SPATIO-TEMPORAL VARIATION IN THE PHYTOBENTHOS AND PHYTOPLANKTON COMMUNITY STRUCTURE AND COMPOSITION OF PARTICULATE MATTER ALONG A RIVER-ESTUARY CONTINUUM ASSESSED USING MICROSCOPIC AND STABLE ISOTOPE ANALYSES

Dissertation submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (SCIENCE)

of

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by

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PREFACE

This thesis comprises a general introduction (Chapter 1), study site description (Chapter 2), results (Chapter 3–7) and a general synthesis (Chapter 8) chapter. The combined reference list at the end of the thesis ensures limited repetition. The result sections were organised as scientific papers, which have either been published, or are *in press, or currently under review* (see below):

Publication list

1. **Dalu T**, Richoux NB, Froneman PW. 2014. An assessment, using multivariate analysis and stable isotopes, of the effects of substrate type on phytobenthos communities. *Inland Waters*. 4(4):397–412.
2. **Dalu T**, Froneman PW, Chari LD, Richoux NB. 2014. Colonisation and community structure of benthic diatoms on artificial substrates following a major flood event: A case of the Kowie River (Eastern Cape, South Africa). *Water SA*. 40(3):471–480.
3. **Dalu T**, Froneman PW, Richoux NB. 2014. Phytoplankton community diversity along a river-estuary continuum. *Transactions of the Royal Society of South Africa*. 69(2):107–116.
4. **Dalu T**, Bere T, Richoux NB, Froneman PW. Use of multimetric and stable isotope analysis to assess the spatial and temporal variation of periphyton communities along a small temperate river system. *South Journal of Botany*. ***Under review***.
5. **Dalu T**, Richoux NB, Froneman PW. Assessing habitat connectivity using stable isotope ratios: contributions of allochthonous and autochthonous materials to suspended particulate matter and detritus along a river-estuarine continuum. *Science of the Total Environment*. ***Under review***.

Sections of the estuary results have also been published as:

6. Bergamino L, **Dalu T**, Richoux NB. 2014. Spatial and temporal patterns in sediment organic matter composition within an estuarine environment: stable isotope and fatty acid signatures. *Hydrobiologia*. 732(1):133–145.
7. Bergamino L, **Dalu T**, Whitfield AK, Carassou L, Richoux NB. 2014. Stable isotope evidence of food web connectivity by a top predator (*Argyrosomus japonicus*: Sciaenidae: Teleostei) in the Kowie Estuary, South Africa. *African Journal of Marine Sciences*. 36(2): 207–213.

Other publications

8. **Dalu T** and Froneman PW. 2014. Can stable isotopes ($\delta^{15}N$, $\delta^{13}C$) and fatty acid signatures be employed to observe changes in phytobenthos composition on artificial substrates? *African Journal of Aquatic Science*. 39(4): 425–433.
9. **Dalu T**, Taylor JC, Richoux NB and Froneman PW. 2015. The morphology and distribution of *Entomoneis paludosa* (W. Smith) Reimer in African waters. *Fottea*. 15(1). **In press.**
10. **Dalu T**, Richoux NB and Froneman PW. Distribution of benthic diatom communities in a permanently open temperate estuary, in relation to physico-chemical variables. Special Issue: *South Africa Journal of Botany.* **Under review.**
11. **Dalu T**, Galloway AWE, Richoux NB and Froneman PW. Effect of substrate type on phytobenthos essential fatty acid availability in an austral temperate river system. *Hydrobiologia.* **Under review.**
12. Richoux NB and **Dalu T**. Fatty acid profiles indicate gradients of terrestrial inputs and nutritional quality in food sources along a river-estuary continuum. Target journal *Hydrobiologia*. **In preparation.**

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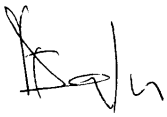
DECLARATION

I, Tatenda Dalu, hereby declare that the work described in this thesis was carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Prof. William Froneman and Dr. Nicole B Richoux. The various components of the thesis comprise original work by the author and have not been submitted to any other university.



Tatenda Dalu

29 August 2014

GENERAL ABSTRACT

Phytoplankton and phytobenthos communities play an important role in lotic systems as primary producers providing essential biomolecules to higher trophic oganisms and are important indicators for environmental or ecological change. In this thesis, field studies (observational and experimental) along a river–estuary continuum were conducted to assess the spatio-temporal variation and development of phytobenthos and phytoplankton communities using a combination of stable isotope and community analyses in a temperate southern African system across four study periods: September (early spring) and November/December (late spring) 2012, and February (summer) and May/June (winter) 2013. Additionally, the sources and composition of the particulate organic matter were also analysed using stable isotope (δ^{15}N and δ^{13}C) analysis.

The effects of substrate type and flood occurrence were assessed through experimental studies at an up- and downstream site of the river after a major flood event that occurred between October and November 2012. Common household tiles were used as artificial substrates to study the development/succession of phytobenthos communities after the flood disturbance. Distinct diatom communities were observed between upstream and downstream sites and at each site, community structure changed with time indicating succession. In addition to recording diatom characteristics on three natural substrates, namely; macrophytes, rocks and sediment, artificial substrates observations were also made on three different types of artificial substrates, namely; brick, brown clay and grey clay tiles. The natural (species richness 78) and artificial substrates (sp. richness 93) had different communities with the latter having greater species richness. Common phytobenthos taxa were not restricted to a single substrate but preference was generally high for the artificial substrates, especially brown tiles (mean sp. richness 47). Results of the redundancy analysis (RDA) analysis indicated that ammonium, conductivity, total dissolved solids, salinity, pH, oxygen reduction potential, phosphate and water depth were the major determinants of the phytobenthos composition at the two sites.

The spatio–temporal variation of phytoplankton and phytobenthos communities and allochthonous organic matter along the river–estuary continuum was assessed at 8 sites using a combination of community and stable isotope analyses. A total of 178 species belonging to 78 genera were recorded with diatoms being predominant, accounting for 81.9 % of the total abundance. The total chl-*a* concentration along the river-estuary continuum increased from spring to a high in summer before decreasing to a low in winter. Periphyton communities were observed to be significantly different across sites ($p < 0.05$) in terms of species richness, abundances and isotopically The high periphytic $\delta^{15}N$ values (range 7.9–15.2 ‰) recorded at the downstream sites compared to the pristine upstream sites ($\delta^{15}N$ values range 4–7 ‰) suggest nutrient enrichment most likely derived from anthropogenic sources. Overall, our results reveal general patterns of periphyton communities and stable isotopes and provide improved information in the use of periphyton $\delta^{15}N$ as an excellent indicator of anthropogenic nitrogen pollution.

Ecologists are interested in the factors that control, and the variability in, the contributions of different sources to mixed organic materials traveling through lotic systems. We hypothesized that the source matter fuelling mixed organic pools in a river-estuary continuum varies over space and time, with the upper reaches of a system characterized by allochthonous-dominated material and autochthonous contributions becoming more important in the lower reaches. Samples of the mixed organic pools and allochthonous and autochthonous source materials were collected during the four study periods. The C:N ratios of suspended particulate matter (SPM) collected during summer and winter indicated that the lower reaches of the system had similar organic matter contributions from the freshwater and terrestrial sources. Stable isotope analysis in R revealed that the contributions of autochthonous organic matter were high in SPM along the entire continuum, and aquatic macrophytes were significant contributors to SPM specifically in the upper reaches. The terrestrial leaves made major contributions to the SPM in the middle regions of the system (i.e. downstream sites of the river, particularly in early and late spring). Bulk detritus had

large allochthonous matter components in the lower reaches (estuary), and the contributions of aquatic macrophytes and benthic algae to bulk detritus were high (> 50 %) in the upper to middle reaches (river), but low (< 20 %) in the lower reaches (estuary).

The current investigation represents the first attempt to assess the validity of the River Continuum Concept (RCC) in a southern African temperate river. The phytoplankton and phytobenthos communities, and chl-*a* concentration followed a trend similar to that proposed for the river continuum concept (RCC). The middle reaches based on the phytobenthos or phytoplankton communities and chl-*a* concentrations which were employed as proxies for primary production, were the most productive, while the upper reaches were the least primary productive. The evaluation of organic matter contributions to the SPM and detritus along the river–estuary continuum provided a baseline assessment of the nature and sources of potential food for consumers inhabiting different locations during different times of the year. Incorporating such spatio-temporal variations in SPM and detritus into food web studies will improve our understanding of the flow of carbon through aquatic systems.

CHAPTER 1

GENERAL INTRODUCTION

Plate 1. View of the Horseshoe where site E1 was located during the current investigation. Photo by Dr. Nicole B. Richoux

General introduction

Accelerated population growth, industrialisation and agricultural practices have contributed to a decline in the health of freshwater ecosystems worldwide (Dudgeon et al., 2006). As a consequence, these systems are demonstrating losses in biodiversity far in excess of those recorded in terrestrial ecosystems (Dudgeon et al., 2006). Indeed, it may be argued that freshwater ecosystems are among the most endangered ecosystems worldwide (Dudgeon et al., 2006; Nel and Driver, 2012). It is anticipated that global climate change will exacerbate the impacts of human activities on aquatic ecosystems (Dudgeon et al., 2006). The National Biodiversity Assessment of South Africa provides a regular high level summary of the state of South Africa's biodiversity (Nel and Driver, 2012). A recent assessment indicates that of the 223 river ecosystems within the subregion, 55 % can be regarded as threatened (Nel and driver, 2012). The poor ecological state of these systems can be ascribed to poor maintenance of sewerage infrastructure, freshwater abstraction, poor agricultural practices, habitat loss and presence of invasive species including both vegetation and fish (Nel and Driver, 2012). There is therefore, an urgent need to establish and implement management and conservation strategies to ensure the sustainability of these ecosystems (Nel and Driver, 2012). Critical to the conservation of these endangered ecosystems is an understanding of the various hydrodynamic, physico-chemical and biological factors that structure these systems and contribute to their ecosystem functioning.

River ecosystems

In local ecosystems, communities that appear discrete are open and connected in myriad ways to outside influences with connectivity varying enormously among systems, from near total isolation to strong mixing (Polis et al., 1997). The dynamics of headwater stream ecosystems are generally regarded as occurring at the interface of aquatic and terrestrial ecosystems, where food web dynamics are influenced in complex ways by both *in situ* primary productivity and allochthonous inputs from the terrestrial landscape (Vannote et al., 1980; Nakano et al., 1999). The

inputs of allochthonous subsidies from terrestrial ecosystems represent an important energy source of production in most headwater streams (Polis et al., 1997).

Several conceptual models such as the river continuum concept (Vannote et al., 1980), flood pulse theory (Junk, 1984), riverine productivity model (Thorp and Delong, 1994; 2002) and more recently, the ecosystem synthesis concept (Thorp et al., 2006) have been proposed to describe the variations and sources of organic matter and primary production in river ecosystems. The river continuum concept hypothesizes that organic matter driving riverine food webs is as a result of the inefficiencies of upstream consumers in processing terrestrially derived organic matter entering headwater and mid-order streams (Vannote et al., 1980). The downstream transport of organic matter shape trophic dynamics of rivers since in-stream primary production is limited by depth and turbidity (Vannote et al., 1980).

The riverine productivity model hypothesizes that production in rivers is fuelled primarily by in-stream autotrophic production such as microalgal (phytoplankton and phytobenthos) production (Thorpe and Delong, 2002). Some autotrophy may originate in the main river channel but the major sources of carbon are derived from primary production in areas of low current velocity and during regular and aperiodic inundation of the floodplain (Thorp and Delong, 1994; 2002). Despite lively discussions on the origin and significance of the various forms of organic matter providing the majority of energy to large lowland river ecosystems, no model has received vigorous empirical testing (Delong and Thorp, 2006). This study using stable food web models seeks to identify whether the particulate organic matter or phytoplankton and phytobenthos are potential primary energy sources directly or indirectly fueling primary production in a river-estuary system.

River Continuum Concept

The river continuum concept (RCC) is based on the idea that a watercourse is an open ecosystem that is in constant interaction with the bank, and hence, is

constantly changing as it moves from source to mouth (Figure 1.1). According to the RCC, headwaters (upstream sites) are small shaded streams where allochthonous inputs of coarse particulate organic matter are the dominant resource for consumers (Vannote et al., 1980; Nilsson et al., 1999), but as the river widens, energy inputs change as the sunlight reaches the benthos and supports significant algal production. The biological processing of coarse particulate organic matter from the headwaters results in the transport of large amounts of fine particulate organic matter to downstream ecosystems (Vannote et al., 1980; Statzner and Higler, 1985; Townsend et al., 1987; Nilsson et al., 1999). Phytoplankton will dominate autochthonous production in the downstream regions, although photosynthetic rates are limited by high turbidity and mixing. Allochthonous inputs in terms of fine particulate organic matter tend to be the energy source for large rivers (Vannote et al., 1980), but recent studies have found that allochthonous carbon is very lipid poor hence autochthonous are important (Brett et al., 2009; Taipale et al. 2014). Middle streams are expected to receive the largest variety of energy inputs and to host the highest biological diversity (Lindeman, 1942; Vannote et al., 1980; Townsend et al., 1987; Statzner and Higler 1985; Carpenter et al., 2005; Marcarelli et al., 2011), since middle streams are more dependent on in-stream primary production i.e. photosynthesis (P) is greater than respiration (R; P>R) and fine particulate organic matter is argued to be the principal carbon source in downstream reaches and much of this is derived from upstream processing (Vannote et al., 1980). Chang et al. (2012) studies showed that the quantity and composition of fine particulate organic matter and coarse particulate organic matter transported downstream along the Lanyang River (China) were generally consistent with the predictions of the RCC. The contribution of leaves to coarse particulate organic matter decreased from the headwater to the estuary while those of periphyton and macrophytes increased (Chang et al., 2012).

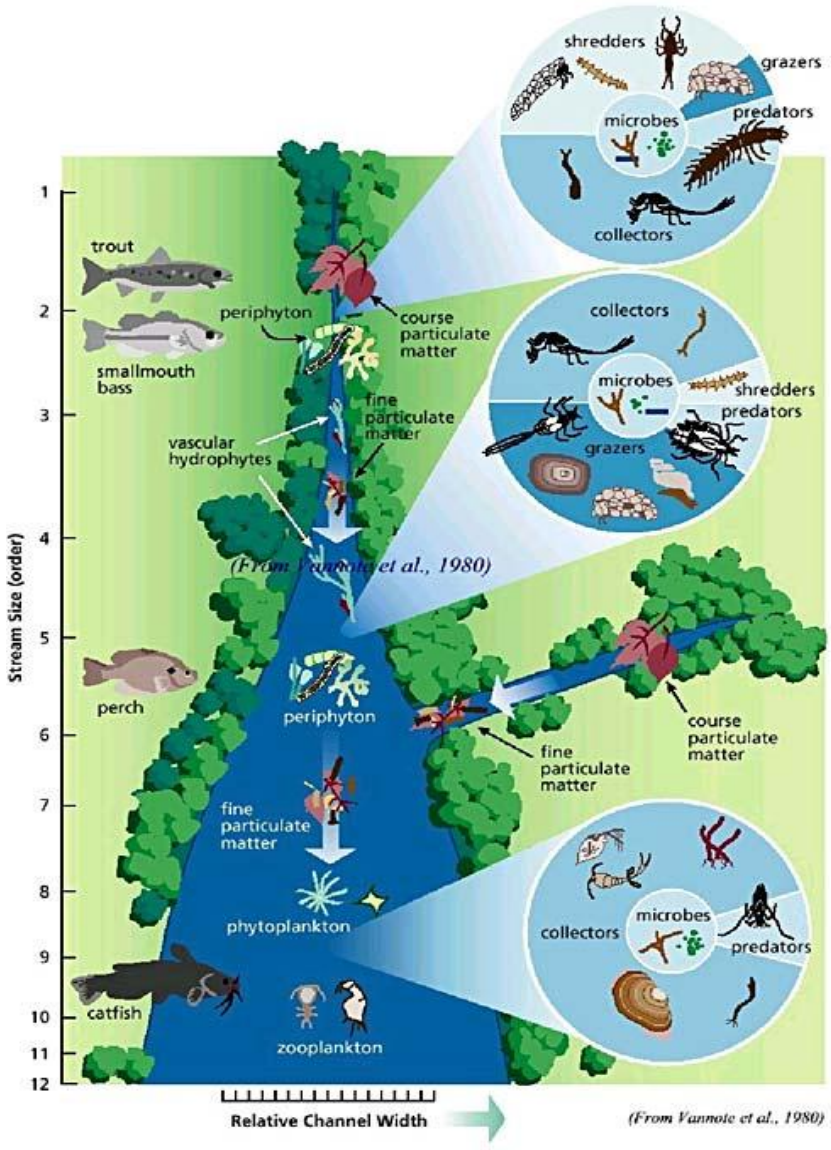


Figure 1.1. Diagram showing the river continuum concept as outlined by Vannote et al. (1980). Source: www.oxbowriver.com/Web_Pages/Stream_Ecology_Pages/Ecology_Riparian/Ecology_RCC.html. Accessed 31 May 2012.

Allochthonous organic matter inputs

Organic subsidies influence faunal and floral population dynamics, community interactions and ecosystem processes through supply of energy (Lindeman, 1942; Carpenter et al., 2005; Marcarelli et al., 2011), and can dominate ecosystem budgets and strongly affect within-ecosystem dynamics (Marcarelli et al., 2011). There is

growing evidence for the significance of cross-boundary subsidies between aquatic (streams, rivers and lakes) and terrestrial (islands and riparian) environments (Carpenter et al., 2005; Shurin et al., 2006; Bartels et al., 2011). Lindeman (1942) suggested two different properties account for the structural discrepancy observed between aquatic and terrestrial ecosystems;

1. The dominance of unicellular primary producers in aquatic ecosystems as opposed to multicellular primary producers in terrestrial ecosystems.
2. Ecosystems that lie low in the landscape receive more organic and inorganic allochthonous matter than ecosystems in high positions (Shurin et al., 2006; Bartels et al., 2012). This is the result of low laying ecosystems accumulating nutrients and detritus through runoff.

Freshwater ecosystems receive inorganic nutrients and organic matter in dissolved and particulate forms from terrestrial ecosystems from which aquatic consumers can potentially benefit (Carpenter et al., 2005). Several field and laboratory experiments suggest terrestrial particulate organic carbon inputs account for a disproportionate portion of secondary production and that autochthonous resources are possibly more important than allochthonous foods (Mantel et al., 2004; Brett et al., 2009; Lau et al., 2009). Recently, Cole et al. (2011) showed that zooplankton diet comprised of ~20–40 % of organic material terrestrial in origin. Organic subsidy fluxes from terrestrial to aquatic environments are largely governed by physical factors such as gravity, run-off, precipitation, wind activity and to a lesser extent by biotic factors such as the dispersal of terrestrial prey organisms (Bartels et al., 2012).

Understanding the spatial linkages between habitats is important for understanding community dynamics (Vannote et al., 1980; Thorpe and Delong, 2002). It is therefore, of paramount importance to understand how organic subsidies link adjacent aquatic and terrestrial habitats. This study will attempt to assess the importance of autochthonous production and allochthonous subsidies from the upstream to downstream locations in relation to variations in in the local physical

conditions and the phytoplankton and phytobenthos communities as conceptualized by Lindeman (1942) and Vannote et al. (1980).

Phytobenthos ecology

Phytobenthos is a general term used to describe algal communities associated with submerged substrates (Bate et al., 2002). Phytobenthos communities in rivers and standing waters are neither simple, nor homogeneous (DARES, 2005), and occur in four distinct habitats: *epiphyton* (supported by aquatic plant surfaces), *epilithon* (stone surfaces), *epipsammon* (sand surfaces) and the *epipelon* (mobile taxa growing among deposited inorganic and organic sediment particles; Bate et al., 2002; DARES, 2005). Under natural conditions, the distribution of phytobenthos in rivers reflects a complex series of interactions between hydrology, water chemistry, substrate type and biotic factors such as grazing by macroinvertebrates and fish (Bates et al., 2004; Schletter et al*.,* 2011). The relative importance of these factors in determining the phytobenthos community structure, however, demonstrates a high degree of both spatial and temporal variability. This variability is exacerbated by human activities as phytobenthos been demonstrated to become particularly abundant where water systems are impacted by anthropogenic influences such as nutrient enrichment or changes in hydrology due to flow modifications (Bates et al., 2002).

The most common phytobenthos in freshwater habitats are blue green algae (Cyanophyta), green algae (Chlorophyta), diatoms (Bacillariophyta) and red algae (Rhodophyta) (Bate et al., 2002). Phytobenthos occur in unicellular, colonial or filamentous growth forms. During the initial stage of colonisation, bacteria and fungi are conspicuous constituents of the benthic community and precondition the surface for early diatom colonisation (Bate et al*.*, 2002; DARES, 2005). The first diatoms to colonise are prostrate species closely attached to the surface (DARES, 2005). Migrating species contribute significantly to the early colonisation of newly immersed surfaces, particularly the motile species which re-suspend easily into the water

column and migrate downstream (Bate et al., 2002; DARES, 2005). In the next stage of succession, larger species that are apically attached or stalked become more conspicuous in the benthic community (Poff et al., 1990; Bere, 2011). Depending on the local growth conditions such as stream velocity and light availability, a stratified community is formed, and loosely attached diatoms are predominant under intermediate currents and high light conditions. Under high current and low light conditions, a largely unstratified community dominated by different species is formed (Poff et al., 1990; Bere 2011).

Phytobenthos play a pivotal role in nutrient cycling processes in streams, also referred to as nutrient spiralling (Newbold et al., 1982). The nutrient spiralling concept deals specifically with the coupled processes of material cycling and transport. Webster and Patten (1979) pointed out that carbon and nutrients undergo continual downstream displacement as they cycle. Nutrient spiralling is therefore, represented by a spiral (Figure 1.2) in which the diameter decreases in size as the biological activity increases (the faster the recycling). As the spirals congregate, the system's retention capacity increases (less transport) (Newbold et al., 1982). Webster and Patten (1979) suggested that the degree of retention and reutilization of nutrients in a stream is associated with the tightness of the spirals.

Sources of nutrients that are utilised by the phytobenthos are derived from the substratum and stream water (Bate et al., 2002; Karthick et al., 2010). Nutrient transformation and remineralisation are other important functions of stream phytobenthos (Bate et al., 2002). Thus, phytobenthos constitute a fundamental link between primary (autotrophic) and secondary (heterotrophic) production and form a vital component of aquatic ecosystems. The total supply of nitrogen can be increased via fixation by endosymbionts such as *Rhopalodia gibba* and *Epithemia turgida* which have been shown to fix atmospheric nitrogen (Bate et al., 2002; Karthick et al., 2010).

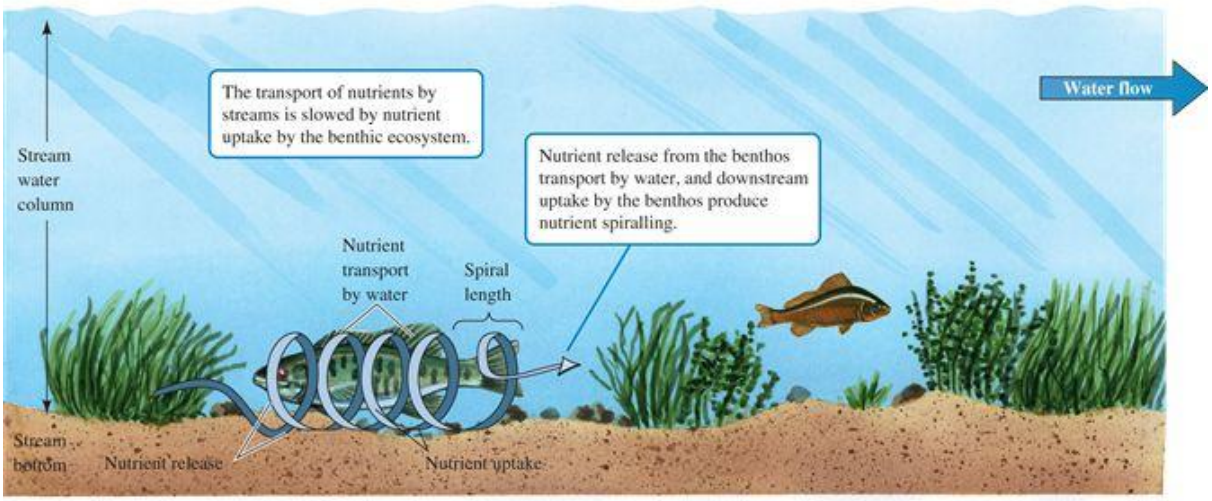


Figure 1.2. Nutrient spiraling between particulate organic matter, including phytoplankton, phytobenthos and the water column proposed in a lotic system. Source: Molles (1998)

In South African river systems, the phytobenthos have been studied extensively for many decades (Giffen, 1963; 1970; 1971; 1973; Schoeman and Archibald, 1976; Schoeman, 1982), and efforts have been made to relate phytobenthos associations to both hydrology and water quality (Bate et al., 2002; 2004; Taylor et al., 2005a, b; Harding et al., 2005; Taylor et al., 2007). These studies indicated that while some species are endemic to the region, the majority of diatoms in the phytobenthos comprise species commonly found in other parts of the world (Harding et al., 2005; Taylor et al., 2007). The taxonomic work undertaken using microscopy by the diatom scholars in South Africa has been of great scientific value and contributed to our understanding of the dynamics of the phytobenthos within southern African rivers (Bates et al., 2002).

Phytobenthic studies have been carried out in many estuaries and coastal environments around South Africa such as St Helena Bay (Giffen, 1973), False Bay (Giffen, 1971), Sea Point (Giffen, 1970), Groot Brak (Adams and Bate, 1999), Nyara (Walker et al., 2001), Mpenjati (Perissinotto et al., 2002), Mhlanga (Iyer, 2004; Perissinotto et al., 2006), Mdloti (Blaber et al., 1984; Mundree, 2001; Perissinotto et al., 2003, 2006; Iyer, 2004; Anandraj et al., 2008), Tongati (Blaber et al., 1984), Van

Stadens (Skinner et al*.,* 2006) and Kowie River (Giffen, 1970). Phytobenthos are considered the main source of primary production in estuaries, streams and river systems (Wetzel, 2001; Perissinotto et al., 2002; Skinner et al*.,* 2006) and are thus functionally important in sequestering and transforming inorganic nutrients into organic forms (Newbold et al. 1982; Stevenson, 1996; Mundree, 2001). Several cyanobacteria or phytobenthos species including *Epithemia* and *Rhopalodia* spp. are capable of converting atmospheric nitrogen into ammonia or amino acids (Borchardt 1996). The phytobenthos themselves constitute a significant source of energy e.g. sugars, fatty acids etc. for higher trophic levels particularly in oligotrophic systems where water column production is low. Like their freshwater counterparts, numerous studies have attempted to employ the phytobenthos community structure, as an indicator of river/estaury water quality (Adams and Bate, 1999; Bate et al., 2002; Carvalho et al., 2002).

Phytoplankton ecology

Phytoplanktons are microscopic unicellular plants that drift in the water currents and are important primary producers in aquatic ecosystems (Piirsoo et al., 2008; Ezekiel et al., 2011, Wu et al., 2011). In large rivers, phytoplankton are often the dominant primary producers and as such play a key role in the biogeochemical cycling of carbon, nitrogen, phosphorus and other elements such as nitrogen, iron, manganese, molybdenum and zinc (Piirsoo et al., 2008; Ezekiel et al., 2011). For the majority of aquatic ecosystems, phytoplankton are the foundation of the food web and provide a nutritional base for zooplankton and subsequently, for other invertebrates and vertebrates (Ezekiel et al., 2011; Wu et al., 2011).

