chlorophyll *a* according to the procedure of Biggs and Kilroy (2000). A Benthic chlorophyll *a* reading was taken at 665 nm and a turbidity reading at 750 nm. The solution was then acidified using 0.30 M hydrochloric acid to lyse all the phaeopigments (dead cells that no longer photosynthesize) and correct for them. Benthic chlorophyll *a* and turbidity were read again at 665 nm and 750 nm respectively and the corrected Benthic chlorophyll *a* reading before acidification subtracted from the corrected Benthic chlorophyll *a* reading after acidification.

2.5.2 Community structure (taxa cell densities)

The 50 ml algal identification sample was blended by shaking the container for 10 seconds and then moving a spoon through the slurry in a zig zag motion to break up strands of algal filaments. Five ml was removed to centrifuge at 3000 rpm for 10 minutes in order to form a 0.5ml periphyton pellet, which was removed for analysis. From this pellet, 0.1 ml was placed onto a Naubauer® haemocytometer grid with a maximum volume of 0.9 mm^3. A Zeiss compound microscope was used to enumerate periphyton taxa using either the entire grid (144 squares) or a given number of squares (1 square = 0.00625 mm^3). The general rule was to count between 250- 400 periphyton cells across a few squares (16, 32, 48 or 64) in highly concentrated pellets, or the entire grid for less concentrated pellets.

2.5.3 Macroinvertebrates

Macroinvertebrates were identified to genus level using the WRC freshwater invertebrate guides by Day et al. (2002), de Moor et al. (2003 a, b) and Stals & de Moor (2007) with an Olympus SZ compound microscope (Model SZ2-ILST).

2.5.4 Nutrients

Nutrient concentrations were determined using a Thermo Spectronic spectrophotometer (model: Helios Epsilon). Samples were first vortexed for five seconds using a Vortex Genie 2 (model: G560E). Nitrite was read at 543 nm, NH_4-N was read at 630nm and PO_4-P was read at 886nm. Nitrate concentrations were determined by reducing the nitrate in the sample to nitrites by running the sample through a reduction agent (cadmium column) using a Gilson Miniplus 2 peristaltic pump. The nitrate concentration was determined by subtracting the original nitrite concentration before the reduction step from the combined concentration of original and reduced nitrite after reduction. The bottles were allowed to thaw to room temperature prior to analysis.

2.6 Data analysis

2.6.1 Determination of spectrophotometric Benthic chlorophyll *a* (mg m^2)

The Benthic chlorophyll *a* as milligram per replicate was calculated according to the following equation:

Benthic chlorophyll *a* (mg) =

$$\frac{final\ absorbance\ reading * 28.66 * total\ jar\ volume\ (ml) * 0.03}{available\ stone\ surface\ area\ (m^2)}$$

where: 28.66 represents the absorbance coefficient of ethanol and 0.03 the volume of ethanol expressed in litres. The total jar volume represents the volume before extraction of biomass and taxon identification sub samples. Available stone surface area represents the area on the cobble that was potentially colonized by periphyton. This was achieved by using Benthotorch® biomass estimations (Chapter 5) to assess which surfaces of the cobble (top, front, bottom, back, left and right) were potentially embedded (readings of 0.01µg cm^2) The approximate area of the embedded surface was subtracted from the total area of the cobble, giving the available stone surface area.

2.6.2 Determination of community structure units (taxon cells.m^2)

The cell density for each taxon was calculated based on how cells were enumerated on a Naubauer® haemocytometer grid, which consists of a grid made up of 144 constituent squares. Each square has a depth of 0.1 mm and area of 0.0625 mm^2, giving a square volume of 0.00625 mm^3 and grid volume of 0.9 mm^3, which equates to 0.009 ml.

The equation is expressed as:

Genus cell density (cell count/m^2) =

$$\frac{taxon\ cell\ count * 0.1 * total\ jar\ volume(ml)}{volume\ sampled(ml) * available\ stone\ area\ (m2)}$$

Where:

0.1: the dilution factor, to correct for cell concentration during centrifuging

Taxa cell densities were weighted according to the largest taxon identified in this study (*Ceratium* = 270 µM) in order to correct for the over representation of smaller taxa during periphyton identification. Weighted taxa cell densities are provided in Appendix 1a and b.

2.6.3 Determination of macroinvertebrate grazer pressure

2.6.3.1 FFG abundance (individuals m^2)

Genera that belonged to functional feeding groups (FFG's) of scrapers, brushers or deposit feeders were enumerated according to length classes. Abundances were summed across length classes to give genus abundance and all genera within an FFG group summed to give FFG abundance. FFG abundance from each replicate was standardised to $1m^2$ according to the available cobble surface area. FFG's were derived from Shael (2005) and modified by Merrit and Cummins (1984).

2.6.3.2 FFG biomass (g m^2)

Genus length-specific abundance was multiplied by it's family length-specific weight and these products where summed across length classes to give genus weights. Genera belonging to the same FFG group were then summed together. FFG biomass from each replicate was standardised to $1m^2$ according to the available cobble surface area. Family length-class weights were provided by Ewart-Smith (2012). Macroinvertebrate abundance and biomass data was then square root transformed, as these metrics were treated as environmental variables and needed to assume multivariate normality in subsequent tests. Appendix 2a and b list grazer FFG abundances and biomass in autumn and summer in the Western Cape and KwaZulu-Natal in 2015.

2.6.4 Derivation of environmental variables

Flow data was obtained from the DWS website (https://www.dwa.gov.za). Hourly solar irradiation and air temperature was provided by the Agricultural Research Council (ARC). Hourly water temperature data was provided from data loggers in the same river reaches from Helen Dallas of the Freshwater Research Centre, based in Kommetjie, Cape Town. Water temperature data were modeled for sites Helen had no data for using daily average air temperatures utilizing the approach of Rivers-Moore et al. (2005) and given by the equation:

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

Where:

WTx = Maximum daily water temperature; β = 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

Environmental variables are listed for the Western Cape and KwaZulu-Natal in Tables 3.2 and 3.3 respectively.

2.6.5 Determination of flow, water temperature and solar irradiation metrics

Flow metrics were used to calculate the frequency (#Flds$_{\geq X}$), duration (#Days$_{\geq X}$) and days since (Since$_{\geq X}$) a particular flood event (x) was experienced. These calculations were based on average daily discharge for a period of five months preceding the date of sampling. The partial duration series (PDS) approach (Shaw 1988) was used to identify all flood magnitudes above a predetermined discharge threshold (m^3/s). The threshold value typically represents those of small commonly occurring floods and was calculated as the median amongst a few observed values from studying the hydrographs. The PDS was further modified to incorporate summer and winter base flow values that were also calculated from medians from these hydrographs. A list of floods for the five-month period was identified of which the highest discharge between every drop to summer or winter flows was selected. These were ranked in descending order of magnitude. The Gringorten recurrence interval equation (Guo 1990) was applied to calculate recurrence intervals for each rank and ultimately identify a flood magnitude that occurs every two years (1:2 year flood event). The DRIFT methodology (King et al. 2003) was then applied to calculate within-year flood category events (Class 4, 3, 2 and 1). Class 4 floods have half the magnitude of 1:2 year floods, Class 3, half the magnitude of Class 4 and so on. Appendix 3 shows the flood class sizes.

Daily metrics for solar irradiation and water temperature were calculated for a period of one month prior to sampling. Solar irradiation metrics used in the analysis included the average daily minimum solar irradiation (Rs_{MIN}) and the cumulative solar irradiation (Rs_{CUM}), which was the sum of hourly daylight readings between 7 am and 6 pm. Water temperature metrics used in the analysis included the average daily coefficient of variation in water temperature (WT_{CV}), the average daily minimum water temperature (WT_{MIN}), the average daily maximum water temperature (WT_{MAX}) and the cumulative average daily water temperature (WT_{CUM}).

2.6.6 Univariate statistical analysis

2.6.6.1 Biomass (Benthic chlorophyll *a*)

Biomass data was non-parametric ($p<0.01$), which is expected considering the spatial and seasonal contrast of this study and the patchy distribution of periphyton (Matthaei et al. 2003; Harris & Graham 2015). Kruskal-Wallis tests were done using R version Wooden Christmas-Tree (R Core Team 2013) to test for significant differences in the medians of biomass across seasons and categories of enrichment and flow alteration. The gradient effects of flow alteration were tested by controlling for the level of enrichment. The gradient effects of enrichment were tested by controlling for the level of flow alteration. Spearman rank correlations were used to test for a relationship between Benthic chlorophyll *a* and invertebrate density (individuals m^2) or invertebrate grazer pressure (g m^2).

2.6.7 Multivariate statistical analysis

The PRIMER v6+PERMANOVA software package (Clarke and Warwick 2001, Clarke and Gorley 2006) was used for all ordination and multivariate analyses (Anderson et al. 2008) which require the construction of Euclidean Distance and Bray-Curtis similarity matrices. Euclidean Distance, which measures the distance ($0 - \infty$) between two samples, is appropriate on continuous data such as environmental measurements. Empty records were treated as missing, rather than absent, to account for not having measured a given variable. Environmental variables were assessed for autocorrelation using Draftsman plots and in the event that a group of variables were correlated, a single variable was selected in order to decrease redundant information in statistical tests. Environmental variables were transformed (square root, fourth root or log) to bring them into normality and then normalised, to bring variables with different measurements into the same scale. Bray Curtis similarity measures the similarity (0-100) between two samples and is appropriate for use on ecological data such as taxon abundances, as it takes taxon identity into account. Empty records were treated as taxa not being present. Taxa were square root transformed to bring abundances into multivariate normality.

2.6.7.1 Site separation

A Principal Components Analysis (PCA) identified environmental variables that varied most between sites. A principal component highlights the underlying structure amongst samples by separating groups

of samples that have different measurements based on the principal component, as well as point to samples that measured the highest based on that principal component. Principal components consist of paired values of Eigenvectors and their Eigenvalues, which describe in what direction the variation occurs, and how much variation is explained respectively The Eigenvector with the highest Eigenvalue is the principal component. Environmental data were prepared as in section 2.6.7. Correlations ranged from -0.6 to 1.

2.6.7.2 Community Structure distributions

Multi-Dimensional Scaling (MDS) using Bray-Curtis similarity matrices were used to assess for similarities between algal community samples when controlling either for enrichment or flow alteration in the Western Cape. MDS allows the user to view underlying dimensions that help to explain similarities or dissimilarities between samples, and is particularly useful as multidimensional data are collapsed for easy interpretation.

2.6.7.3 Relationships between environmental variables and periphyton biomass and community structure

Distance Based Linear Modeling (DISTLM) was used to identify individual, and groups, of environmental variables that best explained variation in periphyton biomass and community structure. Biomass and community structure were plotted graphically onto two dimensional space using a step-wise Distance Based Redundancy Analysis (dbRDA) that was run for 9999 permutations. The dbRDA was used to inform whether patterns in biomass and community structure were evident across gradients in flow alteration and enrichment. Environmental variables were identified as potential proxies for enrichment, where patterns in periphyton followed a gradient in enrichment.

Chapter 3. Spatial patterns in periphyton biomass

3.1 Introduction

Periphyton biomass is regulated by environmental parameters such as the characteristics of the flow and water temperature regimes, the density of grazers and the availability of nutrients and sunlight (Biggs 2000; Larned 2010; Stevenson 2014). Of these parameters, the flow regime, which comprises the timing, frequency and magnitude of flood events, is widely considered to be a primary regulator of periphyton biomass in flood prone rivers (Uehlinger 1979; Clausen & Biggs 1997; Uehlinger et al. 2003; Robinson et al. 2004; Francoeur & Biggs 2006). Water temperature, grazers, sunlight and nutrients are only afforded the opportunity to influence periphyton biomass accrual once flows have subsided to levels where periphyton are no longer dislodged from substrata (Biggs 2000; Stevenson 2014). This is particularly the case for nutrients (Ewart-Smith 2012, Ponsat et al. 2016), such that together, the flow regime and nutrients are able to explain 50% of the annual vairability in periphyton biomass (Biggs & Close 1989). Some workers contend that spatial patterns in periphyton biomass on the regional scale are explained mainly by the flow regime during flooding periods and the availability of nutrients between flood periods (Biggs 1996; Torńs & Sabater 2010). Francoeur et al. (1999) attributed seasonal variation in periphyton biomass to availabilities of nitrates and found that 66% and 86% of the variation in monthly biomass means was explained by floods and nutrients respectively.

During such stable flow periods, many workers regard the stoichiometric[1] ratios of nutrients such as nitrate, ammonia and soluble reactive phosphorous to be key determinants of periphyton biomass (Biggs & Close 1989; Biggs et al. 1998; Ewart-Smith 2012). However, the importance of any given variable which promotes periphyton growth (nutrients, water temperature and sunlight) is based on it's rank of limitation, relative to other variables (Allan and Castillo 2007). For example, Lewis & McCutchan (2010) found that nutrients in oligotrophic montane streams in Colorado did not explain periphyton biomass, but rather water temperature, which was more limiting. Von Schiller et al. (2007) found light to be a primary limiter of algal biomass in Mediterranean streams followed by nutrients. Sections 3.1.1 – 3.1.3 explains how each variable influences periphyton biomass.

3.1.1 The flow regime

Increased base flows decrease periphyton biomass by means of shear force and abrasion (Smolar-Žvanut & Mikoš 2013). Shear force becomes critical when the drag exerted by the current velocity overcomes the tensile strength of periphyton filaments (Biggs & Gerbeaux 1993). Current velocities act

[1] Stoichiometric ratios: The ratio of one molecule relative to another, based on their molar masses.

synergistically with abrasion in rivers that contain fine sediment, whose scouring effect are more effective at removing periphyton biomass than shear stress alone (Francoeur & Biggs 2006). Thomson et al. (2005) for example noted a decrease in periphyton biomass removal after the deconstruction of a dam, due to the sudden mobilization of trapped sediments. Molar action occurs in rivers with unstable cobbles, which remove periphyton by means of toppling over at higher current velocities (Power & Stewart 1987). The effect of the flow regime is also determined by the preflood biomass of periphyton communities, which are more susceptible to dislodgement the greater the biomass (Uehlinger 1979; Biggs & Close 1989).

3.1.2 Flow and Nutrients

Nutrient influences must be understood within the context of the flow regime, as it regulates their availability (Biggs & Close 1989), except in large rivers systems that are hydrologically stable (Chetelat et al. 1999). Periphyton are able to accrue high biomass between flood regimes when nutrient concentrations are high (Biggs 1996; Biggs et al. 1998; Biggs et al. 1999). However, an increase in current velocity in nutrient poor rivers may simulate nutrient pulses and increase periphyton biomass (Dodds et al. 1998; Davies & Bothwell 2012; Townsend et al. 2012)

Stevenson et al. (2006) mentions that periphyton biomass may also be higher during increased velocities because grazers are washed downstream, allowing periphyton to grow unchecked whilst capitilising on increased nutrient availabilities. By contrast, they also believe that periphyton can accrue high biomass in nutrient poor rivers with stable flows, provided that such conditions persist and grazers are absent. Townsend et al. (2012) however did not note any increases in periphyton biomass in nutrient poor rivers, but rather in rivers that were enriched. Sometimes there is no measurable response between periphyton biomass and nutrients, which may rather be reflected in grazer biomass (Bourassa & Cattaneo 1998; Francoeur et al. 1999).

The relative role of nitrogen and phosphorous in shaping periphyton biomass is variable around the world. Grimm et al. (1986) found that nitrogen limited biomass accrual while the addition of phosphorous made no difference in Sonoran desert streams.

3.1.3 Nutrients, sunlight and grazers

In testing for the relative importance of nutrients, sunlight and grazers on periphyton biomass, Rosemond et al. (2000) found that grazers had the strongest effect on periphyton biomass in summer and autumn, which was reflected in grazer biomass. Increases in periphyton biomass due to increases in nutrient availabilities and sunlight were however only observed when grazers were removed. Some studies have found that nutrients are limited under high light conditions, which is rarely the case under low light conditions (Bourassa & Cattaneo 2000; Greenwood & Rosemond 2005).

In their comparison between the relative effects of nutrients and grazers, Hillebrand (2002) found that the removal of grazers had a larger influence on periphyton biomass relative to an increase in nutrients. They concluded that the removal of grazers provides an immediate relief, whereas an increase in nutrients and subsequently nutrient uptake is a time lag response.

3.1.4 Periphyton biomass in South African rivers

Ewart-Smith (2012) identified the end of summer/early autumn (April) as the end of the growing season for periphyton in the Western Cape, when biomass reached a peak. She attributed this due to a long growing season characterized by stable flow conditions, optimal water temperatures and sunlight and sufficient time to outgrow the feeding pressure of grazers. She marked the beginning of the growing season in spring (October), after a winter period of frequent flooding and suboptimal water temperatures and sunlight.

In KwaZulu-Natal, it is proposed that the start and end of the periphyton growing seasons coincide with that of the Western Cape based on water temperature, but that biomass peaks do not. By contrast, less biomass is expected at the end of summer in comparison to spring. The reason for this is disturbance by floods and grazers between flood periods in summer, with flooding also increasing turbidity which attenuates sunlight. Finally, increased base flows in summer may dilute nutrients (Ewart-Smith et al. 2017).

Spring provides a window of opportunity for periphyton growth when flows have stabilised and base flows are lower, which increases water temperature and the clearer water (as a result of slower current velocities) allows for increased sunlight penetration. Nutrient concentrations are also expected to be higher, due to the decomposition of leaf litter during the preceding winter period (Ross-Gillespie pers

comm.). Finally, grazer emergence lags behind periphyton communities, providing a period to grow unchecked when environmental variables are favourable (Ross-Gillespie 2014).

The above literature review highlights the dynamic nature between periphyton biomass and the environment. An understanding of periphyton biomass in South African rivers is however still in it's infancy and this chapter aims to understand the relative importance of the discussed environmental variables, towards regulating periphyton biomass in order to reduce the outbreak of periphyton proliferations and maintain ecological balance. A clear understanding on periphyton biomass would encourage the development of such indices, to be used in South African biomonitoring programs.

The research aims of this chapter are to:

1) Understand the relative strength of nutrients and flow alteration towards regulating periphyton biomass in the Western Cape;
2) Assess if periphyton biomass mirrors the level of enrichment and if so, identifying which environmental variables, if any, act as a proxy for enrichment in the Western Cape and KwaZulu-Natal;
3) Compare which environmental variables explain shifts in periphyton biomass in each region across seasons and levels of enrichment

3.2 Results

3.2.1 Site differences based on environmental variables in the Western Cape

Vector abbreviations are given in the list of abbreviations and in the captions in this section. The PCA for the Western Cape explained 64 % of the overall variation between sites on the first two axes. Electrical conductivity, TIN and PO_4-P were considerably variable between sites within and across seasons. In particular, EC ranged from 8 µS cm^{-1} at BOKR to 179 µS cm^{-1} at EERS in autumn and from 13.4 µS cm^{-1} at BOKR to 709 µS cm^{-1} at KLEIN in spring. TIN ranged from below detectable limits at DWAR to 1.1 mg l^{-1} at EERS in autumn and from 0.023 mg l^{-1} at BERG to 0.339 mg l^{-1} at EERS in spring. PO_4-P ranged from 0.003 mg l^{-1} at BERG and BOK to 0.111 mg l^{-1} at EERS in autumn and from below detectable limits across the majority of sites to 0.220 mg l^{-1} at the EERS site in spring. In terms of flow, HEXR had the driest winter, while KLEIN had the wettest based on the number of class 2 floods experienced over the winter period (Table 3.2).

3.2.1.1 Variation across seasons

EC, PO_4-P and pH are good indicators of seasonal changes, irrespective of the level of enrichment. Within seasons, the highest EC, PO_4-P and pH readings were recorded at the most enriched sites (Figure 3.1a). As Table 3.1a suggests, EC should be considered a primary variable explaining spatial variation based on seasons, followed by PO_4-P and pH.

3.2.1.2 Variation across enrichment levels

The variables NO_3-N, WT_{CUM}, DP_{BMASS} and GR_{BMASS} are good indicators of the level of enrichment, irrespective of season. However, NO_3-N and WT_{CUM} measure greatest at enriched sites in autumn compared to spring (Figure 3.1a). DP_{BMASS}, GR_{BMASS} and #Fld $_{\geq 2}$ measure greatest at nutrient poor sites in spring compared to autumn. As Table 3.1a suggests, WT_{CUM} and NO_3-N should be considered primary variables of spatial variation, based on enrichment, followed by DP_{BMASS} and $GR_{BMASS.}$ These findings suggest that site separation is based on nutrients, water temperature and grazers and that flows contribute minimally to this separation.

3.2.2 Site differences based on environmental variables in KwaZulu-Natal

The PCA for KwaZulu-Natal explained 71% of the overall variation between sites on the first two axes. In contrast to the Western Cape, the range in EC was lower in autumn and spring (59 µS cm^{-1} to 302 µS cm^{-1}) and (79 µS cm^{-1} to 159 µS cm^{-1}) respectively, but the range in TIN was higher (0.101 mg l^{-1} to 3.391 mg l-1) and (0.181 mg l^{-1} to 1.701 mg l^{-1}) for autumn and spring respectively. UMGE 1 and UMLA were the driest while MZIM1 the wettest based on the number of class 2 flood events over the summer season(Table 3.3). Average daily water temperatures in KwaZulu-Natal general fluctuated more so than in the Western Cape, based on WT_{CV} but average daily discharge amongst sites in the Western Cape in the winter rainy season was more variable than for the summer rainy season in KwaZulu-Natal, based on Q_{CV} (Table 3.2 and 3.3).

3.2.2.1 Variation across seasons

#Days $_{\geq 2}$, Since $_{\geq 2}$, #Flds $_{\geq 2}$, WT_{CV}, SCR_{BMASS} and GR_{BMASS} all indicate seasonal site changes, irrespective of the level of enrichment (Figure 3.1b). Of these variables, Table 3.1b suggests that #Days

$_{\geq 2}$, Since $_{\geq 2}$,SCR_{BMASS} and WT_{CUM} explain the most variation seasonally, with #Days $_{\geq 2}$ measuring greatest in autumn and since $_{\geq 2}$,SCR_{BMASS} and WT_{CUM} measuring greatest in spring (Figure 3.1b).

3.2.2.2 Variation across enrichment levels

This study could not find any robust environmental variables that differentiated enrichment levels in KwaZulu-Natal. These findings suggest that site separation in KwaZulu-Natal is not based on nutrients but rather on flows, scrapers and water temperature.

Table 3.1: PCA eigenvector coefficients in the linear combination of environmental variables making up the principial coordinates in a) the Western Cape and b) KwaZulu-Natal for autumn and spring in 2015. EC = electrical conductivity, WTCUM = Cumulative average daily water temperature over the inter-sampling period, WTCV = Coefficient of variation across average daily water temperature for the inter-sampling period, #Fld ≥ 2 = Number of floods equal to a DRIFT class 2 or greater flood over the inter-sampling period, #Days ≥ 2 = Number of days in flood equal to a DRIFT class 2 or greater flood, since ≥ 2 = Number of days since a DRIFT class 2 or greater flood was experienced, QCV = Coefficient of variation across daily average discharge for the inter-sampling period, GRBMASS = Biomass of grazers (scrapers +deposit feeders + brushers), SCRBMASS = Biomass of scrapers, DPBMASS = Biomass of deposit feeders.

Variable	PC1	Variable	PC2
EC	0,416	pH	-0,541
WT_{CUM}	0,391	EC	-0,451
NO_3-N	0,382	#Fld $_{\geq 2}$	-0,366
DP_{BMASS}	-0,381	PO_4-P	-0,308
PO_4-P	0,370	WT_{CUM}	0,208
GR_{BMASS}	-0,357	DP_{BMASS}	-0,180
pH	0,120	Since $_{\geq 2}$	0,159
#Fld $_{\geq 2}$	-0,108	NO_3-N	0,121
since $_{\geq 2}$	-0,055	GR_{BMASS}	-0,063

Variable	PC1	Variable	PC2
#Days $_{\geq 2}$	-0,551	SCR_{BMASS}	0,628
Since $_{\geq 2}$	0,546	WT_{CUM}	0,454
#Fld $_{\geq 2}$	-0,436	WT_{CV}	-0,447
WT_{CV}	0,304	GR_{BMASS}	0,438
GR_{BMASS}	0,272	#Fld $_{\geq 2}$	0,051
Q_{CV}	-0,169	Q_{CV}	-0,047
WT_{CUM}	-0,094	Since $_{\geq 2}$	0,031
SCR_{BMASS}	0,065	#Days $_{\geq 2}$	-0,028

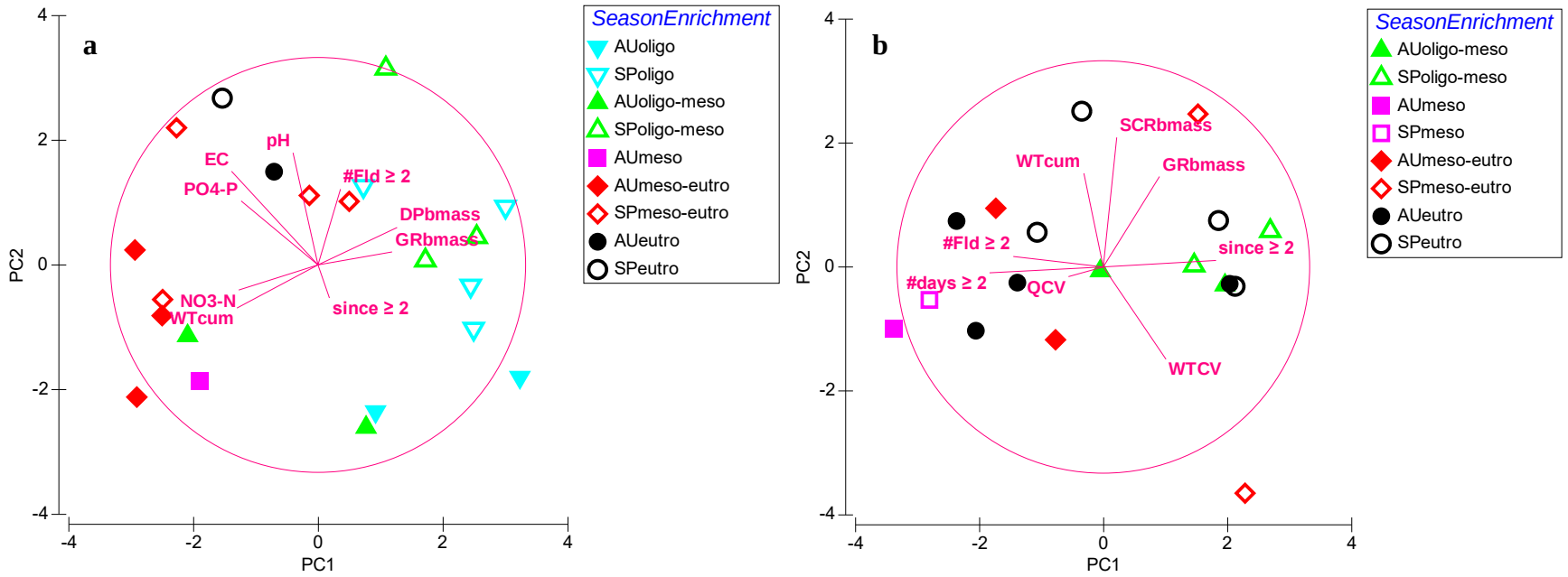


Figure 3.1: PCA ordinations of site separation in the a) Western Cape and b) KwaZulu-Natal for autumn and spring in 2015 factored for enrichment classes based on a priori categorization using Malan and Day (2012). Vectors were derived from the environmental data provided in Table 3.2 and 3.3 and show the eigenvector coefficients between environmental variables and the PCA axes. AU = autumn, SP = spring, oligo = oligotrophic, oligo-meso = oligotrophic-mesotrophic, meso = mesotrophic, meso-eutro = mesotrophic-eutrophic and eutro = eutrophic. EC = electrical conductivity, WTCUM = Cumulative average daily water temperature over the inter-sampling period, WTCV = Coefficient of variation across average daily water temperature for the inter-sampling period, #Fld ≥ 2 = Number of floods equal to a DRIFT class 2 or greater flood over the inter-sampling period, #Days ≥ 2 = Number of days in flood equal to a DRIFT class 2 or greater flood, since ≥ 2 = Number of days since a DRIFT class 2 or greater flood was experienced, QCV = Coefficient of variation across daily average discharge for the inter-sampling period, GRBMASS = Biomass of grazers (scrapers +deposit feeders + brushers), SCRBMASS = Biomass of scrapers, DPBMASS = Biomass of deposit feeders

Table 3.2: Environmental variables and associated measurements for the Western Cape in autumn and spring in 2015. Abbreviations are provided in the list of abbreviations.