Phytoplankton communities in rivers are strongly influenced by pulses of flood and drought, which constitute the predominant forces in the functioning of rivers and integrate the main channel and the alluvial valley in a dynamic system (Junk et al., 1989; Devercelli, 2006; Wu et al., 2011). This permanent pulsing connectivity is

responsible for lateral exchanges of water, suspended solids and dissolved materials. Two different but analogous situations hinder plankton density increases;

1. Episodes of high discharge when phytoplankton are quickly transported embedded in a turbid medium,
2. Episodes of low flow where other constraints such as insufficient inoculums or sedimentary losses may occur (Devercelli, 2006).

Spatial variation of phytoplankton can result from a change in river morphology from the headwaters to low laying streams due to changes in biotic and abiotic factors (de Domitrovic et al., 2007; Piirsoo et al., 2008) Phytoplankton generally attain high abundances in lowland rivers, where residence time and low flow velocity allow sufficient time for growth and reproduction (Devercelli, 2006; de Domitrovic et al., 2007; Piirsoo et al., 2008; Wu et al., 2011). Conversely highly flow rates are often associated with a significant decrease in phytoplankton concentrations within rivers. Phytoplankton succession is mainly determined by changes in hydrological and other abiotic parameters such as water level, dissolved oxygen, run-off, sediment load, light intensity both in space and time (de Domitrovic et al., 2007; Piirsoo et al., 2008). Biotic and hydrochemical factors such as conductivity are rarely reported to affect the development of the phytoplankton community in rivers (Devercelli, 2006; Piirsoo et al., 2008). Although a longitudinal decrease in phytoplankton abundance from the upper to the lower reaches is a well-known phenomenon in most river systems (Reynolds, 1988), population densities can be substantial in the upper and middle reaches, particularly in long slow-flowing rivers (Piirsoo et al., 2008). The balance between the conditions that promote or prevent the development of phytoplankton in rivers varies both temporally and spatially, with temporal changes being rapid after floods or heavy rains due to an increase sediment loads that attenuate underwater light (de Domitrovic et al., 2007; Wu et al., 2011).

Phytoplankton have been studied extensively in lentic fresh waters (lakes and reservoirs), where long residence times and low flow velocities allow sufficient time

for growth and reproduction (Harding, 1992). A detailed description of all phytoplankton studies in South Africa lentic systems was recently published by Van Ginkel (2012). In estuaries, several studies have been carried out by Hilmer and Bate (1991), Thomas et al. (2005), Anandraj et al. (2007), Gama (2008) and van der Molen (2011). In comparison with lakes and estuaries, much less is known about the spatio-temporal development of phytoplankton in South African rivers (Roos et al., 1996; Piirsoo et al., 2008; Wu et al., 2011). The spatio-temporal patterns of phytoplankton communities are important for understanding ecosystem functioning because phytoplankton can affect ecosystem processes, functioning, and stability and their community structure can reflect major shifts in environmental conditions and river health (Suikkanen et al*.,* 2007; Wu et al., 2011).

Biomarker methods

Stable isotopes in phytoplankton and phytobenthos

Stable isotope analysis is a useful tool used by ecologists to address a wide range of questions related to food sources of organisms, the length of food chains and the transfer of contaminants (Yoshii et al., 1999; Marty and Planas, 2008). Consumers assimilate carbon and nitrogen from their diets and preferentially respire the light carbon isotope (δ^{12}C) and excrete the light N isotope (δ^{14}N) (Marty and Planas, 2008). Thus, consumers are generally enriched with heavier isotopes in relation to their food source (Finlay, 2004). Stable isotope tracing offers two potential advantages over other traditional methods in terms of food-web analysis; δ^{13}C and δ^{15}N ratios of animal tissue represent the integration of carbon and nitrogen over a prolonged period and; they are also based on assimilation rather than ingestion (Finlay, 2004). The δ^{13}C value is the average value of all organic compounds (including carbohydrates, fatty acids, other lipids) as well as the δ^{15}N value of all organic nitrogen including compounds (amino acids).

Investigation of food web and ecosystem dynamics with isotopic analysis utilises the abundance of stable isotopes in nature to follow pathways of organic matter through

successive tropic levels (Hill, 2007). Stable isotope ratios in plankton are highly variable in rivers and influenced by multiple biogeochemical, physical and physiological processes throughout watersheds (Finlay and Kendall, 2007). Environmental conditions, such as resource type and food availability demonstrate substantial seasonal and spatial variability and may directly influence carbon and nitrogen stable isotope ratios in consumers, and thus determine when, where, and how stable isotopes may be applied in food web studies (Finlay and Kendall, 2007). The carbon isotopic fractionation of phytoplankton and phytobenthos sugars/carbohydrates is an important biogeochemical process, as fractionation of carbon isotopes during photosynthesis is a key parameter for understanding organic carbon isotope signatures in aquatic ecosystems (Keller and Morel, 1999; Bade et al., 2006).

Studies of trophic ecology within aquatic systems have focused primarily on carbon and nitrogen as both these elements demonstrate predictable changes in isotope ratios when moving from food source to consumer (Werner and Brand, 2001; Finlay, 2004; Richoux and Froneman, 2007). The isotopic signature of an organism provides integrated information about its feeding habits over significant time periods corresponding to taxon-specific organic carbon turnover times (Yoshii et al., 1999; Marty and Planas, 2008). Aquatic ($\delta^{13}C$ = -19.3 to -27.9 ‰) and wetland vascular ($\delta^{13}C$ = -24.4 to -31.4 ‰) plants display the greatest range in isotopic composition while phytobenthos and particulate organic matter are more constrained in their isotope signatures (Maier et al., 2011). Thus, stable carbon isotopes ($\delta^{13}C$) hold much promise as a tool for determining the energy base of aquatic food webs because terrestrial and aquatic plants often have distinct carbon signatures (Finlay et al., 1999).

Bulk particulate organic carbon represents a mixture of live and detrital matter of terrestrial and aquatic origin. Because of the difficulty of separating living and non-living organisms within the POM, previous studies have assumed that the carbon

signature of particulate organic carbon is equivalent to that of phytoplankton (Marty and Planas, 2008)**.** This is likely a valid assumption in systems dominated by high phytoplankton production such as in eutrophic waters (Keough et al., 1998; Finlay et al., 1999; Marty and Planas, 2008). For example, Gu et al. (2006) showed that the average ^{13}C isotope of particular organic matter was -19.3 ‰, consistent with an autochthonous origin from phytoplankton production in Lake Wauberg, Florida. In many oligotrophic freshwater ecosystems, however, terrestrial organic matter often represents a significant portion of bulk particulate organic matter and carbon flow (Carpenter et al., 2005). Bunn et al. (1989) studies showed that the terrestrial detritus carbon signature (-27.8 ‰) was different from algae (-22.6 ‰) in small tributary streams and rapids of the mainstream Koroc River. Moreover, terrestrial detritus was considered the most likely source of energy fuelling the animal communities. Following this, Rounick et al. (1982) showed that allochthonous (-27.3 ‰) and autochthonous (-35 ‰) plant materials were isotopically distinct in small New Zealand streams, thereby providing a basis for identifying food resource utilization by aquatic animals. These results suggest that the sources and composition of POM in riverine systems is strongly linked to hydrology and nutrient status of the waters. The potential variable contribution of terrestrial derived carbon to the POM can be assessed when calculating algal signatures by including the ratio of algal carbon to total particulate organic carbon in mixing models (Finlay et al., 1999; Marty and Planas, 2008).

Aims

The main aims of this thesis are to assess the spatio-temporal patterns in the phytobenthos and phytoplankton community structure and composition of the POM along a river-estuary continuum. The study was conducted along the length of a medium sized lowland temperate river located along the eastern seaboard of South Africa. The main findings of the investigation were then employed to assess the validity of the River Continuum Concept (RCC) within the system

Thesis outline

As highlighted in the preface, the thesis consists of general introduction (Chapter 1), a study site description (Chapter 2), five data chapters (Chapters 3 to 7) and a general conclusions chapter, Chapter 8. Chapters 3 and 4 on the thesis focus on the development and growth of phytobenthos on different substrates and the impact of a flood event on the phytobenthos community structure. Chapters 5 and 6 assess the spatial-temporal variation in the phytoplankton community structure along the river–estuary continuum. Finally in Chapter 7, stable isotopes analyses are employed to examine the spatial and temporal variations in the composition of the POM.

CHAPTER 2

STUDY AREA

Plate 2. Phytobenthos collected from site F1 in the upper reach of the Kowie River during separation from benthic sediment. Photo by Tatenda Dalu

Catchment characteristics

This investigation was conducted in the Kowie River system located in the Eastern Cape Province of South Africa (33º36′10.69"S, 26º54′03.01"E; Figure 2.1). The Kowie River is a permanently open system draining a relatively small catchment area estimated at ~800 km^2 (Heydorn and Grindley, 1982; Whitfield et al., 1994). The system is classified as an intermediate and extends inland as far as the Cape Fold Belt Mountains. The river system is considered ancient, tracing back to the earliest Gondwana drainage region (Heydorn and Grindley, 1982). The Kowie system source is the Grahamstown Heights hills, from where it flows in a south-east direction. The main tributaries are the Bloukrans, Brakrivier, JP De Wet Steyn and Lushington (or Torrens) Rivers. The little Kowie River tributary enters the estuarine portion of the river 14 km from the mouth and multiple streams feed into the river upstream of the sharp estuarine-to-freshwater transition zone at Waters Meeting Nature Reserve (Bathurst). The total length of the river system is estimated at ~70 km. The river system has a meandering course which has cut deeply into the Bokkeveld shale's which make up most of its catchment, with the upper reaches of the tidal water lying in a narrow valley with steep, high and densely wooded slopes. Commercial agricultural activities (pineapples, citrus, chicory, fodder crops, beef cattle and goats) utilize part of the upstream waters of the river (Figure 2.1). The Kowie is one of the longest tidal systems in South Africa, with marine waters to intruding up to about 30 km upstream from the river mouth (Heydorn and Grindley, 1982).

Kowie Estuary

The Kowie Estuary is approximately 21 km in length and varies in width and depth between 30–150 m and 2–8 m, respectively (Heydorn and Grindley, 1982; Whitfield et al., 1994). The upper reach has steep banks, often vegetated right down to the water's edge. The bottom comprises mainly of very fine sand and silt and has a very narrow intertidal zone (<10 m wide). The middle reach broadens out to about 100 m in width and 3 m deep. Intertidal salt marshes and mud banks >50 m wide are found in some areas. The lower reach consists of an artificial channel about 80 m wide,

linked to the Kowie marina which covers an area of 45 ha. The channel and marina canal walls comprise granitic blocks which drop almost vertically to a sandy bottom 24 m deep.

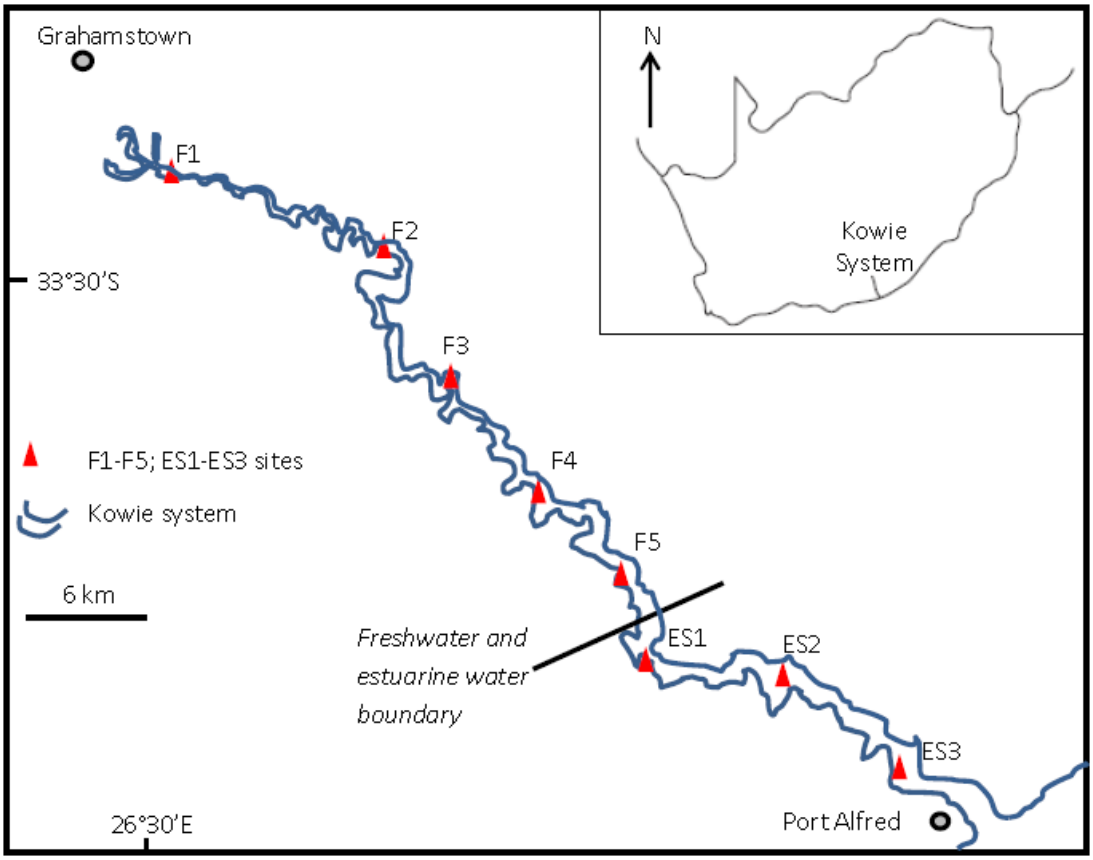


Figure 2.1. Geographic location of the Kowie River and position of the study sites occupied along the riverine-estuarine continuum (September 2012 to May 2013). *F1–F5 and ES1–ES3 indicate riverine and estuarine sites, respectively*.

A large intertidal mud flat exceeding 100 m in width and salt marsh occurs in the Bay of Biscay area, in the lower reach of the estuary. The spring tidal range in the upper reach is about 1.1 m, 1.5m in middle reach and 1.7m in the lower reach (Whitfield et al., 1994). Strong tidal water currents have been recorded in the Kowie estuary with ebb current speeds of 0.12–0.20 m s^{-1}. A very strong flow (>2 m s^{-1}) occurs in the lower reaches of the estuary if river floods coincide with an outgoing spring tide (Heydorn and Grindley, 1982). Episodic river floods in excess of l000 m^3 s^{-1} have

periodically been recorded at the Wolf's Crag gauging station located in the upper reach of the Kowie River. The estimated mean annual river discharge, excluding major flood events is 20×106 m^3 (Whitfield et al., 1994).

The Kowie system water has a high pH (mean = 8.2) and alkalinity (mean = 139–185 ppm $CaCO_3$). The water is usually clear with a mean Secchi disc transparency typically ranging from 0.7–1.03 m although a maximum value of 2.50 m has been recorded during low flows. Light penetration is limited when there is high concentration of dissolved organic material within the water column, typically after flood events (Bok, 1983; Whitfield et al., 1994). River water entering the Kowie Estuary during low flow conditions is brackish ranging in salinity from 2–6 µS cm^{-1} (Heydorn and Grindley, 1982). Salinity in the estuary is generally >30 µS cm^{-1} but may increase to 40 during drought periods. During prolonged river floods, the surface water of the whole estuary is almost fresh, although sea water may be recorded in the mouth region during high tide. Salinity stratification, following flood events, is often strongly developed in the middle and lower estuary reaches. Seasonal water temperatures recorded in the mouth region range from 14 to 22 °C, while in the upper reach it ranges from 11 to 27 °C (Heydorn and Grindley, 1982).

Geology

Most of the Kowie system lies within the Bokkeveld series consisting of shale and subsidiary sandstone bands (Heydorn and Grindley, 1982). Pronounced dipping and folding are evident e.g. in quarries and cuttings of Port Alfred. Successions of thin marine sediments, referred to as the Alexandria formation, are found along the coast overlying and resting on the shale’s whilst the dune rock covers the Alexandria formation. Unconsolidated beach deposits and bare dunes occur right at the coast and along the river, alluvial floodplain deposits are present (Heydorn and Grindley, 1982; Whitfield et al., 1994).

Climate

The Kowie system and its catchment fall into the temperate climate zone, with rainfall occurring mainly during spring and autumn (60%). Mean annual rainfall is estimated at ~650 mm and is evenly distributed over the entire catchment area (Heydorn and Grindley, 1982). Minimum and maximum temperature of Kowie system is about 1.5 °C and 39.8 °C, respectively, with an average daily temperature of 10.6 °C (Bok, 1983). The Kowie system is considered perennial and the flow is very reliable. Although rain may fall in the catchment this does not translate in an increased flow reflecting the flow modifications as a result of human activities (Heydorn and Grindley, 1982; Bok, 1983; Whitfield et al., 1994).

Phytoplankton, phytobenthos and macrophytes

Giffen (1970) collected and described approximately 286 diatom species, with many having a cosmopolitan distribution and previously not recorded from South Africa. Eleven species were new to science. Many diatoms species were recorded in all lagoons adjacent to the river, of these *Ulva* cf *capensis*, *Enteromorpha prolifera* and *Gelidium pristoides* were dominant while *Chondria* sp., *Centrocerus clavulatum* and *Griffithsia* sp. were less conspicuous. Blue green algae, including *Plectonema* sp., *Gracilaria verrucosa* and *Lyngbya majuscule*, were recorded in the lower east bank lagoon and Waters Meeting Nature Reserve area, which could be an indication of high nutrient levels in these waters. Dense mats of red algae and *Lyngbya* sp. from furrows and streams north of the Bay of Biscay were reported (Giffen, 1970). In contrast, Bok (1983) recorded no algal blooms although filamentous green and red algae were at times being abundant in lower estuary littoral areas. Where the filtering function of adjacent reed swamps is disturbed, in the upper east bank lagoon and Waters Meeting Nature Reserve area, algal growth was more profuse (Giffen, 1970).

Aquatic macrophytic vegetation in the Kowie Estuary comprises mainly *Ruppia* spp. and *Phragmites australis* in the upper reaches, with *Zostera capensis* and various

salt marsh plants such as *Spartina maritima, Chenolea diffusa* and *Sarcocornia perennis* dominating in the middle and lower reaches (Whitfield et al., 1994).

Vegetation

The warm temperate coastal forests are well established to the east, dominated by *Sideroxylon inerme* (milkwood), *Mimusops caffra* (red milkwood) and *Brachylaena discolor* (wild silver oak). The coastal forest is more open and stunted near the river channel (Heydorn and Grindley, 1982). Alien trees such as *Eucalyptus globulus* (blue gum) and *Acacia cyclops* (rooikrans) are common along the channel forests. Other species such as *Passerina* sp., *Chrysanthemoides monolifera* and *Rhus crenata* are found near the estuary mouth (Heydorn and Grindley, 1982). The succulent woodland and coastal sub-formation, the euphorbias and other succulents are a common vegetation types as well as *Ptaeroxylon obliquum* (sneeze wood), *Schotia latifolia* (bush boer-bean) and *Cussonia spicata* (cabbage tree). The succulent woodland and coastal sub-formation vegetation type is found adjacent to the river within the catchment, except in areas where the vegetation has been removed by private land owners for agricultural activities (Heydorn and Grindley, 1982).

Study Sites

During this study, the Kowie river system was divided into three sections: upper (sites F1 and F2), middle (sites F3–F5) and the lower (sites E1–E3) reaches for the phytoplankton and stable isotope study. River site F3 was not sampled in September 2012 (spring) due to limited accessibility. Estuary sites (E1–E3) were characterised by clay/silt sediments with cobbles and boulders on the eastern bank. At low tide, all macrophytes were exposed along the banks. All freshwater sites were characterised by silt/clay and sandy bottom sediments plus cobbles, pebbles, boulders and/or bedrock also being observed. Analysis of phytoplankton and phytobenthos composition, allochthonous organic matter using a combination of community and stable isotope analyses was carried out for each site. The study was carried out in

four different seasons/periods: spring (September 2012), summer (November–December 2012), autumn (February 2013) and winter (May–June 2013) on pools along the continuum.

Table 2.1. Geographic co-ordinates of the study sites along the Kowie River estuary system

Site name	Coordinates	
	South	East
F1	33°20’59.2”	026°33’37.6”
F2	33°21’57.7”	026°37’38.0”
F3	33°27’23.4”	026°41’22.0”
F4	33°30’16.0”	026°44’40.9”
F5	33°32’45.6”	026°47’04.2”
E1	33°32’23.6”	026°48’13.0”
E2	33°32’51.3”	026°49’02.3”
E3	33°34’55.8”	026°51’41.8”

CHAPTER 3

COLONISATION AND COMMUNITY STRUCTURE OF BENTHIC DIATOMS ON ARTIFICIAL SUBSTRATES FOLLOWING A MAJOR FLOOD EVENT: A CASE OF THE KOWIE RIVER (EASTERN CAPE, SOUTH AFRICA)

Plate 3. Artificial substrate structure submerged in water. Photo by Tatenda Dalu

Abstract

A major flooding event that occurred during October–November 2012 caused major changes in the Kowie River hydromorphology and aquatic communities. The aim of this study was to identify the environmental variables that structure riverine benthic diatom communities at upstream and downstream locations 25 km apart on the Kowie River, South Africa. This was undertaken using tiles as artificial substrates so that we could study how the communities developed after the flood disturbance. The diatom community structure was assessed over a 28–day period following a flood event in October 2012. The Mann Whitney test indicated that there was a statistically significant difference ($p < 0.05$) in total dissolved solids, salinity, pH and oxygen reduction potential between the two sites. In total, 58 diatom species belonging to 30 genera were identified over the 28–day study. *Achnanthidium minutissimum, Fragilaria biceps*, *F. ulna* var. *acus*, *Pinnularia borealis* and *P. acrosphaeria* were the most numerically dominant on Day 7 and were considered as early colonisers, while on Day 28, *Achnanthidium minutissimum*, *F. capucina*, *Craticula buderi*, *C. vixnegligenda, Diploneis subovalis* and *Gomphonema venusta,* the late colonisers, were dominant. The species richness increased from 13 (upstream location) on Day 7 to 22 (both locations) by Day 21. A redundancy analysis showed that total dissolved solids (TDS), salinity, resistivity, pH and oxygen reduction potential (ORP) were the most significant physico-chemical variables explaining diatom composition. The results from this relatively small-scale tile experiment indicate the complexity of freshwater benthic diatom community structure and development.

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Introduction

Numerous studies have been conducted on the seasonal dynamics of diatom communities in lotic and lentic ecosystems (Ruth, 1977; Gamier et al., 1995; Ha et al., 1998; Lane et al. 2003; Claquin et al., 2006; Wojtal and Sobczyk, 2012), with diatom community structure periodicity being largely described more in lentic than lotic systems (Gamier et al., 1995; Claquin et al*.,* 2006). Stream diatom communities are composed primarily of benthic and epiphytic species, whilst planktonic genera are less prevalent owing to their displacement by water currents which disrupt their population growth and development (Ruth, 1977; Claquin et al*.,* 2006). Although benthic diatoms are dominant contributors to primary production in rivers and streams (Claquin et al., 2006) and are an important food source for many invertebrates (Lane et al., 2003), we know surprisingly little about their community structure and dynamics. Moreover, diatoms are important biological indicators of environmental change as diatoms are sensitive and respond to shifts in physico-chemical parameters in lotic and lentic ecosystems (Bere et al., 2013). As such, diatom community analysis can represent a tool with which to explore and interpret many ecological and environmental changes (Reynolds, 1984; Ács and Kiss, 1993).

An important aspect of community ecology is the identification of patterns in community structure relative to environmental gradients (Soininen et al., 2004). Characteristics of rivers, including unidirectional flow and continuous physical changes, make riverine ecology a unique avenue of study (Giller and Malmqvist, 1998; Soininen et al., 2004). For example, variation in river discharge as a result of freshwater input has a significant influence on primary production by benthic diatoms as shifts in flow affect diatom community composition (Stevenson, 1990). An increase in water flow (current) can positively affect the benthic algal community by increasing turbulent flux and thus the transport of nutrients to individual algal cells, thereby stimulating their metabolism (Miller et al., 1987; Ács and Kiss, 1993; Wehr and Sheath, 2003; Song 2007). Conversely, increased current velocity can also negatively affect benthic algal communities by decreasing immigration rates to the

substrata and increasing the drag forces on attached algae which reduces the rate of colonisation of benthic diatoms and increases the sloughing of algal cells (Song, 2007). Flood effects on benthic diatoms increase with flood intensity, with effects ranging from scouring nearly all algae from substrates to having little effect on standing crops (Stevenson, 1990; Steinman and McIntire, 1990).

River systems, from headwaters to estuaries, represent a continuum of interdependent habitats, so understanding diatom community structure at any place in a river requires an understanding of upstream properties (Gamier et al., 1995). As such, we have incorporated into our study design a spatial aspect which allows upstream and downstream comparisons of diatom community structure. Colonisation and development of diatoms has traditionally been studied by collecting benthic diatoms from a substratum over time (Lane, 2003). Artificial substrata have been used in diatom studies for many years. The advantages of using artificial substrates include decreased habitat disruption and substantially improved sampling precision (Lane et al., 2003; Wojtal and Sobczyk, 2012). The use of artificial substrata also reduces the effects of small-scale habitat variations that inevitably exist in relation to natural substrata and ensures that diatom samples obtained are relatively stable (Lane et al., 2003; Wojtal and Sobczyk, 2012).

Few studies have been done on diatom colonisation and development patterns in South Africa. Therefore, the aim was to identify the environmental factors that affect benthic diatom community structure and development at 2 locations within a temperate South African river. The study was approached by documenting the diatom communities on artificial substrata for 28 days following a major flooding event that occurred during October–November 2012, in order to study how the communities develop after a disturbance such as a flood.

Materials and methods

Study area

A detailed description of the study area is provided for in Chapter 2. The mean monthly rainfall values for the Kowie River in September, October and November 2012 were 38.2 mm, 432.1 mm and 80.5 mm, while discharge rates (and volumes) were estimated at 0.2 $m^3 s^{-1}$ (7.5×10^5 m^3), 1.1 $m^3 \cdot s^{-1}$ (2.4×10^8 m^3) and 0.5 $m^3 s^{-1}$ (5.8×10^6 m^3) (Department of Water Affairs (DWA), 2013). The mean yearly rainfall amounts (and discharge rates) for 2009–2012 were 537.0 mm (0.07 $m^3 s^{-1}$), 638.0 mm (0.09 $m^3 s^{-1}$), 1 029.4 mm (3.6 $m^3 \cdot s^{-1}$) and 989.7 mm (8.4 $m^3 \cdot s^{-1}$) (DWA, 2013; Figure 3.1).

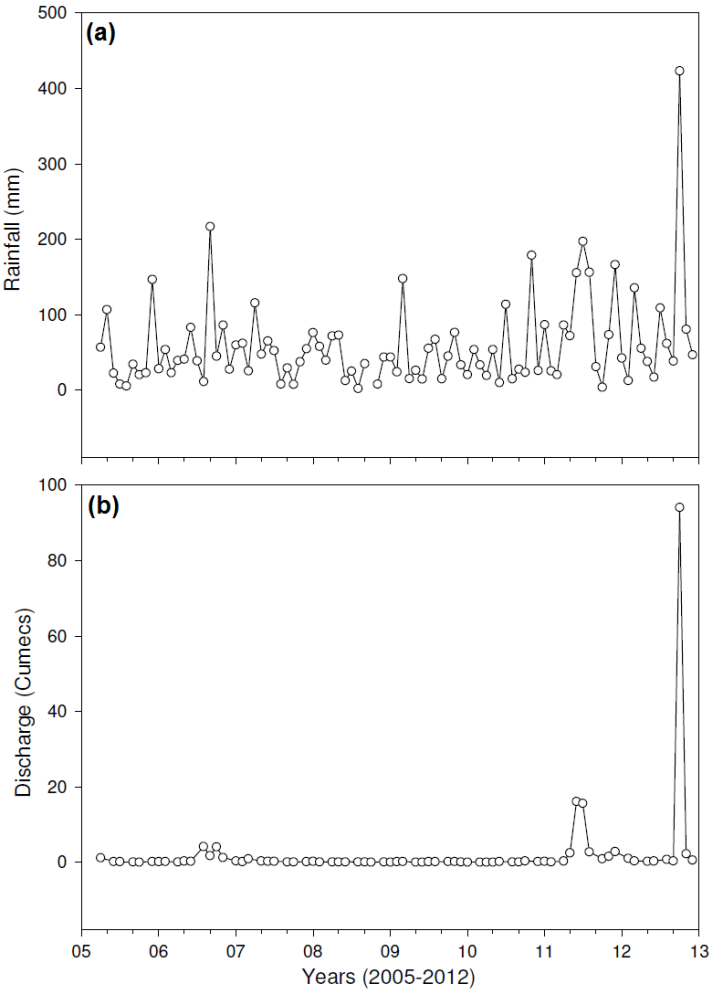

Figure 3.1. Mean (**a**) rainfall and (**b**) discharge trends measured by the Department of Water Affairs (South Africa) at Station P4H001 (Kowie River, Bathurst). One cumec = 1 $m^3 s^{-1}$

Benthic diatom sampling was conducted every 7 days from 16 November to 13 December 2012. The two study sites, 25 km apart, were located upstream (site F1–33°20'59.2"S, 026°33'37.6"E) and downstream (F4–33°30'16.0"S, 026°44'40.9"E) on the Kowie River (Figure 2.1). The sites were chosen based on the river morphology, where upstream areas were narrow and shaded and allochthonous inputs are dominant. As a river widens downstream, energy inputs change as the sunlight reaches the benthos and supports increased autochthonous algal production. The upstream site had a mean water depth of 0.35±0.1 m, a channel width of 1.8±0.3 m and 75 % terrestrial cover from the riparian zone, while the downstream site had a mean water depth of 0.7±0.2 m, a channel width of 7.5±0.2 m and 20 % terrestrial cover.

Physico-chemical parameters

Physico-chemical data (temperature, pH, dissolved oxygen, conductivity, total suspended solids (TDS), oxygen reduction potential (ORP), sodium chloride, resistivity and water transparency) of the sites were measured using a portable probe (Cyber Scan Series 600, Eutech Instruments, Singapore). Flow velocity was measured using a Flo-mate portable flow meter Model 2000 (Marsh McBirney, Maryland). Water samples were collected according to Brendnock et al. (2003) and Wu et al. (2011). At each site (Figure 2.1), 3 water samples, one on either side of the river (littoral zones) and one at the centre, were collected using an 8 L bucket. All three water samples were pooled together in a 30 L bucket and stirred, after which a 500 mL sub-sample was taken as representative of the study site. The 500 mL sample was later processed to determine phosphate, nitrate and ammonia concentrations. Ammonia was analysed using a Nessler method, nitrate–cadmium reduction method and phosphate–amino acid method, using the HI 83203 multi-parameter bench photometer for aquaculture (Hanna Instruments Inc., Rhode Island).

Diatom sampling

The artificial substrata used in this study were tiles (approximately 22×10×0.7 cm thickness). At each study site, 18 tiles were placed vertically in 6 tile structures (30×18 cm) punctured with regular holes to allow the free flow of water around the tiles (Figure 3.2), as per Peterson (1983), Lane et al. (2003) and Bere and Tundisi (2011). Each structure was anchored in the stream using a rope tied to the nearest tree in the riparian zone. When orientated vertically, the smooth surface of the tiles provided a substratum closely resembling the naturally occurring epilithon (Lane et al., 2003), while the rough side represented the rough material (rocks) washed into the river channel. Thus, it was our assumption that the tiles represented the different cleared habitats available for diatom colonisation following flooding events.

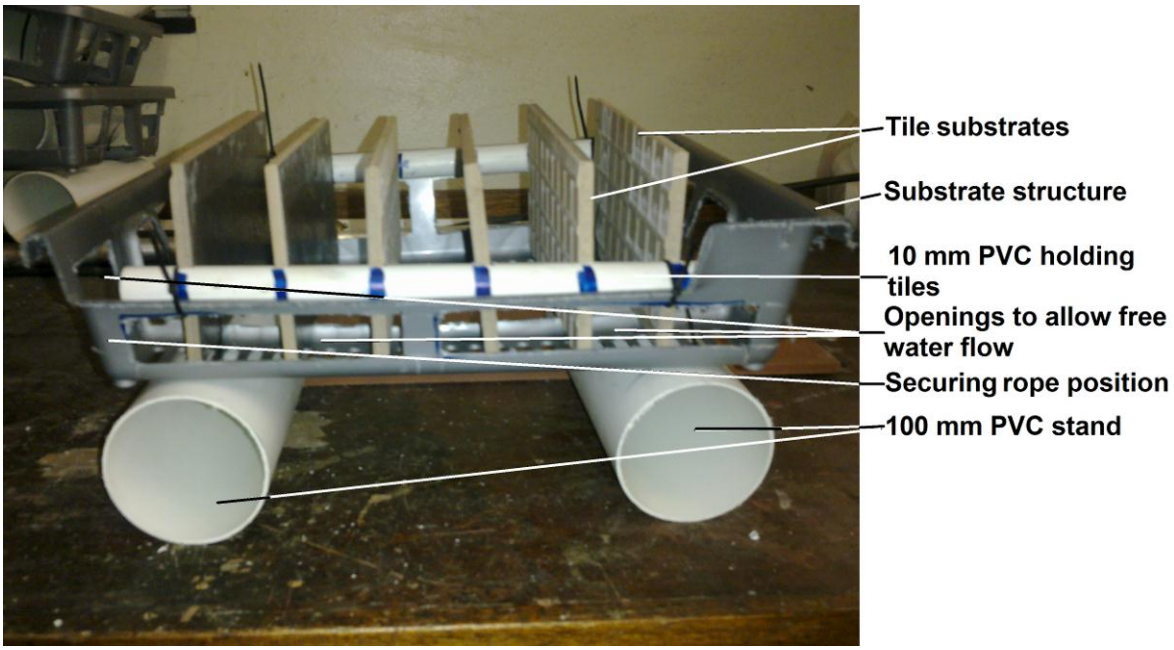

Figure 3.2. Artificial tile structures used for benthic diatom experiments in the Kowie River

At each sampling event, 4 tiles were randomly selected from the 3 tile structures which were haphazardly placed within the river channel at each site. The tiles were carefully removed from the tile structures in the stream and the diatoms were then removed by brushing them with a toothbrush into a container with distilled water to form one integrated sample for that particular site. This procedure reduced the

effects of heterogeneity between tiles that may result from contamination, microhabitat conditions that vary with position in the tray, or natural variation (Lane et al., 2003; Taylor et al., 2005c). The diatom material was then preserved in 70 % ethanol until analysis.

Diatom preparation and analysis

Diatoms were prepared by oxidising organic material in samples with hydrochloric acid and potassium permanganate (Taylor et al., 2005). After 24 hours, the undisturbed diatom samples were further concentrated by decanting the supernatant, taking care not to disturb the settled diatom material at the bottom of the container. The diatom samples were cleaned of organic materials as outlined by DARES (2005) and Taylor et al. (2005). After decanting, 10 mL of well-mixed sample and 10 mL of saturated potassium permanganate solution were placed into a heat-resistant beaker and left for 24 hours. Five mL of concentrated hydrochloric acid (32%) were then added and the beaker was covered with a watch glass and heated on a hot plate at 90°C for 1.5 hours until the solution became clear. After the oxidation of the organic material, 1 mL of hydrogen peroxide was added to assess whether the oxidation process was complete and that no organic material remained. When oxidation was complete, the samples were cooled and transferred to 10 mL centrifuge tubes. The samples were rinsed by centrifuging with distilled water at 2500 rpm for 10 minutes, the supernatant was decanted, and the washing was repeated four more times until the sample was circumneutral. After the last wash, the diatoms were loosened using a jet of distilled water and poured into labelled glass storage vials (Taylor et al. 2005a, c). The concentrated diatom solution was diluted with distilled water until it was only slightly cloudy. About 1.5–2 mL of solution were placed on a cover slip and air-dried. Clean diatom frustules were mounted in a synthetic resin with high refraction index (1.73) and counted and identified to the lowest taxonomic level possible (usually species) using a phase-contrast Olympus CX light microscope with 1000× magnification. Keys by Giffen (1970) and Taylor et

al. (2007) were used for diatom identification. Data were presented as species percentage abundances relative to the total abundance for each sampling occasion.

Data analysis

Diversity index

The Shannon–Weiner (*H*) diversity index (Shannon and Weiner, 1949) was used to assess the diatom community diversity at the two study sites throughout the study period;

$$H = -\sum_{i}^{n} pi \ln pi$$

where *H* is the Shannon–Wiener index and p_i is the proportional abundance of species *i* through *n*.

Redundancy analysis

Redundancy analysis (RDA) is a constrained linear ordination method based on significant ($p < 0.05$) forward selected environmental variables using 999 Monte Carlo permutations (ter Braak, 2002). Only those diatom species that contributed >1 % to the relative abundance (28 taxa in total) were included in the RDA, to eliminate the effects of rare species. All chemical and diatom raw abundance data were log-transformed before RDA analysis. RDA was performed to examine the relationships between diatom species composition and the selected environmental variables measured during the study. Detrended canonical correspondence analysis (DCCA) was employed to determine whether linear or unimodal analysis methods should be used (Leps and Šmilauer, 2003). The gradient lengths from the DCCA output were examined, and, since the longest gradient was between 3 and 4, a linear RDA model was selected (Leps and Šmilauer, 2003). All ordinations were performed using CANOCO version 4.5 (ter Braak and Šmilauer, 2002).

Results

Flood event

Heavy rainfall produced a strong flooding of freshwater (2.7×10^8 m^3) along the Kowie River for 12 days. The total rainfall for this period (17–28 October) was 418 mm. The discharge rate increased from 0.70 m^3 s^{-1} on 16 October to a peak of 699.14 m^3 s^{-1} on 21 October, while the river volume increased from 6.0×10^4 m^3 on Oct 16 to a peak of 6.0×10^7 m^3 on 21 October). Discharge rates and volume remained elevated until 21 November, with values of 1.06 m^3 s^{-1} and 9.16×10^4 m^3, respectively.

Physico-chemical parameters

Dissolved oxygen concentrations at the two sites ranged between 5.33 and 6.54 mg L^{-1} during the study period. Conductivity downstream increased from 2.53 to 3.75 mS cm^{-1}, while it was relatively constant upstream (range 0.26–0.41 mS cm^{-1}) over the study period. The pH values ranged between 7.21 and 8.62 at the downstream site, and 5.5 and 7.51 at the upstream site (Table 3.1). Mean concentrations of phosphates ranged from 0.8 to 19.6 mg L^{-1} downstream and 0.2 to 2.8 mg L^{-1} upstream, while ammonia values were between 0.0 and 1.4 mg L^{-1} downstream and 0.1 and 1 mg L^{-1} upstream. Nitrates were generally high (0–8.8 mg L^{-1} – downstream and 14.9–17.6 mg L^{-1} – upstream) during the last 14 days of the study at the two sites (Table 3.1). Some environmental variables were not spatially distinct (Mann Whitney U tests, $p > 0.05$), but TDS, salinity, resistivity, pH and ORP did show significant differences between the sites ($p < 0.05$).

Diatom communities and succession

Fifty-eight (58) diatom species belonging to 30 genera were identified over the period of study (Table 3.2). The species richness at both sites increased from minima on 13 November (downstream – 17 and upstream – 13) to maxima on 6 December (22 species at both sites). *Achnanthidium, Pinnularia*, *Achnanthes, Brachysira* and *Fragilaria* genera were the most numerically dominant on 23 November downstream, while upstream the diatom community was numerically dominated by *Fragilaria*,

Cocconeis, *Staurosira* and *Tabularia*. These genera could be considered as the early colonisers. On 13 December *Craticula, Diploneis*, *Gomphonema* and *Eunotia* genera dominated downstream, while at the upstream site on 13 December *Achnanthidium*, *Craticula*, *Hantzschia* and *Eunotia* were dominant; these genera represent the intermediate to late colonisers (Table 3.2). No major differences in Shannon–Wiener (*H*) diversity index occurred downstream throughout the study period, as the values ranged from 2.52–2.66, while upstream the *H* index values gradually increased from 23 November (1.92) to Dec 13 (2.67).

Table 3.1. Physico-chemical variables at the study sites sampled in November and December 2012. DO – dissolved oxygen, D – downstream site; TDS – total dissolved solids, U – upstream site, ORP – oxygen reduction potential

Parameter	**16-Nov**		**23-Nov**		**29-Nov**		**06-Dec**		**13-Dec**	
	D	**U**	**D**	**U**	**D**	**U**	**D**	**U**	**D**	**U**
DO (mg L^{-1})	5.33	5.89	6.39	6.54	6.20	5.99	7.02	6.25	5.61	6.03
Conductivity (mS cm^{-1})	2.53	0.41	2.94	0.36	3.18	0.42	3.45	0.39	3.75	0.26
TDS (mg L^{-1})	1.7	0.27	1.97	0.24	2.13	1.16	2.31	0.25	2.52	0.26
Salinity (µS cm^{-1})	1.24	0.2	1.45	0.18	1.56	1.63	1.69	0.18	1.87	0.19
Resistivity (Ω)	215.2	1339	186.3	1482	224.3	170.9	157.6	1450	145.5	1409
pH	8.6	6.5	8.3	6.4	8.9	7.5	7.2	5.6	7.6	5.5
Temperature (°C)	23.5	20.8	22.9	19.9	22.4	23.2	21.2	20.9	22.7	18.1
ORP (mV)	-93.3	-8.9	-78.9	34	-108.7	-33	-10.9	81.2	-36.1	88
Water flow (ms^{-1})	1.71	0.86	0.84	0.29	0.58	0.74	0.68	0.47	0.55	0.28
Ammonia (mg L^{-1})										
NH_3-N	0.05	0.16	0	0.19	0.3	0.09	1.23	0.75	0	0.11
NH_3	0.06	0.2	0	0.23	0.36	0.11	1.47	0.91	0	0.13
NH_4^+	0.06	0.21	0	0.25	0.39	0.12	1.51	0.97	0	0.14
Phosphates (mg L^{-1})										
PO_3^{-4}	1.2	0.7	1.6	0.2	28.4	1.5	6.3	4.1	1.3	2.5
P	0.4	0.2	0.5	0.1	9.3	0.5	2.9	1.3	0.4	0.8
P_2O_5	0.9	0.5	1.2	0.2	21.2	1.1	4.8	3	0.9	1.8
Nitrates (mg L^{-1})										
NO_3^--N	0.5	0	1.8	0	0	3.4	0.1	4	2	3.4
NO_3^-	2.3	0	8	0	0	15.1	0	17.6	8.8	14.9

Table 3.2. Percentage species abundance of diatoms growing on tile substrates at the study sites

Species name	Downstream				Upstream			
	23-Nov	29-Nov	06-Dec	13-Dec	23-Nov	29-Nov	06-Dec	13-Dec
Achnanthes oblongella Østrup	0.8							
Achnanthes standeri Cholnoky	5.0	2.9	1.7	4.1				
Achnanthes subaffinis Cholnoky	1.7	1.4						
Achnanthidium minutissimum (Kützing) Czarnecki	15.8	5.7						11.2
Achnanthes coarctata (Brébisson ex W. Smith) Grunow					4.0			
Amphora pediculus (Kützing) Grunow		3.8						
Asterionella formosa Hassal						2.3		
Brachysira neoexilis (Grunow) DG Mann	5.4					16.1	4.3	2.7
Caloneis schumanniana (Grunow) Cleve							0.4	
Cocconeis placentula Ehrenberg					8.0			
Craticula buderi (Hustedt)					1.1	2.1	2.9	10.9
Craticula cuspidata (Kützing) DG Mann			0.8	3.2				
Craticula vixnegligenda Lange-Bertalot	7.5	14.3	3.9	14.7		16.0	14.0	9.4
Cyclotella meneghiniana Kützing		1.4	1.5	0.9				
Cymbella kappii (Cholnoky) Cholnoky			2.0					
Diatoma vulgaris Bory		0.5	3.4	0.9				
Diploneis subovalis Cleve		10.0	23.7	18.8				
Encyonema caespitosum Kützing				1.8				
Encyonopsis microcephala (Grunow) Krammer								2.1
Epithemia adnata (Kützing) Brébisson				0.6				
Eunotia formica Ehrenberg				5.9			1.7	5.5
Eunotia incisa Gregory					1.1			
Eunotia lunaris (Ehrenberg) Grunow						2.2		
Eunotia minor (Kützing) Grunow								0.9
Fragilaria biceps (Kützing) Lange-Bertalot		1.4			41.4	15.5	16.6	1.8
Fragilaria capucina Desmazières						6.1	5.4	14.5
Fragilaria capucina var. *rumpens* (Kützing) Lange-Bertalot	8.8	3.3	7.9	6.2				5.8
Fragilaria capucina var. *vaucheriae* (Kützing) Lange-Bertalot	3.8					4.6	6.6	9.1
Fragilaria tenera (WM Smith) Lange-Bertalot	5.8	3.3	0.6					3.9
Fragilaria ulna var. *acus* (Kützing) Lange-	1.3				16.7	5.5	19.5	1.5

Bertalot								
Gomphonema acuminatum Ehrenberg	2.1		0.8	2.4		1.9	1.8	1.2
Gomphonema angustatum (Kützing) Rabenhorst					3.4	6.1	3.3	
Gomphonema laticollum Reichart				5.3				
Gomphonema parvulius Lange-Bertalot & Reichardt							2.9	
Gomphonema pseudoaugur Krammer		7.6	3.4					
Gomphonema truncatum Ehrenberg		6.7	4.9	4.4				
Gomphonema venusta Passy, Kociolek & Lowe	3.8	7.6	3.9	10.0				
Gyrosigma acuminatum (Kützing) Robenhorst				0.3				
Hantzschia amphioxys (Ehrenberg) Grunow	2.5						0.4	9.7
Melosira varians Agardh			2.3	0.6			1.7	
Navicula cryptotenelloides						1.7	4.0	
Navicula cryptotenella Lange-Bertalot						2.2	2.1	
Navicula radiosa Kützing								2.7
Navicula rhynchocephala Kützing	4.6	1.9	5.6		0.6	1.2	1.5	2.1
Nitzschia bacillum Hustedt				0.6				
Pinnularia acrosphaeria W Smith	14.2	10.5						
Pinnularia borealis Ehrenberg sensu lato	15.8	11.4	7.9	9.7				
Pinnularia divergens var. *undulata* (Peragallo & Heribaud)						6.6	2.8	2.4
Pinnularia gibba (Ehrenberg) Grunow					1.1			
Pinnularia viridiformis Krammer			4.0		5.2	3.9		
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhityarova		5.2						
Pseudostaurosira brexistriata (Grunow in Van Heurk) Williams & Round			0.6					
Reimeria uniseriata Sala, Guerrero & Ferrario					2.8			
Staurosira elliptica (Schumann) Williams & Round			9.3	7.9	6.4	2.8	1.9	1.2
Stenopterobia delicatissima (Lewis) Brebisson							0.8	
Surirella brebissonii Krammer & Lange-Bertalot	1.3		10.4	1.2			1.7	1.2
Tabularia fasciculata (Agardh) Williams & Round		1.0	0.8	0.6	8.1	3.3	3.7	
Tryblionella apiculata Gregory			0.6					
Species richness	**17**	**19**	**22**	**21**	**13**	**18**	**22**	**20**

Shannon–Wiener	**2.52**	**2.66**	**2.63**	**2.57**	**1.92**	**2.57**	**2.62**	**2.67**
Evenness	**0.73**	**0.75**	**0.63**	**0.62**	**0.53**	**0.73**	**0.62**	**0.72**

The first two axes of the RDA, with eigenvalues of 0.45 in axis 1 and 0.17 in axis 2, accounted for 73.4 % of the species–environment relationship while also accounting for 62.1 % of the variance in the species data (Figure 3.3). Diatom species composition differed over time in both locations (Figure 3.3). Upstream there was a large difference in diatom communities between 23 November and the other three sampling times. In contrast, there were fewer changes in the diatom community through time at the downstream location. There were clear distinctions in the environmental factors associated with diatom community structure at the two locations (Table 3.1). The RDA analysis identified 7 physico-chemical factors that were significant ($p < 0.05$) in affecting diatom community variation, these being nitrates, phosphates (P), TDS, salinity, pH, resistivity and water flow (Figure 3.3).

Discussion

Physico-chemical factors

Discharge rate and volume continued to be high up until 21 November, while rainfall decreased. Along the Kowie River, the upstream location had significantly decreased conductivity, TDS, pH, water flow, salinity, temperature, phosphates and DO due to the pristine nature of the site and minimal human influences compared to the downstream location, which was influenced by agricultural activities (crop and cattle farming) and wastewater discharge. In contrast, ammonia and nitrates were high upstream relative to downstream. This suggests the influence of natural, mature forests which conserve nitrogen and release it through processes of decomposition and leaching (Hallberg and Keeney, 1993).

Downstream of the headwaters, the Kowie River's riparian zone cover begins to decrease as the river passes through several game reserves, cultivated farms and ranches. One of the main tributaries of the Kowie River, the Bloukrans River, joins the main channel and contributes to the high phosphate loads observed downstream

(Whitfield et al., 1994). Riparian buffer strips or zones are observed along the whole river channel, but these are interspersed with game reserves, pineapple plantations and cattle ranching.

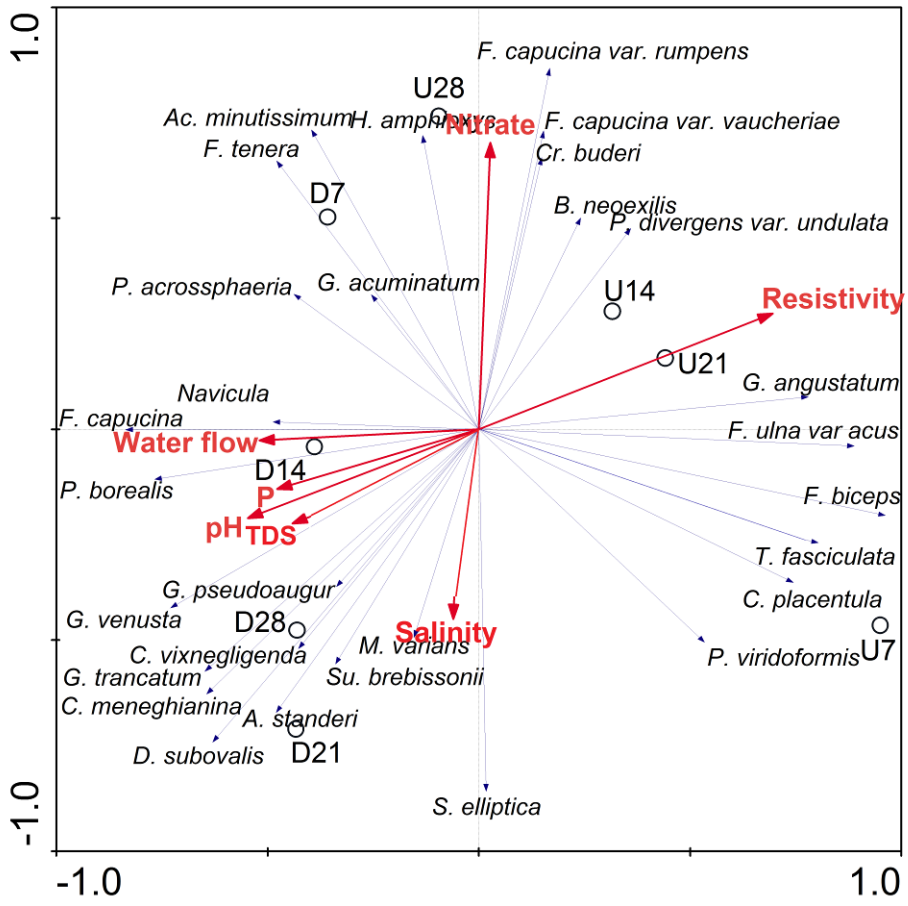

Figure 3.3. RDA ordination showing the relationship between diatom community composition and the significant environmental variable. Letters in sample labels represent sites (U, upstream; D, downstream). Numbers in labels represent sampling dates (23 Nov, 29 Nov, 6 Dec and 13 Dec).

Diatom community structure in relation to environmental variables

The benthic diatom community structure on tiles at both sites was structured by environmental variables, with grouping of sites generally reflecting a change in diatom community composition between sampling periods. Different diatom species and communities responded to changes in TDS, salinity, resistivity, pH, P and water

flow because of their differences in tolerances and adaptations; hence the distinct diatom community composition at different sampling sites.

The upstream location had almost similar diatom communities for Days 14 and 21, but the communities were very different on Day 7 and Day 28. At the downstream site, the diatom communities were significantly different from week to week, which can be a result of natural succession. Water flow likely excluded some algal taxa in our study of Kowie diatom communities, especially the large-celled long filamentous algae such as *Fragilaria* spp. that tend to prefer slow currents with fine sediments as their substrata (Claquin et al., 2006). Current flow indirectly modulates benthic algal community structure by interacting with both the biotic and abiotic factors in different habitats, such as substratum type, size and stability, or by altering the diatom species composition, community and distribution via the control of invertebrate grazers and fish through feeding or physical disturbances (Song, 2007).

Benthic diatom communities are considered to be regulated by local physico-chemical factors rather than by broad-scale climatic factors (Pan et al., 1996), but Mann and Droop (1996) argued that a considerable number of diatoms are endemic and/or show a regionally restricted distribution. The study results support the view of Mann and Droop (1996) that diatom communities are strongly affected by physico-chemical factors, with different diatom communities being recorded at different locations or study sites within the Kowie River. Most taxa in this study were truly cosmopolitan but a number of the species exhibited limited distributions, with TDS, salinity, resistivity, pH, P and water flow playing an important role in the diatom distribution patterns.

Water flow was one important variable structuring diatom communities in the study (Figure 3.3). Low water flow (upstream) was associated with the diatom species generally considered as 'cool adapted' (Ruth, 1977; Bere et al., 2013), including *Craticula buderi, Gomphonema angustatum, Navicula cryptotenelloides, N.*

cryptotenella and *Pinnularia divergens* var. *undulata.* The downstream site, which had high water flow, was associated with *Achnanthes subaffinis, A. standeri, Achnanthidium minutissimum, Cyclotella meneghiniana, Diatoma vulgaris, Diploneis subovalis, Gomphonema pseudoaugur, G. truncatum, G. venusta, Pinnularia borealis* and *P. acrosphaeria* which were considered 'locals'. These species may have developed morphological adaptations to different water flow regimes which enabled them to be better suited to their environment. Ruth (1977) and Claquin et al. (2006) highlighted that smaller diatoms predominate under conditions of high water flow in lotic systems, which was the case in our study – the downstream site was dominated by small-sized diatoms. Song (2007) showed that there was a shift from dominance by large-celled upright filamentous taxa at low water flow to smaller chain-forming taxa at higher water flow, in response to changes in water flow in the Maple River, Michigan.