		AUTUMN									SPRING											
	unit	BREE	HEXR	DWAR	OLIF	BERG	PALM1	DUIW	EERS	BOKR	BREE	HEXR	DWAR	BERG	PALM1	DUIW	EERS	BOKR	KLEIN	PALM2	JONKE	JAND
pH		6,8	7,1	5,1	6,6	5,7	6,8	7,4	7,6	5,7	8	7,4	6,4	6,2	7	5,2	7,7	6,8	7,4	7,1	6,9	6,9
EC	$\mu S.cm^{-1}$	92,3	144,6	21,9	102,3	14,9	70,4	169	179,8	8	197,1	288,5	35,3	19,8	111,4	542,8	395,5	13,4	709	125,1	30,3	33,7
NO2-N	$mg.l^{-1}$	0,002	0,003	0,001	0,001	0,001	0,003	0,007	0,073	0,001	0,005	0,003	0	0,001	0,002	0	0,044	0	0,002	0,001	0,001	0
NO3-N	$mg.l^{-1}$	0,231	0,977	0,001	0,104	0,026	0,546	0,230	0,313	0,006	0,002	0,001	0,002	0,004	0,012	0,014	0,044	0,003	0,014	0,007	0,001	0,001
TIN	$mg.l^{-1}$	0,279	0,98	0,001	0,105	0,026	0,549	0,237	1,107	0,006	0,042	0,028	0,037	0,023	0,052	0,054	0,339	0,038	0,085	0,046	0,03	0,027
PO4-P	$mg.l^{-1}$	0,093	0,042	0,018	0,007	0,004	0,035	0,067	0,111	0,003	0,106	0,043	0,01	0,002	0,023	0,02	0,22	0	0,06	0,005	0,001	0,001
Rs_{min}	$MJ.m^{-2}$	7	13	46	46	25	57	43	19	4	97	65	161	63	113	106	148	51	79	100	59	161
Rs_{cum}	$MJ.m^{-2}$	137253	127610	148514	148514	152809	143531	105605	114967	106108	155714	143129	191021	114503	148813	150265	171752	129876	139621	150581	139663	191021
WT_{CV}		0,103	0,045	0,060	0,034	0,067	0,066	0,036	0,147	0,045	0,099	0,075	0,067	0,157	0,085	0,070	0,105	0,046	0,123	0,073	0,142	0,089
WT_{MIN}	°C	16,0	18,4	16,6	21,4	15,8	17,0	19,4	14,1	12,2	12,5	14,2	12,7	9,0	13,1	15,3	14,1	11,3	15,0	13,3	9,0	13,3
WT_{MAX}	°C	27,4	22,7	20,1	25,2	21,6	23,3	21,9	23,6	14,4	19,5	18,7	16,2	16,4	19,3	21,5	21,3	13,1	26,6	18,1	16,0	20,1
WT_{CUM}	°C	720	667	577	731	599	647	662	517	418	495	493	453	397	466	558	522	374	555	489	380	493
Q_{CV}		1,591	0,325	0,316	0,838	0,715	3,825	1,555	0,498	0,299	2,352	0,607	1,616	1,788	1,136	1,224	1,772	1,071	1,138	0,975	1,411	1,769
$\#Fld_{\geq 2}$		0	0	0	0	0	0	4	0	0	6	0	10	6	3	16	7	6	27	18	19	10
$\#days_{\geq 1}$		0	0	0	5	0	0	6	0	0	15	3	26	18	45	47	16	44	115	91	44	26
$\#days_{\geq 2}$		0	0	0	0	0	0	6	0	0	6	0	12	6	22	22	10	8	54	35	21	12
$since_{\geq 2}$		207	208	209	151	232	205	14	224	227	79	398	63	70	23	0	80	72	7	13	38	63
$since_{\geq 1:2}$		593	1755	263	230	4309	305	467	536	2142	783	1945	446	4488	483	647	706	2303	81	694	698	446
GR_{DENS}	$counts.m^{-2}$	1290	1626	2502	1119	5343	189	859	19493	3138	16997	6483	5660	9079	7853	571	8166	1983	2059	10594	12328	10262
SCR_{DENS}	$counts.m^{-2}$	435	579	767	526	3161	132	274	18739	355	2261	1807	2715	4551	5438	262	5848	1387	1032	7760	2447	7441
BR_{DENS}	$counts.m^{-2}$	341	0	69	0	205	0	0	0	24	22	0	141	7	0	16	0	47	0	0	14	38
DP_{DENS}	$counts.m^{-2}$	514	1047	1665	593	1977	57	586	754	2760	14715	4677	2804	4520	2415	294	2318	549	1028	2834	9867	2784
GR_{BMASS}	$g.m^{-2}$	0,340	0,075	0,103	0,088	0,250	0,069	0,031	0,311	0,568	0,485	0,121	0,202	0,237	0,172	0,012	0,147	0,259	0,050	0,157	0,251	0,427
SCR_{BMASS}	$g.m^{-2}$	0,127	0,041	0,022	0,072	0,083	0,068	0,010	0,248	0,018	0,178	0,022	0,058	0,074	0,070	0,003	0,071	0,197	0,015	0,097	0,041	0,276
BR_{BMASS}	$g.m^{-2}$	0,201	0,000	0,010	0,000	0,107	0,000	0,000	0,000	0,002	0,018	0,000	0,015	0,048	0,000	0,001	0,000	0,004	0,000	0,000	0,001	0,020
DP_{BMASS}	$g.m^{-2}$	0,012	0,034	0,071	0,017	0,060	0,001	0,020	0,062	0,548	0,290	0,099	0,129	0,115	0,102	0,008	0,075	0,058	0,035	0,060	0,210	0,131

Table 3.3: Environmental variables and associated measurements for KwaZulu-Natal in autumn and spring (2015). Abbreviations are provided in the list of abbreviations.

		Autumn									Spring								
	unit	DUZE	MZIM3	MZIM1	CASC	UMGE1	UMGE3	UMLA	UMGE7	MVOT	DUZE	MZIM3	MZIM1	CASC	UMGE1	UMGE3	UMLA	UMGE7	MVOT
pH		7,5	7,8	7,2	7	7,4	6,9	6,7	7,3	6,8	7,4	8	7,3	7	6,9	7,1	7,1	7,6	7,4
EC	$\mu S.cm^{-1}$	302	80	59	120	92	89	65	86	91	37,7	110	7,9	159	117	91	92	118	118
NO2-N	$mg.l^{-1}$	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,05	0,001	0,001	0,001
NO3-N	$mg.l^{-1}$	1,05	0,2	0,23	0,67	0,72	0,05	0,05	0,05	0,05	0,33	0,05	0,15	0,6	0,59	0,14	0,05	0,05	0,05
TIN	$mg.l^{-1}$	3,391	0,251	0,281	0,821	0,771	0,101	0,101	0,101	0,101	1,701	0,181	0,331	0,731	0,741	0,33	0,201	0,211	0,191
PO4-P	$mg.l^{-1}$	0,158	0,006	0,003	0,008	0,022	0,007	0,003	0,003	0,003	0,005	0,003	0,003	0,003	0,003	0,003	0,003	0,003	0,005
Rs_{min}	$MJ.m^{-2}$	3	4	4	1	1	2	2	2	3	30	50	50	24	21	23	38	16	27
Rs_{cum}	$MJ.m^{-2}$	116438	114620	115489	106553	105837	90581	80227	89000	113901	144189	137064	137064	129016	122426	113052	123023	112640	142469
WT_{CV}		0,087	0,143	0,080	0,088	0,120	0,074	0,184	0,128	0,289	0,177	0,233	0,071	0,074	0,235	0,127	0,220	0,175	0,756
WT_{MIN}	°C	17,2	11,3	11,5	9,9	11,6	15,3	8,4	12,0	3,9	13,1	9,4	14,7	12,7	10,9	16,0	8,3	13,9	8,0
WT_{MAX}	°C	24,8	18,8	14,8	15,0	16,8	19,5	18,3	18,3	18,0	29,6	26,3	19,7	16,6	27,6	25,7	23,2	28,4	18,0
WT_{CUM}	°C	628	490	414	431	455	565	452	489	418	670	585	541	467	595	658	486	647	266
Q_{CV}		0,862	0,899	0,926	0,883	0,098	0,298	0,165	0,239	1,549	0,476	0,503	0,537	0,497	0,041	0,287	0,336	0,244	0,605
$\#Fld_{\geq 2}$		16	8	31	16	0	3	0	1	8	4	0	0	3	0	3	0	0	0
$\#days_{\geq 1}$		129	129	127	141	0	173	52	97	68	88	0	0	82	0	120	1	109	0
$\#days_{\geq 2}$		51	87	95	49	0	133	0	3	24	7	0	0	8	0	59	0	0	0
$since_{\geq 2}$		10	66	64	21	765	0	379	65	84	23	199	196	17	886	0	495	186	202
$since_{\geq 1:2}$		846	520	1551	858	846	837	2258	1778	932	986	653	1683	980	967	957	2374	1896	1050
GR_{DENS}	$counts.m^{-2}$	3315	405	932	1968	1395	388	751	1087	1486	4713	638	1738	1566	750	927	408	1008	423
SCR_{DENS}	$counts.m^{-2}$	3298	168	283	166	1097	205	88	60	1226	4711	225	742	781	321	475	41	229	150
BR_{DENS}	$counts.m^{-2}$	0	58	87	0	16	0	58	119	24	0	146	101	0	44	0	63	57	22
DP_{DENS}	$counts.m^{-2}$	17	180	561	1802	283	183	605	908	236	3	266	895	785	386	453	304	722	251
GR_{BMASS}	$g.m^{-2}$	0,048	0,122	0,190	0,066	0,060	0,010	0,159	0,138	0,062	0,289	0,277	0,382	0,107	0,205	0,018	0,144	0,180	0,059
SCR_{BMASS}	$g.m^{-2}$	0,048	0,018	0,100	0,003	0,027	0,003	0,024	0,018	0,032	0,289	0,052	0,205	0,053	0,039	0,007	0,025	0,004	0,002
BR_{BMASS}	$g.m^{-2}$	0,000	0,095	0,061	0,000	0,022	0,000	0,117	0,093	0,015	0,000	0,212	0,103	0,000	0,152	0,000	0,101	0,156	0,038
DP_{BMASS}	$g.m^{-2}$	0,000	0,008	0,029	0,063	0,012	0,007	0,018	0,028	0,016	0,000	0,014	0,074	0,054	0,013	0,011	0,018	0,019	0,018

3.2.3 The effect of nutrient enrichment under natural flows in the Western Cape

Figure 3.2 shows that seasonality is the primary determinant of biomass, within which enrichment levels operate. Benthic biomass increased with an increase in the level of enrichment in both seasons (autumn and spring), but the pattern was much more distinct in autumn. The Kruskal-Wallis rank sum test found no significant difference between the enrichment-season groups (n = 65, df = 62, p = 0.4). A Nemenyi post hoc test indicated a significant difference only between the spring oligotrophic group (O_SP) and the autumn eutrophic group (E_AU), p = 0.0002.

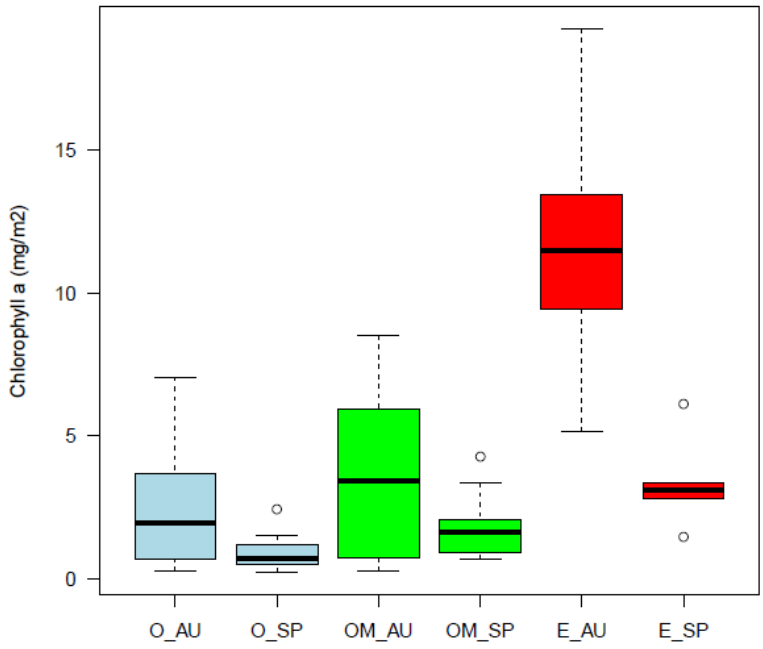


Figure 3.2: Box and whisker plots showing the medians and the interquartile range of Benthic chl *a* biomass (mg m^2) across a range of enrichment categories in rivers with natural flow regimes in the Western Cape in autumn and spring 2015. O = oligotrophic , OM = oligotrophic-mesotrophic , E = Eutrophic , Au = autumn and Sp = spring. Circles represent outliers.

3.2.4 The effect of flows under meso-eutrophic conditions in the Western Cape

Figure 3.3 shows that seasonality is a primary determinant of biomass, within which flow categories operate. Biomass decreased as the level of flow regulation increased. Biomass was greater in rivers with natural wet season flows (ND) compared to rivers with regulated wet season flows (DD). The Kruskal-Wallis rank sum test (n = 38, df = 37, p = 0.4) found no significant difference between the flow-season groups. The interquartile range in rivers with natural wet season flows and decreased dry season flows (ND) in autumn was markedly greater compared to any other group. There is thus some

indication that periphyton communities are able to accrue greater biomass in ND rivers compared to DD rivers.

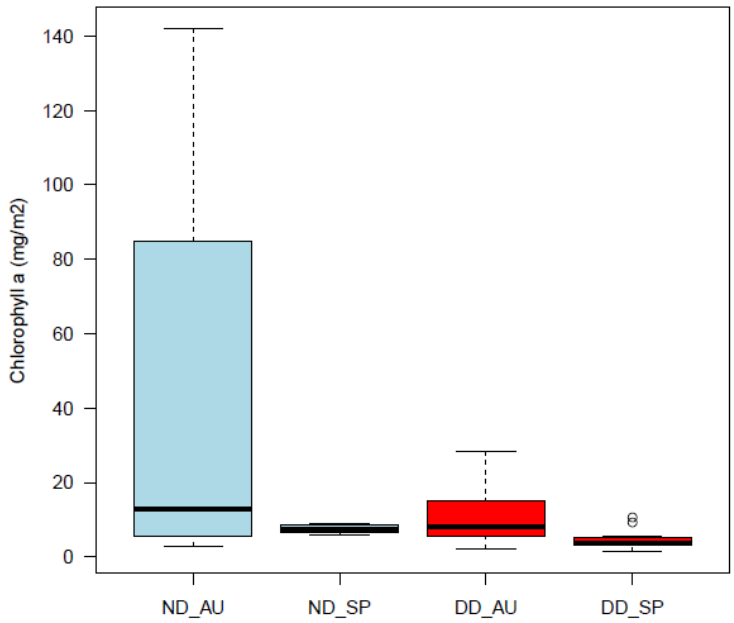


Figure 3.3: Box and whisker plots showing the medians and the interquartile range of Benthic chl *a* biomass (mg m^2) across two flow alteration categories in meso-eutrophic rivers in the Western Cape in autumn and spring 2015. ND = rivers with natural wet season flows but decreased dry season flows and DD = rivers with decreased wet and dry season flows. Au = autumn and Sp = spring.

3.2.5 Links between periphyton biomass and environmental variables

3.2.5.1 Western Cape (autumn and spring)

In the Western Cape in both autumn and spring, the variables explaining the greatest variation in site biomass on their own were WT_{MAX} and NO_3-N followed by EC, PO_4-P and DP_{DENS} (Table 3.4a). The best subset of variables that had significant effects, explained 60 % in the overall variation in site biomass out of 71%, which was explained by all variables. In order of decreasing strength, these comprised of WT_{MAX}, NO_3-N, EC, DP_{DENS} and RS_{CUM}. WT_{MAX} explained approximately 44% of this variation (Table 3.4a).

The distance based redundancy analysis (dbRDA) ordination shows that sites clustered according to their *a priori* assignment of enrichment levels, regardless of season (Figure 3.4a). WT_{MAX}, NO_3-N and EC measured greatest at the enriched sites and separated enriched sites from nutrient poor sites. Closer investigation of the dbRDA plot revealed that WT_{MAX}, NO_3-N and EC separate nutrient poor

(oligotrophic and oligo-mesotrophic) from more enriched sites (mesotrophic, meso-eutrophic and eutrophic), and could potentially be used as proxies for enrichment, based on periphyton biomass.

3.2.5.2 Western Cape (autumn)

In the Western Cape in autumn, the variables explaining the greatest variation in site biomass on their own were TIN and pH followed by EC and WT_{MAX} (Table 3.4b). The best subset of variables that had significant effects, explained 62% in the overall variation in site biomass, out of 65%, which was explained by all variables. In order of decreasing strength, these comprised of TIN, RS_{MIN}, RS_{CUM} and DP_{DENS}. TIN explained approximately 48% of this variation (Table 3.4b).

The dbRDA ordination shows that sites clustered according to their *a priori* assignment of enrichment levels (Figure 3.4b). TIN measured highest at the enriched sites (meso-eutrophic and eutrophic) and separated them from nutrient poor sites (oligotrophic, oligo-mesotrophic and mesotrophic).DP_{DENS} was greatest at nutrient poor sites (oligotrophic and oligo-mesotrophic). Closer investigation of the dbRDA revealed that TIN could be a proxy for enrichment, although, the relationship was weak.

3.2.5.3 Western Cape (spring)

In the Western Cape in spring, the variables explaining the greatest variation in site biomass on their own were WT_{MAX}, EC, PO_4-P, Since $_{\geq 1:2}$ and NO_3-N (Table 3.4c). The best subset of variables that had significant effects, explained 72% in the overall variation in site biomass, out of 78%, which was explained by all variables. In order of decreasing strength, these comprised of WT_{MAX}, Q_{CV}, SCR_{DENS}, pH and PO_4-P. WT_{MAX} explained 48% of this variation (Table 3.4c).

The dbRDA ordination shows that sites clustered according to their *a priori* assignment of enrichment levels (Figure 3.4c). PO_4-P measured highest at enriched sites and separated enriched from nutrient poor sites. WT_{MAX} also measured highest at enriched sites and separated enriched from nutrient poor sites. However, upon closer investigation, none of these environmental variables could be identified as robust proxies for enrichment.

3.2.5.4 KwaZulu-Natal (autumn and spring)

In KwaZulu-Natal in autumn and spring, the variables explaining the greatest variation in site biomass on their own were #Days $_{\geq 1}$, WT_{CV}, #Fld $_{\geq 2}$ and Since $_{\geq 2}$ (Table 3.5a). The best subset of variables that had significant effects, explained 41% in the overall variation in site biomass, out of 63%, which was explained by all variables. In order of decreasing strength, these comprised of #Days $_{\geq 1}$, WT_{MAX}, pH, NO_2-N, Since $_{\geq 1;2}$ and NO_3-N. #Days $_{\geq 1}$ explained approximately 17% of this variation, which was double the % contribution compared to other variables (Table 3.5a).

The dbRDA ordination shows that sites did not cluster according to their *a priori* assignment of enrichment levels (Figure 3.5a).

3.2.5.5 KwaZulu-Natal (autumn)

In KwaZulu – Natal in autumn, the variables explaining the greatest variation in site biomass on their own were #Days $_{\geq 1}$, Since $_{\geq 2}$ and GR_{BMASS} (Table 3.5b). The best subset of variables that had significant effects, explained 63% in the overall variation in site biomass, out of 66%, which was explained by all variables. In order of decreasing strength, these comprised of #Days $_{\geq 1}$, Since $_{\geq 2}$, WT_{CV}, pH and TIN. Since $_{\geq 2}$ and WT_{CV} together explained 40% of this variation.

The dbRDA ordination shows that sites did not cluster according to their *a priori* assignment of enrichment levels (Figure 3.5b)

3.2.5.6 KwaZulu-Natal (spring)

In KwaZulu – Natal in spring the variables explaining the greatest variation in site biomass on their own were Since $_{\geq 2}$, Q_{CV}, #days $_{\geq 2}$, WT_{CV} and #Days $_{\geq 1}$ (Table 3.5c). The best subset of variables, that had significant effects, explained 52% in the overall variation in site biomass, out of 57%, which was explained by all variables. In order of decreasing strength, these comprised of Since $_{\geq 2}$, #Days $_{\geq 2}$, PO_4-P and WT_{MAX} (Table 3.5c). Since $_{\geq 2}$, explained 25% of this variation.

The dbRDA ordination shows that sites did not cluster according to their *a priori* assignment of enrichment levels (Figure 3.5c)

Table 3.4: Relationship between square-root transformed replicate Benthic biomass (mg chl *a* m^2) and environmental variables across sites in a) the Western Cape in autumn and spring, b) the Western Cape in autumn and c) the Western Cape in spring in 2015 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 and ran for 9999 permutations. 'Proportion' indicates the percentage of Benthic chl *a* biomass variation explained by a variable when considered alone 'Cumulative' 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 vector abbreviations are given in the list of abbreviations. Environmental data on which these analyses are based are presented in Table 3.2. The Marginal tests explain how much variation each environmental explains, while the Sequential tests explain how much variation each environmental explains relative to other environmental variables.

a

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
WT_{MAX}	-	35447	93,128	0,0001	43,9%	-	-
NO_3-N	-	33964	86,404	0,0001	42,1%	-	-
EC	-	20505	40,508	0,0001	25,4%	-	-
PO_4-P	-	20325	40,034	0,0001	25,2%	-	-
DP_{DENS}	-	15851	29,068	0,0001	19,6%	-	-
Since $_{\geq 1:2}$	-	7759,8	12,653	0,0001	9,6%	-	-
pH	-	5990,1	9,5359	0,0006	7,4%	-	-
Rs_{CUM}	-	3158,9	4,8453	0,0185	3,9%	-	-

SEQUENTIAL TESTS

+WT_{MAX}	0,434	35447	93,128	0,0001	43,9%	43,9%	119
+NO_3-N	0,516	6886,8	21,158	0,0001	8,5%	52,4%	118
+EC	0,550	2976,4	9,8285	0,0002	3,7%	56,1%	117
+DP_{DENS}	0,571	1963	6,8036	0,0011	2,4%	58,5%	116
+Rs_{CUM}	0,583	1186,8	4,2279	0,0148	1,5%	60,0%	115

b

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
TIN	-	19451	49,014	0,0001	48,5%	-	-
pH	-	18049	42,589	0,0001	45,0%	-	-
EC	-	15713	33,523	0,0001	39,2%	-	-
WT_{MAX}	-	12506	23,578	0,0001	31,2%	-	-
DP_{DENS}	-	9845,9	16,93	0,0001	24,6%	-	-
PO_4-P	-	9828,6	16,891	0,0001	24,5%	-	-
Q_{CV}	-	8861,3	14,757	0,0002	22,1%	-	-
Rs_{MIN}	-	2071,6	2,8336	0,0713	5,2%	-	-
Rs_{CUM}	-	1072,9	1,43	0,2243	2,7%	-	-

SEQUENTIAL TESTS

+TIN	0,475	19451	49,014	0,0001	48,5%	48,5%	52
+Rs_{MIN}	0,528	2418,8	6,7715	0,0007	6,0%	54,6%	51
+Rs_{CUM}	0,574	2108,1	6,543	0,0015	5,3%	59,8%	50
+DP_{DENS}	0,594	1047,7	3,4085	0,0313	2,6%	62,4%	49

MARGINAL TESTS

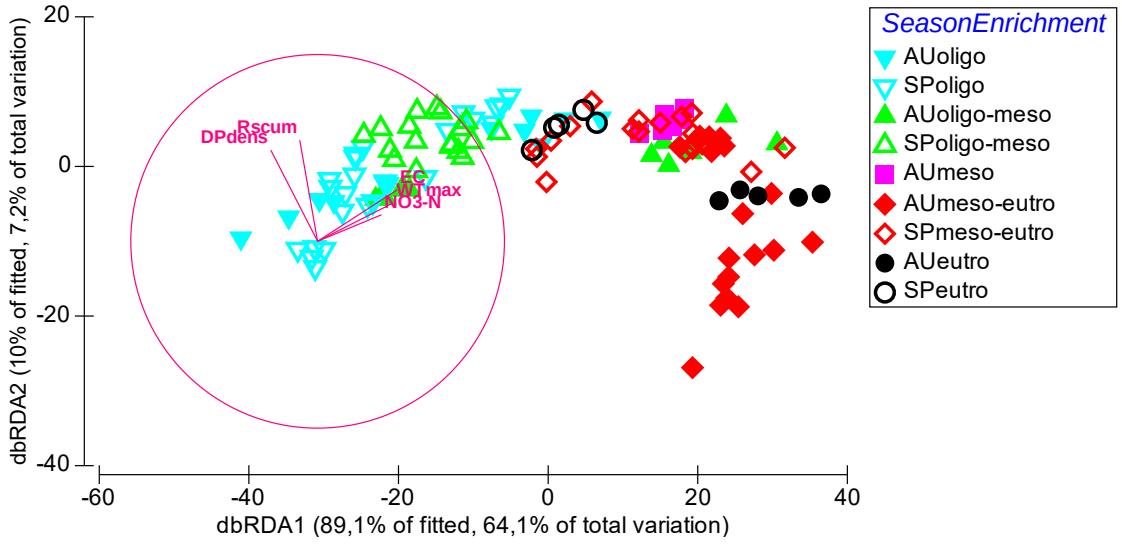
Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
WT_{MAX}	-	14333	57,343	0,0001	46,9%	-	-
EC	-	14317	57,222	0,0001	46,8%	-	-
PO_4-P	-	8296,8	24,201	0,0001	27,1%	-	-
Since $_{\geq 1:2}$	-	6808,5	18,616	0,0001	22,3%	-	-
NO_3-N	-	6786,9	18,54	0,0001	22,2%	-	-
BR_{DENS}	-	4146,5	10,196	0,001	13,6%	-	-
Q_{CV}	-	3129,5	7,4103	0,0053	10,2%	-	-
SCR_{DENS}	-	1183,3	2,6164	0,1006	3,9%	-	-
pH	-	375,25	0,80751	0,3901	1,2%	-	-

SEQUENTIAL TESTS

$+WT_{MAX}$	0,461	14333	57,343	0,0001	46,9%	46,9%	65
$+Q_{CV}$	0,564	3316	16,412	0,0001	10,8%	57,7%	64
$+SCR_{DENS}$	0,614	1661,3	9,2866	0,0011	5,4%	63,1%	63
+pH	0,637	836,8	4,9727	0,0167	2,7%	65,9%	62
$+PO_4$-P	0,702	2015,3	14,604	0,0001	6,6%	72,5%	61

c

a



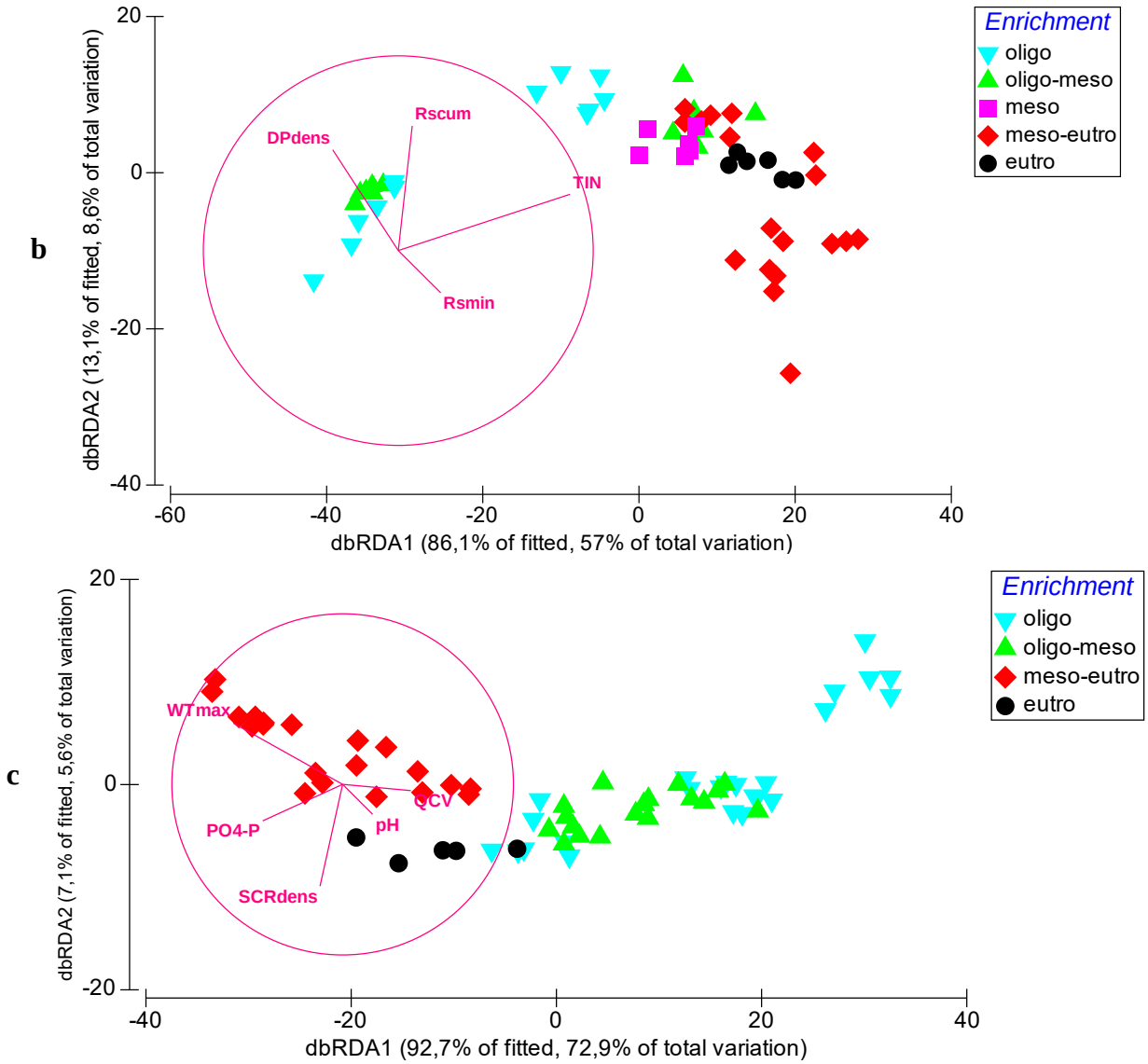


Figure 3.4: dbRDA ordinations of square-root transformed replicate Benthic biomass (mg chl *a* m^2) across sites in the a) the Western Cape in autumn and spring, b) the Western Cape in autumn and c) the Western Cape in spring in 2015 across enrichment categories and based on Malan and Day (2012). Environmental vector abbreviations are provided in the list of abbreviations and show the Spearman correlation between environmental variables and the dbRDA axes. Environmental data on which these analyses are based are provided in Table 3.2. AU = autumn, SP = spring, oligo = oligotrophic, oligo-meso = oligotrophic-mesotrophic, meso = mesotrophic, meso-eutro = mesotrophic-eutrophic and eutro = eutrophic.