The pH levels also played an important role in structuring benthic diatom communities in this study. The pH levels affect diatom motility and adhesion to surfaces, the affinities of diatoms to various cations has not been sufficiently investigated or demonstrated, but is likely to be linked to physiological mechanisms (Bere et al., 2013). The pH level can exert a direct physiological stress on diatoms and also strongly influences other water chemistry variables such as resistivity and conductivity (Bere and Tundisi, 2011). The pH level can exert a direct physiological stress on diatoms and also strongly influences other water chemistry variables such as resistivity and conductivity (Bere and Tundisi, 2011). The pH values at the upstream site were largely alkaline (6.4–8.9), while at the downstream site they were circumneutral; thus most of the species were either alkaliphilic or circumneutral–tolerant. A decrease in pH downstream may have had an influence on diatom population growth and development on *A. standeri*, *F. capucina, G. truncatum, G. venusta* and *P. borealis*, which are weakly alkaline to slightly acidic environment species, while an increase in pH had an influence on upstream species, *F. ulna* var. *acus, F. biceps* and *G. angustatum*, which are alkaliphilic species (Taylor et al.,

2007; Koçer and Şen, 2012). TDS which is commonly used as an indicator of the presence of a broad array of chemical contaminants (Jorgensen, 1996), was also important in structuring diatom communities. The distribution of moderate- to high-electrolyte content species such as *A. standeri*, *D. subovalis, F. capucina, G. truncatum, G. venusta, P. pseudoaugur* and *P. borealis* (Taylor et al*.,* 2007) were influenced by TDS concentrations in this study, as clearly reflected in the RDA graph.

Resistivity levels were also important in structuring diatom community structure with the downstream warmer site associated with low resistivity levels. Resistivity of water is a measure of the ability of the water to resist an electric current, and is directly related to the amount of ionic material, mainly salts, in the water (Jorgensen, 1996). Thus, water with a relatively high TDS will have a low resistivity and a high conductivity (Jorgensen, 1996). Species such as *F. ulna* var. *acus, F. biceps, G. angustatum* and *T. fasciculata,* which are mostly cosmopolitan, inhabit different electrolyte content habitats, have low conductivity and alkalinity tolerances, and hence are greatly influenced by resistivity, which is an indirect measure of these two parameters (Taylor, 2007).

Nitrate concentration was identified as one of the main factors structuring diatom communities within the upstream site. The upstream site was associated with high nitrate concentrations, with Days 7, 21 and 28 being separated from the rest of the time period. Nitrate concentration is an important nutrient required for diatom growth and has an important function in the structuring of benthic diatom communities in river systems (Bere et al., 2013). Diatoms in the upstream sites influenced by nitrate concentration included *Hantzschia amphioxys, G. angustatum, Fragilaria capucina* var. *rumpens, F. capucina* var. *vaucheriae, Brachysira neoexilis* and *P. divergens* var. *undulata*.

Benthic diatom colonisation and development

Benthic diatom colonisation and development on tile surfaces is the direct consequence of immigration, reproduction, disturbance, death, grazing and emigration (Ruth, 1977; Peterson, 1986; Delesalle, 1993; Claquin et al., 2006; Power et al., 2008; Bere and Tundisi, 2011; Koçer and Şen, 2012; Bere et al., 2013). Periods of 3 to 4 weeks have been sufficient for benthic diatom colonisation and development, which are dependent on algal abundance and biomass in the water column (Peterson, 1983; Lane et al., 2003; Bere and Tundisi, 2011; Kralj et al., 2006). The numerically dominant benthic diatom genera found in this study, especially *Cocconeis, Cymbella, Fragilaria, Gomphonema, Pinnularia* and *Epithemia*, are important components of stream diatom communities worldwide (Lane et al., 2003; Claquin et al., 2006; Koçer and Şen, 2012).

In our study, the species richness peaked after 21 days and seemed to stabilise thereafter at both sites. There was significant arrival of new colonisers within the first 14 days, e.g. the *Achnanthes, Achnanthidium* and *Pinnularia* genera (downstream) and *Cocconeis* and *Gomphonema* spp. upstream. All of the observed new coloniser genera at both sites have high immigration rates and respond better than other genera to disturbance, which enables them to populate surfaces before other competitors (Stevenson, 1990). Other pioneer colonists, such as *Fragilaria* spp. observed at both sites, were mostly relatively large and had an advantage during immigration in that they can settle rapidly to the substrate (Stevenson, 1990; Krajl et al., 2006). *Fragilaria* spp., which are non-motile and not very good competitors during later stages of colonisation, were shown to decrease in their abundances with time (Table 3.2). *Craticula, Diploneis*, *Gomphonema, Staurosira* and *Eunotia* spp. at the downstream site, and *Brachysira*, *Craticula*, *Hantzschia, Navicula* and *Eunotia* spp. at the upstream site, were dominant as time progressed. These are considered as intermediate to late colonisers, as most of these genera have low (slow) immigration ability but their small sizes make them fast reproducers, and they are

probably better competitors in nutrient-rich environments than the other species mentioned.

The Shannon–Wiener diversity index increased downstream up to Day 21 before decreasing, but continued to increase upstream. This may be explained by the fact that more new species were still migrating to tiles upstream (Table 3.2) after Day 21, suggesting that the population had not yet reached stability, whereas the downstream site after Day 21represented the late stages of diatom community development, during which competition might be a factor causing a decrease in diversity (Krajl et al., 2006).

Conclusion

Diatom colonisation and development in the Kowie River, Eastern Cape, was governed by physico-chemical factors such as water flow, resistivity, phosphates, pH, salinity, TDS and nitrates. This result corroborates the classic subsidy-stress gradient model of Odum et al. (1979), which states that small-scale perturbations involving usable nutrients would stimulate ecosystem function, while continued perturbation would have a negative impact on community equilibrium and functioning. Since benthic diatom communities recycle nutrients, and use recycled nutrients within the community for some time, this isolates benthic diatom communities from the influence of external nutrient inputs, which can be advantageous to their survival because most of the inputs from the water column can then be used to increase net growth and production (Wetzel, 2001). The flood event in the Kowie River caused a major perturbation which heavily affected the benthic diatom community equilibrium and functioning by affecting nutrient sources and community structure, as most diatoms were washed away by the flood. Long-term monitoring studies are clearly required to better understand the colonisation, development, succession and productivity of diatoms in flowing waters (streams and rivers).

CHAPTER 4

AN ASSESSMENT, USING MULTIVARIATE ANALYSIS AND STABLE ISOTOPES, OF THE EFFECTS OF SUBSTRATE TYPE ON PHYTOBENTHOS COMMUNITIES

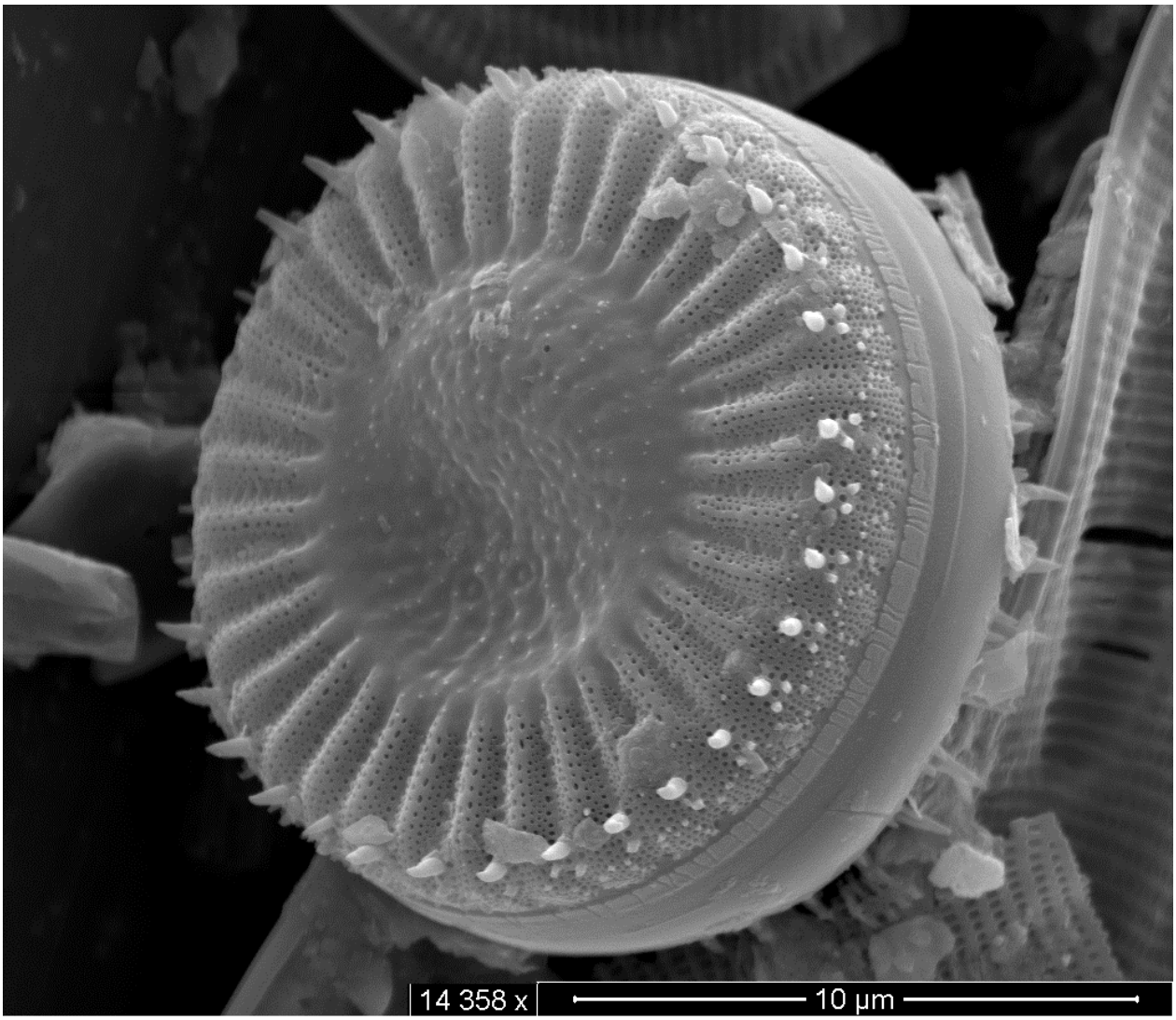

Plate 4. *Cyclotella meneghiniana* Kützing viewed under an electron microscope. Photo by Tatenda Dalu

Abstract

For over a century, artificial substrates have been employed in phytobenthos studies. In the present study, a comparison of phytobenthos community structure in a field experiment over three seasons (summer, autumn and winter) on three types of artificial substrates: brick, and brown and grey clay tiles and three natural substrates: macrophytes, rocks and sediment, in a small temperate river system. A combination of multivariate analyses (cluster, multi-response permutation procedure, indicator species (IndVal) and canonical correspondence analysis (CCA)) and stable isotope analysis was used. A total of ninety-six phytobenthos taxa were identified. Artificial substrates resulted in different substrate communities as shown by stable isotope analysis, cluster analysis and multi-response permutation procedure, with only those communities growing on grey tiles being similar to natural substrate communities. Overall, artificial substrates exhibited slightly high species richness compared to natural substrates over the three seasons although there were no significant differences ($p > 0.05$). Phytobenthos grown on brown tiles, rocks and bricks showed seasonal variability of the $\delta^{13}C$ values using one-way ANOVA ($p < 0.05$). Phytobenthos community structure did not show great seasonal variation. However, water flow, conductivity, ammonium, phosphate and water depth were identified using CCA as important in structuring phytobenthos communities on different substrates. IndVal analysis showed that common phytobenthos taxa were not restricted to a single substrate but preference was generally high for natural substrate, especially rocks, compared to artificial substrates. Substrate microhabitat type appears to influence the communities within the study areas.

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Introduction

Since there are limited overlaps in species composition among regions, or at least some ecological characteristics of the key taxa vary, implementation of research outputs or results from other parts of the world can lead to flawed interpretation of diatom communities and water quality (Lobo et al., 1995; Bere and Tundisi, 2011b). Furthermore, the existence of endemic phytobenthos compounds the complexity of the situation (Taylor et al., 2007a). Thus, diatom ecological assessment should be region and river type specific and express the ecological running water quality.

Stable isotope analysis is potentially a powerful tool for assessing food chain relationships (Fry, 1983; Yoshii et al., 1999; Marty and Planas, 2008; Mejía et al., 2013; Wang et al., 2013). Since different food sources have distinctive carbon isotope ratios, thus, their use in carbon and nitrogen stable isotope analysis can provide much more information on the different sources (Keller and Morel, 1999).

The possible advantages of using artificial substrates studying the development of phytobenthos communities are clear; for instance, the phytobenthos community is observed directly (Peterson, 1986; Lane et al., 2003; Bere and Tundisi, 2011b) and the substrates are the same at all sampling locations (Round, 1991). Therefore, there is a further need to undertake comparative research examining diatom community structure on different substrate types (artificial and/or natural) in different parts of the world. The present study compares the phytobenthos community composition on three types of artificial substrates: brick, and brown and grey clay tiles and three natural substrates: macrophytes, rocks and sediment, in the Kowie River, South Africa, using a combination of stable isotopes analysis and commonly used diatom-based multivariate analysis.

Materials and methods

Study area

A detailed description of the study site is provided for in Chapter 2. The study was carried out in summer (13 Nov to 13 Dec 2012), autumn (25 Apr to 31 May 2013) and winter (10 Jul to 9 Aug 2013) with benthic phytobenthos sampling and physico-chemical factors measurement being carried out on the last day of each season. Two sites, upstream (site F1) and downstream (site F4), were chosen along the Kowie River.

Physico-chemical analysis

A detailed description of tools and sampling techniques used is highlighted in Chapter 3.

Phytobenthos sampling

At each of the two sites, eighteen (9 brown and 9 grey) tiles measuring approximately 22×10×0.7 cm were placed vertically in six brown and grey clay tile structures (30×18 cm) punctured with regular holes to allow the free flow of water around the tiles for 30 days according Peterson (1986). The structure was anchored using a rope. Six bricks were placed at the two sites. At each site, phytobenthos on natural (sediment, macrophytes, and rocks) and artificial (bricks, grey and brown tiles) substrates were collected separately according to Taylor et al. (2005) and DARES (2005) at the end of the 30 day period.

Prior to sampling, all substrates were gently shaken in stream water to remove any loosely attached sediment. Epiphytic phytobenthos (attached to macrophytes) were sampled from *Cyperus* spp. At least 15 *Cyperus* spp. whole stalks comprising stem and leaves were carefully cut and removed from the stream. The phytobenthos were then removed by brushing with a toothbrush. Epipsammic phytobenthos (found on sediment) were sampled using a syringe (Taylor et al. 2005). The contents were then emptied into labelled containers. The tile structure and bricks were gently lifted out of

the stream and all tiles were carefully removed from the tile structures. A toothbrush was used to brush off phytobenthos into labelled containers with distilled water for bricks, brown and grey tiles at each site. The resulting diatom suspensions were stored on ice for stable isotope analysis while samples for microscopic analysis were fixed and preserved in Lugol's Iodine solution.

After a day or two, the undisturbed phytobenthos samples preserved in Lugol's iodine were further concentrated by decanting the supernatant, taking care not to disturb the sedimented phytobenthos material at the bottom. The remaining precipitate was then vigorously mixed and about 5–10 mL was transferred into a petri dish with grid marking for identification and scoring under an inverted Nikon TMS microscope at 1000×.The diatom component of the phytobenthos were identified and quantified after cleaning with hot hydrochloric acid (HCl) and potassium permanganate ($KMnO_4$) method (Taylor et al., 2007a). After cleaning, the concentrated diatom solution was then diluted with distilled water until it was only slightly cloudy. Approximately 1.5–2 mL of solution was then placed on a cover slip and the sample was allowed to air-dry. The cover slip was heated to drive off excess moisture and the sample was mounted with high-resolution mountant Pleurax (Jonathan Taylor, North-West University, Potchefstroom). The phytobenthos were identified and counted using the phase-contrast light microscope at 1000× using the keys of Giffen (1970), John et al. (2002) and Taylor et al. (2007b). Phytobenthos were enumerated as percentage relative abundances.

Stable isotope analysis

From each site, 6 samples (3 from natural substrates and 3 from artificial substrates) in triplicates were collected during each season. Samples excluding, the epiphytes, were first acidified by vortexing for two minutes in 2 M hydrochloric acid in 15 mL Falcon tubes followed by centrifugation for five minutes at 3600 rpm. The process was repeated where carbon dioxide release was not complete after first acidification. Thereafter, samples were washed twice in deionised water, centrifuged, dried at 50

ºC and homogenised in a Retsch Mixer Mill. Stable isotope analyses were conducted using a Europa Scientific 20–20 IRMS linked to an ANCA SL Prep Unit. Carbon and nitrogen isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) were expressed as the relative differences between isotopic ratios in the sample and conventional standards (29×Internal standards: refmix2 = beet sugar and ammonium sulphate and 5×certified protein standard: Casein (calibrated against IAEA-CH-6 and IAEA-N-1)) (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen), using the standard equation:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] x\ 1000$$

where *R* is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Data analyses

A two-way ANOVA was used to test the differences in physico-chemical variables among the two study sites and seasons (H_o: no difference among sites and seasons). Relationships between pairs of physicochemical factors were tested using correlations. The ANOVA and correlation analyses were performed using SPSS 16.0 for Windows software (SPSS Inc., 2007).

Cluster analysis was conducted based on community composition data among the six substrate types (macrophytes, rocks, sediment, bricks, brown and grey tiles) sampled across the seasons to classify the spatial and temporal distribution of benthic diatom communities using the flexible beta method (b = -0.25) with the Sorenson (Bray-Curtis) distance measure (McCune and Grace, 2002). Samples were classified into clusters based on similarities of community composition. A non-parametric method, multi response permutation procedure (MRPP), was used to test for the hypothesis of no difference between two or more groups of entities (Mielke et al., 1976). MRPP has the advantage of not requiring assumptions such as normality and homogeneity of variances that are seldom met with ecological community data (Mielke et al., 1976; McCune and Mefford, 2006). The statistic *A* in MRPP is a

descriptor of within-group homogeneity, compared to the random expectation known as chance-corrected within-group agreement. When all items are identical within groups, then the observed delta = 0 and A = 1 (the highest possible value for A). If heterogeneity within groups equals expectation by chance, then A = 0. On the other hand, if there is less agreement within groups than expected by chance, then A < 0. An MRPP was carried out in PC-ORD version 5.10 (McCune and Mefford, 2006) using Sorensen (Bray-Curtis) as a measure of distance and the weighting of the groups using n/sum (n).

Only phytobenthos taxa which contributed >1 % to the relative abundance were included in a canonical correspondence analysis (CCA) to reduce data skewness and eliminate the effects of rare species (Bere et al., 2013; Dalu et al., 2013). The phytobenthos density values were log (x+1) transformed to stabilize the variance (Dalu et al., 2013). CCA was performed on the diatom datasets to examine the links between diatom species composition and the selected environmental variables. To determine whether to use linear or unimodal methods for the analysis, detrended canonical correspondence analysis (DCCA) was employed. The gradient lengths were examined and since the longest gradients were greater than 4, a unimodal model (CCA) was selected (Leps and Šmilauer, 2003). CCA is a constrained ordination method based on significant (p < 0.05) forward selected environmental variables using 999 Monte Carlo Permutations (ter Braak, 2002). The software Canoco (ver. 4.5) was used for the analysis. Several CCAs were run with one selected environmental variable for each of the six substrates sampled at a time so as to select the environmental variables for use in the CCAs. Eight environmental variables: ammonium, conductivity, channel width, salinity, pH, phosphate concentration, water flow and water depth were chosen based on inspection of their loadings (>0.3 arbitrary) with respect to first and second axes.

The relationship strengths between phytobenthos communities and physico-chemical factors in the CCAs were calculated using the first and second eigenvalues

ratios (λ_1/λ_2). The λ_1/λ_2 ratio measures the strength of the constraining variable with respect to the first unconstrained gradient in the community data (Bere and Tundisi, 2011b). A greater ratio indicates a strong response of phytobenthos communities to physico-chemical factors and the relationship strength is considered very high if $\lambda_1/\lambda_2 > 1$, moderately high if $0.5 < \lambda_1/\lambda_2 < 1$ and weak if $\lambda_1/\lambda_2 < 0.5$ (Leps and Šmilauer, 2003). A non-parametric test, Kruskal–Wallis, with Mann Whitney pairwise comparisons and Bonferroni corrected/uncorrected analysis was carried out to compare λ_1/λ_2 ratios among the different CCA groups using PAST version 2.00 (Hammer et al., 2001).

Data were further analysed with Indicator Species Analysis method (IndVal), which identifies indicator species within the sampling periods and communities. All 96 phytobenthos species identified were used for analysis. Good indicator species are those that are always present at sites in a given group and never occur in other groups. The indicator value ranges from zero (no indication) to 100 (perfect indication). The significance of each taxon was tested using Monte Carlo test with 999 permutations in PC-ORD version 5.10 (McCune and Mefford, 2006). The species with significant indicator value ($p < 0.05$) were considered indicator species.

A two-way ANOVA was used to test for significant differences in $\delta^{13}C$ and $\delta^{15}N$ values of phytobenthos between the two sites, using site and season as the main factors. No transformation was needed to fulfill all statistical assumptions.

Results

Physico-chemical factors variation

Seasonal values of the physico-chemical factors measured across the two study sites of the Kowie River are given in Table 4.1. Water quality was low downstream as the streams passed through agricultural farms and a polluted tributary (Bloukrans River) which drains Grahamstown and has sewage discharged in it joined the main channel (Kowie River). The pH increased slightly down the pristine (upstream) to

agricultural/urban (downstream) gradient, being slightly acidic at the upstream sites and slightly alkaline at downstream sites (Table 4.1). The difference in pH among the three seasons and sites was not statistically significant ($p > 0.05$). Ammonium, conductivity, channel width, dissolved oxygen, nitrates, salinity, temperature, total dissolved solids, water flow and water depth increased at the downstream site, but as in the case of pH, the increase for ammonium, dissolved oxygen, nitrates, temperature and water flow was also not significant ($p > 0.05$, Table 4.1). Oxidation reduction potential (ORP) and phosphate concentration decreased significantly downstream. No significant differences were observed in physico-chemical factors among the three seasons ($p > 0.05$) except for temperature and nitrates (Table 4.1). A relationship between the significant ($p < 0.05$) physico-chemical is highlighted in Table 4.2, with conductivity, channel width, TDS, pH, ORP, water flow and depth being significantly correlated.

Table 4.1. Physico-chemical parameter values measured during the three seasons between November 2012 and August 2013. *Significant differences at p < 0.05 (two-way ANOVA) are indicated in bold.*

Parameters	**Summer**		**Autumn**		**Winter**		**Seasonal**		**Site**	
	US	**DS**	**US**	**DS**	**US**	**DS**	**F-value**	***p*-value**	**F-value**	***p*-value**
DO (mg L^{-1})	6.03	5.61	6.05	8.29	7.32	8.8	2.067	0.273	1.042	0.365
Conductivity (mS cm^{-1})	0.26	3.75	0.04	2.77	0.4	3.3	0.042	0.96	**101.346**	**0.001**
TDS (ppt)	0.26	2.52	0.04	1.81	0.26	2.16	0.056	0.947	**82.45**	**0.001**
Salinity (µS cm^{-1})	0.19	1.87	0.16	1.34	0.17	1.63	0.037	0.964	**88.03**	**0.001**
pH	5.69	7.72	7.01	8.61	6.54	8.33	0.685	0.667	6.491	0.061
Temperature (ºC)	18.1	22.7	12.3	12.9	12.3	12.8	**11.251**	**0.04**	0.249	0.644
ORP (mV)	88	-36.1	-38	-96.9	-13.1	-101.9	1.18	0.419	4.25	0.108
Water flow (m s^{-1})	0.28	0.55	0.23	0.37	0.04	0.28	1.304	0.391	4.031	0.115
Ammonium (mg NH_4^+ L^{-1})	0	0.13	0.02	0.08	0.04	0.23	0.436	0.682	**7.722**	**0.05**
Phosphate (mg PO_4^{3-} L^{-1})	0.4	0.8	1.3	5.4	0.1	0.2	2.121	0.267	0.747	0.436
Nitrate (mg NO_3^- L^{-1})	8.8	14.9	7.5	8.9	0	0	**11.283**	**0.04**	0.238	0.651
Water depth (m)	0.55	1.05	0.3	0.85	0.2	0.7	0.47	0.664	**12.645**	**0.024**
Channel width (m)	1.5	10.1	1.1	8.5	0.9	7.4	0.048	0.953	**87.134**	**0.001**

Table 4.2. Correlation between the physico-chemical factors, only the significant relationships ($p < 0.05$) are shown

Relationships	*r value*	*p–value*
Conductivity – channel width	0.979	0.001
Conductivity – TDS	0.999	0.000
Conductivity – water depth	-0.884	0.019
pH – ORP	-0.991	0.000
TDS – channel width	-0.98	0.001
TDS – water depth	-0.897	0.015
Water depth – channel width	0.938	0.006
Water flow – channel width	0.818	0.047
Water flow – water depth	0.936	0.006

Phytobenthos community structure on different substrates

We identified 96 phytobenthos taxa from the natural and artificial substrates, of which 36 taxa were considered the most common with >1 % relative abundance. From the natural substrates, a total of 78 phytobenthos taxa were identified. In the upstream site, 59 phytobenthos taxa recorded, while the downstream site recorded 63 taxa. Forty-two taxa were common to both sites. From the artificial substrates, 93 taxa were identified, 72 and 77 taxa for the upstream and downstream sites, respectively. Fifty-seven taxa were common for the upstream and downstream sites on the artificial substrates. Twenty-nine taxa on natural substrates occurred at an abundance of >1 % in at least one sample, while from the artificial substrates, a total of 37 diatom taxa was recorded. Average taxa richness recorded on different substrates over the three seasons are highlighted in Figure 4.1. The artificial substrates had high taxa richness compared to the natural substrate, although no significant differences were observed ($p > 0.05$). A higher total number of taxa were found on bricks (upstream) and brown tiles with the least being recorded on sediment during the study (Figure 4.1). Most of the additional taxa observed on the

substrates constituted <1 % of the entire sample. The number of phytobenthos taxa identified varied substantially ($p < 0.05$) between the different substrate types for the upstream and downstream sites (Figure 4.1).

A list of the dominant taxa (>4 %), and their percentage relative abundances for each site and substrate type, is shown in Table 4.3. The phytobenthos communities on natural substrate type were generally dominated by 3–4 taxa (>10 % per taxa) which constituted about 30–60 % of the entire sample per season. The artificial substrate communities were dominated by 5–8 taxa (>5 % per taxa) which constituted about 30–60 % of the entire sample per season (Table 4.3). Eight taxa with a relative abundance >0.5 % were recorded only on artificial substrate namely: *Cocconeis engelbretchii, Craticula vixnegligenda, Diploneis subovalis, Fragilaria capucina* var*. rumpens, Navicula veneta, Pinnularia borealis, Scenedesmus* sp. and *Stigeoclonium* sp. Three were identified only on natural substrates: *Nitzschia reversa, N. sigma* and *Spirulina* sp.