Table 3.5: Relationship between square-root transformed replicate Benthic biomass (mg chl *a* m^2) and environmental variables across sites in a) KwaZulu-Natal in autumn and spring, b) KwaZulu-Natal in autumn and c) KwaZulu-Natal in spring in 2015 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 and ran for 9999 permutations. 'Proportion' indicates the percentage of Benthic chl *a* biomass variation explained by a variable when considered alone 'Cumulative' 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. Vector abbreviations are provided in the ist if abbreviations. Environmental data on which these analyses are based are provided in Table 3.3. The Marginal tests explain how much variation each environmental explains, while the Sequential tests explain how much variation each environmental explains relative to other environmental variables.

a

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
#Days $_{\geq 1}$	-	14413	18,101	0,0001	16,7%	-	-
WT_{CV}	-	10639	12,693	0,0003	12,4%	-	-
#Fld $_{\geq 2}$	-	9235,5	10,817	0,0001	10,7%	-	-
Since $_{\geq 2}$	-	8717,1	10,141	0,0007	10,1%	-	-
WT_{MAX}	-	7463,6	8,5443	0,0008	8,7%	-	-
Q_{CV}	-	5747,1	6,4387	0,0037	6,7%	-	-
NO_2-N	-	3089,6	3,3506	0,0462	3,6%	-	-
Since $_{\geq 1:2}$	-	2450,7	2,6374	0,0775	2,8%	-	-
pH	-	2128,7	2,282	0,1019	2,5%	-	-
NO_3-N	-	1960,9	2,098	0,1256	2,3%	-	-
SEQUENTIAL TESTS							
+#Days $_{\geq 1}$	0,158	14413	18,101	0,0001	16,7%	16,7%	90
+WT_{MAX}	0,209	5085,7	6,7982	0,0042	5,9%	22,7%	89
+pH	0,260	4960,5	7,0841	0,0023	5,8%	28,4%	88
+NO_2-N	0,297	3725,4	5,5982	0,0068	4,3%	32,7%	87
+Since $_{\geq 1:2}$	0,328	3196,7	5,0261	0,0108	3,7%	36,5%	86
+NO_3-N	0,373	4296,6	7,246	0,0025	5,0%	41,4%	85

b

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
#Days ≥ 1	-	4311,2	6,8885	0,0051	13,0%	-	-
Since $_{\geq 1:2}$	-	3807,1	5,9784	0,0125	11,5%	-	-
GR_{BMASS}	-	3606,7	5,6251	0,0153	10,9%	-	-
WT_{CV}	-	1487,9	2,1651	0,1312	4,5%	-	-
TIN	-	1238,5	1,788	0,1781	3,7%	-	-
pH	-	426,63	0,60064	0,4798	1,3%	-	-
Since $_{\geq 2}$	-	329,46	0,46246	0,5548	1,0%	-	-

SEQUENTIAL TESTS

+#Days $_{\geq 1}$	0,111	4311,2	6,8885	0,0056	13,0%	13,0%	46
+Since $_{\geq 2}$	0,307	6813,3	13,952	0,0003	20,6%	33,6%	45
$+WT_{CV}$	0,499	6444	18,255	0,0002	19,5%	53,1%	44
+pH	0,526	1164,1	3,484	0,0475	3,5%	56,6%	43
+TIN	0,587	2156,5	7,4171	0,0036	6,5%	63,1%	42

c

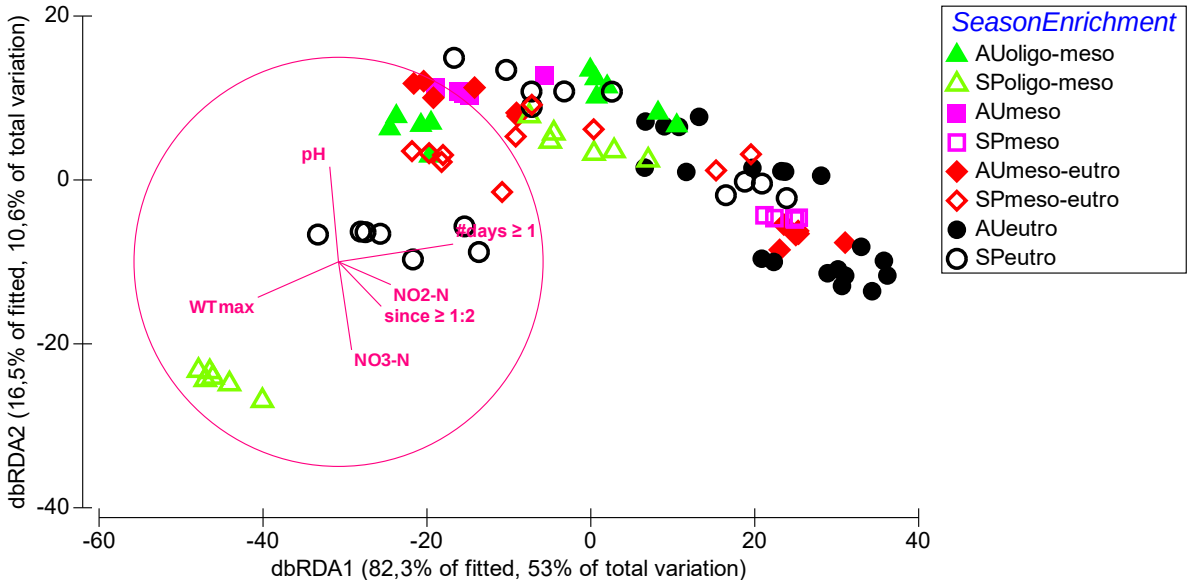
MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
Since $_{\geq 2}$	-	11781	13,984	0,0002	25,0%	-	-
Q_{CV}	-	7728,6	8,2313	0,0015	16,4%	-	-
#Days $_{\geq 2}$	-	6185,5	6,3399	0,0067	13,1%	-	-
WT_{CV}	-	5592,7	5,6505	0,0108	11,9%	-	-
#Days $_{\geq 1}$	-	5220,7	5,2278	0,0129	11,1%	-	-
#Fld $_{\geq 2}$	-	4075,1	3,9722	0,0332	8,6%	-	-
TIN	-	3749,1	3,627	0,0421	7,9%	-	-
pH	-	3593,6	3,4642	0,0465	7,6%	-	-
WT_{MAX}	-	3557,3	3,4263	0,0498	7,5%	-	-
PO_4-P	-	2403,4	2,2552	0,1188	5,1%	-	-

SEQUENTIAL TESTS

+Since $_{\geq 2}$	0,232	11781	13,984	0,0002	25,0%	25,0%	42
+#Days $_{\geq 2}$	0,371	7075	10,247	0,0005	15,0%	40,0%	41
$+PO_4$-P	0,440	3726	6,0631	0,0071	7,9%	47,9%	40
$+WT_{MAX}$	0,475	2112,9	3,6675	0,0324	4,5%	52,4%	39

a



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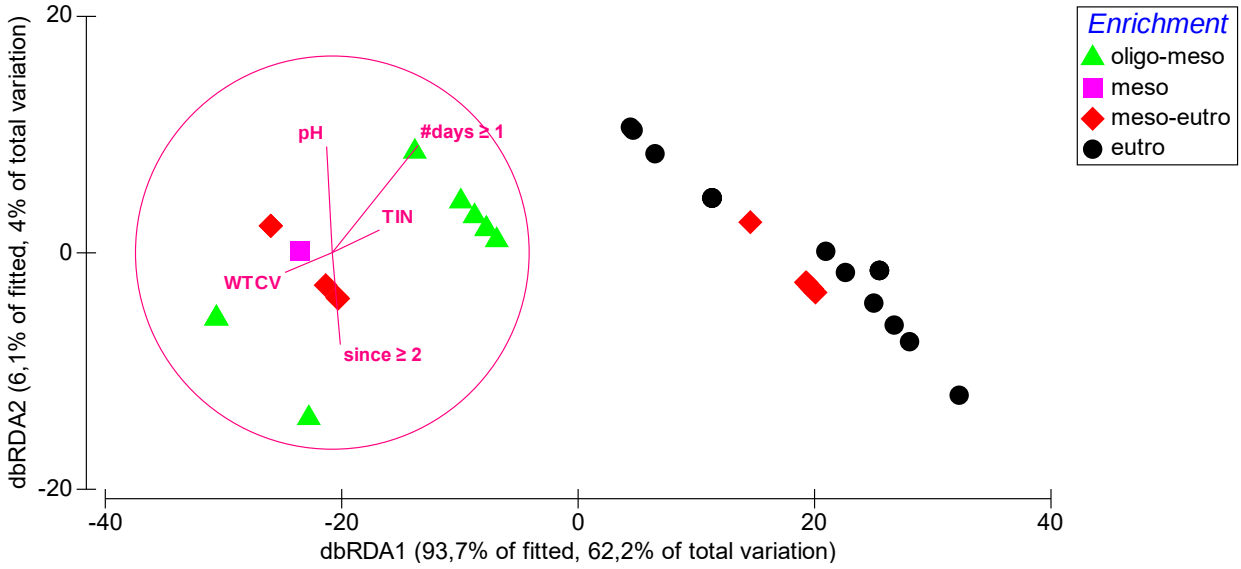


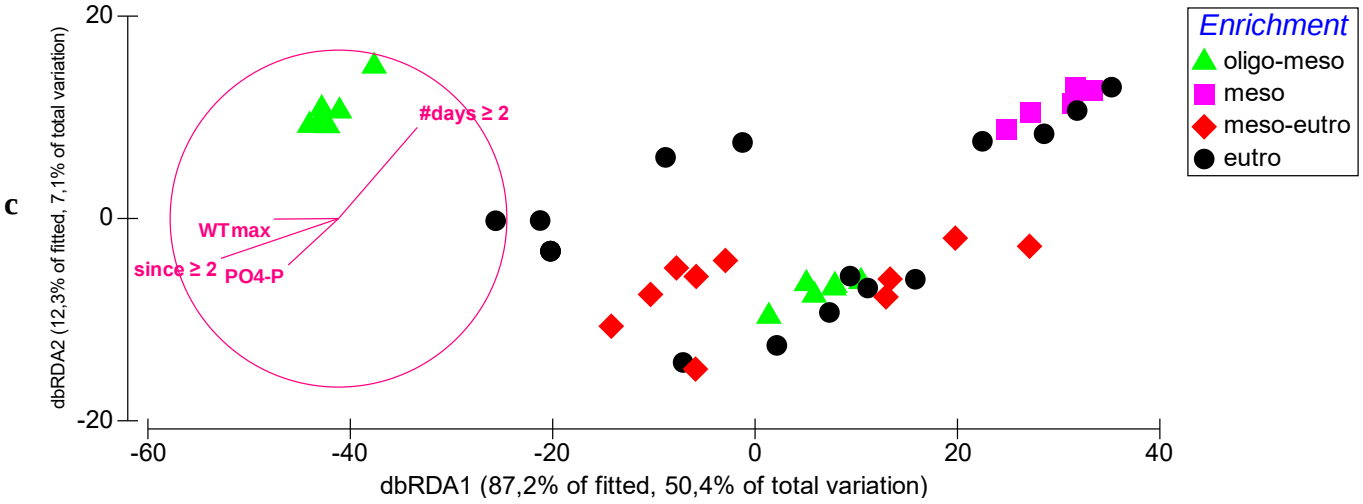


Figure 3.5: dbRDA ordinations of square-root transformed replicate Benthic biomass (mg chl *a*.m^2) across sites in a) KwaZulu-Natal in autumn and spring, b) KwaZulu-Natal in autumn and c) KwaZulu-Natal in spring in 2015 factored for enrichment based on Malan and Day (2012). Environmental vector abbreviations are provided in the list of abbreviations and show the Spearman correlation between environmental variables and the dbRDA axes. Environmental data on which these analyses are based are provided in Table 3.3. AU= autumn, SP = spring, oligo-meso = oligotrophic-mesotrophic, meso = mesotrophic, meso-eutro = mesotrophic-eutrophic and eutro = eutrophic.

3.3 Discussion

South African rivers are susceptible to enrichment (Davies & Day 1998) which can lead to a proliferation of periphytic biomass that degrades aquatic ecosystem functioning and the resource value of freshwater (Biggs 2000). This study aimed to understand regional spatial patterns in periphyton biomass by sampling from a winter rainfall (Western Cape) and summer rainfall (KwaZulu-Natal) area. Sites were sampled in autumn and spring, as these seasons mark the beginning and end of the periphyton growing stage respectively (Ewart-Smith 2012). Peak periphyton biomass was expected in autumn in the Western Cape after a period of stable flow conditions and minimum biomass was expected in spring, after the last spate dislodges any periphyton that persisted through the winter floods. By contrast, peak biomass in KwaZulu-Natal was expected in spring because of stable flow conditions, increases in water temperatures and an absence of grazers. Comparatively less biomass was expected at the end of summer, due to disturbance by flooding during elevated base flows, or by grazers during stable flow conditions. On the regional scale, periphyton biomass is expected to be driven primarily by the flow regime and mediated by nutrients (Biggs & Close 1989; Biggs et al 1998). Sites within each region were grouped according to levels of flow alteration and enrichment in order to understand their separate effects, considering their covariate relationship (Biggs & Close 1989; Townsend et al. 2012). A suite of environmental variables comprising metrics of flows, grazers, water

temperature, sunlight and spot measurements of nutrients, EC and pH were tested to understand their individual and relative influences on periphyton biomass. These environmental variables were also tested for potential use as proxies of periphyton eutrophication.

3.3.1 The effect of enrichment under natural flows

The effect of enrichment under natural flow conditions was not statistically significant, possibly due to comparing a single nutrient rich site to multiple nutrient poor sites. There was however visual evidence to show that biomass was higher in enriched sites compared to nutrient poor sites, irrespective of season, which was also found by Townsend et al. (2012). These findings suggest that periphyton biomass does respond to the level of nutrient availability in the Western Cape and sheds light on the importance of TIN and PO_4-P in biomass accrual, that measured much higher in the eutrophic versus oligotrophic and oligo-mesotrophic sites. Biomass loss may be greatest between seasons at the eutrophic site, owing to having the greatest pre flood biomass, as is suggested by (Uehlinger 1979; Biggs & Close 1989). In addition, high biomass communities are usually dominated by more erect periphyton forms that are more susceptible to increasing flows, compared to low biomass communities of prostate diatoms that tightly clasp to substrates (Power & Stewart 1987; Bourassa & Cattaneo 1998). High biomass communities are also more prone to increased self-shading and nutrient deprivation that weaken the basal cells, promoting dislodgement (Higgins et al. 2008). Because biomass was not significantly different between levels of enrichment, future studies should ensure that at least three sites are sampled per enrichment level.

3.3.2 The effect of flows under meso-eutrophic conditions

The effect of flow alteration (impounded versus non impounded rivers) had no significant effect on periphyton biomass, which may suggest that different flow regimes do not change the influence of nutrients under enriched conditions. However, there is also the possibility that enriched conditions smoothen out the differences in the flow regime which may be reflected rather in community structure so that changes may be recorded in community structure shifts, but not necessarily in biomass (Biggs et al. 1999). Although Dodds et al. (1998) states that an increase in flows pulse nutrients through the water column, this may be more applicable to oligotrophic rivers where periphyton are nutrient limited (Biggs et al. 1998). Unfortunately this study could not test for the effect of flows under oligotrophic conditions, as these sites all belonged to a single flow category (natural flows). Future studies should

identify oligotrophic sites with a range of flow alterations in order to test whether increased flows provide the same effect as an increase in nutrients.

3.3.3 Links between periphyton biomass and environmental variables

3.3.3.1 Western Cape

Periphyton biomass in the Western Cape was explained most consistently by water temperature, nutrients (NO_3-N) and EC across and within seasons, which potentially suggests the use of these variables as predictors of periphyton biomass. Water temperatures were not important in summer, probably because temperature was not a limiting factor. However, in spring, water temperature was a top contributor into explaining spatial variation in periphyton biomass, which may be a result of inter-site variation due to disparities in river volumes and whether they were regulated or not (Rader et al. 2008). In terms of nutrients, TIN may an important predictor of periphyton biomass in summer, perhaps due to increased concentrations during this period when river volumes are low (Ewart-Smith 2012). PO_4-P was more important in spring than summer, whose concentrations may be higher and more variable between sites due to runoff from agricultural land (Oberholster et al. 2016). However, PO_4-P concentrations were similar across sites in spring, which indicates that the snapshot samples may not have captured the true PO_4-P concentrations. PO_4-P has been to shown to often limit periphyton growth (Dodds et al. 2002, Dodds & Smith 2016). Francoeur et al. (1999) found that seasonal periphyton biomass was explained by nitrates and water temperature, which in this study rather separated non enriched from enriched sites, irrespective of season. EC did not explain seasonal variation in periphyton biomass, but rather separated oligotrophic from more enriched sites, irrespective of the season. Sunlight was only important in the summer season, indicating that some sites may be light limited compared to others.

It is surprising that flow in the Western Cape explained very little variation in periphyton biomass, as it is widely recognised to be a primary regulator of periphyton biomass (Biggs & Close 1989; Biggs 1995; Clausen & Biggs 1997; Stevenson et al. 2006; Ewart-Smith 2012). Only in spring after the winter flooding season did Q_{CV} account for approximately 10% of the overall biomass variation, which compared to the percentages explained by WTMAX (~ 47%) in spring and TIN (48%) was small. Possible reasons why Ewart-Smith (2012) found flows to be important could be due to sampling during a year of intense flooding with more class 4 and 1:2 year floods relative to this study (Appendix 4). In addition, her study contained more than double the number of biomass samples, which may have

lended greater capacity from which to draw inferences. There is also the possibility that the flow metrics used in this study were not ecologically meaningful enough to explain spatial variation in periphyton biomass. Hoyle et al. (2016) mention that flow metrics that are calculated on exported flow data can be meaningless, and should be based on the minimal flow discharge that is able to suspend sediments, based on knowledge of the spatio-temporal variability of the bed load. Furthermore, they place emphasis on knowing the local shear stress velocities on periphyton communities, which considers age, type and health. Although grazer biomass appeared to be higher at the pristine sites (BERG, BOK, DWAR, JAND and JONKE), there was no link between grazer biomass and Benthic chlorophyll *a*, perhaps due to use of the length specific biomass data from Ewart-Smith (2012) that was limited to two rivers. Nevertheless, this finding is congruent with Ewart-Smith (2012) according to her grazer exclusion experiments. Feminella & Hawkins (1995) and Hillebrand (2002) by contrast however found that grazers significantly decreased periphyton biomass. Grazer abundance also did not explain periphyton biomass variation, which could be due to a misrepresentation in abundances by not weighting according to length classes that vary in the amount of periphyton they consume.

The author is unaware of any studies that weight macroinvertebrate abundance based on their length classes. Nevertheless, there is some indication that deposit feeders (2,6%) and scrapers (5.4%) do contribute marginally to spatial variation in periphyton biomass in autumn and spring respectively. These findings suggest that periphyton biomass in the Western Cape is explained by bottom up influences rather than disturbances, considering the relatively small proportion of biomass explained by flows and grazers relative to nutrients, water temperature and sunlight.

Although biomass mirrored an enrichment gradient in the Western Cape irrespective of season, it was only EC and to a lesser extent TIN that showed potential to be used as proxies of enrichment.PO_4-P should not be discounted as an enrichment proxy, whose ranges may be too small to noticed by DISTLM's. EC may be a more reliable indicator of nutrient concentrations, as it's concentration does not diminish with increasing periphyton biomass (Biggs & Close 1989). This does not necessarily suggest that nutrients are not a good indicator of enrichment, but rather that reliable inferences can't be drawn from snapshot sampling, given their spatio-temporal variability (Wagenhoff et al. 2011) .

3.3.3.2 KwaZulu-Natal

In contrast to the Western Cape in which periphyton biomass was primarily driven by nutrient availability, water temperature and sunlight, periphyton biomass in KwaZulu-Natal was explained primary by flow and water temperature metrics. The flow regime in KwaZulu-Natal may influence spatial variation in periphyton biomass for two reasons: the first being that periphyton growth occurs in the rainy season, where disparities in the flow regime are expected to differ between sites and influence periphyton loss differentially, secondly, the majority of sites were impounded, whose flow regimes that are expected to be similar, may actually be very different, depending on water management strategies between the sites. The most important flow metrics were Days $_{\geq 1}$ and Since $_{\geq 2}$ which indicate that the duration of class 1 floods and the time between class 2 floods in these regulated rivers explained the most spatial variation in periphyton biomass. Ewart-Smith (2017) suggested that periphyton communities in KwaZulu-Natal have many windows of opportunity to grow in summer between the flood events when flows are more stable, which may explain why the number of days since a class 2 or greater flood was an important flow metric.

Water temperature consistently contributed to spatial variation in periphyton biomass, although was not as influential as in the Western Cape. Nevertheless, the importance of water temperature in Kwa-Zulu-Natal may be attributed to the differences in the thermal regime between regulated and non-regulated rivers, as well as the water release strategies in regulated rivers. The release of impounded water may have profound effects on the thermal regime of the river downstream (Robinson et al. 2004).

Nutrient availabilities were not influential on periphyton biomass in KwaZulu-Natal compared to in the Western Cape. The majority of KwaZulu-Natal's rivers chosen in this study were enriched, which may suggest that there was no spatial disparity in nutrient availabilities with which to explain spatial differences in periphyton biomass. By contrast, sites in the Western Cape ranged from nutrient poor to enriched, which could account for the high spatial variability explained in this region.

Sunlight also accounted very little for spatial variation in periphyton biomass and may be a result of higher turbidity (Dodds & Cole 2007) in KwaZulu-Natal compared to the Western Cape.

3.4 Conclusion

Speculation still exists as to whether spatial differences in periphyton biomass are influenced more by differences in nutrient availabilities or differences in flow alteration. Future studies should test for the

effects of flow alteration in oligotrophic rather than enriched rivers, where an increase in flow may be reflected in periphyton communities that are not already saturated with nutrients. These tests should also ensure that each treatment (enrichment or flow alteration category) contains a sufficient and equal number of replicates. This study highlighted the shifting importance of environmental variables in KwaZulu-Natal and the Western Cape. The most influential variables were those that varied in availability and character across sites and were limiting to periphyton growth. Regional comparative studies that wish to periphyton biomass to environmental variables should ensure that a range of sites within regions are chosen that represent a range of environmental conditions. This will ensure that the best possible inferences can be drawn on the relative effects of environmental variables. From the observations of this study, future efforts to manage periphyton biomass should focus on keeping nutrient levels low and allow high flow events to maintain periphyton biomass at acceptable levels.

Chapter 4. Spatial patterns in periphyton community structure

4.1 Introduction

Chapter 3 focused on understanding the relative effects of nutrient enrichment against a control of flow alteration, and the relative effects of flow alterations against a control for enrichment on periphyton biomass in the Western Cape. The individual and relative influences of environmental variables in the Western Cape and KwaZulu-Natal were also tested, and where periphyton biomass followed a gradient in enrichment, a suite of environmental variables were tested for potential proxies of eutrophication. Chapter 4 also focuses on these objectives, but in terms of patterns in the periphyton community structure.

The periphyton community structure is a description of the assemblage of periphyton taxa and their attributes such as the periphyton group (diatoms, green algae, cyanobacteria) or growth form (single celled, colonial, filamentous). Each periphyton taxon has a preferred window of environmental conditions (Stevenson 2014). The group to which it belongs gives a sense of nutritional value to consumers (Chester & Norris 2006), and it's growth form gives an indication of the successional state of the community (Ewart-Smith 2012). A combination of group and growth form however puts the periphyton successional state into context of the season of occurrence, nutrient availabilities and characteristics of the flood regime (Ewart-Smith 2012). For example, single celled diatoms are usually found in high abundance during early succession, while filamentous green algae proliferate during late succession (Davie et al. 2012).

Many authors advocate the monitoring of shifts in periphyton community structure over biomass because environmental changes are not always reflected in biomass, but rather in the underlying periphyton assemblage, that shifts to adapt to the new environment (Biggs et al. 1999).

Shifts in periphyton biomass are associated with shifts in periphyton community structure according to a continuum of succession, which is determined by the environment and predicts what genera, groups and growth forms the periphyton community is made up of (Davie et al. 2012; Stevenson et al. 2006). Early successional periphyton taxa are represented by prostrate/adnate carbohydrate-rich single-celled diatoms, which occur in oligotrophic-natural flowing conditions (Poff & Ward 1995; Piggott et al. 2015) Late successional periphyton are characteristically non nutritious or unpalatable filamentous green algae and cyanobacteria (Chester & Norris 2006). As such, late successional periphyton taxa skew the flow of energy to higher trophic guilds and may not be able to satisfy the energetic demands of the entire food web (McCormick et al. 1997; Dodds 2006; Li et al. 2010; Davie et al. 2012). It is

therefore important to understand how the environment determines the rate of periphyton succession, to maintain good water quality and a healthy aquatic ecosystem. What follows is an introduction to the various periphyton groups and growth forms, where they place in the succession continuum, and how they are influenced by the environment.

4.1.1 Periphyton groups and growth forms

Single celled growth forms usually become outcompeted for nutrients and light during later succession and ensure that they persist through the floods, or recolonize substrata faster than other growth forms. The single celled growth forms have low growing structures that minimize the shear force of current velocities, unlike erect filaments that extend into the water column, and experience more abrasion and drag by the current (Stevenson 1992; Stevenson & Stevenson 2009; Hart et al. 2013). These structures are also able to clasp tightly to cobbles and grow within their crevices, as an additional means to avoid being dislodged (Bergey 1999). Some single celled diatom species grow within mucilaginous tubes, which is another strategy to minimize shear force when current velocities increase. Filamentous green algae have been found to proliferate under stable flow conditions that are enriched (Biggs & Thompson et al 1995; Biggs 1996; Gaizer et al 2005; Ewart-Smith & King 2012; Smolar-Žvanut & Mikoš 2013), but also under moderate flow velocities, which provides nutrients at a faster rate and thins out the boundary layer of these thick mats, in order to assimilate these nutrients faster (Dodds et al. 1998; Battin et al. 2003; Dodds 2006; Stevenson & Stevenson 2009). Cyanobacteria can dominate nitrogen rich waters when they are not able to fix nitrogen, while nitrogen fixing diatoms can dominate waters that are much lower in nitrogen (Henry & Fisher 2003).

4.1.2 Influence of the environment on periphyton community structure

4.1.2.1 The flow regime and enrichment

The flow regime has differential effects on periphyton taxa and as a consequence determines the assemblage of periphyton groups and growth forms (Francoeur & Biggs 2006). Diatoms typically form the largest proportion of periphyton communities, especially during early succession after flood disturbance (Ewart-Smith & King 2012). During this time, single celled diatoms are the first colonisers of the periphyton mat, because they are able to persist through floods by reducing shear force as a result of their low growing structures and colonization within crevices. These diatoms adopt the R selection life history strategy, by having small cells that colonise substrate early and grow fast to

achieve low biomass (Biggs 1998). A shift to filamentous diatoms is made under stable flow conditions that have good water quality, whose abundance is dependent on the length of the dry season. Filamentous diatoms adopt the S selection life history strategy, by having small to medium cells that are slow to colonise substrate, slow to grow and achieve low biomass (Ewart-Smith 2012). An increase in nutrient availabilities then gives rise either to green algae or cyanobacteria. In the Western Cape at least, it is hypothesized that colonial green algae are found under enriched conditions directly after spring floods that are at least a class 2 in magnitude, and adopt the R-selection life history (Ewart-Smith 2012). Filamentous green algae prevail under enriched stable flow conditions, whose biomass is determined by the length of the dry season. Filamentous green algae adopt the C-S or C selection life history, which have large cells, colonise substrate slowly and grow slowly, but achieve high eventual Benthic biomass (Vos 2015). Single celled blue cyanobacteria can become abundant in winter in the absence of floods, whose abundance is determined by the frequency of flooding, and adopt the S strategy. Colonial cyanobacteria exist under elevated summer base flow conditions, which increase the net transfer of nutrients and are therefore either C or S life history strategists. Filamentous cyanobacteria that form gelatinous masses occur in late summer/autumn when base flows are very low and water temperatures are high. These are late succession species that adopt the C life history strategy.

4.1.2.2 Grazers

Grazers prefer diatom taxa over green algae and cyanobacteria because diatoms are more palatable and nutritious (Chester & Norris 2006). Preferential grazing encourages succession in periphyton communities, as diatoms become outcompeted by filamentous green algae and cyanobacteria (Townsend et al. 2012)

4.1.2.3 Water temperature, nutrients and sunlight

Diatoms prefer cooler temperatures compared to green algae and cyanobacteria and have a lower requirement for nutrients and sunlight (Passy 2007; Villeneuve et al. 2009).