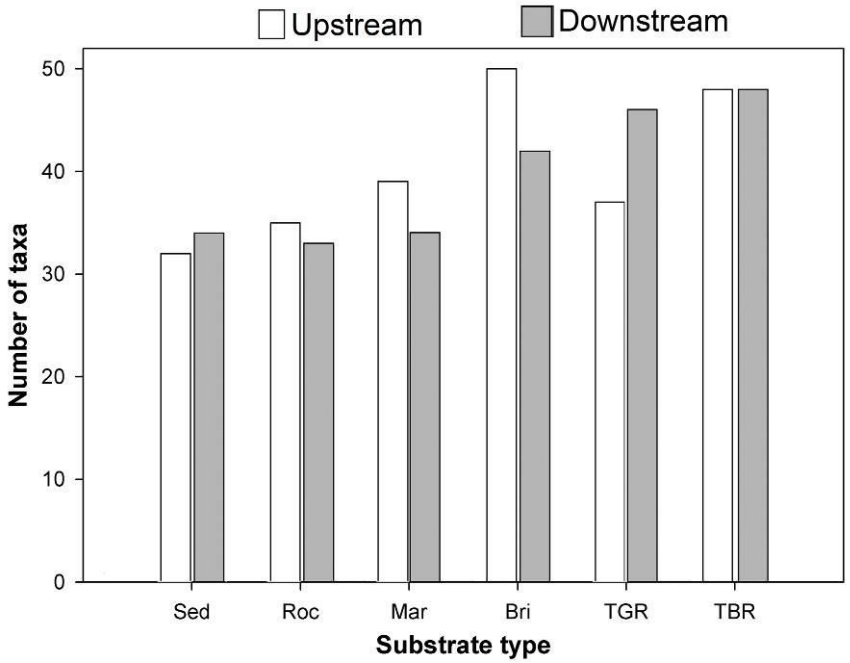

Figure 4.1. Average taxa richness recorded on different substrates in the Kowie River. *Abbreviations: sed – sediment, roc – rock/stone, mar – macrophytes, bri – brick, TGR – grey tiles and TBR – brown tiles*

Table 4.3. Average dominant percentage taxa abundance of phytobenthos (>4 %) found growing on artificial and natural substrates over three seasons (summer, autumn, winter).

Species	Natural substrate						Artificial substrate					
	Sediment		Rocks		Macrophyte		Brick		Grey tiles		Brown tiles	
	DS	US	DS	US	DS	US	DS	US	DS	US	DS	US
Achnanthes sp.			7.8	21.6				4.3		8.1		
Achnanthes standeri												4.2
Achnanthidium eutrophilum			18						4.7			
Achnanthidium minutissimum	16.2	5.9	25.1		13.5			7.5	5.4	8.8	5.1	10.5
Achnanthidium sp. 1	6.8		16.9	10.4	11.7	13.7	11.5		8.4	5.4	8.2	
Achnanthidium sp. 2		8.9					8.7		4.9			
Audouinella sp.		7.1				6.4						
Craticula buderi										5.6		
Entomoneis sp.	7.9											
Eolimna minima			6.9		6				8.7			
Fragilaria biceps		10.6			4.1	7.5		8.3				9.5
Fragilaria capucina										5.9		6.5
Fragilaria capucina var. *rumpens*										5.5		
Fragilaria nanana	14.7					8.1						
Fragilaria tenera						6.2						
Fragilaria ulna								8.2				8.8
Fragilaria ulna var. *acus*						8.7		6.8				6.3
Gomphonema gracile				9.2								
Gomphonema venusta										4.7		4.1
Gyrosigma sp.	4.5											
Kirchneriella sp.				5.5								
Monoraphidium sp.					11							
Navicula cryptocephala		10			10		9.6	8.6			12.9	
Navicula cryptotenella							8.8		11.9	4.4	7.1	
Navicula gregaria									8.2			
Navicula radiosa		10			6.7							
Navicula sp.			5.3		12.3				5.3		8.2	
Navicula trivialis	5											
Navicula veneta							6.5					
Pinnularia divergens										4.7		
Pinnularia viridiformis		4		7.6								
Sellaphora sp.			4.3				6.1					
Staurosira elliptica			3.8	12.8			11.8					
Surirella brebissonii	7.5					5.3						
Surirella ovalis	18.5											
Tabularia fasciculata							4.1					

Phytobenthos-based site classification

Based on similarities and differences of phytobenthos community composition among the six substrates and two sites sampled plus among seasons, cluster analysis classified two major groups or clusters (Figure 4.2). The first group was divided into four subgroups while Group 2 was divided into five subgroups. MRPP indicated statistically significant differences among the sites (A = 0.047, p < 0.001), season (A = 0.029, p = 0.015) and substrate type (A = 0.056, p = 0.006). Similarities among grey tiles and all natural substrates: macrophyte (A = 0.018, p = 0.209), rocks (A = 0.026, p = 0.184) and sediment (A = 0.035, p = 0.096) were very high. Group and cluster differences or similarities were associated with differences in the sampling sites' geographical location and physico-chemical factors. Using cluster analysis, phytobenthos communities from different substrates sampled on the same site were slightly similar as they were grouped close to each other in most cases (Figure 4.2). Phytobenthos communities growing on most of the artificial and natural substrates from downstream were included in Group 1 while three communities from this site growing on artificial (brown and grey tiles) and natural (rocks/stone) substrates were placed in Group 2. Phytobenthos communities from Group 2 were characterised by upstream site communities. Phytobenthos from the different substrates were not clearly separated by cluster analysis (Figure 4.2).

Substrates phytobenthos community structure in relation to physico-chemical factors

The strength relationships (λ_1/λ_2 ratios) between phytobenthos communities and selected physico-chemical factors were not significantly different (p = 0.379) among the different substrates (Figure 4.3). All of the λ_1/λ_2 ratios were very high (>1) for all the different substrates, with the highest being observed on rocks and lowest on bricks. The strength relationship for selected physico-chemical factors (water depth and water flow) and phytobenthos communities was high (>0.7) and ammonium (0.43).

The results of the CCA for the six substrates types showed that the first two CCA axes accounted for >55 % of the taxa (species) data variance while also accounting for >62 % of the species-environment variance. The CCA analysis for macrophytes was different from the rest of the CCA plots in terms of physico-chemical factors that structure the communities (Figure 4.3). The difference observed could be attributed to the biological nature of the substrate compared to the other substrates which were inorganic. In CCA plots, the natural substrates, at upstream sites were separated along the axis 1 while in the artificial substrates, especially bricks and grey tiles, the separation was not very clear. In the CCA plots, sampling sites were associated with different physico-chemical factors, with taxa associated with different sites being different from one substrate to another (Figure 4.3).

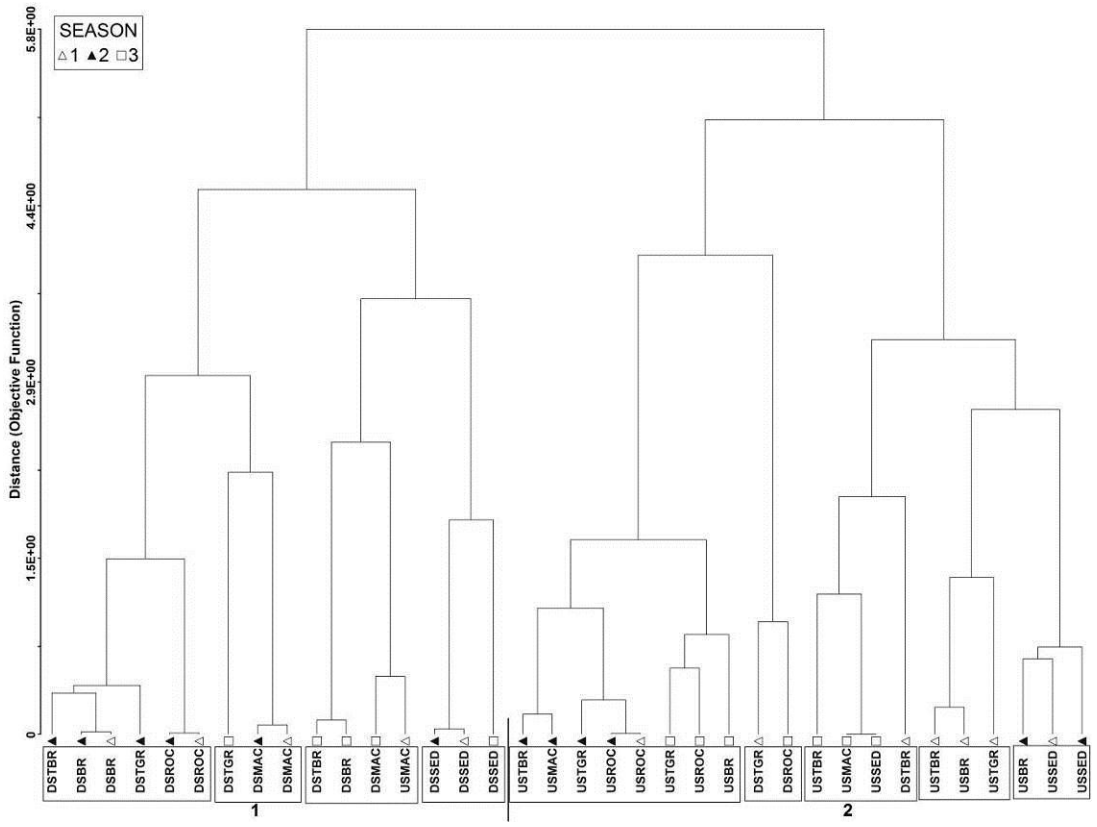

Figure 4.2. Dendrogram of phytobenthos-based taxa classification based on community data sampled across three seasons from different substrates on two sites. *Abbreviation: US and DS – upstream and downstream, for more abbreviations see Figure 4.1*

In the brick substrate CCA plot, all downstream site taxa was mostly associated to conductivity, water flow and depth (Figure 4.3a). The downstream sites were also associated with the small sized phytobenthos taxa such as *Achnanthes* sp., *Navicula cryptocephala, Navicula radiosa, Achnanthidium* sp. and *A. standeri*. Upstream sites in this CCA were associated with larger species such as *Fragilaria capucina, F. ulna, F. nanana*, *Surirella ovalis* and *S. brebissonii*. In the CCA based on macrophyte substrate communities, upstream summer (USS) and downstream winter (DSW) sampling sites were more associated with ammonium, while phosphate, conductivity and water depth were more associated with upstream autumn (USA), downstream autumn (DSA) and summer (DSS). Upstream winter (USW) was associated with *Cylindrocapsa* sp. and *Audouinella* sp. (Figure 4.3b).

The CCA plot rocks/stones substrate communities, DSS and DSA sampling sites were more associated with conductivity, water depth and flow (Figure 4.3c). These two sites were associated with such taxa as *Navicula* sp., *A. standeri*, *Fallacia* sp., *Sellophora* sp. and *Eolimna minima*. Of the communities growing on sediment, the CCA plot had DSW, DSA and DSS being associated with conductivity, pH and water depth. Sites USA and USS were associated with taxa such as *Pinnularia viridiformis, Fragilaria tenera, Navicula cryptotenella* and *Achnanthidium* sp. (Figure 4.4d). In the CCA based on grey tile substrate communities, USA and DSW sites were more associated with water flow and depth, while the DSS and USW sites were associated with channel depth and water flow (Figure 4.3e). Site DSA was associated with taxa such as *Achnanthidium* sp., *A. standeri, Fallacia* sp., *Gomphonema gracile* and *Achnanthidium minutissimum* (Figure 4.3e). The brown tile substrate communities CCA plot, site DSS and DSW were associated with conductivity, water depth, and depth (Figure 4.3f). Site USS, USW and USA were associated with taxa such as *A. standeri, F. capucina, F. biceps, G. venusta, F. capucina, P. borealis, N. cryptotenella, S. elliptica* and *S. brebissonii* (Figure 4.3f).

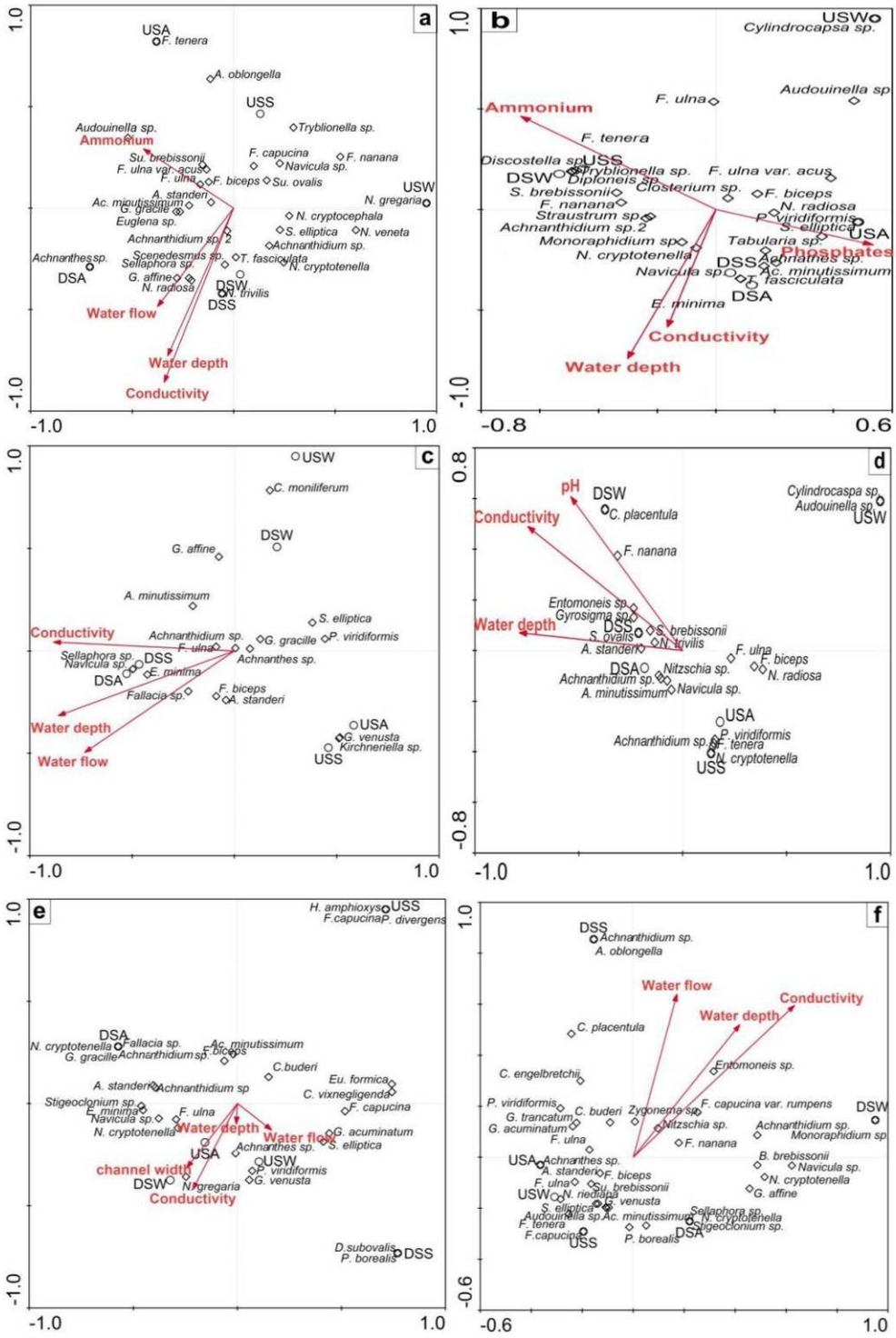

Figure 4.3. CCA plots showing the frequently occurring diatom taxa (>1 %) on (**a**) brick, (**b**) macrophytes, (**c**) rocks/stones, (**d**) sediment, (**e**) grey tiles and (**f**) brown tiles in relation to selected environmental variables. *Abbreviations: US and DS – upstream and downstream, the letters that follow: A, S and W – autumn, summer and winter*

Substrate type indicator species

Different indicator species were recorded on different substrates as highlighted by the IndVal species analysis (Table 4.4). Significant differences ($p < 0.05$) were observed for indicator species, on different substrate types, season and sites (Table 4). Natural substrates had significantly ($p < 0.05$) more indicator species compared to the artificial substrates, with rocks/stones substrate having the highest number of indicator species (4), sediments (3) and macrophytes (1). For example, *Achnanthes standeri* (IV = 60.7 %), *Euglena* sp. (IV = 63.8 %), *Surirella ovalis* (IV = 62.7 %) had the highest indicator values for rocks/stones, brick and sediment respectively. Brown tile substrates did not have any significant ($p > 0.05$) indicator species (Table 4). Seasonal variation of indicator species across substrate types was greater for the summer with six indicator species and autumn having the least (2). The IndVal for the season ranged between 31.7 % and 56.8 %, with *Fragilaria ulna* var. *acus* in autumn having the highest indicator value. The upstream and downstream site had 8 and 6 indicator species, respectively (Table 4.4). Two species, *F. ulna* and *Gomphonema acuminatum* found in the upstream site were indicator species for summer and winter respectively. *Achnanthidium* sp.1 and *Entomoneis* sp. were both indicator species for rock/stone substrate for the downstream site, with *A. standeri* being the indicator species for the rock/stone substrate in autumn (Table 4.4).

Phytobenthos stable isotopes values

In the downstream site, the $\delta^{13}C$ phytobenthos values were lower in the summer: macrophytes, rocks, brown tiles, grey tiles and bricks (Figure 4.4). Phytobenthos on sediment had a high $\delta^{13}C$ with a value of -16.4±0.23 ‰ (Figure 4.4). In autumn, all the $\delta^{13}C$ values were higher, with a range of -20±0.11 ‰ to -25.7±0.09 ‰. From summer to autumn, sediment had the highest $\delta^{13}C$ values, with -23±0.26‰ from -16.4±0.23 ‰. During winter, the $\delta^{13}C$ values for the different were less variable (-18.2±1.23 ‰ to -25.6±0.25 ‰) than in autumn, with rocks substrate phytobenthos $\delta^{13}C$ values increasing (Figure 4.4). There were significant differences in

phytobenthos communities (F = 43.27, $p < 0.001$) within substrate type $\delta^{13}C$ values for the downstream site across seasons.

For the upstream site, the brown tile substrate phytobenthos values were more depleted in $\delta^{13}C$ and were lower in summer and winter with value ranges of -25.9±0.64 ‰ to -28.7±0.23 ‰ and -24.5±0.12 ‰ to -28.2±0.57 ‰, respectively. In summer, phytobenthos on bricks had high $\delta^{13}C$ values while macrophyte phytobenthos had even higher $\delta^{13}C$ values. In winter, phytobenthos on macrophytes and sediment had high $\delta^{13}C$ values while rocks/stones substrate phytobenthos had low $\delta^{13}C$ values (Figure 4.4).

During autumn, the $\delta^{13}C$ values among the different substrates were slightly more enriched and ranged between -23.3±2.12 ‰ and -26.5±0.08 ‰, with rock substrate phytobenthos being high in $\delta^{13}C$ while greys tiles and sediment substrate phytobenthos had low $\delta^{13}C$ (Figure 4.4). There were significant differences (F = 36.12, $p < 0.001$) within the substrate type $\delta^{13}C$ values for the upstream site across seasons. Looking at site variation, sediment was significantly different (F = 23.28, $p < 0.05$) and the rest were not significantly different ($p > 0.05$). Significant seasonal variation for phytobenthos on brown tiles (F = 19.281, $p < 0.05$), rocks (F = 12.2, $p = 0.001$) and bricks (F = 7.85, $p = 0.005$) was found at both sites.

For all the substrates, the $\delta^{15}N$ values of the phytobenthos were higher at the downstream, than the upstream sites (Figure 4.4). The $\delta^{15}N$ values for the downstream site ranged between 9.3±0.08 ‰ and 13.7±0.35 ‰ in summer. In autumn and winter, the average $\delta^{15}N$ values were slightly more enriched, ranging from 10.3±0.23 ‰ to 13.9±0.33 ‰ and from 11.9±0.29 ‰ to 13.6±0.92 ‰, respectively. In summer, phytobenthos on sediment had a high $\delta^{15}N$ value, while in autumn, rocks, brown and grey tiles substrate phytobenthos had a high $\delta^{15}N$ values.

Table 4.4. List of significant ($p < 0.05$) indicator values for phytobenthos taxa sampled on different substrates, season and sites

Taxa	Seasonal			Substrate type			Site		
	IndVal (%)	*p*-value	Season	IndVal (%)	*p*-value	Sub. type	IndVal (%)	*p*-value	Site
Achnanthes standeri	47.5	0.008	autumn	60.7	0.001	rock/stone			
Achnanthidium sp. 1				36.7	0.048	rock/stone	60.5	0.023	downstream
Achnanthidium sp. 2				34.6	0.047	brick			
Audouinella sp.							52.1	0.003	upstream
Cocconeis engelbretchii				50.0	0.021	sediment			
Craticula cuspidata	33.3	0.029	summer						
Cyclotella meneghiniana							39.7	0.026	downstream
Encyonopsis microcephala	33.3	0.027	summer						
Entomoneis sp.				46.0	0.048	rock/stone	46.1	0.008	downstream
Eolimna minima							38.7	0.015	downstream
Euglena sp.				63.8	0.001	brick			
Fragilaria capucina	41.7	0.008	summer						
Fragilaria capucina var. *rumpens*				32.7	0.039	grey tiles			
Fragilaria tenera							38.6	0.026	upstream
Fragilaria ulna	49.0	0.036	summer				54.1	0.002	upstream
Fragilaria ulna var. *acus*	56.8	0.005	autumn						
Gomphonema acuminatum	34.6	0.049	winter				43.1	0.005	upstream
Gomphonema gracile				40.0	0.022	rock/stone			
Gomphonema laticollum	33.3	0.029	summer						
Gomphonema truncatum	37.6	0.047	summer						

Gyrosigma sp.							32.5	0.029	upstream
Kirchneriella sp.							27.8	0.039	upstream
Monoraphidium sp.				49.2	0.01	macrophyte			
Navicula erifuga	31.7	0.041	winter						
Navicula gregaria	33.2	0.021	winter						
Navicula sp.							60.9	0.003	upstream
Nitzschia reversa				35.7	0.041	sediment			
Pinnularia viridiformis							55.6	0.003	upstream
Scenedesmus sp.				48.2	0.009	brick			
Surirella ovalis				62.7	0.014	sediment			

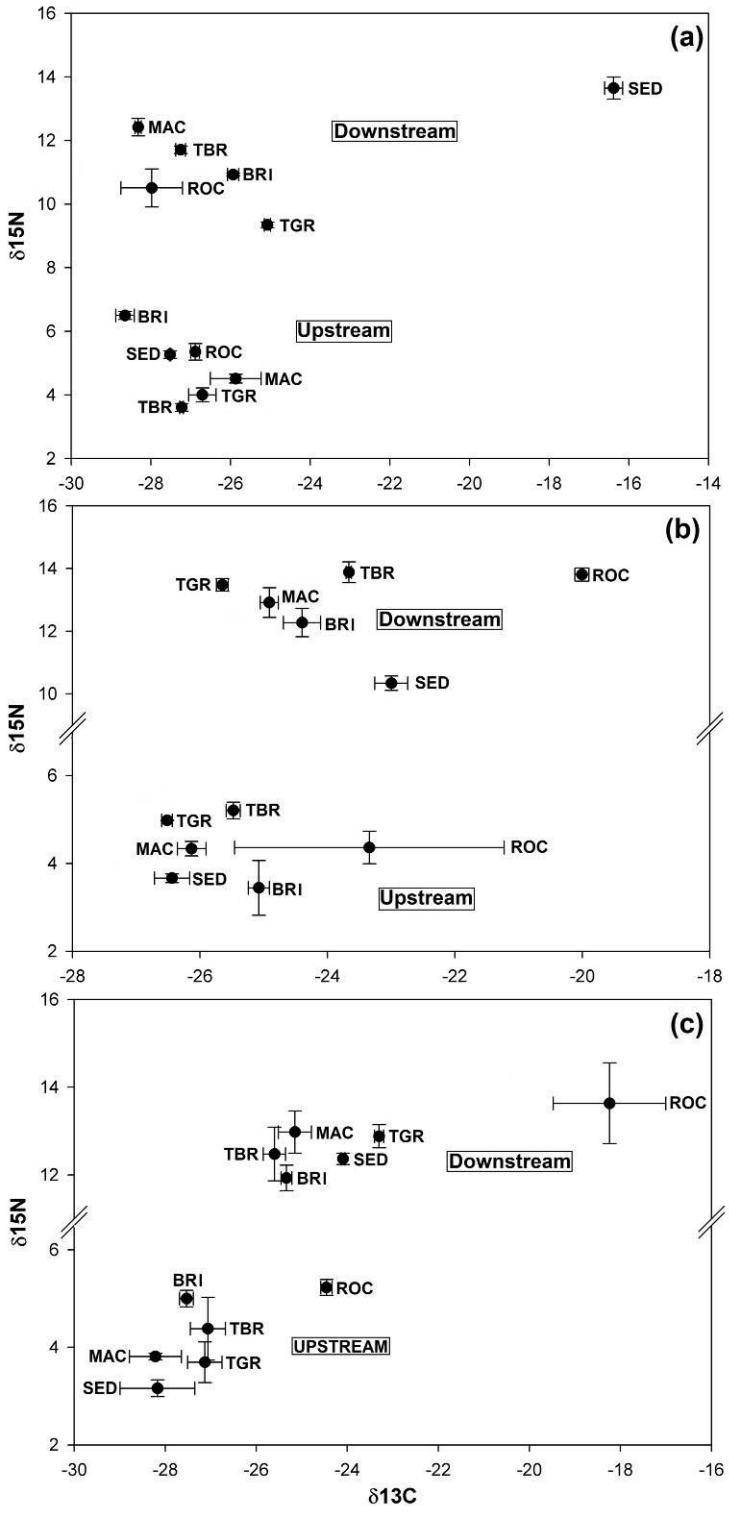

Figure 4.4. Average $\delta^{15}N$ and $\delta^{13}C$ values (± standard deviation) of phytobenthos collected on different substrates: **(a)** summer, **(b)** autumn and **(c)** winter. Abbreviations *see Figure 4.1*

In winter, phytobenthos on rocks had high $\delta^{15}N$ values while brick substrate phytobenthos had low $\delta^{15}N$ values (Figure 4.4). For the upstream site, $\delta^{15}N$ values ranged from 3.6±0.04 ‰ to 6.5±0.12 ‰ in summer, from 3.44±0.62 ‰ to 5.21±0.19 ‰ in autumn and from 3.16±0.17 ‰ to 5.23±0.16 ‰ during winter. Phytobenthos on bricks (summer), brown tiles (autumn) and rocks/stones (winter) had higher $\delta^{15}N$ values for the upstream site (Figure 4.4). $\delta^{15}N$ values were found significantly higher within site variation for phytobenthos on brown tiles ($F = 379.64$, $p < 0.05$), grey tiles ($F = 126.22$, $p < 0.05$), macrophytes ($F = 100.17$, $p < 0.05$), sediment ($F = 164.8$, $p < 0.05$), rocks/stones ($F = 135.702$, $p < 0.05$) and bricks ($F = 29.262$, $p < 0.05$) but no significant variation among seasons ($p > 0.05$).

Discussion

Community structure in relationship to physico-chemical factors

In this study, both cluster analysis and CCA showed that various multi-scale factors were important in explaining variations in the community structure of phytobenthos assemblages on the different substrates of Kowie River. Using cluster analysis, no distinct seasonal patterns between the two sites were observed, with upstream sites being separate from downstream sites. A similar pattern was evident in the number of taxa, with upstream sites generally characterised by a high number of taxa. The results generally reflected a change in community composition along altitudinal or longitudinal gradients as there were fewer species upstream than downstream plus taxa composition differed. This altitudinal gradient may not be due to a single factor (Hwang et al., 2011; Bere et al., 2013) because a downstream gradient is likely to be complex and associated with other factors, such as nutrient levels, land use, channel width, depth and water flow, which in-turn affected the different phytobenthos communities on different substrates.

The CCA showed that phytobenthos assemblages in the Kowie River were also associated with physical habitat conditions such as channel width and depth which

explained most of the variability in the phytobenthos data set. It is not surprising that physical habitat conditions accounted for most of the variability in the phytobenthos assemblages, as Pan et al. (2006) found that stream diatom assemblages in the Central Valley ecoregion were mainly affected by river or stream physical conditions. A study in the upper Han River basin also highlighted that immediate factors such as substratum embeddedness or land use and cover also influence the structure and distribution of phytobenthos (Tan et al., 2014).

Water flow was an important factor in structuring phytobenthos communities in the study area. The CCA indicated that water flow (current) was the most important factor separating groups in most of the substrates and water flow has been reported to affect significantly taxa composition and biomass of phytobenthos in rivers and streams (Leland and Porter, 2000; Song, 2007; Hwang et al., 2011).The importance of water flow in structuring phytobenthos communities has also been reported by several other researchers (e.g. Potapova and Charles, 2003; Bere and Tundisi, 2011a; Wu et al., 2011; Hwang et al., 2011; Dalu et al., 2014a). Water flow effects seem to vary with the trophic status of rivers and streams (Bere and Tundisi, 2011a; Wu et al., 2011; Hwang et al., 2011), probably due to a negative association between biomass accumulation and detachment, which increases with flow. Water flow may play an important role in phytobenthos community structure and this structuring varies with substratum type among other factors (Leland and Porter, 2000).

The phytobenthos taxa on the different substrates in the study area responded differently also to ammonium, conductivity and phosphate. The phytobenthos community composition at different locations provides useful information about how they grow and develop on different substrates. Hustedt (1957) noted that phytobenthos responded mostly to osmotic pressure, and not the concentration of a particular salt. As a result, conductivity may explain much of the variation among phytobenthos assemblages as they integrate several watershed processes,

indicating the watershed's geological nature. The pH levels exert a direct physiological stress on phytobenthos, while also strongly influencing other water chemistry factors (Bere and Mangadze, 2014).

Phosphate was an important factor structuring phytobenthos communities on the macrophyte substrate and phosphate was shown to structure the downstream (autumn and summer) and upstream (autumn) sites when phosphate availability in water was highest. In this study, since the macrophyte substrate *Cyperus* sp. is a rooted macrophyte, it was potentially able to re-mobilize nutrients, mainly the phosphate stocked in the sediments, making it available to the phytobenthos community. Díaz-Olarte et al. (2007) showed that periphyton abundance associated with a macrophyte, *Utricularia foliosa* depended mostly on the changes in the water phosphate concentration. Burkholder and Wetzel (1990), Guariento et al. (2009) and Ferragut et al. (2010) showed that macrophytes influence the availability of phosphate to the phytobenthos. Burkholder and Wetzel (1990) showed that macrophytes, in addition to supplying 25–60 % of the phosphate supply to the phytobenthos, can also influence them via the release of labile compounds.

Comparison of natural and artificial substrates

The number of significant indicator values was higher on natural substrates compared to artificial substrates, with the upstream site having the most indicator taxa due to the stability in physico-chemical factors. Both taxa richness and diversity were low on natural substrates especially during autumn and winter compared to artificial substrates with the differences being greater than five taxa in most cases. The results contradict what others have found e.g. Barbiero (2000) and Lane (2003) as they found high taxa richness and diversity in natural substrates.

These differences could likely to be due to the artificial flora of the brown tiles and bricks being selected for by physico-chemical properties of the substrate and perhaps positioning of substrate in relation to the water flow or currents. Bere and

Tundisi (2011a) showed that substrate properties and orientation or placement in water had an effect on the communities observed. Another important factor could be that the phytobenthos communities on the artificial substrate were indicative of a successional process hence the recommendation of leaving artificial substrates for a year before sampling to allow the diatom communities to progress from a colonisation community to a stable community reflecting environmental conditions typical of natural communities (Komárek and Sukacová, 2004). The results show that the introduction of artificial substrates provided a colonisation habitat that was not as distinct as that of natural habitats, but the 30 day incubation period used in this study should have been sufficient to allow a stable diatom community to develop (Peterson, 1986; Lane et al., 2003).

The similarities among artificial substrates (grey tiles) and all natural substrates were very high. The similarities observed between the grey tiles and rock substrate communities could be related to physico-chemical properties of the clay grey tiles which we believe were closely related to the rock substrate. The phytobenthos taxa sometimes preferentially select one substrate over another (Lane, 2001). This could explain their preference for artificial grey tiles over brown tiles and bricks. The habitat differentiation allowed the development of a stable community similar to that found on the natural environment (rocks/stones). This study therefore clearly highlights the importance of the type of artificial substrates that can be accurately used to represent the natural community.

Phytobenthos stable isotope values

The rock, brick and brown tiles showed seasonal variation in $\delta^{13}C$ values which may suggest a seasonal change in phytobenthos communities (Pond et al., 1998). Mejía et al. (2013) showed that centric diatoms (40-70 µm) had $\delta^{13}C$ values up to 5 ‰ higher than those of pennate diatoms of similar size from the same sample, with the higher values, indicating less extreme fractionation during photosynthesis. In this study, the downstream site was dominated by small sized phytobenthos compared to

the upstream site which had larger taxa which was similar to observation by Mejía et al. (2013). The patterns could also result from seasonal changes in the isotopic signature of the inorganic carbon source (Napolitano et al., 1997; Pond et al., 1998; Mejía et al., 2013). The downstream sites will have higher concentrations of HCO_3^-, as inferred from pH and conductivity, and use of this source of inorganic carbon is linked to higher $\delta^{13}C$ values. The thickness of the boundary layer over the periphyton also affects the discrimination against ^{13}C; discrimination is greater when boundary layers are thick (Wang et al., 2013). The phytobenthos communities from the upstream site were exposed to greater water movement as the channel was narrow and shallow compared to the wide and deep downstream site channel hence this might have dramatically reduced the thickness of the boundary layer resulting in even more severe depletion.

The $\delta^{15}N$ values of the phytobenthos were positively correlated with total nutrient concentrations (ammonium and nitrate). This suggests that changes in the $\delta^{15}N$ values of the phytobenthos may be caused by anthropogenic inputs of nitrogen from livestock waste, synthetic fertilisers and sewage containing more enriched $\delta^{15}N$ values (Ning et al., 2013).

The relative abundance and common occurrence of phytobenthos in Kowie River, which assimilate nutrients directly from the water, make phytobenthos excellent potential indicators of pollution. From this study, most of the artificial substrates plus rock substrate phytobenthos communities can be used as nitrogen pollution indicators using $\delta^{15}N$ values. These phytobenthos communities tended to show greater variation in $\delta^{15}N$ values compared to macrophyte and sediment substrate communities. The $\delta^{15}N$ values did not show a seasonal variation as the pristine upstream site remained relatively unchanged or undisturbed and the downstream site continued to be polluted. Using stable isotope analysis, I was able to identify that the communities on different substrates were different ($p < 0.001$) even though

microscopic analysis identified some common taxa although there were not significant.

Conclusion

The selective use of either brown tiles or bricks appeared to offer no particular advantage in this study, in that both artificial substrates provided similar results, and there was no significant difference in the community composition on either substrate type. However, grey clay tiles may be more representative of the microtopography of the natural substrates making them a suitable substrate for future studies. This study leads to future research of how different sized phytobenthos on different substrates react to pollution and this will help in identifying the best substrate to use in water monitoring studies.

CHAPTER 5

PHYTOPLANKTON COMMUNITY DIVERSITY ALONG A RIVER–ESTUARY CONTINUUM

Plate 5. Selected phytoplankton species recorded along the Kowie river–estuary continuum viewed under the light and electron microscope. Photo by Tatenda Dalu

Abstract

In this chapter an examination of the aspects of phytoplankton communities along a river–estuary continuum in the Kowie system, Eastern Cape (South Africa) is presented. This study aims to relate the phytoplankton population community structure to physicochemical parameters and estimate the different chlorophyll-*a* concentrations for the Kowie system. Eight sampling sites along a 70 km reach of the Kowie system were sampled over a one year period. One hundred and seventy-eight species belonging to 78 genera were recorded within the riverine-estuarine continuum. Diatoms were predominant accounting for 81.9 % of the total abundance. The estuary had 98 species (55 genera) recorded whereas the river had 141 species (67 genera). Using a two-way ANOVA analysis, species richness was significantly different among seasons. The chl–*a* concentration along the river–estuary continuum increased from spring to a high in summer (river mean = 7.9 mg m^{-3} and estuary mean = 3.3 mg m^{-3}) before decreasing to a low in winter. Kruskal–Wallis analysis showed significant differences among chl–*a* concentration with seasons and sites. Redundancy analysis identified five factors; salinity, water depth, aerial cover, nitrates and ammonia that were significant ($p < 0.05$) in affecting phytoplankton variation. The relatively small study indicates the need for further monitoring to gain a better understanding of Kowie system phytoplankton.

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Introduction

Phytoplankton includes cyanobacteria and algae are microscopic and drift in the water currents, and they are important primary producers in aquatic ecosystems. Although a longitudinal decrease in phytoplankton abundance from the upper to the lower reaches is a well-known phenomenon in most river systems (Reynolds, 1988), population densities can be substantial in the upper and middle reaches, particularly in long slow-flowing rivers (Piirsoo et al., 2008). The balance between the conditions that promote or prevent the development of phytoplankton in rivers varies both temporally and spatially, with temporal changes being rapid after floods or heavy rains increase sediment loads that attenuate underwater light (de Domitrovic et al., 2007; Wu et al., 2011).

Spatial variation of phytoplankton can result from a change in river morphology from the headwaters to low-lying streams due to changes in biotic and abiotic factors e.g. light, temperature, radiation (de Domitrovic et al., 2007; Piirsoo et al., 2008). This study will seek to evaluate how spatial variation in phytoplankton occurs linking with the riverine continuum concept (RCC) and riverine productivity model (RPM). Phytoplankton can attain high abundances in lowland rivers, where residence time and low flow velocity allow sufficient time for growth and reproduction (Devercelli, 2006; de Domitrovic et al., 2007; Piirsoo et al., 2008; Wu et al., 2011).

The spatio-temporal patterns of phytoplankton communities are important for understanding ecosystem functioning, because phytoplankton can affect ecosystem processes, functioning, and stability of aquatic ecosystems food web and structure and their community structure can reflect major shifts in environmental conditions (Suikkanen et al., 2007; Wu et al., 2011). Understanding the spatial linkages between habitats is important for understanding phytoplankton community structure. This study aims to describe the importance of autochthonous production focusing on phytoplankton communities, from the upstream to downstream locations using the RCC model as conceptualized by Lindeman (1942) and Vannote et al. (1980). I also

aim to relate the phytoplankton population community structure to physicochemical parameters and estimate the different chlorophyll-*a* concentrations for the Kowie system.

Materials and methods

Study area

A detailed description of the study area is provided for in Chapter. Eight sites were chosen along the riverine-estuarine continuum of the Kowie system, five sites were river (freshwater) and three estuary (saline water) sites (Table 5.1). The river–estuary continuum was divided into three sections, the upper (sites F1 and F2), middle (sites F3–F5) and the lower (sites E1–E3) reaches. The study was carried out in four different seasons; spring (September 2012), summer (November–December 2012), autumn (February 2013) and winter (May–June 2013) on pools along the continuum, with phytoplankton sampling being carried out once a season.

Physico-chemical parameters

A detailed description of tools and sampling techniques used is highlighted in Chapter 3.

Chlorophyll-*a* analysis

Chlorophyll–*a* (chl–*a*) measurements in the water column were done to give a proxy of the phytoplankton concentrations or biomass present and 500 mL water samples were collected in triplicates. In the laboratory, 250 mL of each water sample were serially filtered (vacuum <5 cm Hg) through a 20 µm nylon mesh filter (microphytoplankton size range >20 µm), a 2 µm isopore membrane (nanophytoplankton 2–19.9 µm), and a 0.7 µm glass fibre filter (picophytoplankton <1.9 µm). Each filter was extracted in 90 % acetone at -20 °C for 24 hours in the dark.

Table 5.1. Morphometric features of the riverine-estuarine continuum of the Kowie system

Site name	Elevation (m)	Macrophyte species	Macrophyte cover range (%)	Channel width (m)	Aerial cover range (%)
F1	367	*Cyperus sp.*	5–15	1.3±0.3	55–90
		Lagarosiphon muscoides	0.5–2		
F2	237	*Cyperus eragrostis*	10–30	2.5±0.5	45–60
F3	112	*Cyperus eragrostis*	18–55	12.3±1.7	28–35
		Cyperus alopecuroides	2.5–3.5		
F4	53	*Cyperus eragrostis*	20–50	9.1±1.1	16–20
F5	27	*Phragmatis australis*	15–60	9.5±1.3	5–7
		Potamogeton pectinatus	45–85		
		Schoenoplectus brachycerus	1.5–4		
		Jancus sp.	2–6		
ES1	27	*Schoenoplectus brachycerus*	5–25	32.2±3.5	3.0–4.5
		Phragmatis australis	30–72		
ES2	8	*Schoenoplectus brachycerus*	30–45	46.8±3.2	0.5–1.2
		Phragmatis australis	3–12		
ES3	7	*Spartina maritima*	40–75	69.2±5.8	0

Chl-*a* concentration was then determined fluorometrically using a Turner 10AU fluorometer according to Froneman (2000). After 24 hrs, test tubes were centrifuged at 5000 rpm for 5 mins. After centrifugation, care was taken not to mix the solution with supernatant and ~6 mL of the sample (in acetone) into a glass vial. The glass vial was wiped off any finger prints and readings were taken using a Turner 10AU fluorometer. The vial was removed from the fluorometer and 2 drops of hydrochloric acid were added and the vial was inverted to mix. A second as above was taken as above. Chl–*a* was calculated using the formula:

$$\text{Chl} - a\ (\text{mg m}^{-3}) = \text{Vol. of water filtered} \times (\text{No acid reading} - \text{Acid reading}) \times 0.325$$

Phytoplankton sampling

Horizontal phytoplankton net-tow samples were collected using a 20 µm mesh net. Horizontal tows were carried out at about 2 knots for 2 min. All collected

phytoplankton was then fixed in Lugol's iodine solution. After a day or two, the undisturbed phytoplankton samples preserved in Lugol's iodine were further concentrated by removing or decanting the supernatant, taking care not to disturb the sedimented material at the bottom which contained the phytoplankton. The remaining precipitate was then vigorously mixed, which was about 5–10 mL to allow mixing before being transferred into a petri dish with grid marking for identification and scoring under an inverted Nikon TMS microscope at 1000×. If the sample, contained a lot of diatoms, diatom slides were prepared after oxidizing the organic material using the hot hydrochloric acid and potassium permanganate method (Taylor et al., 2007a) and a minimum of 300 valves were counted for each sample using a phase-contrast Olympus CX light microscope at 1000× under oil immersion. All phytoplankton were identified to the lowest taxonomic level possible using the keys of Giffen (1970), Taylor et al. (2007) and John et al. (2002). Phytoplankton was presented as relative percentage abundances for each sampling occasion.

Data analysis

Due to the presence of a large number of rare species, individual taxa chosen for analyses occurred at more than one site and had a total relative abundance 0.5 % when all sites were summed. This effect reduced the number of taxa in the analysis from 179 to 103. The relationship between phytoplankton species relative abundances and fifteen environmental factors along the riverine-estuarine continuum sites were explored using Redundancy Analysis (RDA). To eliminate the influence of extreme values on ordination scores, phytoplankton species abundances and physico-chemical data were log (x+1) transformed before RDA analysis.

To evaluate changes in phytoplankton community and among site separation, *n*-MDS was used. Ordination stress is a measure of departure from monotonicity in the relationship between the dissimilarity (distance) in the original *p*-dimensional space and distance in the reduced *k*-dimensional ordination space (Kruskal and Wish, 1978). The *n*-MDS analysis was carried out using PC-ORD version 5.10 (McCune

and Mefford, 2006) in autopilot mode so as to allow the program to choose the best solution at each dimensionality and Sorenson (Bray–Curtis) similarity was used as a measure of distance.

Diversity indices (Simpsons, Shannon-Weiner and Margalef), species richness and evenness were calculated using the whole dataset (179 species) using PAST version 2.0 (Hammer et al., 2001). A non–parametric test, Kruskal–Wallis, was used to test for differences in chl-*a*, diversity indices and evenness among sites and seasons using SPSS 16.0 for Windows software (SPSS Inc., 2007). Pearson rank correlations were evaluated to determine the relationships that existed between the physico-chemical factors and chl-*a* concentration, species richness and diversity indices.

Results

Physico-chemical parameters

Physico-chemical factors along the riverine-estuarine continuum varied greatly, with most of the factors increasing from upstream to downstream. Conductivity ranged from 0.4 to 3.4 mS cm^{-1} (mean 2.0 mS cm^{-1}) in the river while the estuary ranged from 4.1 to 36.8 mS cm^{-1} (mean 16.1 mS cm^{-1}). Salinity ranged from 0.1 to 1.8 ppt (river mean 1.0) and 2.0 to 27.4 ppt (estuary mean 9.6 ppt), while TDS ranged 0.2 to 7.7 ppt (river mean 1.8 ppt) and 2.8 to 29.1 (estuary mean 11.0 ppt). Stream depth along the continuum ranged from 0.4 to 6. 6 m (riverine – 0.37 to 1.5 m and estuary – 2.1 to 6.6 m) and stream width ranged from 0.8 to 16.4 m (riverine – 0.8 to 7.8 and estuary – 8.5 to 16.4 m). The estuarine sites had high levels of physico-chemical factors. Ammonia concentration was higher in the estuarine sites (0.04 to 1.04 mg L^{-1} and mean 0.4 mg L^{-1}) than in the riverine sites (0 to 0.5 mg L^{-1} and mean 0.2 mg L^{-1}). Nitrate concentration ranged from 0 to 37.4 mg l^{-1} (river mean 5.1 mg L^{-1}) and 0 to 24.4 mg L^{-1} (estuary mean 5.0 mg L^{-1}). Phosphate concentration ranged from 0 to 9.3 mg L^{-1} (river mean 1.1 mg L^{-1}) and 0 to 6.2 mg L^{-1} (estuary mean 1.0 mg L^{-1}; Table 5.2). The highest ammonia and nitrate concentrations were recorded in the

autumn. The main physico-chemical means and Kruskal–Wallis analysis results are summarized in Table 5.2.

Three physico-chemical factors (dissolved oxygen, temperature and water flow), showed significant differences ($p < 0.05$) among the four seasons, whereas five variables, conductivity, total dissolved solids (TDS), salinity, water depth, and ammonia were significantly different ($p < 0.05$) amongst study sites (Table 5.2). Pearson rank correlation showed that most of physico-chemical variables except dissolved oxygen, ORP, water flow and phosphate were all significant at $p < 0.05$ (Table 5.3).

Phytoplankton communities

One hundred and seventy-eight species belonging to 78 genera were recorded within the riverine-estuarine continuum. Diatoms were predominant with 81.9 % of the total abundance. The estuary sites had 98 species (55 genera) recorded whereas the river had 141 species (67 genera). The species richness was slightly higher in the estuarine than in the river (Table 5.2). The most dominant phytoplankton species are highlighted in Table 5.4. The highest species richness was observed in spring, ranging from 29 to 31 and 27 to 33 for the estuary and river, respectively. Species richness was lowest in autumn (range = 23 to 26) and winter (range = 17 to 25) for the estuarine and riverine sites. Using a Kruskal–Wallis analysis, species richness was found to differ significantly amongst seasons ($F = 6.43$, $p = 0.002$) but was not significantly different among sites ($F = 0.21$ $p = 0.652$). The diversity measures, Shannon-Weiner, Simpsons, evenness and Margalef were not significantly different ($p < 0.05$) among sites and seasons (Table 5.2).

The *n*-MDS ordination of phytoplankton communities indicated a seasonal trend for the estuarine sites although there was no clear trend for the riverine sites (Figure 5.1). From spring to winter, there was a separation for estuarine and riverine sites

along axis 2. This separation between the estuarine and riverine sites was very distinct throughout the four different seasons (Figure 5.1).

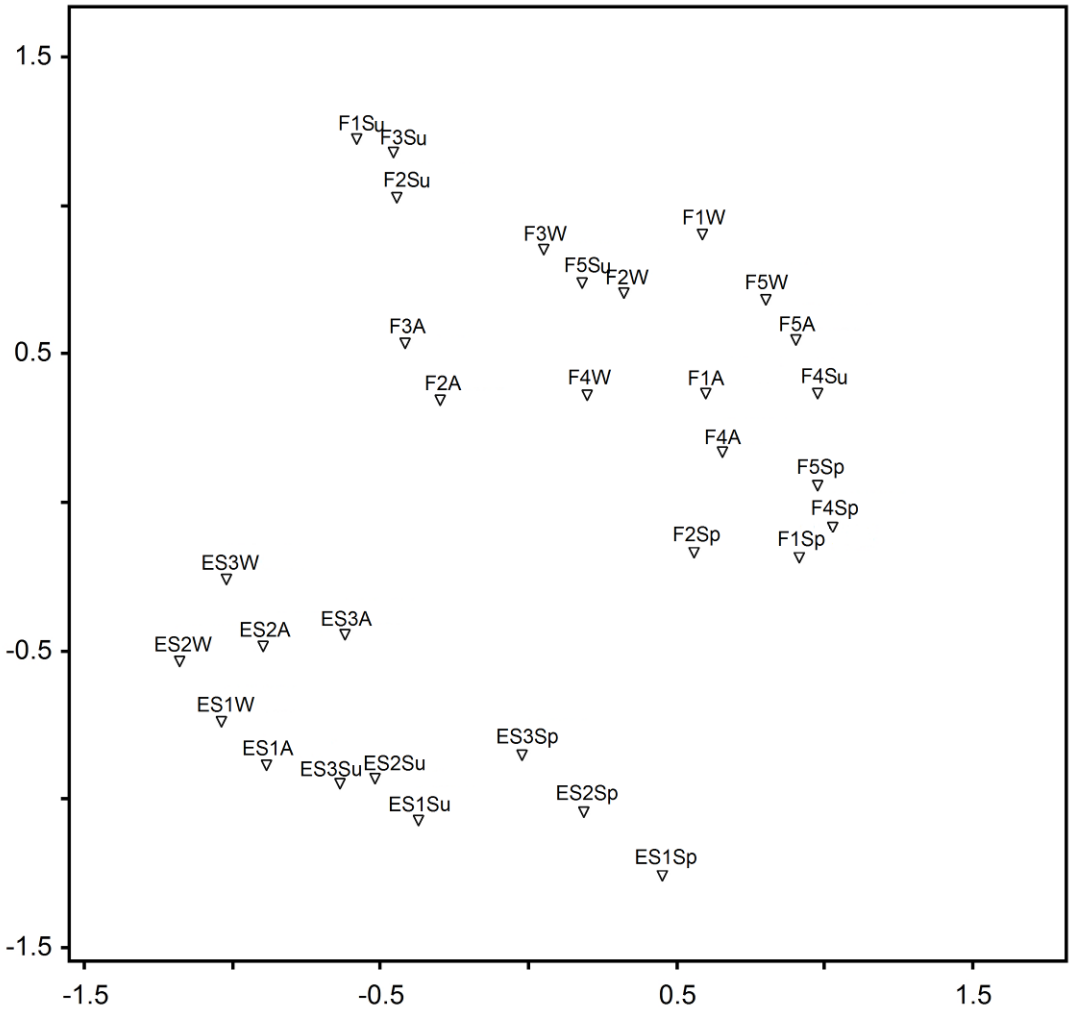

Figure 5.1. *n*-MDS ordination of phytoplankton community along the riverine-estuarine continuum (Kowie system) throughout the study. *Abbreviations: F1–F5 = riverine site 1–5 and ES1–ES3 = estuarine site 1–3, with the letters Sp., Su, A and W representing the study months spring, summer, autumn and winter*

Table 5.2. Mean (±standard error) and Kruskal–Wallis ANOVA summary of physico-chemical factors and diversity indices at different sites and season along the riverine-estuarine continuum (Kowie system). *F-values with significance levels in parentheses; significant differences at p < 0.05 are indicated in bold*. *Cond – conductivity, DO – dissolved oxygen, ORP – oxygen reduction potential, TDS – total dissolved solids*

Parameters	**Spring**		**Summer**		**Autumn**		**Winter**		**Kruskal–Wallis Analysis**	
	Estuarine	**Riverine**	**Estuarine**	**Riverine**	**Estuarine**	**Riverine**	**Estuarine**	**Riverine**	**Season (df = 3)**	**Site (df = 1)**
DO (mg L^{-1})	6.64±0.42	6.55±0.41	4.76±0.12	6.23±0.31	5.83±0.23	5.56±0.49	7.33±0.12	7.36±0.46	**7.08 (0.001)**	0.13 (0.721)
Cond (mS cm^{-1})	13.16±7.24	1.69±0.45	14.73±10.4	2.43±0.54	16.62±9.79	1.83±0.42	19.72±8.79	1.90±0.47	0.12 (0.949)	**20.80 (0.000)**
TDS (ppt)	8.68±4.88	2.63±1.49	9.86±6.94	2.07±0.15	11.11±6.51	1.23±0.28	14.50±7.42	1.24±0.31	0.08 (0.970)	**15.94 (0.000)**
Salinity (ppt)	7.24±4.39	0.85±0.23	8.51±6.34	1.50±0.11	9.55±6	0.91±0.2	13.05±7.27	0.88±0.23	0.20 (0.896)	**18.73 (0.000)**
Resistivity (Ω)	75.63±32.9	1928±1488.7	88.39±36.5	446.66±259	62.21±27.5	556.1±303	42.16±18.7	539.2±279	0.62 (0.606)	0.01 (0.927)
pH	8.04±0.01	7.48±0.38	6.96±0.28	7.81±0.35	7.51±0.13	7.1±0.35	8.06±0.01	7.78±0.32	1.42 (0.260)	2.43 (0.131)
Temperature (ºC)	19±0.42	15.7±0.61	25.1±0.40	21.76±0.71	25.17±0.96	22.28±1.39	13.23±0.47	13.96±0.45	**39.15 (0.000)**	1.42 (0.245)
ORP (mV)	-68.6±0.92	-29.83±19.89	22.27±29	-45.4±21.3	-85.07±8.43	-61.02±22.3	-79.87±0.7	-64.4±17.4	2.61 (0.073)	2.50 (0.126)
Depth (m)	3.56±0.78	0.68±0.10	4.66±0.98	0.92±0.19	3.56±0.77	0.77±0.18	3.37±0.82	0.76±0.2	0.48 (0.698)	**45.47 (0.000)**
Water flow (m s^{-1})	0.13±0.06	0.15±0.01	0.21±0.06	0.52±0.1	0.24±0.04	0.36±0.09	0.26±0.09	0.16±0.09	**3.72 (0.024)**	4.18 (0.051)
Ammonia (mg L^{-1})	0.43±0.31	0.10±0.05	0.29±0.05	0.15±0.05	0.55±0.09	0.26±0.06	0.63±0.12	0.10±0.05	0.99 (0.411)	**13.25 (0.001)**
Phosphate (mg L^{-1})	2.13±2.03	0.95±0.54	0.23±0.07	2.12±1.8	0.13±0.09	0.68±0.31	1.33±0.79	0.68±0.2	0.39 (0.761)	0.01 (0.910)
Nitrate (mg L^{-1})	0.00	0.5±0.31	3.67±1.86	10.58±7.3	13.33±5.54	3.18±0.91	2.97±0.97	5.2±3.21	1.25 (0.311)	0.14 (0.707)
Chl–*a* (mg m^{-3})	2.73±0.04	3.78±0.65	3.26±1.11	7.85±2.66	2.34±0.54	5.30±2.03	1.76±0.58	1.09±0.53	**3.68 (0.025)**	**9.97 (0.004)**
Sp. richness	30±0.58	31.25±1.55	29±1.53	26±0.89	24.67±0.88	24±1.79	27.67±2.6	22±1.34	**6.43 (0.002)**	0.21 (0.652)
Shannon-Wiener	2.61±0.07	2.68±0.12	2.36±0.15	2.34±0.1	1.95±0.57	2.73±0.07	2.23±0.29	2.66±0.05	0.75 (0.533)	1.09 (0.306)
Simpsons	0.88±0.01	0.89±0.02	0.81±0.04	0.85±0.01	0.64±0.16	0.91±0.01	0.76±0.08	0.91±0.01	0.63 (0.604)	2.35 (0.137)
Evenness	0.45±0.02	0.48±0.06	0.38±0.06	0.41±0.04	0.40±0.23	0.65±0.03	0.4±0.16	0.66±0.03	1.55 (0.226)	0.69 (0.415)
Margalef	4.54±0.09	4.88±0.14	4.78±0.21	4.26±0.19	4.47±0.52	4.25±0.22	4.63±0.22	4.03±0.2	1.35 (0.280)	1.71 (0.203)