The focus of this Chapter is to explain patterns in spatial variability of periphyton community structure at a broad regional scale by:

1) Broadening the knowledge on periphyton taxa in South African rivers
2) Improving the understanding of the relative strength of nutrients and flow alteration towards shaping periphyton communities
3) Assessing whether community structure mirrors the level of enrichment and identifying environmental variables that act as a proxy for enrichment
4) Improving on the understanding of which environmental variables explain shifts in community structure across and within seasons

4.2 Results

4.2.1 Community Structure Patterns

Periphyton representing 51 taxa were identified from both regions, of which 28 were diatoms (Bacillariophyta), 13 green algae (Chlorophyta) 6 cyanobacteria (Cyanophyta), 2 euglenophytes (Euglenophyta), 1 dinoflagellate (Dinogflagellata) and 1 golden brown algae (Chrysophyta). Forty taxa were described in the Western Cape of which 22 were diatoms, 11 green algae and 5 cyanobacteria, with 17 of these taxa not occurring in KwaZulu-Natal. Thirty four taxa were identified in KwaZulu-Natal of which 19 were diatoms, 8 green algae and 5 cyanobacteria, with 11 of these taxa not occurring in the Western Cape, concluding that 28 taxa were not found in both regions. A list of algal taxon densities (cells m^2) which are length weighted and averaged per site for each season and region is provided in Appendix 1a and b.

4.2.1.1 Western Cape

Total cell densities were generally greater in autumn compared to spring, and most distinct at the enriched sites (HEXR, PALM1, DUIW, EERS). Diatoms dominated in both seasons, irrespective of the level of enrichment, while filamentous green algae were more abundant at enriched sites in autumn (Figure 4.1). A breakdown of these algal groups into their respective growth forms shows that single celled diatoms dominate the oligotrophic (BERG, BOKR, JONKE, PALM2), oligo-mesotrophic (DWAR, JAND, BREE) and mesotrophic (OLIF) sites, followed by colonial diatoms and single celled cyanobacteria, By contrast, the enriched sites (HEXR, PALM1, DUIW, KLEIN, EERS) contained higher densities of branched and unbranched filamentous greens (Figure 4.2).

Fragilaria sp., *Navicula* sp., *Gomphonema* sp. and *Achnanthidium* sp. represented the highest diatom densities in autumn while *Navicula* sp., *Achanthidium* sp. and *Achnanthidium oblongella* represented the highest densities in spring. *Fragilaria* sp. and *Gomphonema* sp. were orders of magnitude lower in spring and taxa such as *Eunotia* sp. and *Aulocoseira* sp. increased during this season. In terms of green algae, *Oedogonium* sp. densities were orders of magnitude greater than other green algae in autumn, while *Stigeoclonium* sp. was orders of magnitude greater in spring. Cyanobacteria were represented by *Chamaesiphon* sp. and *Lyngba* sp. in autumn, and by *Chamaesiphon* sp., *Dichotrix* sp. and *Phormidium* sp. in spring.

4.2.1.2 KwaZulu-Natal

Total cell densities were greater in spring compared to autumn, and not necessarily more distinct at the enriched sites, as in the Western Cape. Diatoms were also the most common group in this region, albeit not as dominant as in the Western Cape, owing to filamentous green algae and cyanobacteria being more common in this region (Figure 4.1). However, when looking at the growth forms it is evident that branched filamentous green algae and branched filamentous cyanobacteria are more abundant at the mesotrophic (UMGE3), meso-eutrophic (MZIM1, MVOT) and eutrophic sites (MZIM3, UMLA) (Figure 4.2).

Gomphonema sp., *Navicula* sp., *Encyonopsis leei*, *Fragilaria* sp. and *Achanthidium* sp. represented the highest diatom densities in both seasons. *Stigeoclonium* sp., *Mougeotia* sp., *Scenedesmus* sp. and *Oedogonium* sp. represented the highest green algae densities in autumn and spring. *Heteroleibleinia* sp., *Chamaesiphon* sp., *Phormidium* sp. and *Dichothrix* sp. represented the highest cyanobacteria densities in autumn and spring, with the addition of *Lyngba* sp. in spring. *Dinobryon* sp., a brown alga was found only at UMLA in autumn.

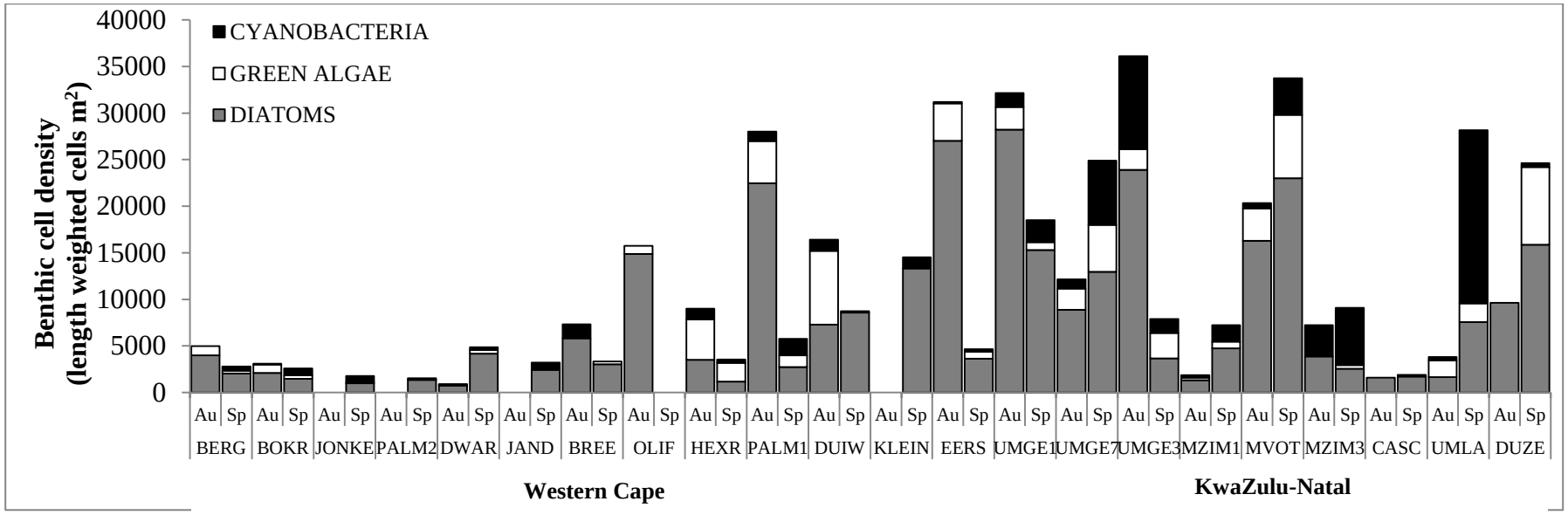


Figure 4.1: Community structure (algal group) shown across sites and seasons in the Western Cape and KwaZulu-Natal. Benthic cell densities were length weighted and averaged per site. Empty spaces indicate that the site of interest was not sampled during that season. Au = autumn and sp = spring.

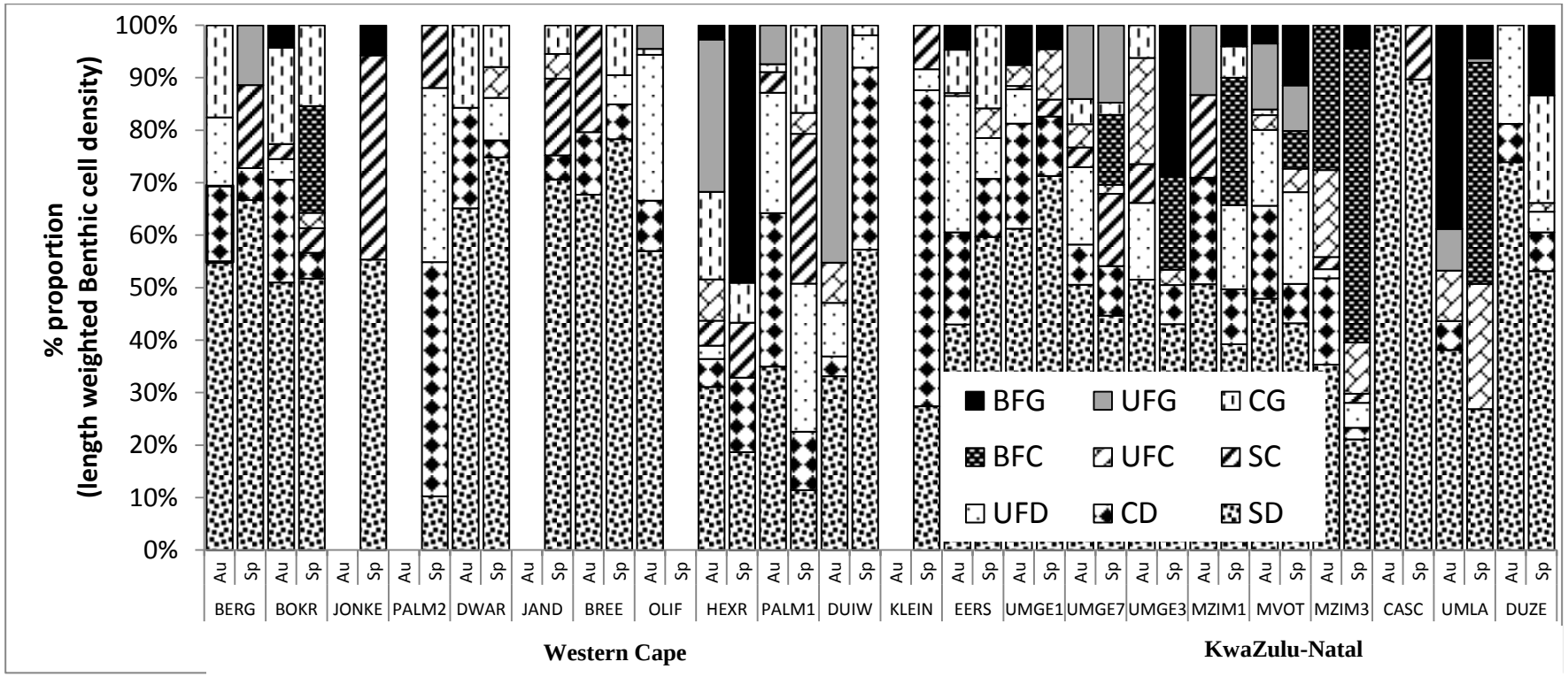


Figure 4.2: Community structure (algal group and growth form) shown across sites and seasons in the Western Cape and KwaZulu-Natal in terms of the relative proportion of Benthic cell densities per site. Proportions were calculated from length weighted densities which were averaged per site. SD = single celled diatoms, CD = colonial diatoms , UFD = unbranched filamentous diatoms, SC = single celled cyanobacteria , UFC = unbranched filamentous cyanobacteria, BFC = branched filamentous cyanobacteria, CG = colonial greens, UFG = unbranched filamentous greens and BFG = branched filamentous greens. Au = autumn and sp = spring

4.2.2 The effect of nutrient enrichment under natural flows in the Western Cape

A comparison of community structure in the Western Cape across levels of enrichment (oligotrophic, oligo-mesotrophic, eutrophic) in naturally flowing rivers shows three patterns. Firstly, community structure is similar at the majority of oligotrophic and oligo-mesotrophic sites, besides for the oligo-mesotrophic site BREE (shown in group b). Secondly, community structure at the oligotrophic and oligo-mesotrophic sites (group a) regardless of season was different compared to the eutrophic site (group c). Thirdly, seasonal differences in community structure were most evident at the eutrophic site (Figure 4.3).

A SIMPER analysis was performed to test for community structure differences between a) nutrient poor and b) more enriched sites, which revealed a 94% dissimilarity. *Eunotia* sp., *Eunotia rhomboidea* and *Desmococcus* sp. were only found at the nutrient poor sites while *Navicula* sp.,*Cocconeis* sp., *Gomphonema* sp., *Sellophora* sp. and *Scenedesmus* sp. were only found at more enriched sites (group b), (Figure 4.4).

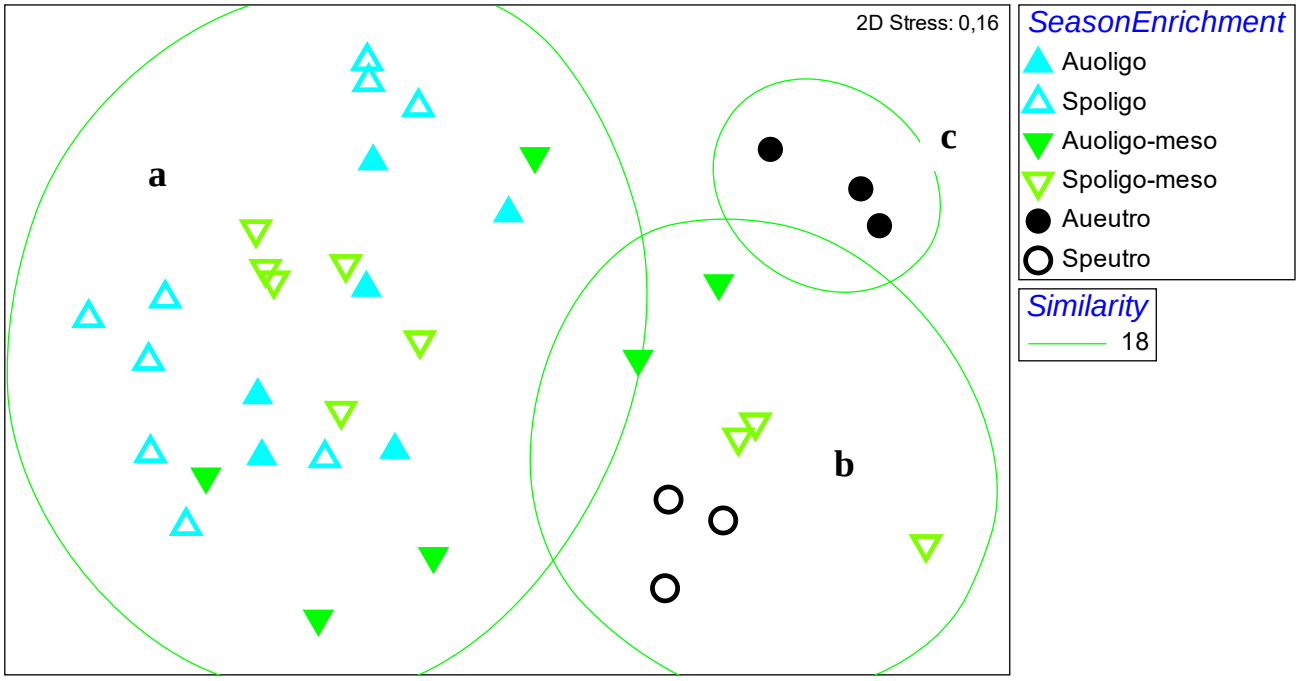


Figure 4.3: MDS plot of the community structure based on length weighted and square root transformed Benthic cell densities in the Western Cape in naturally flowing rivers across levels of enrichment in 2015. Oligo = oligotrophic, oligo-meso = oligo-mesotrophic and eutro = eutrophic. Sites within an oval border are 18% similar. Au = autumn and SP = spring.

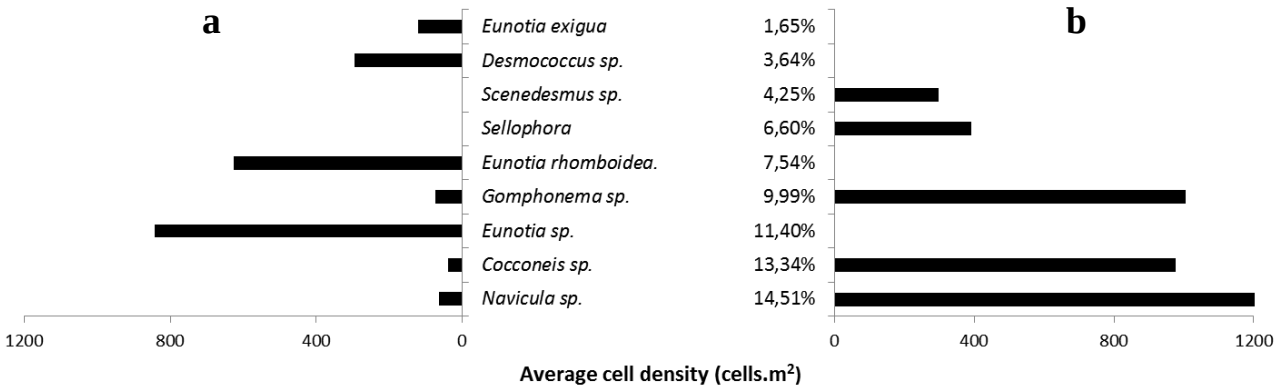


Figure 4.4: SIMPER results of periphyton taxa abundances based on length weighted and square root transformed Benthic cell densities. The Bray-Curtis dissimilarity compared taxa between group a) oligotrophic/oligo-mesotrophic sites and group b) oligo-mesotrophic/eutrophic sites in naturally flowing rivers in the Western Cape in 2015. Average taxa dissimilarities between groups are given in percentages with the total dissimilarity between group a and b being 94%. Community structure data are based on Appendix 1.

A SIMPER analysis was performed to test for community structure differences under eutrophic conditions across seasons revealed an 85% dissimilarity (Figure 4.5). Autumn communities had much higher densities of *Navicula* sp., *Fragilaria* sp. and *Gomphonema* sp. and taxa such as *Diadesmis* sp. were entirely absent in spring.

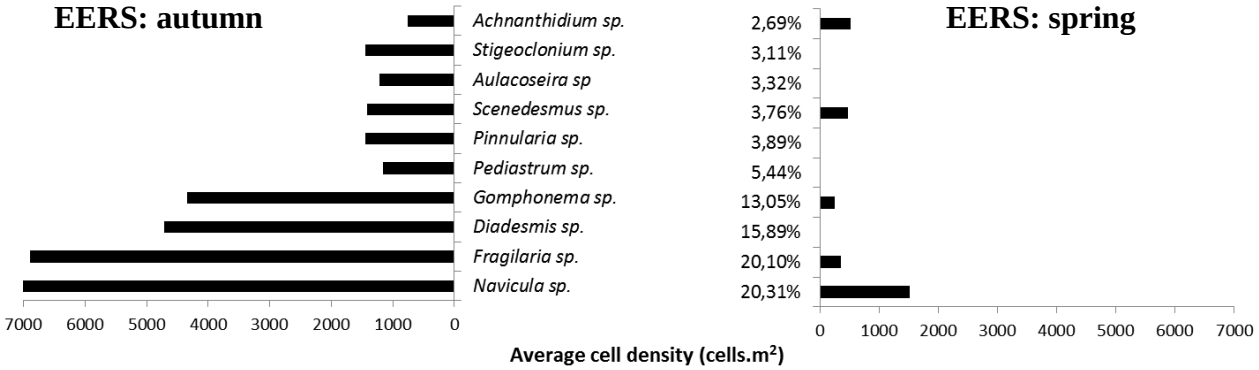


Figure 4.5: SIMPER analysis of periphyton taxa abundances based on length weighted and square root transformed Benthic cell densities. The Bray-Curtis dissimilarity compared taxa between the EERS site in autumn and spring in naturally flowing rivers in the Western Cape in 2015. Average taxa dissimilarities between groups are given in percentages with the total dissimilarity between autumn and spring being 85%. Community structure data are based on Appendix 1.

4.2.3 The effect of flows under meso-eutrophic conditions in the Western Cape

Figure 4.6 shows that community structure does not vary due to flow alteration, but rather due to large discrepancies in flood events over the wet season. In autumn, PALM1 and HEXR, which are both

impounded, were more similar before the winter rains compared to after. Appendix 5 shows that PALM1 experienced class 2 and 3 floods in the wet season (June – October 2015), while HEXR barely experienced class 1 floods. The SIMPER analysis (Figure 4.7) further showed that taxa such as *Aulocoseira* sp and *Chamaesiphon* sp. occurred at PALM1 while taxa such as *Stigeoclonium* sp occurred at HEXR.

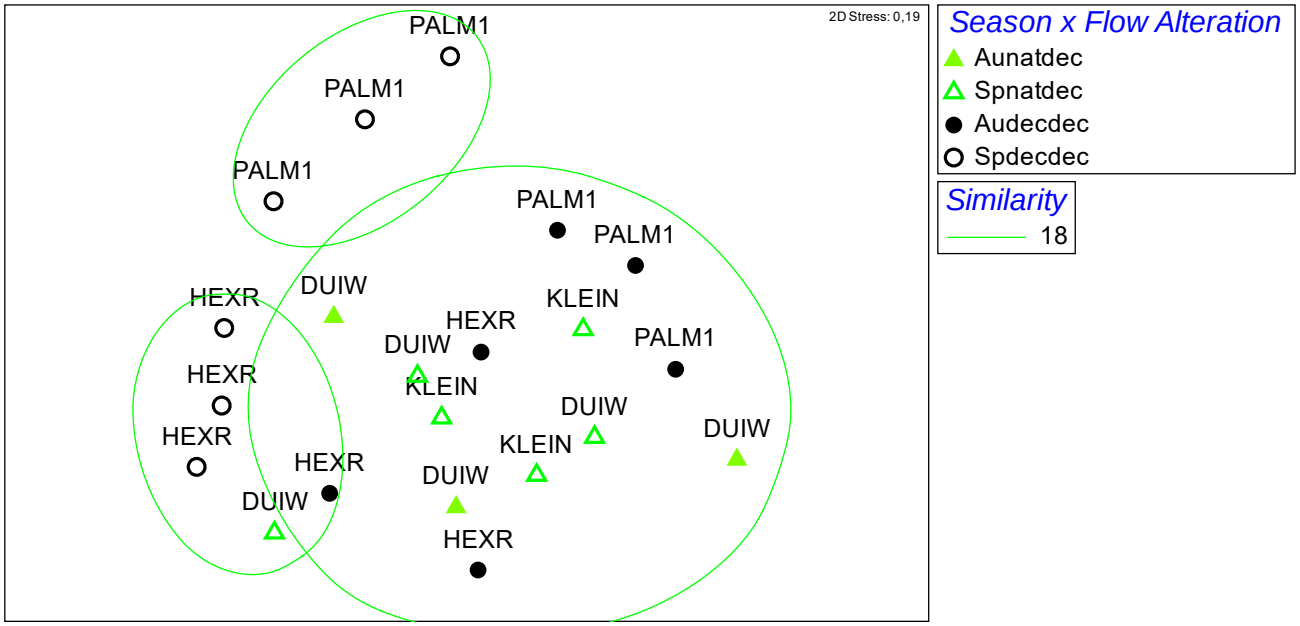


Figure 4.6: MDS plot of the periphyton taxa abundances based on length weighted and square root transformed Benthic cell densities in the Western Cape in meso-eutrophic rivers across levels of flow alteration in 2015. Natdec = rivers with natural wet season flows but decreased dry season flows due to abstraction and decdec = rivers with decreased wet and dry season flows, due to impounding and abstraction. Au = autumn and Sp = spring. Sites within an oval border are 18% similar. Community structure data are based on Appendix 1.

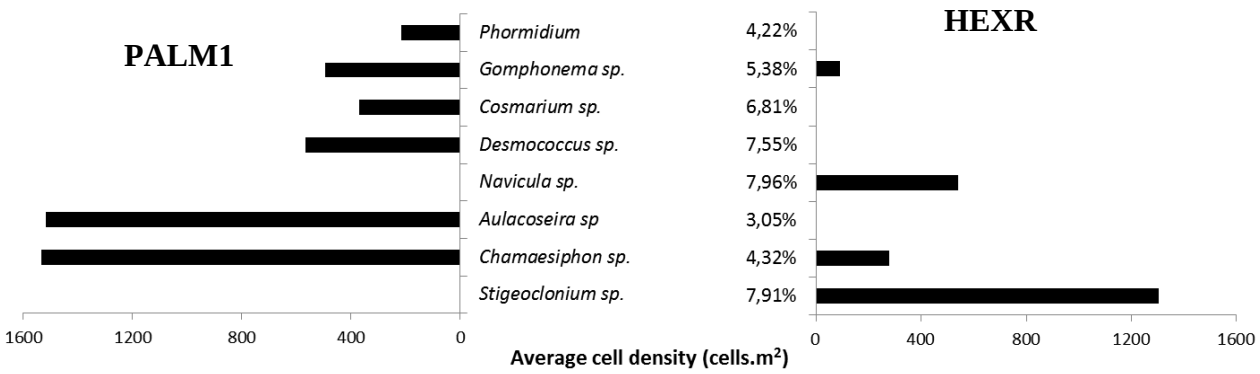


Figure 4.7: SIMPER results of periphyton taxa abundances based on length weighted and square root transformed Benthic cell densities. The Bray-Curtis dissimilarity compared taxa between sites PALM1 and HEXR in the Western Cape in 2015. These sites are mesotrophic, impounded and abstracted. Average dissimilarities in taxa between these sites are given as percentages with the total dissimilarity between them being 85%. Community structure data are based on Appendix 1.

4.2.4 Links between periphyton community structure and environmental variables

4.2.4.1 Western Cape (autumn and spring)

The variables explaining the greatest variation in community structure on their own were EC, PO_4-P, TIN and WT_{MAX} (Table 4.1a). The best subset of variables that had significant effects explained 57% in the overall variation in community structure. In order of decreasing contribution, the top five contributors comprised of EC, NO_3-N, WT_{CV}, PO_4-P and DP_{DENS} (Table 4.1a). The dbRDA ordination explained 45% of the fitted variation on 10 PCA axes, of which 30% was explained on the first two dbRDA axes (Figure 4.8a). The ordination separated sites based on their *a priori* level of enrichment, irrespective of season. The EC, NO_3-N and PO_4-P vectors separated enriched (mesotrophic, meso-eutrophic and eutrophic) from nutrient poor sites (oligotrophic and oligo-mesotrophic), but closer investigation revealed that only NO_3-N shows potential to be used as a proxy for enrichment.

4.2.4.2 Western Cape (autumn)

The variables explaining the greatest variation in community structure on their own were TIN, pH, EC, PO_4-P and WT_{MAX} (Table 4.1b). The best subset of variables that had significant effects explained 57% in the overall variation in community structure. In order of decreasing contribution, these comprised of TIN, since $_{\geq 1:2}$, WT_{MAX}, EC, NO_3-N and WT_{CUM}, of which TIN alone explained almost 22% (Table 4.1b). The dbRDA ordination explained 55% of the fitted variation on 10 PCA axes, of which 35% was explained on the first two dbRDA axes (Figure 4.8b). The ordination separated sites based on their *a*

priori level of enrichment, of which EC was the best proxy that explained community structure differences between oligotrophic and more enriched sites.

4.2.4.3 Western Cape (spring)

The variables explaining the greatest variation in community structure on their own were EC, PO_4-P, WT_{CUM} and TIN (Table 4.1c). The best subset of variables that had significant effects explained approximately 63% in the overall variation in community structure. EC was the top contributor, which was followed by flow and water temperature metrics (#days $_{\geq 1}$, $Q_{CV,}$ Since $_{\geq 1:2}$; #Fld $_{\geq 2}$) and water temperature (WT_{CV}, WT_{CUM}, WT_{MIN}) (Table 4.1c). EC however accounted for twenty two percent of this variation alone in the model. The dbRDA explained 52% of the fitted variation on 10 PCA axes, of which 37% was explained on the first two axes (Figure 4.8c). However, the ordination did not reflect community structure shifts along a gradient of enrichment, unlike in autumn where this pattern was distinct.

4.2.4.4 KwaZulu-Natal (autumn and spring)

All variables accounted for similar variations according to the marginal tests (Table 4.2a) which was also true for the sequential test. The best subset of variables explained 61% in the overall variation in community structure. These comprised of all variable types, i.e. flows, grazers, solar irradiation, water temperature and nutrients (Table 4.2a). The dbRDA ordination explained 44.4% on 10 PCA axes, of which 30.5% was explained on the first two axes (Figure 5.9a). According to the dbRDA ordination, community structure did not shift along a gradient of nutrient enrichment.

4.2.4.5 KwaZulu-Natal (autumn)

The variables accounting for the greatest variations according to the marginal tests included WT_{MAX}, #Fld $_{\geq 2}$, BR_{BMASS}, WT_{CUM} and EC (table 5.2b). The best subset of variables explained 71% in the overall variation in community structure, of which WT_{MAX}, #Fld $_{\geq 2}$, and BR_{BMASS} were top contributors (Table 4.2b). The dbRDA ordination explained 52% in the fitted variation on 10 PCA axes, of which 38% was explained on the first two axes (Figure 4.9b). There was however no pattern of community structure along a gradient of enrichment.

4.2.4.6 KwaZulu-Natal in spring

The variables explaining the greatest variation alone included Rs_{MIN}, TIN, PO_4-P and #days $_{\geq 1}$ (Table 4.2c). The best subset of variables explained 53% in the overall variation in community structure.

These comprised of Rs_{MIN}, PO_4-P, Q_{CV}, NO_3-N, WT_{MIN} and TIN (Table 4.2c). The dbRDA plot again showed no community structure shifts according to a gradient in enrichment, but explained 53% on 10 PCA axes, of which 33% was explained on the first two axes (Figure 4.9c).