Table 5.3. Pearson correlation coefficient between physico-chemical factors and diversity indices along a riverine-estuarine continuum. *The * and ** indicate significant level at p < 0.05 and p < 0.01, respectively. Abbreviations: COND – conductivity, DO – dissolved oxygen, WF – water flow, TEMP – temperature, PHOS – phosphate, AMM – ammonia, Chl-a – chlorophyll-a, SW – Shannon-Weiner, SIMP – Simpsons, Even – evenness and SR – Species richness*

	TDS	**Salinity**	**COND**	**DO**	**ORP**	**DEPTH**	**WF**	**TEMP**	**PHOS**	**AMM**	**PH**	**Chl-*a***	**SW**	**SIMP**	**Even**	**SR**
TDS																
Salinity	**0.989****															
COND	**0.984****	**0.990****														
DO	0.035	0.031	0.014													
ORP	-0.174	-0.147	-0.164	-0.224												
Depth	**0.827****	**0.834****	**0.868****	-0.168	-0.224											
WF	-0.133	-0.114	-0.137	-0.078	-0.163	-0.187										
Temp	0.059	0.055	0.091	**-0.663**	0.043	0.292	**0.439***									
PHOS	0.161	0.174	0.166	0.154	-0.193	0.085	0.132	-0.019								
AMM	**0.541****	**0.525****	**0.554****	0.122	-0.292	**0.570****	-0.185	0.118	0.284							
PH	0.178	0.133	0.150	0.266	**-0.807****	0.083	0.164	-0.139	0.286	0.284						
Chl-*a*	-0.179	-0.193	-0.187	-0.147	-0.210	-0.150	**0.571***	**0.364***	**0.407***	-0.117	0.314					
SW	0.120	0.143	0.117	-0.039	0.088	-0.093	-0.053	-0.237	0.019	**-0.451***	-0.290	-0.029				
SIMP	0.057	0.078	0.044	0.035	0.142	-0.183	-0.018	-0.277	0.081	**-0.519****	-0.080	0.058	**0.958****			
Even	0.12	0.168	0.13	0.116	-0.006	-0.151	-0.048	-0.248	-0.09	-0.355	-0.090	-0.159	**0.856****	**0.814****		
SR	0.066	0.006	0.043	-0.309	0.075	0.230	-0.078	0.09	0.128	0.062	0.225	0.171	0.013	-0.073	**-0.463****	
Margalef	0.350	0.312	0.348	-0.282	0.065	**0.397***	0.020	0.119	-0.032	0.047	0.116	0.035	0.354	0.190	0.007	**0.747****

Table 5.4. Dominant phytoplankton species relative percentage abundances collected in four different seasons along the riverine-estuarine continuum (Kowie system)

Species	Spring	Summer	Autumn	Winter
Estuary				
Diploneis vacillans		3.3		
Entomoneis sp.		4.1	3.3	
Gyrosigma attenuatum	3.9			
Kirchneriella sp. 2				4.5
Melosira dubia	6.1			
Nitzschia sp. 1			3.2	
Nitzschia reversa	4.7			
Pleurosigma elongatum	16.0	39.8	2.6	
Pleurosigma salinarum	5.4	10.7	64.3	47.6
Phacus sp.				7.2
Tabularia fasciculata				3.1
Trachelomonas hispida				5.4
Staurosira elliptica	3.1	5.4		
Surerilla brebissonii	12.5			
Surerilla ulva	8.0			
Surirella ovalis	0.0	4.7		
River				
Achnanthidium exiguum	3.1			
Cyclotella meneghiniana		10.3	4.8	3.0
Cyclotella ocellata		5.8		
Cymbella kappii			4.4	
Fragilaria biceps	18.9	5.5	9.5	4.4
Fragilaria nanana	5.0			
Fragilaria tenera	3.1		2.2	
Fragilaria ulna	13.2	2.6	4.5	2.1
Fragilaria ulna var. *acus*	8.6	9.4	14.9	16.0
Gyrosigma acuminatum			4.4	
Gomphonema venusta	3.7			
Kirchneriella sp. 1		6.9	2.6	3.7
Monoraphidium sp.		17.8		3.6
Navicula cryptocephala				8.9
Navicula cryptotenella				8.3
Nitzschia draveilensis				3.6

Perinidium lomnicki			4.5	2.3
Gyrosigma scalproides	4.1		7.9	4.6
Scenedesmus communis		5.6		
Staurosira elliptica		8.5	3.0	

Chlorophyll-*a* concentration

The chl-*a* concentration at the nine study sites along the estuarine-riverine continuum increased from spring to a high in summer (river mean = 7.9 mg m^{-3} and estuary mean = 3.3 mg m^{-3}) before decreasing to a low in winter (Figure 5.2). Total chl-*a* concentration ranged from 1.1 to 7.9 mg m^{-3} (river mean 4.5 mg m^{-3}) and 1.8 to 3.3 mg m^{-3} (estuary mean 2.5 mg m^{-3}; Table 5.2). Kruskal–Wallis ANOVA analysis showed significant differences among chl-*a* concentration and season ($F = 3.68$, $p = 0.025$) and sites ($F = 9.97$, $p = 0.004$; Table 5.2). Pearson rank correlation analysis showed that chl-*a* concentration was significantly correlated with water flow, temperature and phosphate ($p > 0.05$; Table 5.3). Throughout the study, total phytoplankton biomass was dominated by nanophytoplankton which comprised of mean 51 % and 60 % of the total for riverine and estuarine sites, respectively. The concentration of nanophytoplankton ranged from 0.7 to 4.7 mg m^{-3} (river) and 1.0 to 2.3 mg m^{-3} (estuary). Picophytoplankton biomass ranged between 0.3 and 2.4 mg m^{-3} (river) and 0.05 and 0.7 mg m^{-3} (estuary; Figures 3). Microphytoplankton contribution to total biomass was less than mean < 32 % of the total for both the estuarine and riverine sites (Figure 5.2).

The measure of phytoplankton standing crop included in this study was chl-*a* concentration due to its widespread use in the literature. Comparisons of chl-*a* concentration at eight sites illustrate differences in spatial and temporal patterns of chl-*a* concentration in phytoplankton standing crop within the four seasons (Figure 5.3). The inter-seasonal changes in chl-*a* concentration reflect that phytoplankton standing crop was high during the summer and autumn. Spatial differences in the chl-*a* concentration were also observed over the riverine-estuarine continuum. Low chl-*a* concentration for

the phytoplankton standing crop in spring and winter was recorded (Figure 5.3), while the middle section had a high chl-*a* concentration (Figure 5.3).

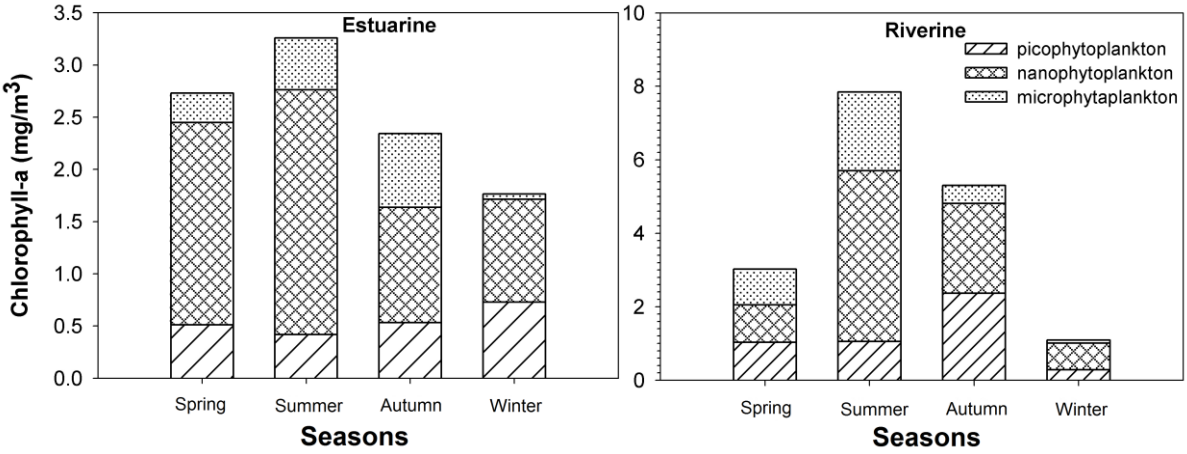

Figure 5.2. Size fractionated chlorophyll-*a* concentration among the four seasons in the Kowie system in 2012 to 2013

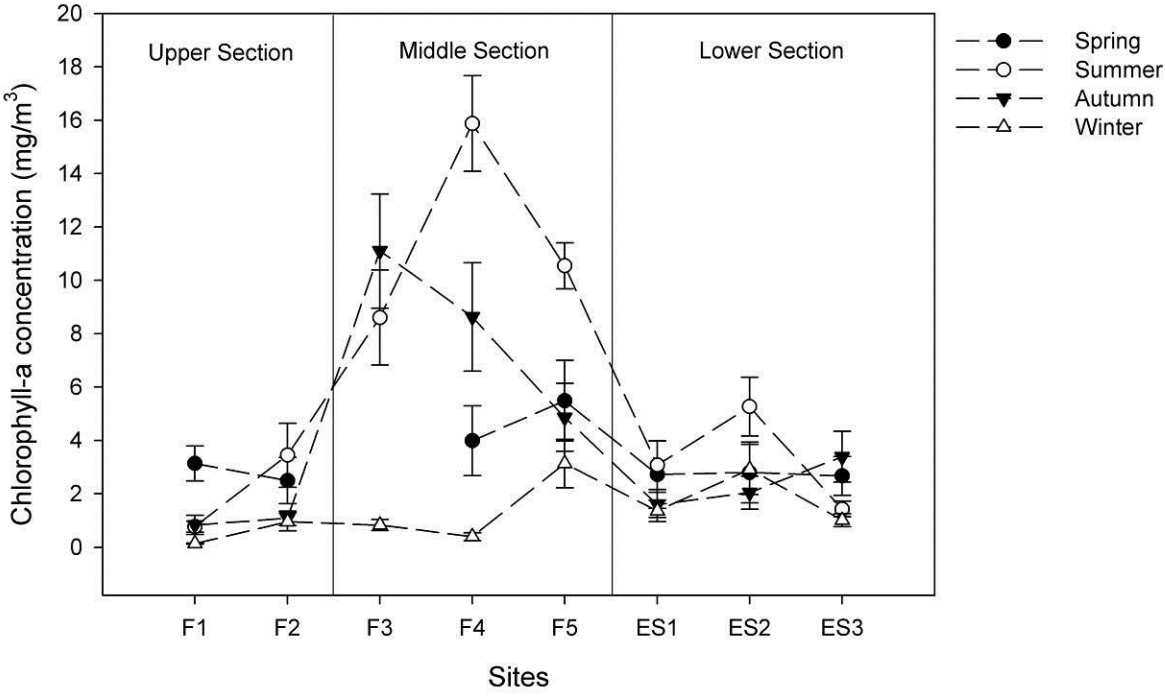

Figure 5.3. Chlorophyll-*a* concentration (± standard error) along the riverine-estuarine continuum in the Kowie system in four seasons

Phytoplankton community structuring in relation to environmental variables

The relationship between measured phytoplankton communities and environmental variables along the riverine-estuarine continuum was explored using RDA. The analysis identified five factors that were significant ($p < 0.05$) in affecting phytoplankton variation being major nutrients (nitrates – F = 1.97, $p = 0.006$ and ammonia – F = 1.49, $p = 0.044$), chemical factor (salinity – F = 1.77, $p = 0.012$) and hydrological variables (water depth – F = 4.79, $p = 0.002$, aerial cover – F = 5.21, $p = 0.011$; Figure 5). The first two axes (1 and 2) accounted for 48.9 % and 26.8 % of the species-environment and species (phytoplankton) variation, respectively. The species–environment correlations were 0.964 (eigenvalue = 0.176) for Axis 1 and 0.892 (eigenvalue = 0.092) for Axis 2. The estuarine sites were mostly structured by salinity, ammonia and depth which are very important for phytoplankton species (Figure 5.5) and this was reflected by the species that were present in the estuarine sites (Figure 5.4).

Discussion

Phytoplankton communities

The study of phytoplankton communities along a riverine-estuarine continuum indicated two main groups the estuarine and riverine plankton communities both dominated by diatoms (Figure 5.4; Table 5.4). The dominance of diatoms in the Kowie River system was similar to studies by Taylor et al. (2007a, b), Salmaso and Zignin (2010), Wu et al. (2011) and Fonge et al. (2013) who found that diatoms dominated the phytoplankton group. *Pleurosigma salinarum* was found throughout the four seasons (Table 5.4) in the estuary. *Pleurosigma salinarum* is predominantly found in brackish and saline inland waters (Taylor et al., 2007a), thus its abundance was expected to increase as the water became more saline as it is more saline tolerant. Another similar species, *P. elongatum* was very dominant from spring to autumn with the highest abundances begin in summer. This species was mostly dominant in the estuary closer to the ocean in highly saline waters (brackish) and its abundance decreased with an increase in *P. salinarum*. Each study season had species that were unique to it (Table 5.4). The river was mostly

dominated by *Fragilaria, Cyclotella, Gyrosigma, Navicula* and *Kirchneriella* genera (Table 5.4). Most of these species were only found within the river section. *Fragilaria biceps, F. ulna* and *F ulna* var*. acus* where found throughout the study and were mostly dominant in the upstream sites where water velocity was low. The dominance of these *Fragilaria* species upstream was due to their large size and at high water velocity they are prone to being washed away (Song, 2007).

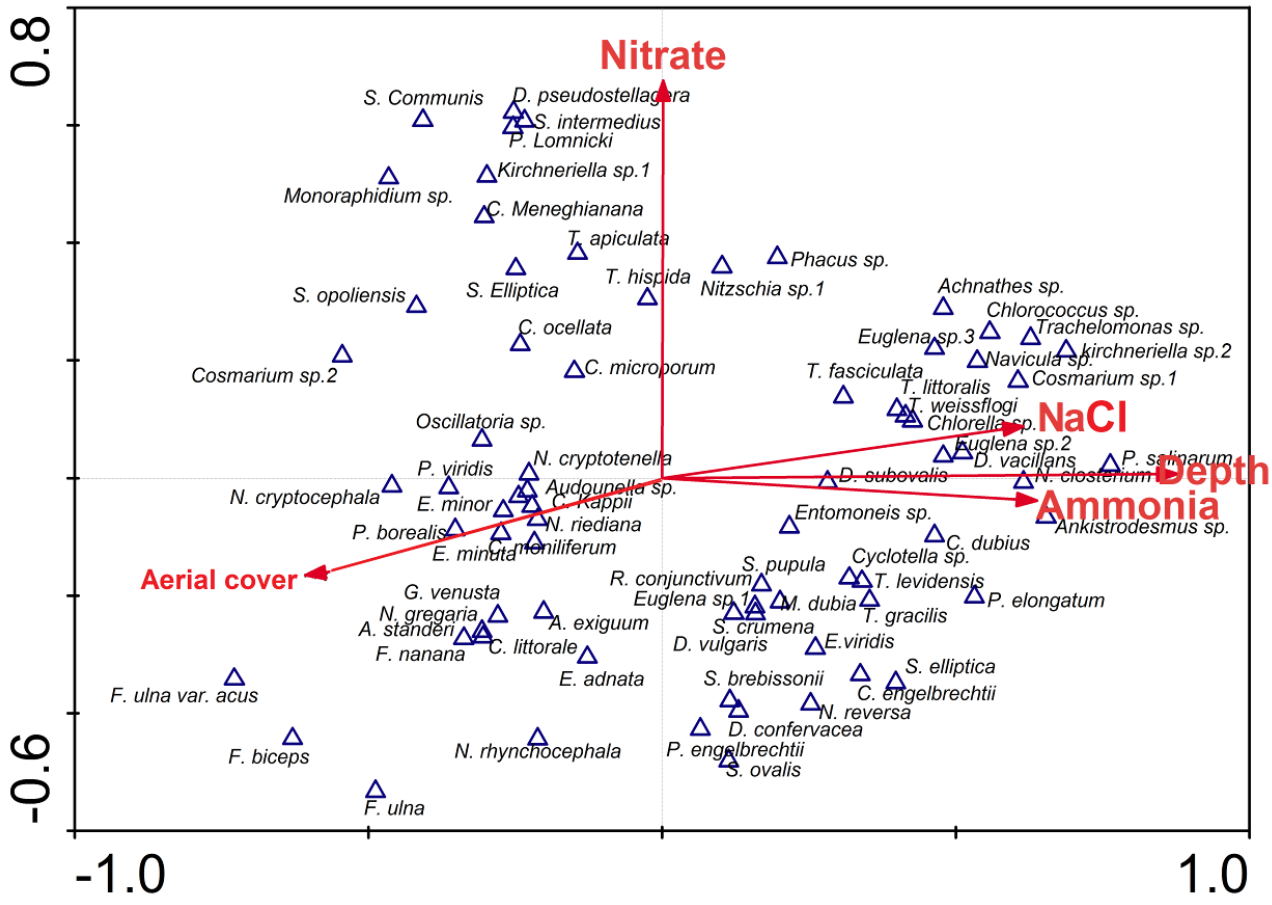

Figure 5.4. RDA ordination bioplots of the phytoplankton species and associated significant physico-chemical factors collected along the riverine-estuarine continuum (Kowie system) throughout the study

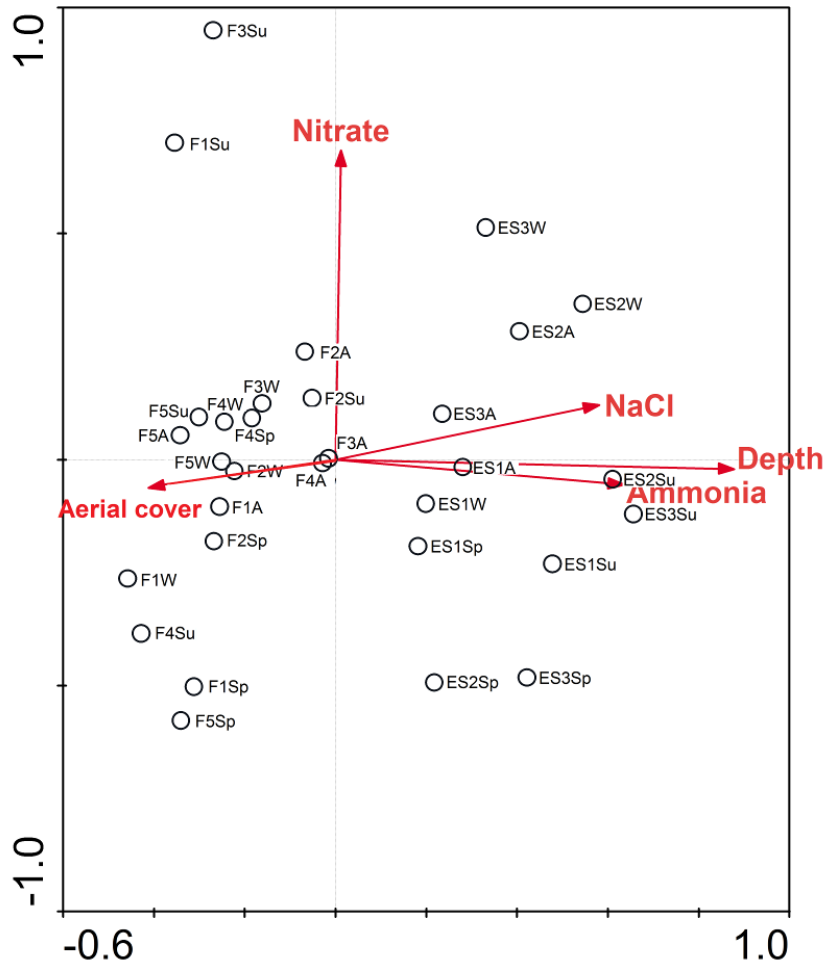

Figure 5.5. RDA ordination of the sampling sites and the significant physico-chemical factors of the phytoplankton samples collected along the riverine-estuarine continuum (Kowie system) throughout the study. *Abbreviations are highlighted in Figure 5.2.*

The total phytoplankton biomass based on chl–*a* concentration recorded during study had a river and estuary mean of 4.5 mg m^{-3} and 2.5 mg m^{-3}. The estuary mean chl–*a* concentration was found to be within the mean range observed in an early study of the Kowie Estuary convergence zone of mean 1.8 to 2.2 mg m^{-3} (Kruger and Strydom 2011). These chl-*a* concentration recorded in the Kowie Estuary were within similar ranges to concentrations recorded within the region such as the Kariega Estuary (Froneman and McQuaid, 1997; Froneman, 2000; Froneman 2002a), Nyara Estuary (Walker et al., 2001) and Kasouga Estuary (Froneman, 2002a, b). Even though the chl-

a concentrations were similar to other studies, they were substantially lower than those obtained in the freshwater dominated estuaries such as the Sundays (~29 mg m^{-3}) and Fish Rivers (~210 mg m^{-3}; Adams et al., 1999). High chl-*a* concentrations were observed in the middle estuary, with a high of 5.27 mg m^{-3} in autumn, could have been as result of a more stable water column and increased nutrient availability. The high salinity levels observed in the Kowie Estuary mouth might have resulted in low chl-*a* concentrations recorded. Adams *et al.* (1999) showed that when freshwater input is low and salinity is high, this result in limited nutrient loading which in turn lowers chl-*a* concentration. The highlighted scenario might explain the low chl-*a* concentrations observed in the Kowie Estuary.

In the riverine sites, the chl-*a* concentration increased from upstream to downstream and the concentrations were higher in the river than those observed in the estuary (Figure 5.2). The high river chl-*a* concentration could be a result of low nutrient levels and changes in physico-chemical factors concentrations (Table 5.2). In winter, the chl–*a* concentrations were very low (<1 mg m^{-3}) resulting in low phytoplankton abundances in the river section and this? could be attributed to low water temperatures (seasonal variation $F = 39.15$, $p = 0.000$) that lower phytoplankton growth and a change in water stability as a result of changes in salinity, conductivity, ammonia, water depth and TDS concentrations.

Phytoplankton community structuring in relation to environmental variables

Results of the RDA indicated the phytoplankton assemblage significantly correlated with hydrological factors (water depth) and physico-chemical factors (ammonia, salinity and nitrates) were important in structuring phytoplankton community in the Kowie system (Figures 5.4 and 5.5). This study highlighted the importance of above factors in controlling the structure of estuarine phytoplankton community. RDA analyses clearly distinguished samples from estuarine and riverine habitats (Figure 5.4 and 5.5). The four RDA axes explained only 35.7 % of the species data variance and the sum of all

canonical eigenvalues ratio was 0.547, suggesting four physico-chemical and hydrological factors have an important influence on phytoplankton community characteristics. Ammonia, salinity and water depth were very important in structuring estuarine communities. Species richness and abundance decreased with increase in salinity and high species numbers were recorded in spring for the estuary and river. The decrease in the number of phytoplankton species with increased salinity might have been due to the fact that most freshwater species do not tolerate high salinity concentrations (Fonge et al., 2013). *Campylodiscus clypeus, Cyclotella ocellata, Entomoneis* sp.*, Surirella brebissonii, S. ovalis, Staurosira elliptica, F. ulna,* and *F. ulna* var*. acus* were the only species that could tolerate both estuarine and riverine habitats.

Of the dissolved substances that possibly influence phytoplankton diversity, ammonia and nitrates appear to be of particular importance for the estuary (Table 5.2, Figures 5.4 and 5.5). Inorganic nitrates and ammonia limit phytoplankton growth (Claquin et al., 2006; Wu et al., 2011; Fonge et al., 2013), and their ecological significance in rivers and streams has been well documented (Pieterse and van Zyl, 1988; Palmer and O'Keeffe, 1990; Claquin et al., 2006; Taylor et al., 2007a, b; Wu et al*.,* 2011; Fonge et al., 2013). Ammonia concentration was positively correlated ($p < 0.01$) with TDS, salinity, conductivity and water depth. Phytoplankton abundance and production are controlled by discharge, which is related to residence time, channel depth, and dilution rate and affects water transparency and sedimentation (Wehr and Descy, 1998) thereby affecting phytoplankton biomass.

The Kowie system, chl–*a* concentration and the Riverine Continuum Concept

The phytoplankton standing crop in terms of chl–*a* concentrations and abundances in the Kowie system do conform to the general principles of the RCC that phytoplankton will main primary producer in the middle reaches, as originally defined by Vannote et al. (1980). In the upper reaches, sites F1 and F2 of the Kowie system were narrow and lined by thick riparian vegetation which prevented the light penetration thereby

minimizing the rate of primary production. The upper reaches had low phytoplankton abundances, chl-*a* concentration and macrophyte diversity and abundance (Tables 5.1 and 5.4), which resulted in low autochthonous production within the system. Most of the organic matter that made its way into the system was from riparian vegetation that fell in. Moving to the middle reaches to sites F3 to F5, the river became wide and deep. The substratum was dominated by rocks which played an important role as a supplier of organic material e.g. periphyton. The middle reaches of the Kowie system showed that the greatest phytoplankton biodiversity and chl–*a* concentration within the system increased significantly (Figure 5.3) as well as macrophyte composition and abundances (Table 5.1). Allochthonous organic material still played an important role in the upper reaches were shredders were dominant (S. Moyo, *unpublished data*) although it was not as significant as autochthonous production. More light could penetrate the system as the riparian vegetation cover had reduced (Table 5.1), thereby promoting more primary production within the system. In the lower reaches in sites E1 to E3, there is a large change in particulate material and also a decrease in chl–*a* concentration which affected autochthonous production. Phytoplankton abundance and diversity generally declined in the downward direction, indicating that primary production is frequently outweighed by sedimentation, respiration, senescence, grazing and washout (Phlips et al., 2000). The decrease in phytoplankton abundance and diversity resulted in a decrease in chl-*a* concentration mainly due to increase in total dissolved solids, salinity (Table 5.1) and factors highlighted above. Even though the channel width had increased, light penetration that increases autochthonous production was hampered by the high amount of total dissolved solids. The macrophyte abundance was slightly lower than that of the middle reaches but its effect on autochthonous production was reduced due to tidal action which left them exposed. Further research will aim at measuring particulate organic matter, primary production and respiration within the Kowie system so that we can further test the RCC model.