Table 4.1: Relationship between length weighted and square-root transformed replicate community structure (Benthic cells m^2) and environmental variables across sites in a) the Western Cape in autumn and spring, b) the Western Cape in autumn and c) the Western Cape in spring in 2015 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 and ran for 9999 permutations. 'Proportion' indicates the percentage of community structure variation explained by a variable when considered alone 'Cumulative' is the cumulative percentage variation explained for each additional co-variate in the sequential tests, for which only significantly different ($p \leq 0.05$) relationships are shown. Environmental vector abbreviations are provided in the list of abbreviations and environmental data on which these analyses are based are presented in Table 3.2. The Marginal tests explain how much variation each environmental explains, while the Sequential tests explain how much variation each environmental variable explains relative to a group of environmental variables.

a

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
EC	-	34301	10,806	0,0001	15,0%	-	-
PO_4-P	-	32031	9,9737	0,0001	14,1%	-	-
TIN	-	27168	8,2546	0,0001	11,9%	-	-
WT_{MAX}	-	24997	7,5138	0,0001	11,0%	-	-
DP_{DENS}	-	12229	3,4584	0,0003	5,4%	-	-
Since $_{\geq 1:2}$	-	9749,1	2,7257	0,0036	4,3%	-	-
WT_{CV}	-	8754,9	2,4366	0,0091	3,8%	-	-
#Fld $_{\geq 2}$	-	7565,1	2,0941	0,022	3,3%	-	-
SCR_{BMASS}	-	7182,6	1,9848	0,0264	3,2%	-	-
Q_{CV}	-	6811,4	1,879	0,0407	3,0%	-	-

SEQUENTIAL TESTS

+EC	0,137	34301	10,806	0,0001	15,0%	15,0%	61
+NO_3-N	0,201	17279	5,8788	0,0001	7,6%	22,6%	60
+WT_{CV}	0,227	8672,3	3,0514	0,0002	3,8%	26,4%	59
+PO_4-P	0,255	8901,6	3,2516	0,0001	3,9%	30,3%	58
+DP_{DENS}	0,273	6354,1	2,3762	0,003	2,8%	33,1%	57
+NO_2-N	0,289	6048,2	2,3139	0,0031	2,7%	35,8%	56
+TIN	0,308	6495,6	2,554	0,0016	2,8%	38,6%	55
+Rs_{CUM}	0,323	5572,9	2,2406	0,0085	2,4%	41,1%	54
+#Fld $_{\geq 2}$	0,337	5090,7	2,088	0,0111	2,2%	43,3%	53
+#Days $_{\geq 2}$	0,358	6516,3	2,7616	0,0011	2,9%	46,2%	52
+Rs_{MIN}	0,373	5194,4	2,2545	0,0081	2,3%	48,4%	51
+Since ≥ 1:2	0,387	4805,3	2,1319	0,0125	2,1%	50,6%	50
+DP_{BMASS}	0,399	4348,9	1,9667	0,0235	1,9%	52,5%	49
+Q_{CV}	0,410	4173,8	1,9231	0,0271	1,8%	54,3%	48
+SCR_{BMASS}	0,420	3940,7	1,8477	0,039	1,7%	56,0%	47
+GR_{DENS}	0,429	3720,7	1,7733	0,0461	1,6%	57,7%	46

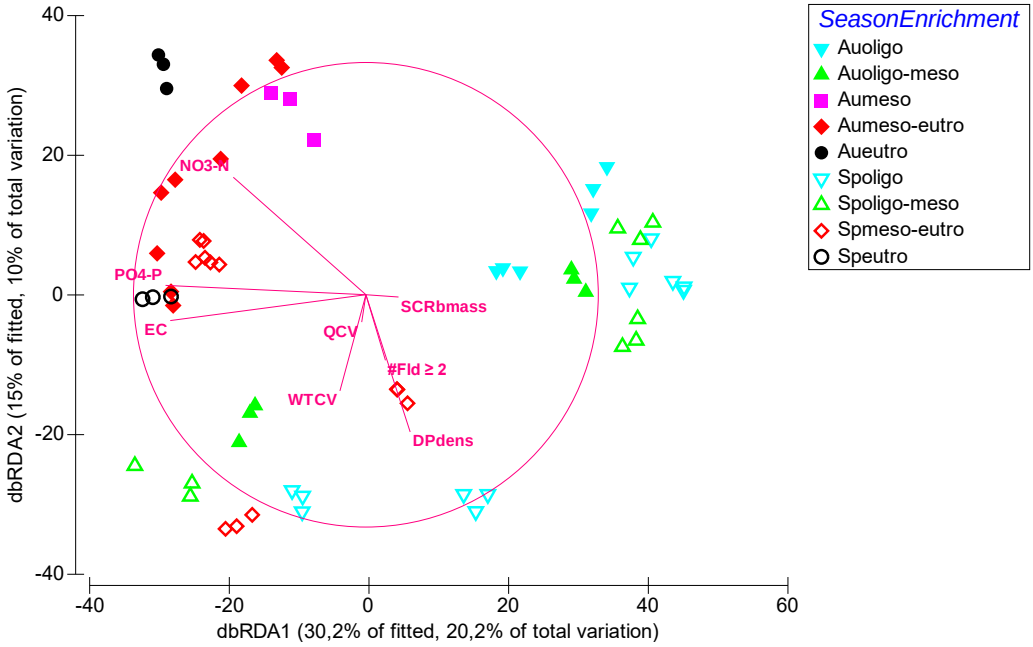
MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
TIN	-	19704	6,9911	0,0001	21,9%	-	-
pH	-	19600	6,9441	0,0001	21,7%	-	-
EC	-	19080	6,7102	0,0001	21,2%	-	-
PO_4-P	-	14102	4,635	0,0001	15,6%	-	-
WT_{MAX}	-	13886	4,5509	0,0001	15,4%	-	-
SCR_{BMASS}	-	9612,7	2,9833	0,0033	10,7%	-	-
DP_{DENS}	-	9477,4	2,9364	0,0032	10,5%	-	-
BR_{DENS}	-	9454,2	2,9284	0,0039	10,5%	-	-
Since $_{\geq 1:2}$	-	8887,1	2,7335	0,007	9,9%	-	-
WT_{CUM}	-	8486,2	2,5974	0,0098	9,4%	-	-
Q_{CV}	-	8353,4	2,5526	0,0076	9,3%	-	-
SEQUENTIAL TESTS							
+TIN	0,187	19704	6,9911	0,0001	21,9%	21,9%	25
+Since $_{\geq 1:2}$	0,244	7558,7	2,8839	0,0008	8,4%	30,2%	24
+WT_{MAX}	0,291	6317,5	2,5678	0,0018	7,0%	37,2%	23
+EC	0,340	6234,1	2,7239	0,0018	6,9%	44,2%	22
+NO_3-N	0,386	5635,1	2,6464	0,0024	6,2%	50,4%	21
+WT_{CUM}	0,443	6054,2	3,1319	0,0005	6,7%	57,1%	20

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
EC	-	28266	9,7632	0,0001	22,3%	-	-
PO_4-P	-	23399	7,7012	0,0001	18,5%	-	-
WT_{CUM}	-	21080	6,7855	0,0001	16,6%	-	-
TIN	-	15558	4,7595	0,0001	12,3%	-	-
BR_{DENS}	-	12501	3,7217	0,0005	9,9%	-	-
Since $_{\geq 1:2}$	-	11431	3,3715	0,0012	9,0%	-	-
SCR_{BMASS}	-	10993	3,23	0,0024	8,7%	-	-
Q_{CV}	-	9527,3	2,7645	0,0066	7,5%	-	-
#Days $_{\geq 1}$	-	8817,7	2,5432	0,011	7,0%	-	-
#Fld $_{\geq 2}$	-	8506,7	2,447	0,0103	6,7%	-	-
DP_{DENS}	-	6561,9	1,857	0,0556	5,2%	-	-
WT_{CV}	-	4653,1	1,2962	0,2161	3,7%	-	-
C							
SEQUENTIAL TESTS							
+EC	0,200	28266	9,7632	0,0001	22,3%	22,3%	34
+#Days $_{\geq 1}$	0,250	8798,2	3,239	0,0003	6,9%	29,3%	33
+Q_{CV}	0,300	8528,8	3,3649	0,001	6,7%	36,0%	32
+DP_{DENS}	0,350	8203,7	3,4883	0,0005	6,5%	42,5%	31
+Since $_{\geq 1:2}$	0,383	5867,6	2,6258	0,0042	4,6%	47,1%	30
+WT_{CV}	0,422	6309,6	3,0131	0,0013	5,0%	52,1%	29
+#Fld $_{\geq 2}$	0,452	5167,9	2,6044	0,0045	4,1%	56,1%	28
+WT_{CUM}	0,475	4251,3	2,2371	0,0162	3,4%	59,5%	27
+WT_{MIN}	0,500	4272,8	2,3619	0,0122	3,4%	62,9%	26

a



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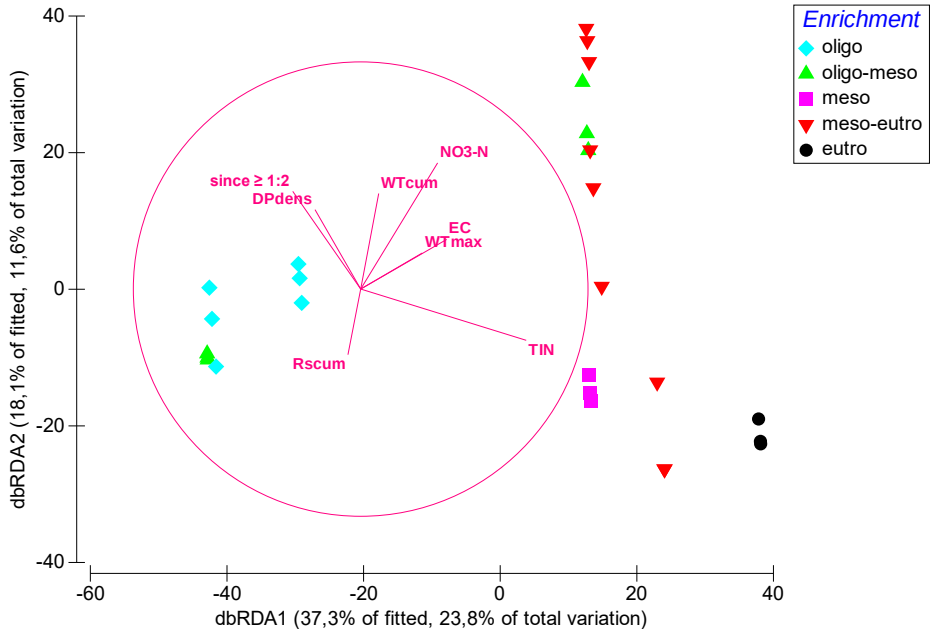

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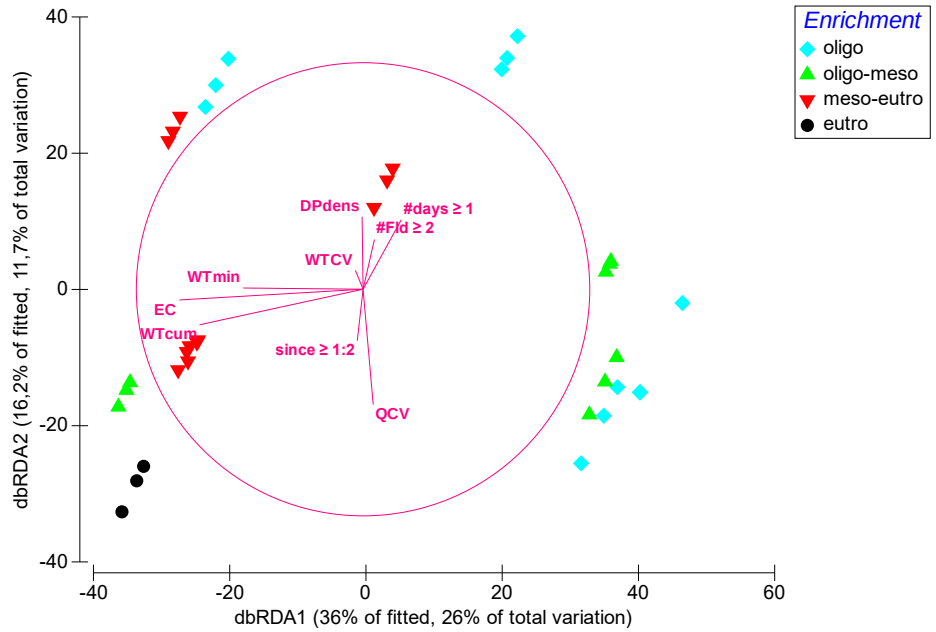


Figure 4.8: dbRDA ordinations of length weighted and square-root transformed replicate taxa Benthic cell densities (cells m^{-2}) across sites in a) the Western Cape in autumn and spring, b) the Western Cape in autumn and c) the Western Cape in spring in 2015 across enrichment categories and based on Malan and Day (2012). Environmental vector abbreviations are provided in the list of abbreviations and show the Spearman correlation between environmental variables and the dbRDA axes. Environmental data on which these analyses are based are provided in Table 3.2. AU = autumn, SP = spring, oligo = oligotrophic, oligo-meso = oligotrophic-mesotrophic, meso = mesotrophic, meso-eutro = mesotrophic-eutrophic and eutro = eutrophic.

Table 4.2: Relationship between length weighted and square-root transformed replicate community structure (Benthic cells m^2) and environmental variables across sites in a) KwaZulu-Natal in autumn and spring, b) KwaZulu-Natal in autumn and c) KwaZulu-Natal in spring in 2015 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 and ran for 9999 permutations. 'Proportion' indicates the percentage of community structure variation explained by a variable when considered alone 'Cumulative' is the cumulative percentage variation explained for each additional co-variate in the sequential tests, for which only significantly different ($p \leq 0.05$) relationships are shown. Environmental vector abbreviations are provided in the list of abbreviations and environmental data on which these analyses are based are presented in Table 3.3. The Marginal tests explain how much variation each environmental explains, while the Sequential tests explain how much variation each environmental variable explains relative to a group of environmental variables.

a

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
DP_{DENS}	-	10635	3,5031	0,0004	6,3%	-	-
#Days $_{\geq 1}$	-	9502,2	3,1077	0,0008	5,6%	-	-
WT_{CV}	-	8938	2,9129	0,001	5,3%	-	-
#Fld $_{\geq 2}$	-	8752,7	2,8492	0,0022	5,2%	-	-
pH	-	8041,9	2,6062	0,0042	4,8%	-	-
PO_4-P	-	7671,1	2,4803	0,0056	4,6%	-	-
Since $_{\geq 1:2}$	-	7671,2	2,4803	0,0073	4,6%	-	-
Q_{CV}	-	7514,2	2,4272	0,0072	4,5%	-	-
EC	-	7288	2,3508	0,0084	4,3%	-	-
TIN	-	7259	2,3411	0,0088	4,3%	-	-
Rs_{MIN}	-	6291,1	2,0168	0,0236	3,7%	-	-
SEQUENTIAL TESTS							
+DP_{DENS}	0,045	10635	3,5031	0,0002	6,3%	6,3%	52
+#Days $_{\geq 1}$	0,094	10940	3,7976	0,0002	6,5%	12,8%	51
+pH	0,144	10892	4,0034	0,0001	6,5%	19,3%	50
+TIN	0,181	8491,9	3,2626	0,0003	5,0%	24,3%	49
+Q_{CV}	0,213	7408,6	2,9602	0,0008	4,4%	28,7%	48
+Since $_{\geq 1:2}$	0,241	6645,6	2,7523	0,0025	3,9%	32,6%	47
+NO_2-N	0,260	5289,7	2,249	0,0162	3,1%	35,8%	46
+PO_4-P	0,277	4828,3	2,102	0,02	2,9%	38,7%	45
+Rs_{MIN}	0,303	5861,7	2,6451	0,0041	3,5%	42,1%	44
+Rs_{CUM}	0,351	8734,5	4,231	0,0001	5,2%	47,3%	43
+WT_{CUM}	0,374	5150,1	2,5868	0,0039	3,1%	50,4%	42
+Since $_{\geq 2}$	0,393	4521	2,3434	0,0083	2,7%	53,1%	41
+EC	0,425	5949,3	3,2532	0,0002	3,5%	56,6%	40
+WT_{MIN}	0,443	4126,8	2,3318	0,0074	2,4%	59,0%	39
+#Days $_{\geq 2}$	0,457	3372,8	1,9523	0,0361	2,0%	61,0%	38

b

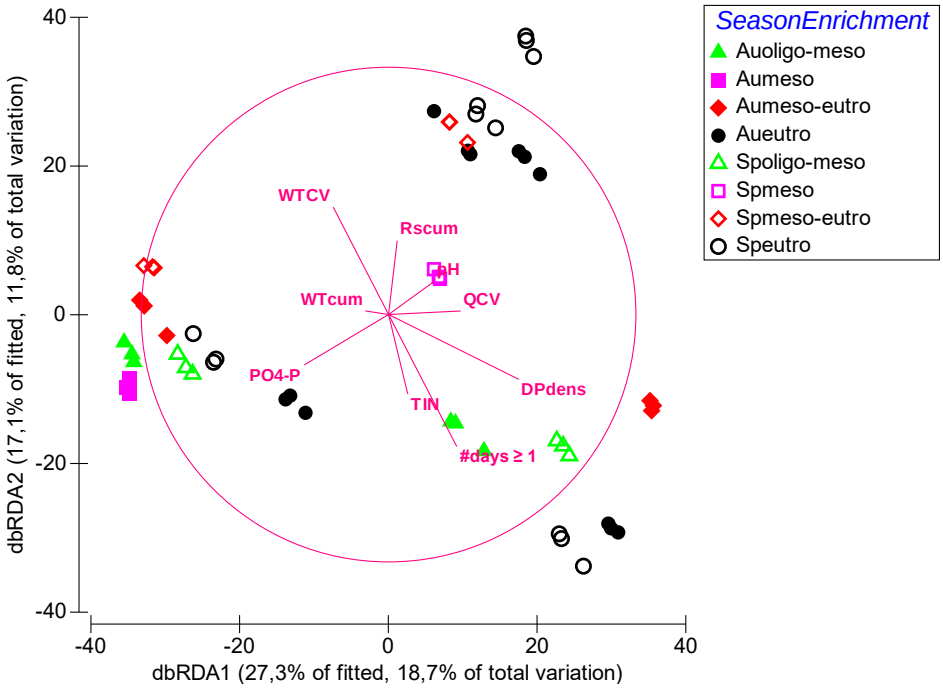
MARGINAL TESTS

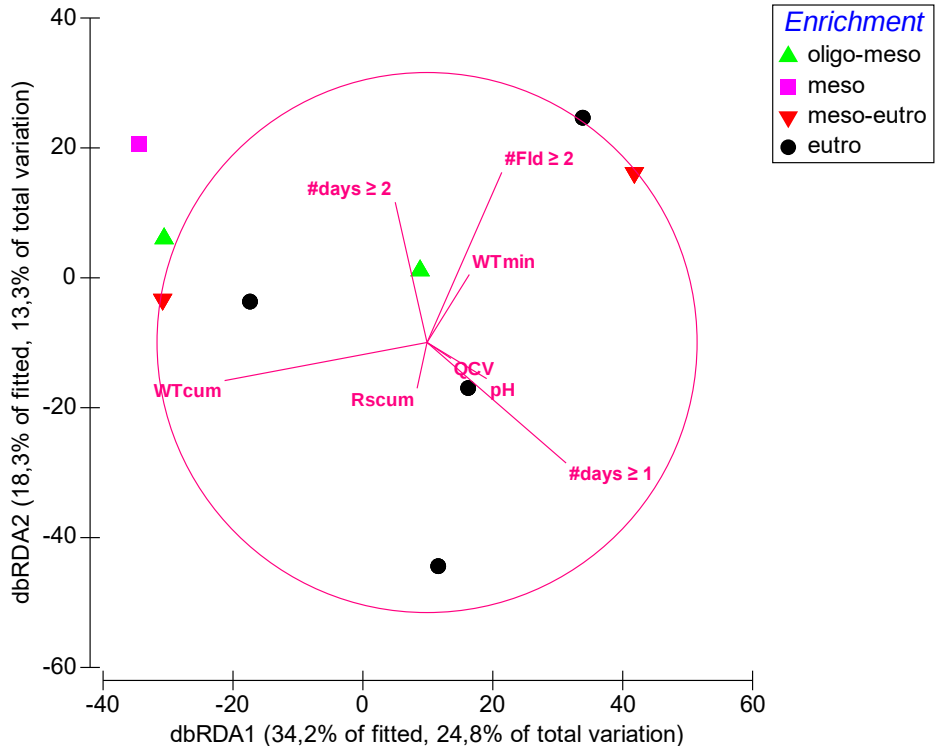
Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
WT_{MAX}	-	10155	3,5525	0,0001	12,4%	-	-
#Fld $_{\geq 2}$	-	9165,7	3,1625	0,0017	11,2%	-	-
BR_{BMASS}	-	8328,1	2,8407	0,0048	10,2%	-	-
WT_{CUM}	-	8058,9	2,7388	0,006	9,9%	-	-
EC	-	7473,2	2,5197	0,0079	9,2%	-	-
Since $_{\geq 1:2}$	-	7432,2	2,5045	0,0078	9,1%	-	-
Rs_{CUM}	-	7387,8	2,488	0,0108	9,1%	-	-
#Days $_{\geq 2}$	-	7187,6	2,4141	0,0144	8,8%	-	-
#Days $_{\geq 1}$	-	6532,6	2,1749	0,018	8,0%	-	-
Q_{CV}	-	6378,9	2,1195	0,0238	7,8%	-	-
pH	-	5097,6	1,6654	0,0847	6,2%	-	-
SEQUENTIAL TESTS							
+WT_{MAX}	0,089	10155	3,5525	0,0005	12,4%	12,4%	25
+#Fld $_{\geq 2}$	0,176	9349,4	3,6123	0,0008	11,5%	23,9%	24
+BR_{BMASS}	0,246	7677,3	3,2436	0,0019	9,4%	33,3%	23
+Q_{CV}	0,305	6406,9	2,9345	0,0009	7,8%	41,2%	22
+#Days $_{\geq 2}$	0,367	6326,5	3,1855	0,0012	7,8%	48,9%	21
+#Days $_{\geq 1}$	0,425	5608,1	3,1072	0,0021	6,9%	55,8%	20
+Rs_{CUM}	0,520	7439,2	4,932	0,0001	9,1%	64,9%	19
+pH	0,583	5094	3,8911	0,0003	6,2%	71,1%	18

c

MARGINAL TESTS

Variable	Adjusted R^2	SS(trace)	Pseudo-F	P	Proportion	Cumulative	Residual df
Rs_{MIN}	-	11107	3,8329	0,0002	13,3%	-	-
TIN	-	9185,8	3,0882	0,001	11,0%	-	-
PO_4-P	-	9045,1	3,0351	0,002	10,8%	-	-
NO_3-N	-	8628,1	2,8791	0,0015	10,3%	-	-
#Days $_{\geq 1}$	-	8565,3	2,8557	0,0023	10,3%	-	-
#Fld $_{\geq 2}$	-	7542,9	2,481	0,0075	9,0%	-	-
WT_{CV}	-	7409,1	2,4327	0,0055	8,9%	-	-
WT_{MIN}	-	7020,1	2,2933	0,0145	8,4%	-	-
Rs_{CUM}	-	6802,6	2,2159	0,016	8,1%	-	-
Q_{CV}	-	4562,3	1,444	0,1523	5,5%	-	-
SEQUENTIAL TESTS							
+Rs_{MIN}	0,098	11107	3,8329	0,0001	13,3%	13,3%	25
+PO_4-P	0,176	8862,9	3,3456	0,0005	10,6%	23,9%	24
+Q_{CV}	0,240	7418,6	3,0382	0,0005	8,9%	32,8%	23
+NO_3-N	0,312	7545,9	3,4148	0,0004	9,0%	41,8%	22
+WT_{MIN}	0,364	5696,1	2,7871	0,0028	6,8%	48,6%	21
+TIN	0,393	3908,9	2,0041	0,0345	4,7%	53,3%	20





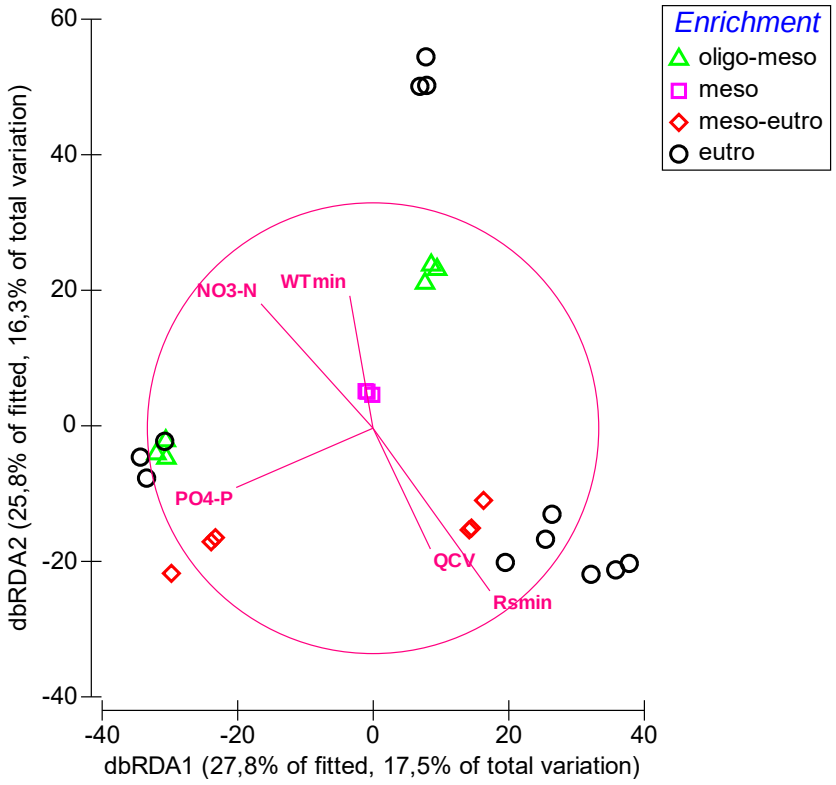


Figure 4.9: dbRDA ordinations of length weighted and square-root transformed replicate taxa cell densities (Benthic cells.m^{-2}) across sites in a) KwaZulu-Natal in autumn and spring, b) KwaZulu-Natal in autumn and c) KwaZulu-Natal in spring in 2015across enrichment categories and based on Malan and Day (2012). Environmental vector abbreviations are provided in the list of abbreviations and show the Spearman correlation between environmental variables and the dbRDA axes. Environmental data on which these analyses are based are provided in Table 3.3. AU = autumn, SP = spring, oligo = oligotrophic, oligo-meso = oligotrophic-mesotrophic, meso = mesotrophic, meso-eutro = mesotrophic-eutrophic and eutro = eutrophic.

4.3 Discussion

This Chapter aimed to broaden the knowledge on periphyton community structure in South African rivers, particularly those of the winter rainfall region of the Western Cape and summer rainfall region of KwaZulu-Natal. Distinct communities were expected between these regions, largely due to climatic, geomorphological and land use differences. Indeed, out of 55 taxa described, 28 were not shared between regions. Periphyton community structure was tested for a response to enrichment by controlling for the level of nutrient input, and for a response to flow alteration, by controlling for the level of enrichment. Furthermore, the separate and combined influences of environmental variables of flow, grazers, nutrients, water temperature and sunlight were assessed in order to explain variation in periphyton community structure. Where community structure followed a gradient of enrichment, environmental variables were further scrutinized to be considered as potential proxies of enrichment.

4.3.1 Community Structure in South African rivers

4.3.1.1 Cell densities

Taxa cell densities in the Western Cape decreased between autumn and spring across all sites, which is suspected to be associated with changes in the flow regime across seasons. Within seasons, cell densities were highest at the enriched sites, indicating that enrichment is a secondary influencer of periphyton community structure. There was also a seasonal difference in taxa cell densities in KwaZulu-Natal, except cell densities increased between autumn and spring. However, cell densities did not increase with enrichment, suggesting that enrichment does not explain spatial variation within seasons (more detail in section 4.3.4.2). By contrast, cell densities were found to be higher in oligotrophic-mesotrophic sites which could suggest a limitation at the enriched sites due to anoxic conditions that favour heterotrophic organisms (Dodds & Cole 2007), especially bacteria when waters are warm and rich in carbon (Villeneuve et al. 2011).

4.3.1.2 Growth form and taxa

Diatoms were the dominant group in both regions across seasons and levels of enrichment, suggesting not only their cosmopolitan distribution, but importance in periphyton communities. Diatoms such as *Navicula* sp. and *Gomphonema* sp. were the most common taxa found in this study under enriched conditions. *Navicula* sp. however was more tolerant of natural flow conditions while *Gomphonema* sp. enjoyed regulated flows, based on cell densities Single celled diatom growth forms in the Western Cape were well represented in autumn and spring, but in autumn were replaced by more elaborate growth forms of filamentous green algae (*Oedogonium* sp.) and cyanobacteria (*Lyngba* sp. and *Chamaesiphon* sp.).

In KwaZulu-Natal, there was no clear pattern in community structure shifts, based on algal group alone, but a combination of periphyton group and growth form revealed that branched filamentous cyanobacteria identified as *Dichothrix* sp. became more abundant in spring, which in KwaZulu-Natal represents a period of low flows after the dry winter season. *Dichothrix* is known to tolerate low flows and even desiccation (Keshari & Adhikary 2014). There was also an indication that single celled diatom communities shifted to communities of branched and unbranched filamentous green algae and branched cyanobacteria. This suggests that periphyton growth forms reflect enrichment changes better than periphyton groups.

4.3.2 The effect of enrichment under natural flow conditions in the Western Cape

When assessing for the effect of enrichment in naturally flowing rivers, it is evident that periphyton community structure is similar between nutrient poor sites, but different when comparing nutrient poor to enriched sites, irrespective of the season. Periphyton taxa such as *Eunotia* sp. were found in unenriched rivers, which is characteristic (Soininen 2002), while *Navicula* sp. (Teittinen et al. 2015) and *Gomphonema* sp. were found in enriched rivers, as expected (Dalu et al. 2015). A seasonal effect was only detected at the enriched site, suggesting that periphyton communities are susceptible to change between the end of summer (April) and early spring (October). The greater concentration of nutrients could potentially explain very high densities of diatoms such as *Navicula* sp., *Gomphonema* sp., *Diadesmis* sp. and *Fragilaria* sp., that were almost absent after the winter rains. The high loss in densities of taxa such as *Stigeoclonium* sp. and *Fragilaria* sp. could be attributed to their filamentous growth forms that extend into the water column, and experience increased drag force during the winter flooding season (Larned et al. 2004). This concludes that there is no seasonal effect on periphyton communities across space, but rather an enrichment effect because the assemblage of taxa between nutrient poor and rich sites was very different, irrespective of the season. Seasonality seems to rather act within an enrichment group and becomes more evident the greater the enrichment. This may be due to increased susceptibility of periphyton growth forms to seasonal changes in the environment between autumn and spring, where periphyton communities consist of different groups and growth forms during autumn and spring.

4.3.3 The effect of flow alteration in the Western Cape

When assessing for the effect of flow alteration in meso-eutrophic rivers, it was found that periphyton community structure was not responsive to various categories of flow alteration (see section 2.2), but rather large discrepancies in the flow regime (flood classes) over the wet season. The literature posits that faster flow velocities do not pulse nutrients in rivers that are already enriched due to saturation within periphyton mats Townsend et al. (2012). The HEXR and PALM1 belonged to the same flow category, yet contained very different periphyton communities due to the difference in the number of floods experienced. HEXR did not experience any floods in comparison to PALM1. After the winter season, HEXR was dominated by *Stigeoclonium* sp. that prefers stable low flow conditions, while PALM1 was dominated by *Chamaesiphon* sp. and *Aulocoseira* sp. that prefer nutrient poorer waters (Hill et al. 2003; Schneider & Lindstrøm 2011). However, prior to the winter season, PALM1 contained taxa representative of impacted conditions (*Gomphonema* sp., *Navicula* sp., *Fragilaria* sp.,

Achanthidium sp. and *Oedogonium* sp.) (Dalu et al. 2015). This suggests that the flood regime is able to reset periphyton communities to early succession, irrespective of the taxa that were established prior to flooding, and highlights the importance of allowing high flow events in order to flush out. undesirable periphyton that do not sufficiently contribute to the food web.

4.3.4 Links between community structure and environmental variables

4.3.4.1 Western Cape

Periphyton community structure in the Western Cape was explained by similar subsets of variables that explained biomass, indicating that the variables responsible for changes in Benthic biomass, are also responsible for changes in the community structure.

EC was a prominent variable in the Western Cape, as it explained spatial variation in biomass in autumn and in both seasons in terms of community structure. EC measures the ability of a body of water to conduct electricity, based on it's dissolved salt and ion content. This measurement is therefore nonspecific to nutrient availabilities, although should be considered a proxy (Biggs & Close 1989), as it indicates the total nutrient content, whether nutrients are in the water column, or harbored inside biota (Stevenson et al. 2006). High EC was noted at sites that were enriched, which had higher biomass and a different assemblage of periphyton compared to nutrient poor sites. The level of enrichment explained spatial patterns in periphyton biomass and community structure in autumn but not spring, probably because periphyton communities are much more similar during the beginning of succession after flood disturbance. NO_3-N was found to be the relevant component of these enrichment categories. The dbRDA showed that NO_3-N explained the shift in periphyton community structure along an enrichment gradient across seasons. NO_3-N was also observed to be a good separator of nutrient poor from nutrient rich sites, suggesting that NO_3-N could be a key limiting nutrient in the rivers of the Western Cape. TIN was the single most influential environmental variable on periphyton Benthic biomass and community structure in autumn, the growing season for periphyton (Ewart-Smith 2012) and suggests that TIN is an important predictor of periphyton communities.

Water temperature metrics also consistently had an effect on periphyton communities, but was more influential on periphyton community structure than biomass. WT_{MAX} appears to be the most ecologically meaningful water temperature metric, which probably reflects the differences in river sizes in this region. WT_{MAX} explained spatial variability in periphyton biomass in spring and community

structure in autumn, but also spatial variability across seasons. The importance of WT_{MAX} in autumn on community structure could relate to the differences in temperature tolerances by diatoms, green algae and cyanobacteria, with diatoms being out competed by filamentous green algae and cyanobacteria when temperatures exceed 21°C (DeNicola 1996). The importance of WT_{MAX} in spring on periphyton community structure could relate to spatial differences in the rate of growth accrual, which is more limited the colder the water temperature (Morin et al. 1999).

Flow metrics were more influential on periphyton communities compared to their biomass, which may indicate that periphyton taxa are more sensitive to the flow regime compared to biomass. Periphyton community structure could shift to accommodate different flow regimes, whilst biomass stays constant (Biggs et al. 1999). In autumn, periphyton communities were explained by the length of the growing season since a 1:2 year flood was experienced, which may produce communities of various successional states as a result (Ewart-Smith 2012). In spring, the duration of class 1 floods and Q_{CV} represented ecologically meaningful aspects of the flow regime. These findings do not necessarily suggest that class 1 floods were the most influential, as class 3, 4 and 1:2 year floods metrics were removed from anaylsis due to autocorrelation. The finding does however suggest that large disparities in the duration of these class 1 floods does produce spatial patterns in periphyton communities. Q_{CV} also explained spatial patterns in periphyton biomass in spring, suggesting that periphyton communities are susceptible to changes in flow velocities. Nevertheless, the surprisingly relatively low importance of the flow regime in the Western Cape may simply be a result of not producing flow metrics that are sufficiently ecologically meaningful (Hoyle et al. 2016). Overall, macroinvertebrates and sunlight accounted very little towards spatial variation in perphyton biomass and community structure, either because the metrics chosen were not ecologically meaningful, or simply because there was not sufficient spatial variability with which to tie to patterns in periphyton biomass and community structure. The observation should still however be noted that deposit feeder and scraper density and biomass does not differ as much between sites in spring compared to in autumn, which may be due to periphyton communities being more similar after disturbance by floods. These FFGS's were much more abundant in spring, probably due to the higher abundance of nutritious diatoms after disturbance (Guo et al. 2016). In autumn, deposit feeders indicate good quality food sources, as their densities and biomass were higher in nutrient poor sites that had more diatoms relative to more enriched sites. Scrapers were also an important FFG component, but were not limited to nutrient poor sites as the highest scraper density and biomass was found at a eutrophic site that had a high proportion of single celled, colonial and unbranched filamentous diatoms.

4.3.4.2 KwaZulu-Natal

Periphyton community structure and biomass in KwaZulu-Natal seem to be influenced primarily by water temperature and the flow regime. WT_{MAX} was also identified as an ecologically meaningful metric in KwaZulu-Natal, as it explained spatial variation in community structure in autumn and in biomass in spring, as in the Western Cape. It is not surprising that WT_{MAX} differed between sites in autumn, considering differences in the flow regime that may tie rather to the timing of the various water release schemes in impounded rivers, than to disparities in the number and duration of floods. WT_{MAX} during the growing season in South Africa should be considered a robust indicator of periphyton communities in autumn and of periphyton biomass in spring. Metrics of the flow regime are more prevalent in KwaZulu-Natal periphyton communities probably because the majority of sites were enriched, which by default places relevance on these metrics. The importance of flow metrics may also simply be attributed to the fact that periphyton grow during the summer season, and would naturally be effected by elevated base flows. Periphyton biomass and community structure did not show patterns that were consistent with enrichment categories and were as important in explaining these indicators in DISTLM tests compared to the Western Cape. However periphyton communities could be PO_4-P limited in KwaZulu-Natal in spring, which may be a result of increasing base flows that mobilise nutrients from decomposed leaf litter during the winter season (Ross-Gillespie 2014). PO_4-P was also important to periphyton biomass in the Western Cape in spring, probably due to runoff from various landuses during the winter wet season. Macroinvertebrates (deposit feeders) appear to influence periphyton assemblages, but not biomass. However, metrics based on macroinvertbrates were much less prevalent in Kwa-Zulu-Natal than the Western Cape, irrespective of whether this was based on community structure or biomass. This may suggest that periphyton food sources are of a poorer quality. KwaZulu-Natal has higher proportions of cyanobacteria, which are not as nutritious as diatoms (Power 1996).

Sunlight was most important in spring, suggesting that these are limiting variables during the start of the growing season. The rivers of KwaZulu-Natal are expected to be more turbid (Allanson et al. 1990) and may attenuate sunlight. Overall, periphyton community structure did not shift according to gradients of enrichment in KwaZulu-Natal, suggesting either that the enrichment categories assigned are incorrect or periphyton are poor indicators of enrichment in KwaZulu-Natal. Sites in KwaZulu-Natal may all be more enriched than categorized, meaning that a range in enriched conditions was not provided in this region.

4.4 Conclusions

Spatial variation in the Western Cape was explained by nutrient availabilities and water temperature and in KwaZulu-Natal by flows and water temperature. Periphyton community structure reflected the gradient in enrichment in the Western Cape with NO_3-N being a potential proxy for enrichment in autumn and spring, and EC a proxy in autumn. Periphyton community structure did not reflect a gradient of enrichment in KwaZulu-Natal. We attribute this to rivers in this region all being at least moderately enriched, whereas the Western Cape contained rivers that were pristine. Periphyton communities in the Western Cape were similar in nutrient poor sites, regardless of the season but different compared to more enriched sites. Seasonality shifted periphyton community structure in more enriched sites. The level of flow alteration did not shift periphyton communities in meso-eutrophic rivers in the Western Cape. However, community shifts could take place where there is a large difference in the flooding regime between two sites with the same level of flow alteration and enrichment. Periphyton – nutrient relationship studies can be improved upon by ensuring that a range of variously enriched sites are chosen, that nutrient ratios are used instead of absolute nutrient concentrations, and that ammonia is included in analyses, as it is considered an important nutrient of periphyton communities. Nutrient proxies such as percentage landuse should also be addressed at they are a more reliable indicator of their availabilities (Omernik & Ewart-Smith et al. 2017)

Chapter 5. Validation of an *in situ* tool (Benthotorch®) as a quantifier of periphyton biomass and community structure

5.1 Introduction

South African rivers are susceptible to enrichment and subsequent eutrophication through proliferation in periphyton growth. Eutrophication of water bodies leads to water quality decreases and hampers the flow of energy through the trophic web (Cashman et al. 2013). Water resource managers could benefit from early warning signs that indicate favourable conditions for eutrophication, by avoiding the cost and labour implications of remedying rivers from periphyton blooms. Having such information on hand would require a rapid assessment monitoring tool that provides an immediate measurement of periphyton biomass and community structure, with which to inform the trophic status and effectiveness of the Ecological Reserve in terms of the timing, magnitude, frequency and water quality of flow events that maintain ecosystem health.

Periphyton are not currently included in Ecological Reserve determinations because sample collection, processing and data analysis is a much more demanding procedure in terms of time and money compared to the current suite of biota used in the REMP programme. This Chapter introduces a newly developed *in situ* tool called the Benthotorch® and validates it's use against conventional techniques of periphyton biomass and community structure estimation.

Conventional approaches of estimating periphyton biomass are performed using spectrophotometry, Ash Free Dry Weight (AFDW) and High-Performance Liquid Chromatography (HPLC), while estimations of community structure involves the identification and enumeration of periphyton cells in the lab. Not only are these procedures costly and tedious to perform, they are likely to be prone to greater estimation error, due to risks associated with transportation and handling, which may affect sample integrity

In situ estimations have been performed on phytoplanktonic biomass (Kring et al. 2014), but no such tools have been available for the estimation of periphyton community structure. However, the methods employed for measuring phytoplankton biomass are not necessarily applicable to periphyton, due to the confounding effects of their three dimensional mat. At high biomass (thick periphytic mats), excitation energy exposure does not reach the basal periphyton cells. This is known as self-shading and leads to underestimations in biomass. By contrast excitation energy in thin mats at low biomass sites often penetrates through the mat and is reflected from the underlying substratum. This leads to

overestimation because the fluorometer incorrectly calculates biomass based on reflective energy from periphyton pigments and the substratum.

The Benthotorch® (bbe Moldaenke GmbH) addresses the challenges associated with various degrees of periphyton mat thickness. The Benthotorch® is a handheld pulse amplitude magnification fluorometer that energises the photosynthetic pigments of three photosynthetic periphyton groups in riverine ecosystems, namely diatoms, green algae and cyanobacteria. The reflective energy that is returned to the probe is group specific so that biomass estimations (µg cm^2) are obtained for each group. Their collective biomass provides an estimate of the total biomass of the replicate, which is the typical estimation provided by a spectrophotomer or HPLC machine. The results are available within a matter of seconds, allowing the user to maximise sampling efforts in the field with minimal effort.

Carpentier (2014) tested the efficacy of the Benthotorch® as a rapid tool for the estimation of periphyton biomass, relative to conventional lab approaches (fluorometry, spectrophotometry and HLPC). She found that the Benthotorch® overestimated biomass in thinner algal mats (< 2mm) and underestimated biomass in thicker mats (>2mm), relative to conventional methods. She attributed overestimation to additional reflectance energy from underlying substrate in thin mats and challenges in removing all the biomass in thin mats, so that conventional results were an underestimation. Underestimations in thick mats were attributed to the inability to penetrate these mats, as excitation energy becomes more attenuated the thicker the mat.

Echenique-Subiabre et al. (2016) found a greater agreement between the Benthotorch® and the spectrophotometer in thin mats (<2mm) compared to thick mats (>2mm) and attributed this to the self-shading effect. Carpentier (2014) suggests that periphyton comparisons between the Benthotorch® and conventional approaches should be done at moderate levels of periphyton biomass, to avoid the influence of substratum reflection in thinner mats and self-shading in thicker mats.

Studies by Kahlert & McKie (2014) and Harris & Graham (2015) validated the Benthotorch® more critically by assessing how accurately the Benthotorch® proportioned periphyton groups (as a % of biomass) against identified and enumerated periphyton groups (as a % of cell densities). Both studies found an agreement between the total biomass estimated by the Benthotorch® and spectrophotometer in oligotrophic streams, but a discrepancy when comparing the periphyton group proportions between the Benthotorch® and periphyton identification and enumeration techniques In particular, Harris &

Graham (2015) reported that the Benthotorch® found cyanobacteria that were not identified by the taxonomist. Similarly, Kahlert & MacKie (2014) and Enchinque-Subiabre et al. (2016) found that the Benthotorch® generally overestimated the diatom proportion %, relative to the proportion % of diatom densities that were calculated by the taxonomist.

These preliminary studies suggest that the Benthotorch® shows potential thus far to be a good relative measure of total periphyton biomass, but that there is potential to incorrectly proportion periphyton groups, or not identify them at all.

The purpose of this study was therefore to evaluate the potential use of the Benthotorch® as a tool for the rapid determination of periphyton biomass and community structure for application in South African rivers. Estimations of periphyton biomass by the Benthotorch® were compared with biomass estimations of a spectrophotometer. Benthotorch® proportion (as a %) of periphyton groups (calculated from biomass) were compared with proportions (as a %) of periphyton groups (calculated from cell densities) that were provided by a taxonomist. To demonstrate the effectiveness of periphyton removal by a toothbrush, the Benthotorch® was used to measure *in situ* periphyton biomass before and after scrubbing. The experiment was intended to help explain the large discrepancies in periphyton biomass estimation between the Benthotorch® and spectrophotometer.

We hypothesize that:

1) Discrepancies exist in biomass estimations between the Benthotorch® and spectrophotometer at low and high periphtyon biomass, but that there is an overall relationship between these methods.
2) Larger proportions (as a %) of biomass are left behind at low biomass sites, compared to high biomass sites after scrubbing with a toothbrush.
3) Discrepancies exist between the % proportioning of periphyton groups by Benthtorch® biomass and manually calculated cell densities by a taxonomist.

5.2 Materials and Methods

5.2.1 Data acquisition

Spectrophotometric biomass data was determined as in sections 2.4, 2.5.1 and 2.6.1. Benthotorch® biomass data was determined as in section 5.2.2. Periphyton cell densities were determined from the procedures detailed in sections 2.4, 2.5.2 and 2.6.2.

5.2.2 Benthotorch® sampling approach

Six estimations were taken on each of six replicates per site in autumn and spring, giving 234 *in situ* readings to be compared with the spectrophotometer in terms of biomass, and 117 comparisons to be made with periphyton identification samples. Biomass on the various cobble surfaces was measured in the order of top, front, bottom, back, left and right (Benthotorch® estimation time set to 10 seconds).

5.2.3 Data analysis

5.2.3.1 Benthotorch® biomass

The six biomass readings per cobble were averaged in order to give an estimation of replicate biomass which is comparable with the spectrophotometer. Surfaces that measured very low biomass (0.01µg m^2) were excluded from average calculations, to correct for embededdness. Benthotorch biomass readings in µg cm^2 were standardised to mg m^2 by multiplying by 10.

5.2.3.2 Univariate statistics

Kendall's tau rank correlations were used to determine the relationship between biomass from the Benthtorch® and spectrophotometer. Kendall's tau assumes non parametric data distributions and takes into account the possibility that a non-monotonic[2] relationship exists between two independent variables, which is represented as a concordance or inversion. The tau statistic is calculated based on the number of concordances and inversions present in the data. A concordance is noted when an increase or decrease in one variable, compared to its previous measurement is also true for the other variable (if $x_i < x_j$ then $y_i < y_j$). An inversion is noted when an increase or decrease in one variable

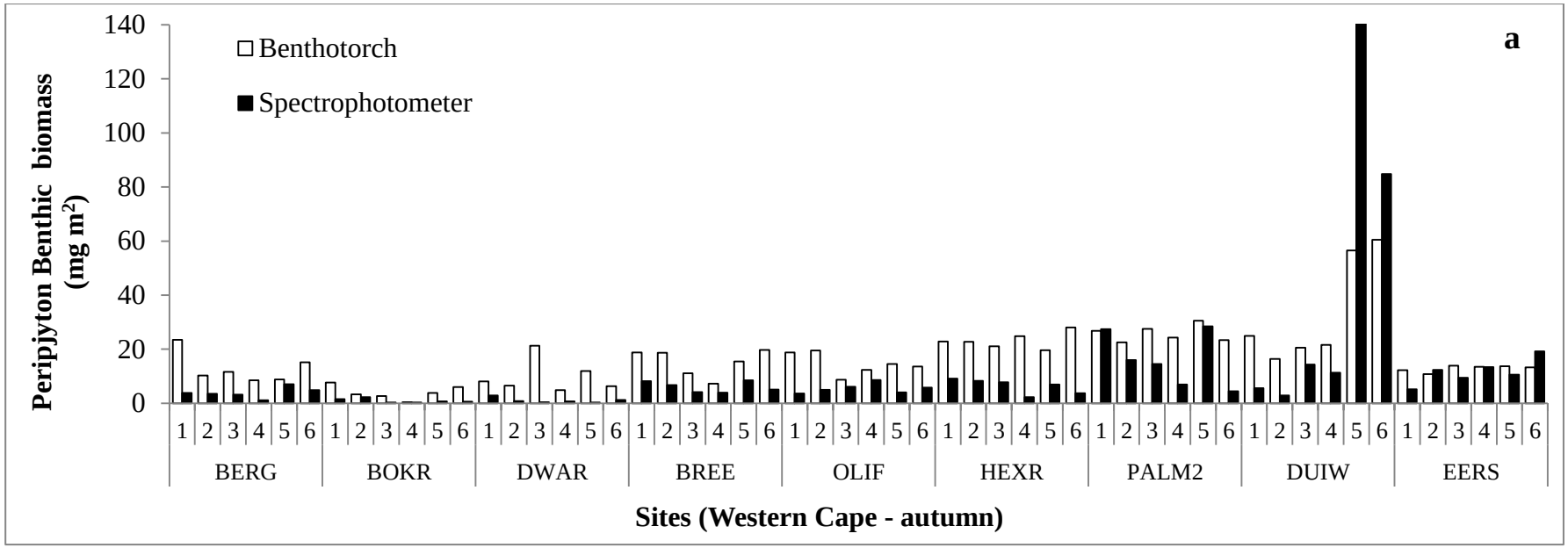
[2] When two variables either don't decrease or increase together in consecutive measurements

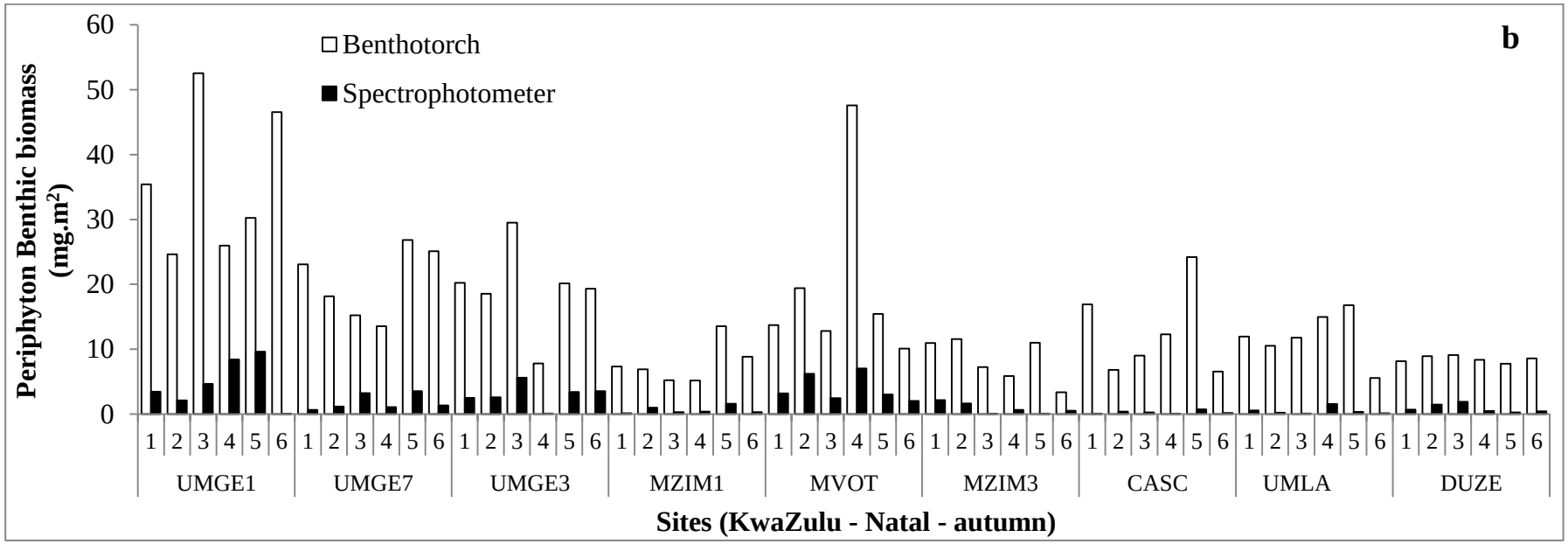
compared to its previous measurement is not true for the other variable (if $x_i < x_j$ then $y_i > y_{j)}$. These variables refer to the Benthotorch® and spectrophotomet readings. Stacked bar charts were used to make site comparisons based on the relative proportions (%) of periphyton groups using Benthotorch® *in situ* biomass and cell densities.

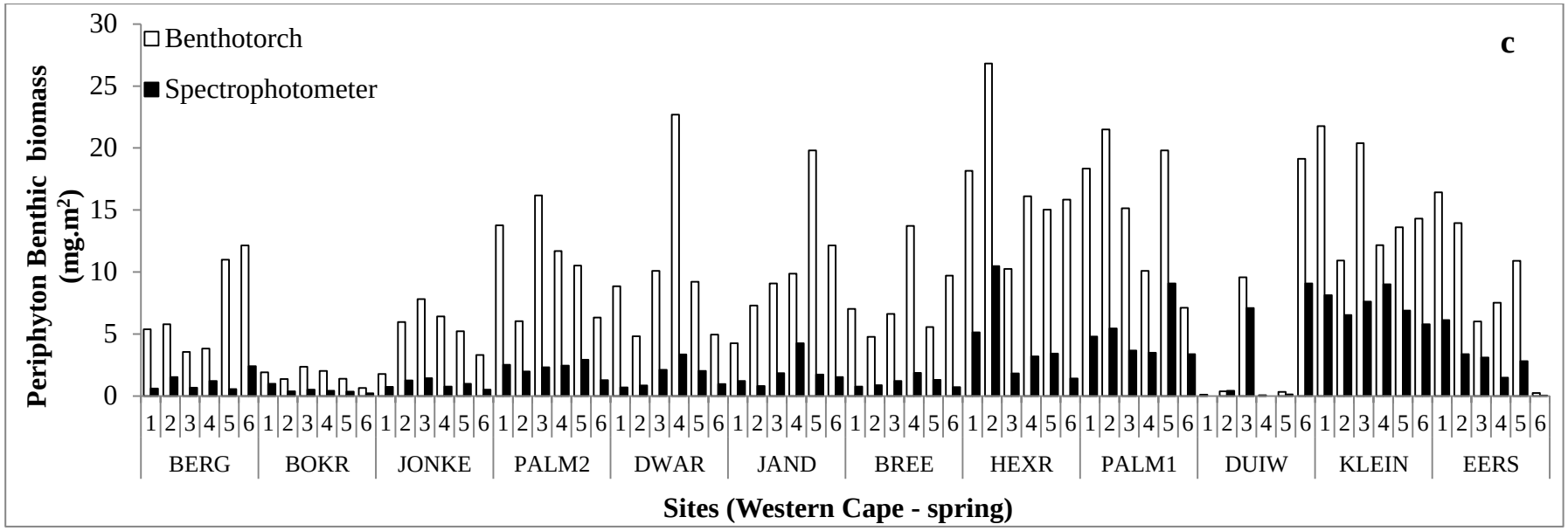
5.3 Results

5.3.1 Site specific biomass comparisons

The Benthotorch® generally estimated higher periphyton biomass compared to the spectrophotometer, irrespective of region and season. This discrepancy was most distinct in KwaZulu-Natal (Figure 5.1b and d), with the Benthotorch® often measuring biomass where the spectrophotometer did not. The spectrophotometer only estimated a higher biomass on two cobbles on the DUIW site in the Western Cape in autumn (Figure 5.1a) and a cobble on the DUZE site in KwaZulu-Natal in spring (Figure 5.1d).







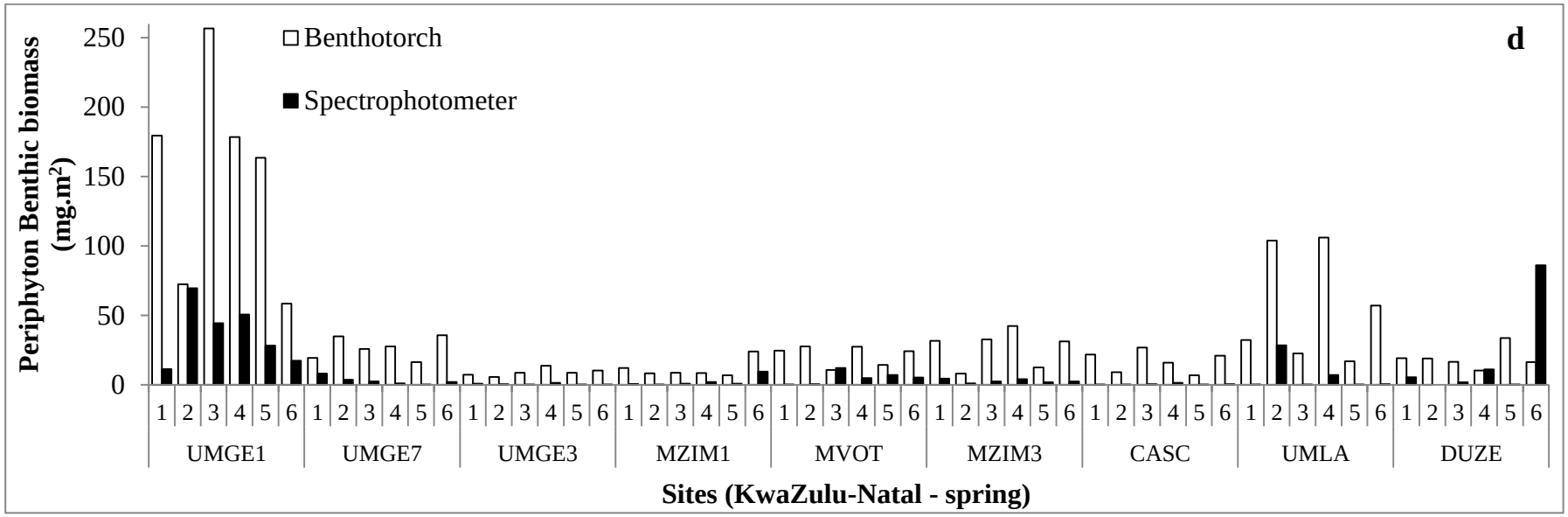


Figure 5.1: Replicate comparisons (cobbles 1-6) of periphyton Benthic biomass (mg m^2) per site between the spectrophotometer and the Benthotorch® in a) the Western Cape in autumn, b) KwaZulu-Natal in autumn, c) Western Cape in spring and d) KwaZulu-Natal in spring.

5.3.2 The relationship between spectrophotometric and Benthotorch® Benthic biomass

Table 5.1: Kendall's tau rank correlation between the spectrophotometer and the Benthotorch®. All = all measurements in the Western Cape and KwaZulu-Natal in autumn and spring, WC = Western Cape, KZN = KwaZulu-Natal, Au = autumn and SP = spring.

Statistic	All	WC	WC-au	WC-sp	KZN	KZN-au	KZN-sp
n	234	126	54	72	108	54	54
tau	0,383	0,599	0,439	0,657	0,362	0,440	0,311
Z	8,723	9,945	4,685	8,167	5,539	4,640	3,306
p	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001

Table 5.1 shows that all relationships between the spectrophotometer and Benthotorch® were highly significant. The strongest relationships, based on the tau statistic were found in the Western Cape, particularly in spring.

5.3.3 Periphyton Benthic biomass removal success

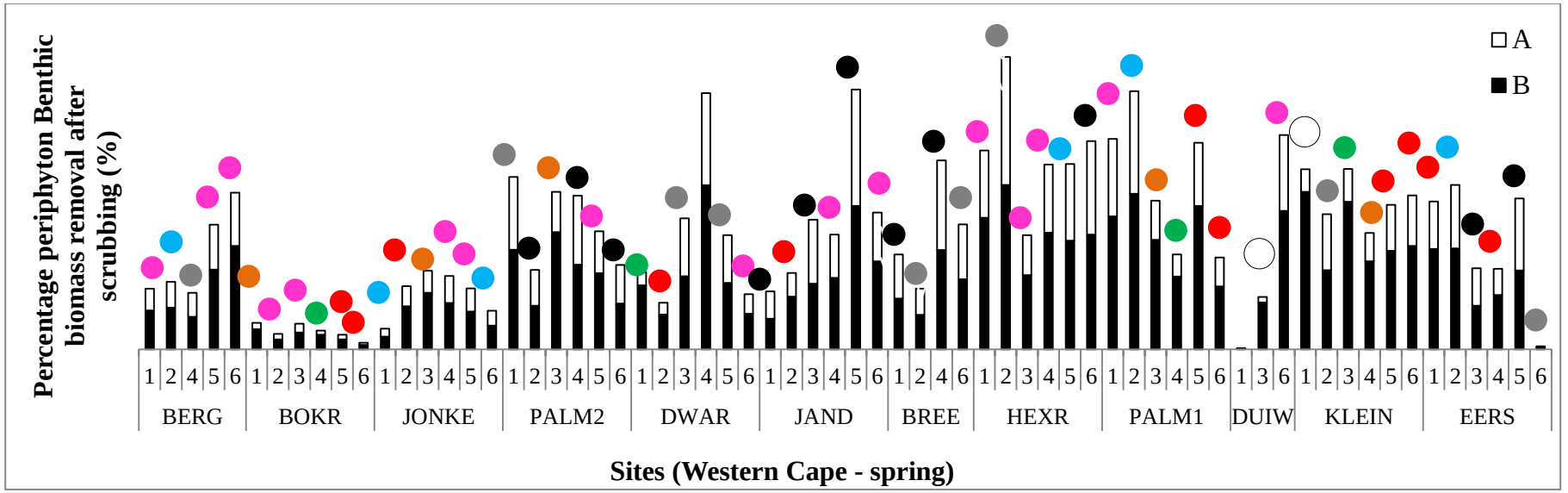
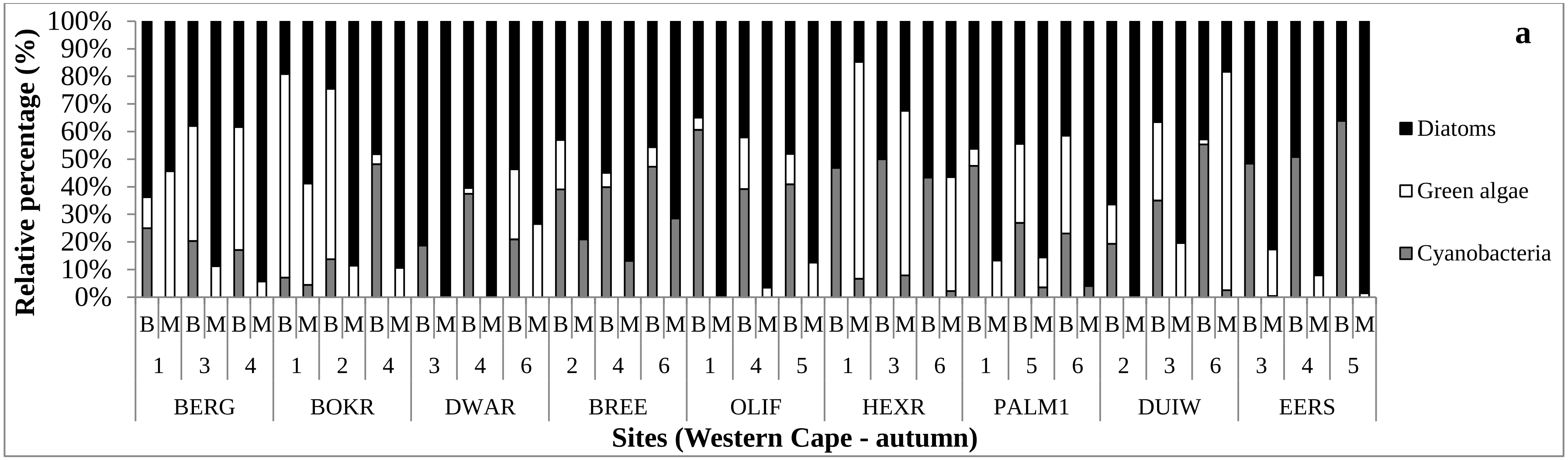
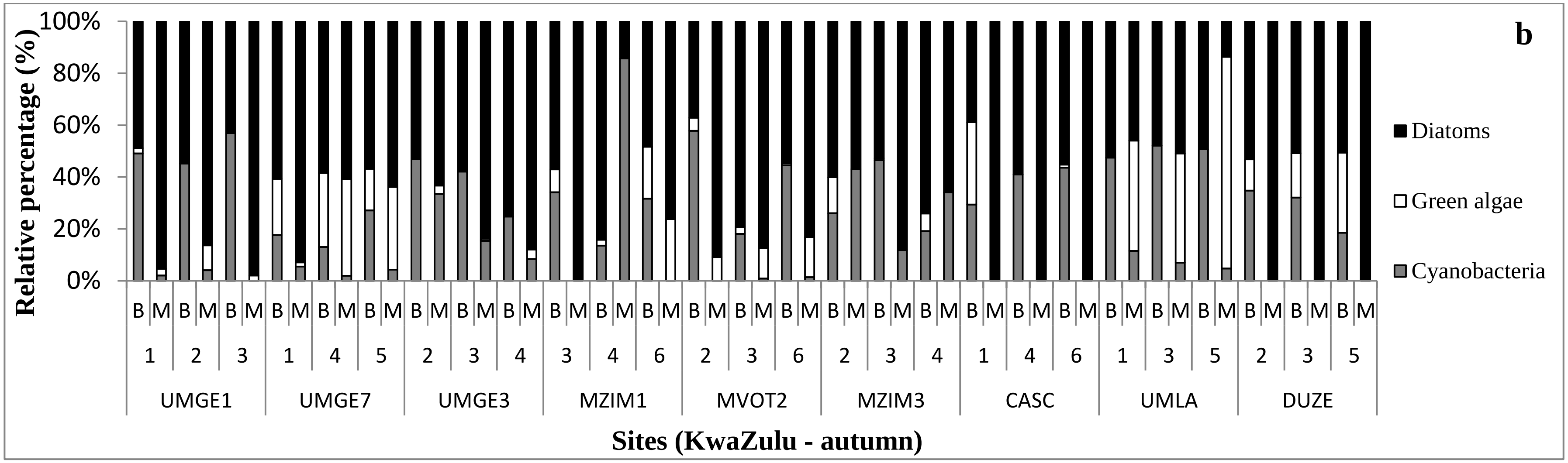


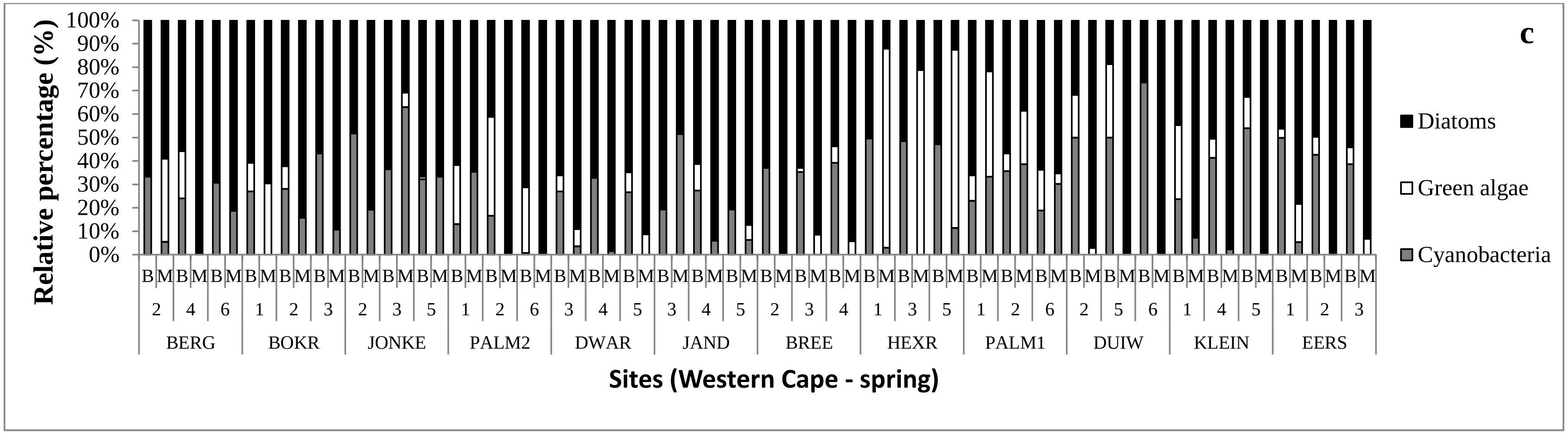
Figure 5.2: Percentage periphyton Benthic biomass removal based on the difference between the initial biomass before scrubbing and after scrubbing at sites in the Western Cape in spring in 2015. A = periphyton biomass after scrubbing and B = biomass before scrubbing. Black = 10-20% , grey = 20-30% ,blue = 30-40%, pink = 40-50%, red = 50-60%, orange = 60-70%, green = 70-80% and transparent = 80-90%.

Figure 5.2 shows that it is seldom that more than 60% of periphyton biomass is removed after scrubbing with a toothbrush. The percentage removal was not necessarily linked to the initial biomass on the cobble, as a large percentage of biomass was removed from sites with low (BERG, BOKR and JONKE) and high (PALM1 and DUIW) biomass.

5.3.4 Comparisons between periphyton group proportions derived from Benthotorch® Benthic biomass and periphyton cell densities







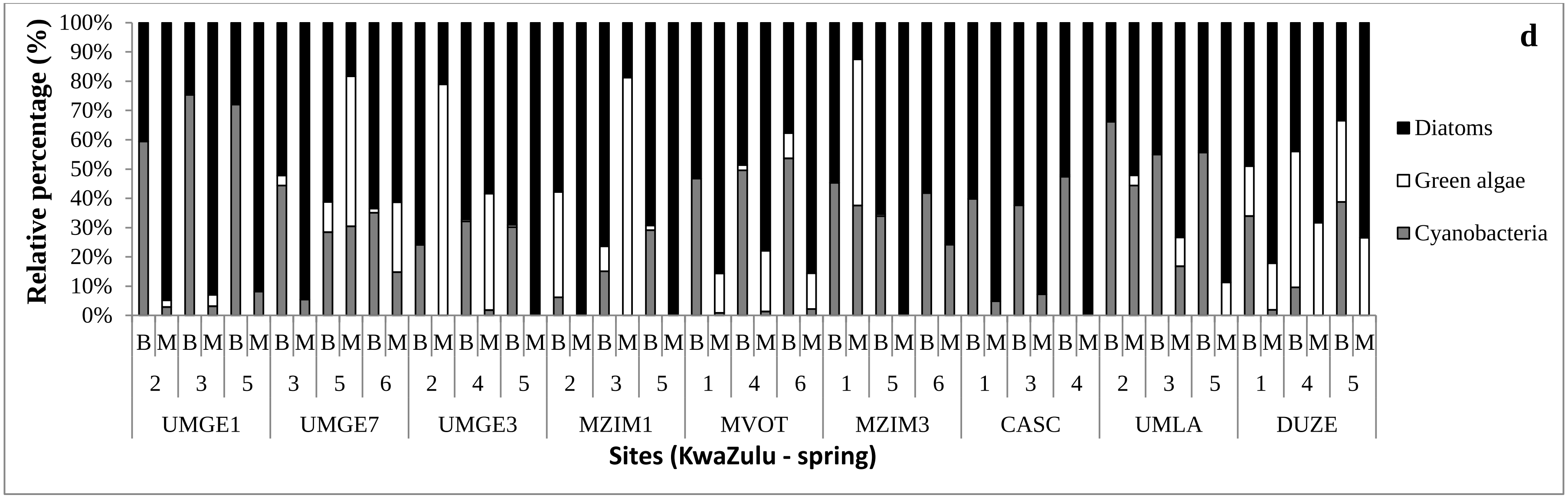


Figure 5.3: Relative percentages of periphyton groups (diatoms, green algae and cyanobacteria) per replicate across sites as calculated from Benthotorch® (B) and microscopic (M) results in the a) Western Cape in autumn, b) KwaZulu-Natal in autumn, c) Western Cape in spring and d) KwaZulu-Natal in spring. Benthotorch® and microscope percentages are based on relative Benthic biomass (mg m^2) and cell density (cells m^2) respectively.

5.3.4.1 Western Cape in autumn

The Benthotorch® and taxonomist both identified diatom taxa, whose proportions were markedly greater under microscopic identification. The Benthotorch® generally overestimated green algae, especially at the oligotrophic sites (BOKR, BERG), but at enriched sites (HEXR, EERS) did not identify them at all, where a taxonomist did. The Benthotorch® overestimated cyanobacteria taxa relative to the microscope, even compared to microscopic samples where no cyanobacteria were present (BERG, DWAR and EERS), Figure 5.3a.

5.3.4.2 KwaZulu-Natal in autumn

The Benthotorch® and microscope both identified diatom taxa, however the proportion of diatoms was markedly greater using the microscope. The Benthotorch® either under or overestimated green algae relative to identification and identified green algae at sites where none were identified under the microscope (DUZE), or didn't identify green algae at sites where there was (UMLA). The Benthotorch® overestimated cyanobacteria relative to identification, even in microscopic samples that contained no cyanobacteria (CASC and DUZE), Figure 5.3b.

5.3.4.3 Western Cape in spring

The Benthotorch® and taxonomist both identified diatom taxa, however the proportion of diatoms was markedly greater in identified samples. The Benthotorch® measured green algae that were not identified in microscopic samples (PALM2) or did not identify any algae at sites that contained green algae (HEXR). The Benthotorch® mostly overestimated cyanobacteria relative to the microscopic techniques, Figure 5.3c, even when no cyanobacteria were present.

5.3.4.4 Kwazulu-Natal in spring

The Benthotorch® and taxonomist both identified diatom taxa, however the proportion of diatoms was markedly greater in microscopic samples. The Benthotorch® often underestimated green algae relative to microscopic techniques (UMGE7) and at UMGE3 did not estimate green algae that were present in the microscopic sample. The Benthotorch® overestimated cyanobacteria relative to microscopic techniques, even when these samples contained no cyanobacteria (MZIM1 and DUZE), Figure 5.3d.

5.4 Discussion

5.4.1 The Benthotorch® as a quantifier of peripyton Benthic biomass

Kendall's tau statistic showed a highly significant relationship between the Benthotorch® and spectrophotometer for the full complement of data, as well as within region and season. The strongest agreements between the Benthotorch® and spectrophotometer were found in the Western Cape, particularly in spring. We attribute this to our findings that diatoms were the most abundant group during this time (Figure 4.1) and that the Benthotorch® does appear to correctly identify diatom groups, considering the consistent relationship between the Benthotorch® and taxonomist in terms of periphyton group proportioning (Figure a-d). In terms of site specific biomass comparisons in autumn in the Western Cape, we noticed that biomass estimations were most similar at the EERS and PALM1 site, which had the highest proportion of diatom taxa compared to other sites (Figure 4.2). The stronger relationship in KwaZulu-Natal in autumn relative to spring could also be explained by having higher proportions of diatoms, particularly single celled growth forms (Figure 4.2). This study is in agreement with the work of (Kahlert & McKie 2014; Echenique-Subiabre et al. 2016) that the Benthotorch® is at best a relative measure of biomass compared to conventional methods. The Benthotorch® generally overestimated biomass, except at a few enriched sites (DUIW, PALM1 and DUZE), where biomass was underestimated Our biomass removal success experiment showed that scrubbing with a toothbrush generally removed 40-50% of periphyton biomass, with only a few cases of removal success above 60%. As such, a more effective technique is required to remove periphyton biomass before future comparisons regarding periphyton biomass are made.

5.4.2 The Benthotorch® as an identifier of periphyton groups

Diatoms were the only group to be consistently identified by both the Benthotorch and taxonomist. Green algae were either under or overestimated by the Benthotorch®, which in extreme cases identified green algal taxa where the taxonomist identified none, or did not identify them when the taxonomist did. Cyanobacteria were consistently overestimated by the Benthotorch®, even in cases where the taxonomist did not identify any. These findings are in agreement with the literature that the Benthotorch does not recognize taxa representative of green and cyanobacteria groups. It is possible that the Benthotorch mistakes periphyton taxa for other taxa, leading to incorrect periphyton group assignments (Kahlert & McKie 2014; Harris & Graham 2015; Echenique-Subiabre et al. 2016).

5.5 Conclusion

This work is concurrent with other studies that show the Benthotorch® to be a relative measure of periphyton biomass against traditional techniques. However, how the Benthotorch® compartmentalizes this biomass is still questionable, with periphyton groups either being under or overestimated, or incorrectly identified. The strong relationship in the Western Cape in spring could be attributed to the greater abundance of diatoms, which the Benthotorch® identifies much better than other groups. Future studies wishing to compare the Benthotorch® to the spectrophotometer could be improved by sampling from sites with moderate biomass, to eliminate the over and under estimation errors associated with low and high biomass respectively. Biomass removal should be done using ethanol instead of a toothbrush, which will successfully extract Benthic chlorophyll *a*, as it is evident that 50% of periphyton biomass often remains behind after scrubbing. Future studies wishing to validate Benthotorch® periphyton group proportions should increase the number of measurements on the sample, in order to account for the potentially patchy distribution of periphyton taxa. The taxonomic samples contained taxa from the entire surface area of cobbles while the Benthotorch® estimates only observed taxa from 6 positions on the cobble.

Chapter 6. Main findings and recommendations

Periphyton biomass in the Western Cape

- ✓ Spatial patterns in periphyton biomass across seasons were influenced by WT_{MAX} , NO_3-N , EC, DP_{DENS} and Rs_{CUM}
- ✓ In autumn, spatial variability was primarily driven by the availability of TIN, Rs_{CUM} and DP_{DENS}
- ✓ In spring, spatial variability was primarily driven by WT_{MAX}, Q_{CV}, SCR_{DENS}, pH and PO_4-P

These results suggest that enrichment by nitrogen compounds should be minimized in order to avoid periphyton proliferations. Emphasis is placed on the influence of enrichment, as periphyton biomass followed gradients in enrichment in both seasons, suggesting there is a spatial response to the availability of nutrients. This was also a finding when testing for the relative effects of enrichment and flows on periphyton biomass, with biomass increasing with the level of enrichment in both seasons, but no significant difference observed across flow alterations. The importance of macroinvertebrate grazing should be considered, with deposit feeder regulation in autumn and scraper regulation in spring. The absence of flow metrics in spring after the wet season calls for the development of metrics that are more ecologically meaningful. However, the absence of these metrics may not necessarily mean they are meaningless. Perhaps the small contribution of flow metrics to periphyton spatial variation can also be attributed to limitations such as 2015 being a particularly dry year in terms of the number and duration of flood events. This study may have drawn on the importance of flows if periphyton samples were taken monthly, suggesting that flow metrics are granted more meaning in temporal studies, as they were in the temporal study of Ewart-Smith (2012). PO_4-P appears to be an important nutrient in spring, which could be a result of spatial disparity in PO_4-P concentrations due to runoff over various land use types. WT_{MAX} was influential in spring, which may reflect different river sizes and whether they were impounded or not and is a good predictor of periphyton Benthic biomass

Periphyton Benthic biomass in KwaZulu-Natal

- ✓ Spatial patterns in periphyton biomass across seasons were influenced by $Days_{\geq 1}$, WT_{MAX}, pH and NO_2-N
- ✓ In autumn, spatial variability was influenced by $Days_{\geq 1}$, $Since_{\geq 2}$, WT_{CV}, pH and TIN
- ✓ In spring, spatial variability was influenced by $Since_{\geq 2}$, $Days_{\geq 2}$, PO_4-P and WT_{MAX}

It was surprising to find that spatial variation in periphyton biomass was not strongly influenced by enrichment, as in the Western Cape. We contend that nutrient ranges in KwaZulu-Natal were not as great as in the Western Cape, which probably explains why periphyton biomass in KwaZulu-Natal did not follow gradients in enrichment. Flow metrics in KwaZulu-Natal were however important compared to the Western Cape, which may simply be a result of periphyton growth in autumn, which marks the wet season in KwaZulu-Natal. Nevertheless, the contribution of flows to spatial patterns in periphyton biomass were very low in both KwaZulu-Natal and the Western Cape, which again may suggest their applicability in temporal rather than spatial studies. WT_{MAX} explained seasonal variability in periphyton biomass and was also significantly influential in spring. WT_{MAX} was also important in spring in the Western Cape, suggesting that the spatial disparity between sites has an effect on periphyton biomass accrual rates. PO_4-P was also important in spring in KwaZulu-Natal as in the Western Cape, and efforts to prevent eutrophication in summer should involve mitigating against PO_4-P discharge into rivers.

Periphyton Community structure in the Western Cape

- ✓ Spatial patterns in periphyton community structure across seasons were influenced by EC, NO_3-N, WT_{CV}, PO_4-P and DP_{DENS}
- ✓ In autumn, spatial patterns in periphyton community structure were influenced by TIN, $Since_{\geq 1:2}$, WT_{MAX}, EC, NO_3-N and WT_{CUM}
- ✓ In spring, spatial patterns in periphyton community structure were influenced by EC, $Days_{\geq 1}$, Q_{CV}, DP_{DENS} and WT_{CV}

Periphyton community structure followed a gradient of nutrient enrichment in autumn but not in spring, which suggests that periphyton assemblages are more similar in spring as a result of flood disturbance. TIN was also found to be influential to spatial variation in periphyton biomass and should be considered a key variable in the management of periphyton proliferation in the Western Cape. However, the comparatively smaller variation explained by orthophospate in periphyton community structure across sites and seasons should not preclude it when managing periphyton. Orthophospate may be so abundant that it does not feature as a driver of eutrophication in statistical analyses, but is favourable for eutrophication of water bodies. EC may be an important predictor of periphyton communities in spring, suggesting that different periphyton taxa can be expected as EC increases. Flow metrics were more important for periphyton community structure compared to biomass which may

indicate that a change in the flow regime prompts changes in periphyton assemblages whilst keeping biomass constant. Deposit feeders were also influential on periphyton biomass and other than flows should be considered as an influential form of disturbance. WT_{MAX} was found to be important for periphyton community structure, but not biomass and appears to influence succession in periphyton communities.

Periphyton community structure in KwaZulu-Natal

- ✓ Spatial patterns in periphyton community structure across seasons were influenced by DP_{DENS}, $Days_{\geq 1}$, pH, TIN, Q_{CV} and $Since_{\geq 1:2}$
- ✓ In autumn, spatial patterns in periphyon community structure were influenced by WT_{MAX}, $Fld_{\geq 2}$, BR_{BMASS}, Q_{CV}, $Days_{\geq 2}$ and $Days_{\geq 1}$
- ✓ In spring, spatial patterns in periphyton community structure were influenced by Rs_{MIN}, PO_4-P, Q_{CV}, NO_3-N, WT_{MIN} and TIN

Although periphyton community structure did not follow a gradient of enrichment, there seems to be an indication that periphyton taxa are more responsive to nutrient availabilities compared to biomass. This was observed in spring, at the start of the growing season when variables such as sunlight, nutrients and water temperature are important for periphyton succession. We recommend that nutrient concentration be lowered, in order to avoid peripyton proliferations in spring.

Recommendations for use of the Benthotorch®

The Benthotorch® is most applicable in rivers after flood disturbance when periphyton communities are dominated by diatoms, as it was noticed that that Benthotorch® estimations of periphyton Benthic biomass and community structure were most similar to conventional methods at sites that were dominated by diatoms. However, it is not clear whether the Benthtorch® is not able to accurately estimate periphyton biomass in rich sites due to the self-shading effect, or because it does not recognize periphyton taxa here. There were instances where the Benthotorch® did not identify green algae that were evident in samples, which suggests that the Benthotorch® has difficulty or is unable to identify these taxa.

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Appendices

Appendix 1a: Length weighted taxon cell densities in autumn and spring in the Western Cape in 2015

			AUTUMN									SPRING											
Taxon	**Division**	**Growth Form**	**BREE**	**HEXR**	**DWAR**	**OLIF**	**BERG**	**PALM1**	**DUIW**	**EERS**	**BOKR**	**BREE**	**HEXR**	**DWAR**	**BERG**	**PALM1**	**DUIW**	**EERS**	**BOKR**	**KLEIN**	**PALM2**	**JONKE**	**JAND**
Adlafia sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis sp.	**Bacillariophyta**	**single cells**	2577228	0	0	0	0	0	0	0	0	1744224	0	0	0	0	0	26506	0	0	0	0	0
Cymbella sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	36727	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbopleura sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis leei	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia exigua*	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	496306	0	0	0	165630	0	0	28973	0
Eunotia rhomboidea. *	**Bacillariophyta**	**single cells**	139075	0	0	5177972	6528615	0	0	0	0	0	0	1882714	0	0	269370	0	0	1258127	0	648282	847521
Eunotia sp.*	**Bacillariophyta**	**single cells**	0	0	250577	1110766	104487	3857055	0	0	1720288	0	0	3942973	1535248	45281	97904	0	1211770	0	0	0	1542196
Frustulia sp.	**Bacillariophyta**	**single cells**	0	0	0	104108	0	0	36729	997100	0	0	0	172763	0	0	0	0	0	0	0	0	0
Gomphonema acuminatum*	**Bacillariophyta**	**single cells**	0	0	0	0	0	281128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema sp.	**Bacillariophyta**	**single cells**	8061203	341384	0	32610227	364011	13640106	3286761	22846100	0	299965	43495	0	0	413160	282838	182513	0	0	0	24337	86619
Gyrosigma sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hippodonta sp.*	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33287	0	0	0	0	0	0
Navicula spp.	**Bacillariophyta**	**single cells**	2345999	3279036	42120	7317733	0	25968214	24303177	51808217	82010	470880	311326	181959	0	0	22464179	2724656	0	5957397	73656	0	0
Nitzchia sigma	**Bacillariophyta**	**single cells**	0	190673	0	0	0	0	0	0	16402	0	0	0	0	0	278412	130366	31804	874998	0	0	76698
Nitzchia spp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia sp.	**Bacillariophyta**	**single cells**	0	165010	0	0	0	0	0	4165935	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurosirella sp. *	**Bacillariophyta**	**single cells**	0	26817	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellophora sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	338451	0	0	0	0	0	536498	0	0	0	0	0
Surirella sp.*	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	78323	0	0	0	0	0	0
Achnanthidium oblongella.	**Bacillariophyta**	**colonial**	1454277	0	20218	3069887	7983	1065709	5509	0	0	75341	205687	0	0	131415	1960614	0	0	25240094	499839	0	65239
Achnanthidium sp.	**Bacillariophyta**	**colonial**	94937	472345	24624	338350	826537	59833921	463693	1744924	527271	0	11791	72784	0	134621	6783023	545276	47706	22387250	0	0	0
Diadesmis sp.*	**Bacillariophyta**	**colonial**	0	0	0	0	0	0	0	24071477	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema sp	**Bacillariophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	85763	27169	40405	0	0	0	0	0	0
Aulacoseira sp	**Bacillariophyta**	**unbranched filaments**	0	0	0	0	0	0	0	2889592	0	0	0	0	0	6902268	0	0	0	0	166772	0	0
Fragilaria sp.	**Bacillariophyta**	**unbranched filaments**	0	160905	0	36642654	37615	24879292	5123219	62107732	0	102401	0	0	0	0	467030	386592	0	1000874	88387	0	0
Melosira sp.	**Bacillariophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabellaria sp.*	**Bacillariophyta**	**unbranched filaments**	0	0	0	0	562383	1959726	0	0	37084	0	0	460701	0	0	0	0	0	0	0	0	0
Actinotaenium spp.*	**Chlorophyta**	**single cells**	0	0	0	0	0	0	0	0	55625	0	0	0	0	0	0	0	0	0	0	0	0
Cosmarium sp.*	**Chlorophyta**	**single cells**	0	0	0	0	0	6440499	2739265	0	46472	0	0	0	0	0	209957	0	0	0	0	0	0
Penium sp.*	**Chlorophyta**	**single cells**	0	0	0	0	71051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurastrum sp.	**Chlorophyta**	**single cells**	0	0	0	0	0	87852	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coelastrum sp.	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crucigenia sp.	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desmococcus spp.	**Chlorophyta**	**colonial**	0	476682	60140	104108	784130	0	0	0	286389	0	0	220844	0	493023	80811	0	472045	0	0	0	92806
Pediastrum sp. *	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	4036555	0	0	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus sp.	**Chlorophyta**	**colonial**	0	1226295	0	0	0	427431	0	3331564	0	152067	109473	0	0	321036	0	456053	0	0	0	0	0
Sphaerocyctis sp.*	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	206183	0	0	0	0	0
Mougeotia sp	**Chlorophyta**	**unbranched filaments**	0	0	0	409604	0	0	0	0	0	0	0	0	303278	0	0	0	0	0	0	0	0
Oedogonium sp.	**Chlorophyta**	**unbranched filaments**	0	10288525	0	330834	0	10970739	129850438	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stigeoclonium spp.	**Chlorophyta**	**branched filaments**	0	178783	0	0	0	0	0	6314965	43739	0	3318160	0	0	0	0	0	0	0	0	31009	0
Chamaesiphon sp.	**Cyanophyta**	**single cells**	2724333	544534	0	0	0	1681730	0	0	19682	0	212792	0	346828	3719267	0	0	44526	3142315	99231	529885	353763
Dichothrix sp	**Cyanophyta**	**branched filaments**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heteroleibleinia spp.	**Cyanophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lyngbya sp.	**Cyanophyta**	**unbranched filaments**	0	697841	0	0	0	0	4189464	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria sp. *	**Cyanophyta**	**unbranched filaments**	0	0	0	0	0	0	0	86497	0	0	0	0	0	0	0	0	0	0	0	0	0
Phormidium sp.	**Cyanophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	0	0	0	123093	0	140802	0	206183	17014	0	0	0	69028
Euglena spp.*	**Euglenophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37109	0	0	0	0	0
Phacus sp.*	**Euglenophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50362	0	0	0	0	0
Ceratium sp.	**Dinoflagellata**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinobryon spp.	**Chrysophyta**	**colonial in gelatenous masses**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	828150	0	0	0	0

*** : Taxa only occuring in the Western Cape**

Appendix 1b: Length weighted taxon cell densities in autumn and spring in KwaZulu-Natal in 2015

			AUTUMN									SPRING								
Taxon	**Division**	**Growth Form**	**DUZE**	**MZIM3**	**MZIM1**	**CASC**	**UMGE1**	**UMGE3**	**UMLA**	**UMGE7**	**MVOT**	**DUZE**	**MZIM3**	**MZIM1**	**CASC**	**UMGE1**	**UMGE3**	**UMLA**	**UMGE7**	**MVOT**
Adlafia sp.*	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	469998	0	0	0	0	0	0	0	0	21504	0
Cocconeis sp.	**Bacillariophyta**	**single cells**	199244	499967	724371	1059463	3241801	0	0	1667412	359478	739089	102989	61918	117962	730676	113503	99611	1960401	612633
Cymbella sp.	**Bacillariophyta**	**single cells**	204521	0	0	0	0	0	0	60889	1800616	99465	0	104698	0	0	0	0	0	0
Cymbopleura sp.*	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	270155	0	0	0	0	0	0	0	0	0
Encyonopsis leei*	**Bacillariophyta**	**single cells**	79153	150561	0	596780	3165735	31994345	244085	2409609	113952	0	909020	187443	0	1733269	2089055	0	2458157	2422844
Eunotia exigua	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia rhomboidea.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia sp.	**Bacillariophyta**	**single cells**	0	0	0	0	1021264	0	0	0	0	0	0	0	0	0	0	0	0	130123
Gomphonema acuminatum	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema sp.	**Bacillariophyta**	**single cells**	1247018	1256771	74129	0	36100854	96630042	1009026	1908462	26959300	80644248	1950297	4407971	134458	26538608	92965	116792163	0	71444658
Gyrosigma sp.*	**Bacillariophyta**	**single cells**	366874	0	0	0	865108	0	0	142967	0	306486	0	0	165903	0	0	0	0	0
Hippodonta sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula spp.	**Bacillariophyta**	**single cells**	10628230	369261	0	155327	82135863	30326376	146808	1109372	6037704	21092966	0	196310	1134180	42287905	3383874	0	952273	23901082
Nitzchia sigma	**Bacillariophyta**	**single cells**	0	0	0	0	683739	0	0	52900	0	4835582	0	0	0	172951	0	0	0	0
Nitzchia spp.*	**Bacillariophyta**	**single cells**	52769	0	0	0	0	0	0	0	151936	0	0	0	0	0	0	0	0	0
Pinnularia sp.	**Bacillariophyta**	**single cells**	2663261	154438	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurosirella sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellophora sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	1953828	0	246041	0	0	0	0	0	0
Surirella sp.	**Bacillariophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthidium oblongella.	**Bacillariophyta**	**colonial**	0	6311	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Achnanthidium sp.	**Bacillariophyta**	**colonial**	52769	1124136	220114	0	20378296	0	130601	74592	7895680	4058811	121672	664200	0	1777535	327959	0	126712	6563163
Diadesmis sp.	**Bacillariophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema sp	**Bacillariophyta**	**colonial**	355593	42299	0	0	5363794	0	0	946078	720289	0	0	0	0	1384475	0	0	411341	0
Aulacoseira sp	**Bacillariophyta**	**unbranched filaments**	0	0	0	0	0	2266950	0	3318594	0	0	0	0	0	0	0	0	0	0
Fragilaria sp.	**Bacillariophyta**	**unbranched filaments**	3675261	47330	0	0	3180046	20245351	0	0	9432320	2768490	559397	4004718	0	0	0	0	0	47756426
Melosira sp.*	**Bacillariophyta**	**unbranched filaments**	0	0	0	0	1323992	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabellaria sp.	**Bacillariophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinotaenium spp.	**Chlorophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cosmarium sp.	**Chlorophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penium sp.	**Chlorophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurastrum sp.	**Chlorophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	625718	0	163446	0
Coelastrum sp.*	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	0	0	3601920	0	0	0	0	0	0	0	0
Crucigenia sp.*	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	39782	0	0	0	0	0	0	0	0	0	0
Desmococcus spp.	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	423205	0	0	0	557641	0	0	0	0	0	0
Pediastrum sp.	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus sp.	**Chlorophyta**	**colonial**	0	0	0	0	0	5632611	0	24864	137659	15087858	0	0	0	0	0	0	101164	0
Sphaerocyctis sp.	**Chlorophyta**	**colonial**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mougeotia sp	**Chlorophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	7148784	0	0	0	0	0	0	0	0	12302921
Oedogonium sp.	**Chlorophyta**	**unbranched filaments**	0	0	185322	0	0	0	274042	4403594	0	0	0	0	0	0	0	143678	2088601	2428966
Stigeoclonium spp.	**Chlorophyta**	**branched filaments**	0	0	0	0	6021589	0	2230641	0	759759	20299110	486687	247841	0	1121491	6600457	7654086	0	20456241
Chamaesiphon sp.	**Cyanophyta**	**single cells**	0	84597	261635	0	127658	21344153	0	313970	0	0	74586	0	56837	1062676	0	0	1459740	0
Dichothrix sp	**Cyanophyta**	**branched filaments**	0	0	0	0	0	0	113125	0	0	0	0	0	0	0	0	0	0	0
Heteroleibleinia spp.*	**Cyanophyta**	**unbranched filaments**	0	421622	0	0	1072327	21893164	0	149183	0	0	1235188	0	0	0	0	96272816	0	0
Lyngbya sp.	**Cyanophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1758625	0	0
Oscillatoria sp.	**Cyanophyta**	**unbranched filaments**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phormidium sp.	**Cyanophyta**	**unbranched filaments**	0	753048	0	0	1417511	12598762	209139	151007	557574	511699	0	0	0	3767072	136204	0	56771	2665130
Euglena spp.	**Euglenophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus sp.	**Euglenophyta**	**single cells**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratium sp.*	**Dinoflagellata**	**single cells**	0	0	0	0	0	0	0	476106	0	0	0	0	0	0	0	0	0	0
Dinobryon spp.*	**Chrysophyta**	**colonila in gelatenous masses**	0	4613367	0	0	0	0	0	0	0	0	31631412	6026494	0	0	4980267	364204509	3397689	9072290

*** : Taxa only occuring in the KwaZulu-Natal**

Appendix 2a: Macroinvertebrate abundance and biomass summed across families and FFG's in autumn and spring in the Western Cape in 2015

		AUTUMN									SPRING										
Family	**FFG**	**BREE**	**HEXR**	**DWAR**	**OLIF**	**BERG**	**PALM1**	**DUIW**	**EERS**	**BOKR**	**BREE**	**HEXR**	**DWAR**	**BERG**	**PALM1**	**DUIW**	**EERS**	**BOKR**	**KLEIN**	**PALM2**	**JONKE**	**JAND**
											FFG ABUNDANCE (individuals.m^2)										
Ancylidae	Scraper	891	492	0	0	0	18	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	Scraper	0	0	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmid larvae:long	Scraper	0	0	1226	1419	191	0	0	0	20	28	0	1228	0	0	59	0	0	0	0	49	1657
Elmid larvae:tear drop	Scraper	0	0	484	65	396	0	321	0	137	30	0	221	53	0	0	0	294	0	246	28	1950
Elmidae adult	Scraper	0	0	42	22	110	0	10	0	203	28	0	359	32	0	0	0	1485	0	21	13	3120
Hydraenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psephenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptilodactylidae	Scraper	0	0	61	0	0	0	22	0	0	0	0	155	0	0	0	0	0	0	0	0	36
Blepharaceridae larvae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45	0
Chironomidae	Scraper	1635	2978	2793	1535	18197	630	1279	112390	1767	13165	10844	14326	27080	32630	1512	35089	3152	6192	46291	14436	37714
Ephydridae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossosomadidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	127	0	0	0	3392	0	0	108	0
Hydroptilidae	Scraper	85	0	0	118	73	102	0	43	0	315	0	0	18	0	0	0	0	0	0	0	168
Helodidae	Brusher	0	0	275	0	232	0	0	0	144	0	0	765	0	0	94	0	281	0	0	85	154
Heptageniidae	Brusher	2046	0	141	0	998	0	0	0	0	129	0	83	43	0	0	0	0	0	0	0	71
Baetidae	Deposit Feeder	3064	6282	6931	3289	9348	341	3169	4521	3072	88114	28059	14167	26499	14489	1763	13907	403	5461	16930	58621	12569
Caenidae	Deposit Feeder	19	0	0	245	0	0	293	0	0	175	0	0	0	0	0	0	0	673	28	0	0
Leptophlebiidae	Deposit Feeder	0	0	144	0	2121	0	10	0	1448	0	0	100	320	0	0	0	317	0	0	250	628
Teloganodidae	Deposit Feeder	0	0	1900	0	142	0	0	0	1819	0	0	0	173	0	0	0	2029	0	47	69	35
Notonemouridae	Deposit Feeder	0	0	999	0	102	0	0	0	416	0	0	1693	59	0	0	0	54	28	0	239	3282
Leptoceridae	Deposit Feeder	0	0	14	23	149	0	42	0	9806	0	0	865	71	0	0	0	492	0	0	24	189
	Total Scraper	2612	3471	4602	3155	18968	793	1642	112433	2127	13566	10844	16289	27308	32630	1571	35089	8324	6192	46558	14680	44646
	Total Brusher	2046	0	416	0	1230	0	0	0	144	129	0	846	43	0	94	0	281	0	0	85	225
	Total Deposit feeder	3083	6282	9989	3558	11860	341	3513	4521	16560	88289	28059	16826	27122	14489	1763	13907	3295	6165	17005	59203	16703
	Total Grazers	7742	9754	15009	6713	32057	1134	5156	116955	18830	101983	38900	33961	54473	47119	3427	48995	11900	12356	63563	73966	61574
											FFG BIOMASS (g.m^2)										
Ancylidae	Scraper	0,507	0,209	0,000	0,000	0,000	0,033	0,012	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Physidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,084	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Elmid larvae:long	Scraper	0,000	0,000	0,053	0,075	0,008	0,000	0,000	0,000	0,001	0,001	0,000	0,053	0,000	0,000	0,003	0,000	0,000	0,000	0,000	0,002	0,072
Elmid larvae:tear drop	Scraper	0,000	0,000	0,029	0,004	0,035	0,000	0,032	0,000	0,011	0,004	0,000	0,026	0,003	0,000	0,000	0,000	0,016	0,000	0,014	0,002	0,147
Elmidae adult	Scraper	0,000	0,000	0,007	0,003	0,024	0,000	0,002	0,000	0,075	0,015	0,000	0,057	0,005	0,000	0,000	0,000	0,255	0,000	0,003	0,002	0,496
Hydraenidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Psephenidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Ptilodactylidae	Scraper	0,000	0,000	0,003	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,030	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,010
Blepharaceridae larvae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,028	0,000
Chironomidae	Scraper	0,021	0,036	0,039	0,021	0,225	0,008	0,016	1,369	0,025	0,164	0,132	0,182	0,346	0,421	0,018	0,428	0,040	0,090	0,564	0,183	0,459
Ephydridae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Glossosomadidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,039	0,000	0,000	0,000	0,874	0,000	0,000	0,028	0,000
Hydroptilidae	Scraper	0,236	0,000	0,000	0,328	0,205	0,284	0,000	0,120	0,000	0,882	0,000	0,000	0,050	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,473
Helodidae	Brusher	0,000	0,000	0,013	0,000	0,013	0,000	0,000	0,000	0,011	0,000	0,000	0,057	0,000	0,000	0,004	0,000	0,024	0,000	0,000	0,004	0,008
Heptageniidae	Brusher	1,203	0,000	0,049	0,000	0,630	0,000	0,000	0,000	0,000	0,106	0,000	0,034	0,288	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,111
Baetidae	Deposit Feeder	0,075	0,203	0,136	0,078	0,197	0,008	0,091	0,374	0,063	1,735	0,593	0,300	0,557	0,612	0,045	0,451	0,009	0,147	0,333	1,155	0,297
Caenidae	Deposit Feeder	0,000	0,000	0,000	0,014	0,000	0,000	0,016	0,000	0,000	0,003	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,063	0,001	0,000	0,000
Leptophlebiidae	Deposit Feeder	0,000	0,000	0,116	0,000	0,101	0,000	0,000	0,000	0,178	0,000	0,000	0,003	0,061	0,000	0,000	0,000	0,060	0,000	0,000	0,053	0,073
Teloganodidae	Deposit Feeder	0,000	0,000	0,067	0,000	0,005	0,000	0,000	0,000	0,064	0,000	0,000	0,000	0,041	0,000	0,000	0,000	0,091	0,000	0,027	0,009	0,034
Notonemouridae	Deposit Feeder	0,000	0,000	0,099	0,000	0,010	0,000	0,000	0,000	0,041	0,000	0,000	0,168	0,006	0,000	0,000	0,000	0,005	0,003	0,000	0,035	0,325
Leptoceridae	Deposit Feeder	0,000	0,000	0,008	0,007	0,045	0,000	0,016	0,000	2,942	0,000	0,000	0,301	0,027	0,000	0,000	0,000	0,180	0,000	0,000	0,007	0,057
	Total Scraper	0,765	0,246	0,131	0,431	0,497	0,409	0,061	1,489	0,111	1,066	0,132	0,348	0,443	0,421	0,021	0,428	1,185	0,090	0,581	0,245	1,657
	Total Brusher	1,203	0,000	0,062	0,000	0,643	0,000	0,000	0,000	0,011	0,106	0,000	0,092	0,288	0,000	0,004	0,000	0,024	0,000	0,000	0,004	0,119
	Total Deposit feeder	0,075	0,203	0,426	0,099	0,358	0,008	0,123	0,374	3,288	1,738	0,593	0,772	0,692	0,612	0,045	0,451	0,345	0,212	0,362	1,259	0,786
	Total Grazers	2,043	0,448	0,619	0,530	1,499	0,417	0,184	1,863	3,410	2,911	0,725	1,212	1,423	1,033	0,070	0,879	1,554	0,302	0,943	1,508	2,562

Appendix 2b: Macroinvertebrate abundance and biomass summed across families and FFG's in autumn and spring in KwaZulu-Natal in 2015

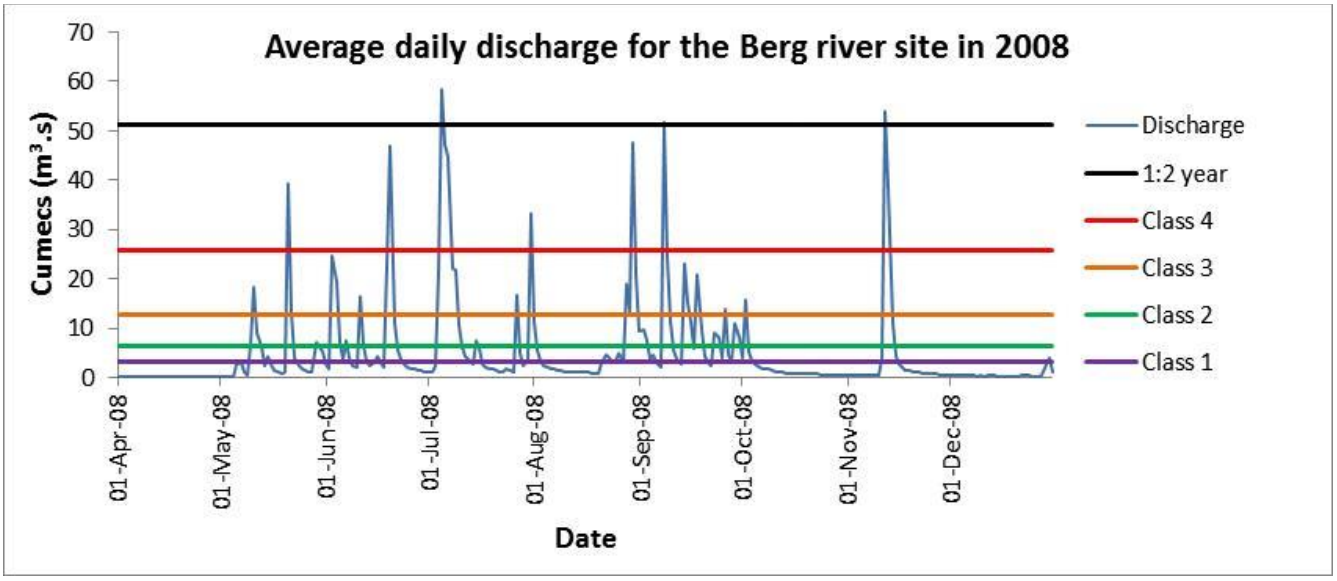
		AUTUMN									SPRING								
Family	**FFG**	**DUZE**	**MZIM3**	**MZIM1**	**CASC**	**UMGE1**	**UMGE3**	**UMLA**	**UMGE7**	**MVOT**	**DUZE**	**MZIM3**	**MZIM1**	**CASC**	**UMGE1**	**UMGE3**	**UMLA**	**UMGE7**	**MVOT**
		FFG ABUNDANCE (individuals.m^2)																	
Ancylidae	Scraper	0	90	1195	0	25	0	18	0	0	0	569	1753	479	217	0	8	21	0
Physidae	Scraper	0	0	0	0	0	0	0	0	0	2365	23	0	180	0	0	0	0	0
Elmid larvae:long	Scraper	0	0	21	0	113	0	0	0	0	0	40	79	0	127	0	58	0	26
Elmid larvae:tear drop	Scraper	21	53	324	0	297	0	48	0	18	0	39	2145	0	230	0	60	0	15
Elmidae adult	Scraper	0	108	0	0	12	0	42	0	0	0	243	17	0	0	0	15	0	0
Hydraenidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0
Psephenidae	Scraper	0	0	67	0	0	0	17	14	0	0	0	91	0	27	0	27	0	0
Ptilodactylidae	Scraper	0	0	0	0	0	0	0	0	0	0	72	0	0	0	0	0	0	0
Blepharaceridae larvae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	Scraper	19769	757	82	973	5999	1228	357	346	7138	25901	362	364	4027	1323	2836	78	1352	861
Ephydridae	Scraper	0	0	0	24	119	0	0	0	197	0	0	0	0	0	0	0	0	0
Glossosomadidae	Scraper	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydroptilidae	Scraper	0	0	12	0	12	0	42	0	0	0	0	0	0	0	0	0	0	0
Helodidae	Brusher	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0
Heptageniidae	Brusher	0	349	522	0	93	0	350	714	145	0	876	608	0	264	0	368	339	133
Baetidae	Deposit Feeder	102	894	2485	9089	1700	1099	3508	5247	709	15	1240	4034	4480	2302	2687	1286	4334	1014
Caenidae	Deposit Feeder	0	83	0	1211	0	0	0	0	705	0	177	178	230	13	28	14	0	374
Leptophlebiidae	Deposit Feeder	0	76	882	513	0	0	106	200	0	0	125	1098	0	0	0	524	0	117
Teloganodidae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonemouridae	Deposit Feeder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	Deposit Feeder	0	25	0	0	0	0	17	0	0	0	55	57	0	0	0	0	0	0
	Total Scraper	19790	1007	1699	997	6580	1228	525	360	7355	28266	1350	4449	4687	1924	2848	244	1372	902
	Total Brusher	0	349	522	0	93	0	350	714	145	0	876	608	0	264	0	379	339	133
	Total Deposit feeder	102	1078	3366	10813	1700	1099	3631	5448	1414	15	1598	5367	4709	2315	2715	1824	4334	1505
	Total Grazers	19892	2431	5589	11810	8370	2326	4505	6521	8914	28280	3825	10425	9397	4501	5563	2445	6045	2538
		FFG BIOMASS (g.m^2)																	
Ancylidae	Scraper	0,000	0,058	0,478	0,000	0,010	0,000	0,004	0,000	0,000	0,000	0,182	0,716	0,122	0,145	0,000	0,005	0,008	0,000
Physidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	1,286	0,014	0,000	0,071	0,000	0,000	0,000	0,000	0,000
Elmid larvae:long	Scraper	0,000	0,000	0,001	0,000	0,005	0,000	0,000	0,000	0,000	0,000	0,002	0,003	0,000	0,005	0,000	0,002	0,000	0,001
Elmid larvae:tear drop	Scraper	0,003	0,005	0,025	0,000	0,025	0,000	0,006	0,000	0,001	0,000	0,005	0,222	0,000	0,016	0,000	0,007	0,000	0,001
Elmidae adult	Scraper	0,000	0,034	0,000	0,000	0,002	0,000	0,007	0,000	0,000	0,000	0,094	0,003	0,000	0,000	0,000	0,002	0,000	0,000
Hydraenidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,003	0,000	0,000	0,000
Psephenidae	Scraper	0,000	0,000	0,058	0,000	0,000	0,000	0,002	0,100	0,000	0,000	0,000	0,283	0,000	0,049	0,000	0,130	0,000	0,000
Ptilodactylidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,009	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Blepharaceridae larvae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Chironomidae	Scraper	0,285	0,011	0,003	0,012	0,077	0,019	0,005	0,006	0,131	0,449	0,005	0,005	0,127	0,021	0,041	0,001	0,016	0,013
Ephydridae	Scraper	0,000	0,000	0,000	0,006	0,011	0,000	0,000	0,000	0,060	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Glossosomadidae	Scraper	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Hydroptilidae	Scraper	0,000	0,000	0,033	0,000	0,034	0,000	0,118	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Helodidae	Brusher	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,001	0,000	0,000
Heptageniidae	Brusher	0,000	0,573	0,366	0,000	0,130	0,000	0,701	0,560	0,088	0,000	1,270	0,619	0,000	0,913	0,000	0,606	0,937	0,231
Baetidae	Deposit Feeder	0,003	0,032	0,079	0,180	0,069	0,043	0,084	0,112	0,026	0,000	0,036	0,089	0,278	0,077	0,058	0,043	0,116	0,032
Caenidae	Deposit Feeder	0,000	0,007	0,000	0,145	0,000	0,000	0,000	0,000	0,069	0,000	0,020	0,018	0,045	0,002	0,006	0,000	0,000	0,047
Leptophlebiidae	Deposit Feeder	0,000	0,002	0,096	0,055	0,000	0,000	0,021	0,053	0,000	0,000	0,009	0,317	0,000	0,000	0,000	0,062	0,000	0,029
Teloganodidae	Deposit Feeder	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Notonemouridae	Deposit Feeder	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Leptoceridae	Deposit Feeder	0,000	0,008	0,000	0,000	0,000	0,000	0,005	0,000	0,000	0,000	0,019	0,017	0,000	0,000	0,000	0,000	0,000	0,000
	Total Scraper	0,288	0,108	0,598	0,018	0,163	0,019	0,141	0,105	0,192	1,735	0,310	1,233	0,320	0,236	0,044	0,148	0,025	0,015
	Total Brusher	0,000	0,573	0,366	0,000	0,130	0,000	0,701	0,560	0,088	0,000	1,270	0,619	0,000	0,913	0,000	0,608	0,937	0,231
	Total Deposit feeder	0,003	0,049	0,176	0,380	0,069	0,043	0,110	0,165	0,095	0,000	0,083	0,441	0,323	0,079	0,064	0,106	0,116	0,108
	Total Grazers	0,290	0,729	1,139	0,398	0,363	0,062	0,952	0,830	0,375	1,736	1,663	2,293	0,643	1,228	0,108	0,861	1,077	0,353

Appendix 3: Flood size classes for sites in the Western Cape and KwaZulu-Natal in 2015

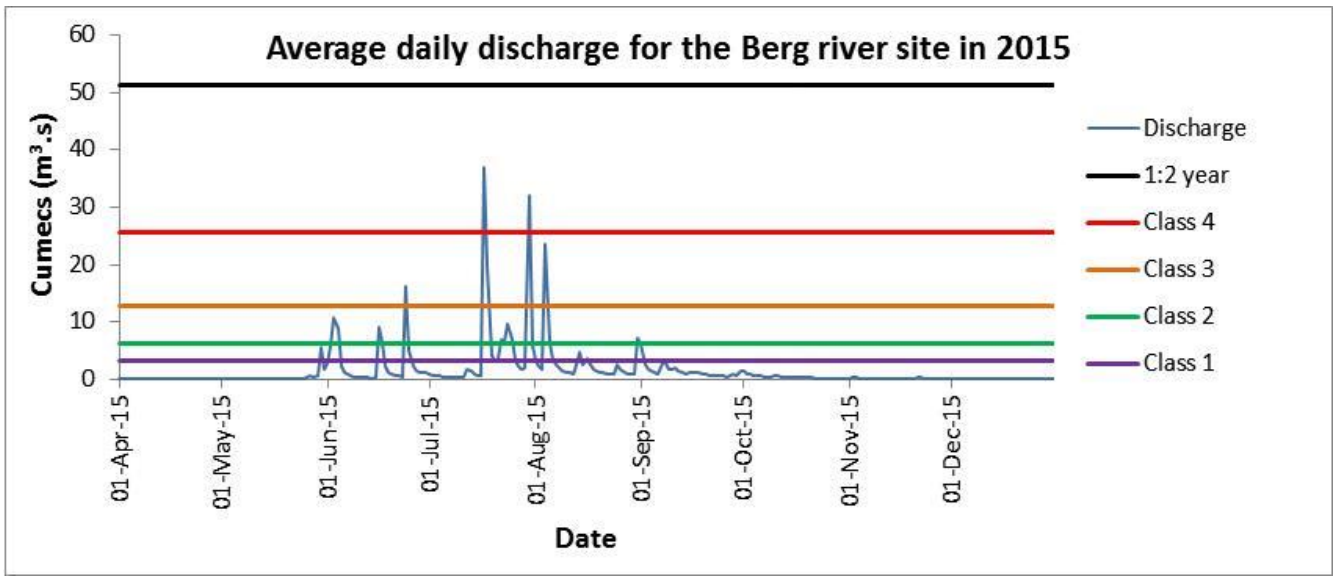
	Western Cape													KwaZulu-Natal								
SITES	**BREE**	**HEX**	**DWAR**	**OLIF**	**BERG**	**PALM1**	**DUIW**	**EERS**	**BOKR**	**KLEIN**	**PALM2**	**JONK**	**JAND**	**DUZE**	**MZIM3**	**MZIM1**	**CASC**	**UMGE1**	**UMGE3**	**UMLA**	**UMGE7**	**MVOT**
	Discharge ($m3.s^{-1}$)																					
Class 1	11,24	4,72	0,24	17,41	3,21	2,69	5,02	1,32	0,09	1,09	4,63	0,77	1,83	2,24	12,84	4,38	0,02	2,23	2,16	0,18	7,89	0,38
Class 2	22,48	9,44	0,47	34,82	6,41	5,38	10,04	2,64	0,18	2,18	9,25	1,54	3,66	4,49	25,68	8,75	0,05	4,46	4,33	0,35	15,78	0,76
Class 3	44,95	18,88	0,94	69,65	12,83	10,75	20,09	5,28	0,37	4,36	18,50	3,09	7,31	8,98	51,37	17,51	0,10	8,93	8,65	0,71	31,56	1,52
Class 4	89,90	37,75	1,89	139,29	25,65	21,50	40,18	10,56	0,74	8,72	37,00	6,18	14,62	17,95	102,73	35,01	0,19	17,85	17,30	1,41	63,12	3,04
1:2 year	179,80	75,50	3,77	278,58	51,30	43,00	80,35	21,12	1,48	17,44	74,00	12,35	29,24	35,90	205,46	70,03	0,39	35,70	34,60	2,82	126,23	6,09

Appendix 4: Average daily discharge at the Berg site for a) 2007-2008 and b) 2015

a



b



Appendix 5: Hydrographs comparing the average daily discharge between PALM1 and HEXR from October 2013 – October 2015