CHAPTER 6

USE OF MULTIMETRIC AND STABLE ISOTOPE ANALYSIS TO ASSESS THE SPATIAL AND TEMPORAL VARIATION OF PERIPHYTON COMMUNITIES ALONG A SMALL TEMPERATE RIVER SYSTEM

Plate 6. View of the Kowie River channel at site F1 showing the rocky substratum and a few marginal macrophytes, *Cyperus* sp. Photo by Tatenda Dalu

Abstract

Periphyton communities and stable isotope values have been identified as potential indicators of anthropogenic nitrogen inputs to aquatic ecosystems; an increasingly important challenge in aquatic systems. The aim of the study was to assess the spatio-temporal periphyton taxa and stable isotope variation in order to identify potential anthropogenic nitrogen pollution sources in Kowie River, South Africa. Periphyton was collected once a season from five sites between September 2012 and May 2013. Diversity indices, multimetric and stable isotope analyses were employed for describing the spatial and temporal dynamics of periphyton community assemblages. Water depth, salinity, resistivity, oxygen reduction potential, nitrates, velocity and conductivity were the major factors affecting periphyton distribution. Eighty-seven periphyton taxa belonging to 43 genera were recorded in throughout the study sites and periods. Periphyton species richness, abundances and stable isotopes differed significantly among sites but not among seasons. The lower sections of the Kowie River were polluted by anthropogenic sources as indicated by the high periphytic isotopic nitrogen values (7.9–15.2‰) compared to the pristine upstream sites. Overall, our results reveal general patterns of periphyton communities and stable isotopes and provide improved information in the use of periphyton $\delta^{15}N$ as an excellent indicator of anthropogenic nitrogen pollution. But, periphyton was not identified as indicators of the relative importance of individual nitrogen sources and periphyton $\delta^{13}C$ in isotopic mixing models in lotic food web studies. A combination of the study of periphyton communities and stable isotopes is recommended to achieve the best results in environmental monitoring and food web studies.

This chapter is currently under review as:

Dalu T, Bere T, Richoux NB, Froneman PW. Use of diversity indices, multimetric and stable isotope analysis to assess the spatial and temporal variation of periphyton communities along a small temperate river system. *South African Journal of Botany*.

Introduction

Periphyton consists mainly of algae, bacteria, protozoa and fungi, found on rock substratum in all lotic systems, with algae making up the largest proportion (Ewart–Smith and King, 2012). Periphyton plays an important role in aquatic ecosystems as a main source of food for higher trophic organisms (Azim et al., 2005). Periphyton community assessments can therefore provide information on the trophic structure and ecological status of a system. They are also widely used for monitoring purposes because of their value as indicators of acidification, eutrophication and organic pollution (Azim et al., 2005; Morin et al., 2010).

Intensive research has been devoted to the understanding of periphyton communities in relation to biotic and abiotic factors (Suren et al., 2003; Uehlinger et al., 2003; Larned, 2010; Ewart-Smith and King, 2012; Tan et al*.,* 2014). A complex interplay of physico-chemical and biological factors such as velocity, temperature and nutrients regulates periphyton communities (Vis et al., 1998; Chételat et al., 1999; Passy et al*.,* 1999). Water chemistry, current velocity, temperature and grazing influence periphyton community distribution, and anthropogenic factors e.g. agricultural runoff, sewage discharge, impact periphyton communities (Chételat et al*.,* 1999; Passy et al., 1999; Ferragut et al*.,* 2010; Larned, 2010; Bere and Tundisi, 2011a). Of the multitude of physico-chemical factors that are known to influence periphyton in rivers and streams, flood disturbances and nutrients are considered the primary drivers of periphyton community structure and biomass (Biggs and Thomsen, 1995; Jowett and Biggs, 1997; Murdock et al*.,* 2004).

Periphyton is particularly responsive to water flow and nutrient concentration changes (Azim et al., 2005), thus periphyton communities are suited to environmental change monitoring. Thus, an understanding of how periphyton communities respond to environmental changes will greatly improve our ability to efficiently manage water resources. Regardless of the great number of freshwater studies on algae, there is still no comprehensive investigation on periphyton communities in temperate African rivers.

Despite the important potential realized in using periphyton to predict and assess ecosystem integrity in other countries (Biggs and Thomsen, 1995; Vis et al., 1998; Biggs and Kilriy, 2000; Suren et al., 2003; Finlay, 2011), very little research on periphyton has been undertaken in African waters. In many countries, for instance, periphyton is currently not recognized as a tool in environmental monitoring programs.

Recent studies have indicated that periphyton stable nitrogen isotopes ($\delta^{15}N$) can be a useful tool in tracing nitrogen sources in river systems (Anderson and Cabana, 2005, 2006; Vander Zanden et al., 2001; Diebel et al., 2009; Ning et al., 2013). Dissolved inorganic nitrogen sources to lotic aquatic ecosystems tend to have distinct isotopic values (Anderson and Cabana, 2005, 2006; Vander Zanden et al., 2001). Different anthropogenic nitrogen sources have different initial isotope values, thus allowing their identification from $\delta^{15}N$ values observed in inorganic nitrogen fractions, algae, plants and finally the consumers. For example, human wastewater and livestock waste nitrogen have high $\delta^{15}N$ values of >10 ‰, while synthetic fertilizers typically have low $\delta^{15}N$ of -3 to 3 ‰ (Vander Zanden et al., 2001; Anderson and Cabana, 2005, 2006). Biogeochemical transformations of nitrogen such as denitrification and ammonia volatilization can result in substantial isotopic fractionation. When periphyton or other plants in aquatic ecosystems take up dissolved organic nitrogen, their tissues will have an isotope reflective of nitrogen source (Lake et al., 2001). Stable carbon isotopes ($\delta^{13}C$) are widely used to discriminate periphytic from terrestrial production in lotic ecosystems (Finlay et al., 1999; Ishikawa et al., 2012). While the use of $\delta^{13}C$ has many advantages as a natural tracer, growing awareness of the large spatial and temporal variability in $\delta^{13}C$ values of lotic periphyton suggest an intrinsic limitation to $\delta^{13}C$ applications under specific circumstances (Ishikawa et al., 2012). The large variability in periphyton $\delta^{13}C$ values can introduce doubt in the determinations of the isotopic baseline of food webs and therefore influences food web analyses accuracy (Finlay et al., 1999; Ishikawa et al., 2012).

The aim of this study was to fill these research gaps and enhance our knowledge on periphyton distribution, communities and as potential indicators of anthropogenic impacts and further aim to relate the communities to physico-chemical variables. We further explored whether any community and biomass changes supported the principles of the RCC e.g. how the periphyton communities relate to changes in stream characteristics and impacts (Vannote et al., 1980) and how record floods in 2012 impacted the periphyton communities. We also investigated the spatial and temporal characteristics of periphytic $\delta^{15}N$ and $\delta^{13}C$ values along the river and related the stable nitrogen signatures to potential sources of nitrogen pollution along the river system so as to identify potential sources of pollution. Due to inadequate or unavailable data necessary for sound and informed decision on water and environmental monitoring in Africa, we hypothesized that periphyton communities and stable isotope values are complementary indicators of anthropogenic nitrogen inputs to aquatic ecosystems.

Materials and Methods

Study area

A detailed description of the study area is provided for in Chapter 2 and a detailed description of the flood events are highlighted in Chapter 3. The study was conducted on 10–14 September 2012 (spring), 26–30 November 2012 (summer), 20–24 February 2013 (autumn) and 25–29 May 2013 (winter). Periphyton samples were collected along the Kowie River at five different sites: 2 sites (F1 and F2) in the relatively un-impacted forested headwaters to act as reference sites and 3 sites (F3, F4 and F5) are polluted due to the Bloukrans River which drains Grahamstown (population 125000) and intensive farmlands along the Belmont Valley area (Figure 2.1). The sampling sites were chosen so as to obtain a pollution gradient from relatively unpolluted headwaters to polluted downstream sites and also highlight a river continuum gradient. No samples were collected in spring for site F3 due to accessibility problems.

Physico-chemical factors

A detailed description of physico–chemical sampling and analysis is provided in Chapter 5.

Periphyton sampling and stable isotope analysis

At each site, epilithon was sampled by brushing stones/rocks with a toothbrush. Prior to sampling, all rock substrata were gently shaken in stream water to remove any loosely attached sediments and non-periphytic algae. At least ten pebble (> 2–64 mm) to cobble (> 64–256 mm) sized rocks/stones were randomly collected at each sampling site and the top surface brushed. The periphyton for microscopic examination were preserved in Lugol's iodine, while another six samples per site collected as above were placed on ice for biomass and stable isotope analysis.

In the laboratory, periphyton biomass measurements and analysis were performed according to Bahls (1993). Periphyton samples preserved in Lugol's iodine were set aside for sedimentation for approximately two days before identification according to Dalu et al. (2014b). The sample was concentrated by decanting the supernatant, taking care not to disturb the sedimented periphyton material at the bottom. The remaining precipitate was then vigorously mixed and about 5–10 mL was transferred into a petri dish with grid marking for identification and scoring under an inverted microscope (Nikon TMS) at 1000×. The diatom component of the periphyton were identified and quantified after cleaning with hot hydrochloric acid (HCl) and potassium permanganate ($KMnO_4$) method (Taylor et al., 2007). The periphyton was then identified and counted to the species level using a phase-contrast light microscope (Olympus CX) at 1000×. All periphytic taxa were identified to genus/species level according to the classifications of Biggs and Kilriy (2000), John et al. (2002) and Taylor et al. (2007).

Samples intended for stable isotope analysis had all visible non-periphytic particles manually removed and samples were then filtered through a 63 µm mesh to remove any

leftover material and zooplankton, followed by filtration onto pre-combusted and pre-weighed Whatman glass-fibre filters (pore size 0.7 µm). The samples were placed in pre-ignited foil envelopes and freeze dried for at least 24 hours using a VirTis benchtop K, model 2KBTES-35 (SP Industries, USA). Freeze-dried material was scraped off the filters and ground to a fine, homogeneous powder using mortar and pestle. A detailed description of acidification process and stable isotope analysis is provided in Chapter 4.

Data analysis

Differences in means of physico-chemical variables among the five study sites and four seasons were compared using Kruskal-Wallis test in SPSS 16.0 for Windows software (SPSS Inc., 2007). Diversity indices (Shannon-Wiener, Simpsons and Margalef) for the periphyton dataset were calculated using PAST version 3.01 (Hammer et al. 2001). Periphyton taxa were included in the canonical correspondence analysis (CCA) only when they contributed >0.5 % relative abundance to reduce data skewness and eliminate the effects of rare species (Wu et al. 2011). Periphyton density values were log (x+1) transformed to stabilise the variance (Dalu et al., 2013).

To determine whether to use linear or unimodal methods for the analysis, detrended canonical correspondence analysis (DCA) was employed. The gradient lengths were examined and since the longest gradients were greater than 4, a unimodal model, CCA was selected (Leps and Šmilauer, 2003). CCA was performed on the periphyton datasets to examine the relationships between diatom taxa composition and selected physico-chemical variables using 999 Monte Carlo permutations at $p < 0.05$ significance level in Canoco (version 4.5) software (ter Braak, 2002). Preliminary CCAs were used to identify collinear variables and a subset was selected on inspection of variance inflation factors that were less than 20 (ter Braak & Šmilauer, 2002). Exploratory variables were subjected to step wise forward selection procedure in CANOCO using 999 unrestricted Monte Carlo permutations so as to identify statistical significance ($p < 0.05$) variables.

The strength of the relationship between periphyton communities and physico-chemical variables in the CCA were calculated using the first and second eigenvalues ratios (λ_1/λ_2), which measure the strength of the constraining variables with respect to the first unconstrained gradient in the community data (Bere and Tundisi, 2011b). Pearson correlations of periphyton biomass with physico-chemical variables were carried out using SPSS 16.0 for Windows software (SPSS Inc., 2007) to test if the physico-chemical factors were related to periphyton biomass.

The Indicator Species Analysis method (IndVal) method (Dufrêne and Legendre, 1997) was used to identify periphyton indicator species between the five sites and four seasons using all species (87) identified. The IndVal method combines species' relative abundance and relative frequency of occurrence in the different samples. Indicator species are defined as the most representative species for each site/season, found mostly in a single site/season and never occur in abundance in other groups. The indicator value ranges from 0 % i.e. no indication or same occurrence and abundances in all sites or seasons to 100 % that is perfect indication or just confined to one group of substrate. The significance of each taxa was tested using Monte Carlo test with 999 permutations in PC-ORD version 5.10 (McCune and Mefford, 2006) and species which had significant ($p < 0.05$) indicator values were considered as indicator species for the study sites and/or seasons.

A two-way ANOVA with Tukey post-hoc tests was used to test for significant differences in stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of periphyton among the five study sites and four seasons. Correlation among physico-chemical variables with $\delta^{13}C$ and $\delta^{15}N$ values was also carried out to assess how the physico-chemical variables are related to stable isotope variables. All statistical tests were performed using SPSS 16.0 for Windows (SPSS Inc., 2007).

Results

Physico-chemical variables

Water quality tended to deteriorate from upstream to downstream as the river passed livestock and citrus farms and where the polluted (agricultural, sewage and domestic effluent) Bloukrans River joined the Kowie (Table 6.1). Nitrate concentrations for site F3 were generally high, as the site was below the Bloukrans River mouth. The pH was acidic to neutral at upstream sites (6.77±0.37 to 6.93±0.17) and alkaline at downstream sites (7.76±0.25 to 8.22±0.15). Temperature, conductivity, total dissolved solids (TDS), stream width and canopy cover increased from upstream to downstream. The mean concentration of ammonia was constant over the study area, and phosphates fluctuated among the study sites. The concentrations of nitrates in the water samples increased from upstream site F1 to site F3 before decreasing (Table 6.1). The mean channel width, water depth and riparian zone terrestrial cover increased from upstream site F1 to the downstream site F5 (Table 6.1). Spatial variation was a significant ($p < 0.05$) factor in affecting physico-chemical factors among the study sites compared with temporal variation ($p > 0.05$). Conductivity, salinity, pH, oxygen reduction potential (ORP), canopy cover and water depth differed significantly ($p < 0.05$) among the study sites. Temperature and current velocity were significantly different ($p < 0.05$) across seasons.

Periphyton communities

Eighty-seven periphyton taxa belonging to 43 genera were recorded in all samples collected. Among the 87 taxa observed, 66 taxa were considered as frequent in the study area with a >1 % relative abundance (Table 6.2). IndVal analysis showed that common periphyton species were mostly restricted to a single site or season (Table 6.3). No significant ($p > 0.05$) indicator species were identified for autumn, winter and site F1 and F4.

Table 6.1. The minimum (min), maximum (max) and mean (± standard error (SE)) values of physico-chemical factors measured at different sites during the four seasons. * *No samples were collected during spring, DO – dissolved oxygen, TDS – total dissolved solids, ORP – oxygen reduction potential*

Parameters	**F1**			**F2**			**F3***			**F4**			**F5**		
	Min	**Max**	**Mean±SE**	**Min**	**Max**	**Mean±SE**	**Min**	**Max**	**Mean±SE**	**Min**	**Max**	**Mean±SE**	**Min**	**Max**	**Mean±SE**
DO (mg L^{-1})	5.7	8.99	7.27±0.55	4.21	6.34	5.43±0.47	5.05	8.25	6.80±0.94	5.7	7.99	6.21±0.19	5.17	7.34	6.48±0.46
Conductivity (mS cm^{-1})	0.31	2.4	0.86±0.52	0.37	1.94	1.28±0.33	1.81	3.38	2.43±0.48	2.4	3.18	2.65±0.18	2.54	3.1	2.79±0.12
TDS (ppt)	0.21	1.61	0.57±0.35	0.92	2.46	1.40±0.36	1.21	2.27	1.61±0.33	1.61	2.13	1.76±0.12	1.83	2.71	2.36±1.45
Salinity (ppt)	0.14	1.14	0.40±0.25	0.64	1.77	1.03±0.26	0.92	1.65	1.17±0.24	1.14	1.56	1.29±0.1	1.23	1.51	1.36±0.06
Resistivity (Ω)	226.6	7080	2666.5±1515	282.7	1482	635.7±283.2	161.1	302.7	243.3±42.5	170.9	221.6	172.7±51.1	178.2	218.5	198.1±8.3
pH	5.77	7.35	6.77±0.37	6.5	7.31	6.93±0.17	7.5	8.27	7.76±0.25	7.35	8.85	8.1±0.33	7.81	8.53	8.22±0.15
Temperature (ºC)	12.7	23.2	16.95±2.34	13.3	21.3	17.38±1.85	14.2	23	19.9±2.84	14.3	23.7	19.08±2.33	15.3	26.2	19.9±2.36
ORP	-33	20.8	-6.23±10.99	-49.8	15.8	-17.5±16.1	-91.2	-11.3	-62.2±25.5	-108.7	-40.7	-83.15±14.8	-104.4	-78.1	-89.9±5.43
Water depth (m)	0.18	0.69	0.36±0.12	0.5	0.81	0.68±0.06	1.2	1.45	1.35±0.08	0.8	1.2	1.01±0.09	0.37	0.9	0.67±0.11
Velocity (m s^{-1})	0.11	0.74	0.28±0.15	0.03	0.22	0.14±0.04	0.07	0.67	0.42±0.18	0.19	0.71	0.32±0.13	0.17	0.51	0.39±0.08
Ammonia (mg L^{-1})	0.05	0.33	0.15±0.06	0	0.25	0.15±0.05	0	0.25	0.13±0.07	0.2	0.45	0.21±0.1	0	0.26	0.14±0.05
Phosphate (mg L^{-1})	0.4	1.2	0.63±0.19	0	2.8	1.03±0.67	0.5	1	0.7±0.15	1.2	9.3	2.53±2.26	0.2	1.6	0.6±0.34
Nitrate (mg L^{-1})	0	4.5	1.13±1.17	0	4.6	1.65±1.04	0	37.4	18.4±10.8	0	4.6	1.85±1.04	1.5	15.1	5.81±3.17

Some species tended to prefer certain sites or seasons as indicated by their high indicator values, with high preference being observed for the downstream site F5 (Table 6.3). The big sized species such as *Fragilaria biceps, F. ulna* and *F. ulna* var. *acus* which prefer slow flowing waters were mostly associated with the upstream site F2 (Table 6.3).

The periphyton community structure in Kowie system was significantly different ($p < 0.05$) between upstream and downstream, but similar among seasons ($p > 0.05$), with 2–5 taxa being dominant. Even though taxa richness varied seasonally, the species richness was not significant to cause differences in the communities. In spring, the highest taxa richness was recorded (25) at site F5, with the lowest being recorded in summer at site F4 (10). Diversity indices and evenness tended to be high downstream (F3–F5) compared to the upstream sites (F1 and F2, Figure 6.1), except in autumn. Species diversity indices were high during spring and winter. Species diversity decreased from site F1 to F4 over autumn before increasing as indicated by the Shannon-Wiener and Margalef indices (Figure 6.1a, b). Diversity indices were significantly different ($p < 0.05$) across sites and were similar across the four seasons ($p > 0.05$).

Seven physico-chemical variables (conductivity, pH, water depth, oxygen reduction potential (ORP), salinity and velocity) were important in structuring periphyton communities (Figure 6.2). The first and second axes of CCA explained 24.1 % of the periphyton taxa variance, while also accounting for 32.5% of the physico-chemical (environment) and periphyton taxa variation. The sum of the first and second axis canonical eigenvalues was 0.55, and accounted for 24.1 % of the total variance in periphyton taxa composition. The λ_1/λ_2 ratio was 1.41, suggesting a very strong relationship between periphyton communities and physico-chemical factors.

Table 6.2. The list of most frequently occurring (>1%) periphyton taxa on different sites across four seasons. Meaning of symbols + (1–4.9%), ++ (5–9.9%), +++ (>10%)

Species	F1	F2	F3	F4	F5
Achnanthes standeri Cholnoky	+	+		++	+
Achnanthes subaffinis Cholnoky					+
Achnanthes crassa Hustedt				+	
Achnanthidium minutissimum (Kützing) Czarnecki	+		+	+	+
Amphora ovalis (Kützing) Kützing				+	
Amphora pediculus (Kützing) Grunow				+	
Ankirostrodesmus sp.			++		++
Aulacoseira granulata (Ehrenberg) Simonsen				+	
Cladophora sp.		+			
Chrococcus sp.				+	
Cocconeis engelbretchtii Cholnoky					+
Cocconeis placentula Ehrenberg					+
Cyclotella meneghianana Kützing			+		+
Cylindrocapsa sp.			++		
Cymbella cymbiformis Agardh		+		+	+
Cymbella neocistula Krammer		+			
Cymbella kappii (Cholnoky) Cholnoky				+	
Diploneis elliptica (Kützing) Cleve	+				
Diploneis subovalis Cleve					++
Encyonopsis minuta Krammer & E.Reichardt	+			+	
Entomoneis sp.				+	
Eolimna minima (Grunow) Lange-Bertalot				+	+
Eunotia bilunaris (Ehrenberg) Mills			+		
Eunotia formica Ehrenberg	+				
Eunotia minor (Kützing) Grunow	+				
Fragilaria biceps (Kützing) Lange-Bertalot	+	+	+	+	+
Fragilaria capucina var. *rumpens* (Kützing) Lange-Bertalot			+		
Fragilaria nanana Lange-Bertalot		+			+
Fragilaria tenera (WM Smith) Lange-Bertalot	+	+++	+	+	+
Fragilaria ulna (Nitzsch) Lange-Bertalot	++	+++	++	+	++
Fragilaria ulna var. *acus* (Kützing) Lange-Bertalot	++	+++		+	+
Gomphonema acuminatum Ehrenberg	+	+			
Gomphonema laticollum Reichart	+++	+			
Gomphonema parvulum (Kützing) Kützing sensu stricto	+	+			
Gomphonema truncatum Ehrenberg	+++	+			

Gomphonema venusta Passy, kociolek & Lowe	+				
Gyrosigma attenuatum (Kützing) Cleve		+			
Hantzschia amphioxys (Ehrenberg) Grunow	+++			+	
Navicula cryptocephala Kützing				+	
Navicula cryptotenella Lange-Bertalot			+	+	+
Navicula erifuga (OF Müller) Bory	+	+	+	+	++
Navicula longicephala Hustedt					+
Navicula gregaria Donkin				+	
Navicula radiosa Kützing	+				
Navicula reidiana Lange-Bertalot & Rumrich			+		+
Navicula rhynchocephala Kützing			+		
Navicula veneta Kützing			+	+	
Navicula venusta Janisch ex Cleve			+		
Nitzschia closterium (Ehrenberg) W Smith				+	
Nitzschia linearis (Agardh) W Smith				+	
Nitzschia sigma (Kützing) W Smith			+		
Nitzschia palea (Kützing) W Smith				+	
Nitzschia umbonata (Ehrenberg) Lange-Bertalot					+
Pinnularia viridiformis Krammer	+		+		
Planothidium engelbretchtii (Cholnoky) Round & Bukhityarova					+
Pleurosigma salinarum Grunow		+			+
Pleurosigma elongatum W Smith		+			
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot				+	
Scenedesmus sp.			++	++	++
Staurosira elliptica	+		+	+	++
Staurostrum sp.	+				
Stigeoclonium sp.			+++	+	+
Surirella brebissonii Krammer & Lange-Bertalot		+			
Trachelomonas sp.			+		
Tribonema sp.	+	+	+		
Tryblionella apiculata Gregory					+

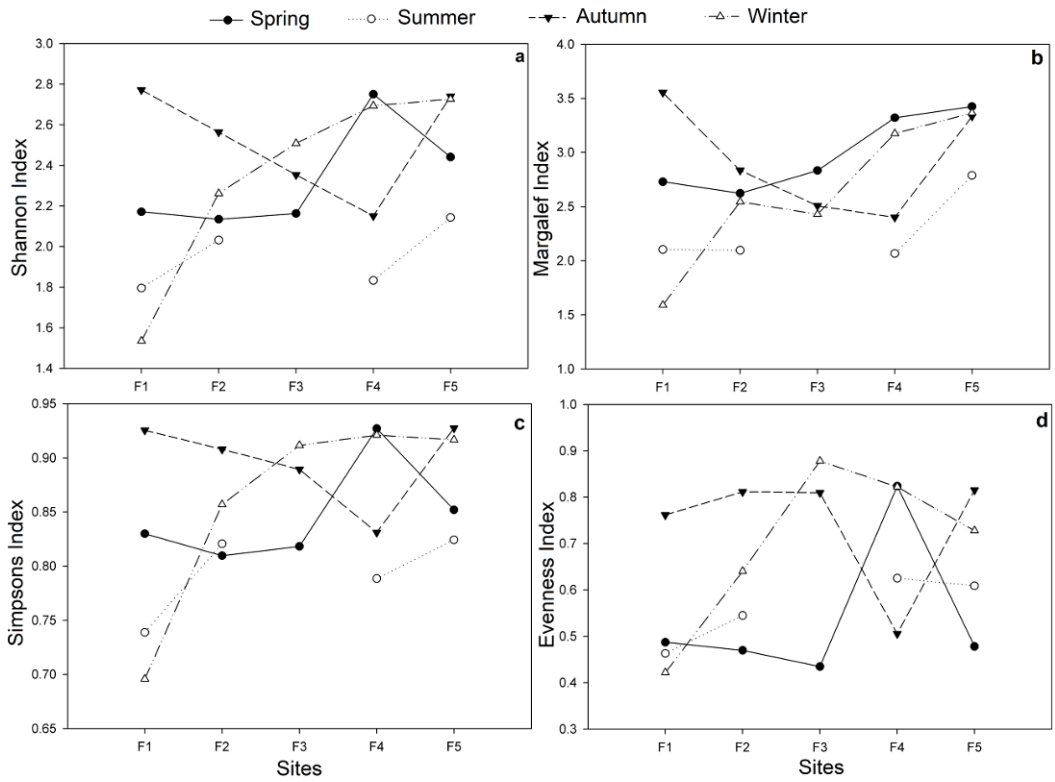

Figure 6.1. The diversity values measured at all the sites during the four sampling periods: (**a**) Shannon-Wiener (**b**) Margalef (**c**) Simpsons and (**d**) evenness

Table 6.3. List of significant ($p < 0.05$) indicator values for periphyton taxa sampled

Taxa	**Site**			**Season**		
	IndVal (%)	***p*-value**	**Site**	**IndVal (%)**	***p*-value**	**Season**
Diploneis subovalis	61.1	0.0196	F5			
Fragilaria biceps	34.8	0.0042	F2			
Fragilaria ulna	30.7	0.0149	F2			
Fragilaria ulna var. *acus*	42	0.0233	F2			
Navicula erifuga	43.5	0.0062	F5			
Navicula reidiana				60	0.036	Spring
Scenedesmus sp.	50.4	0.0436	F3			
Staurosira elliptica				56.8	0.0159	Summer
Tryblionella apiculata	61.2	0.0443	F5			

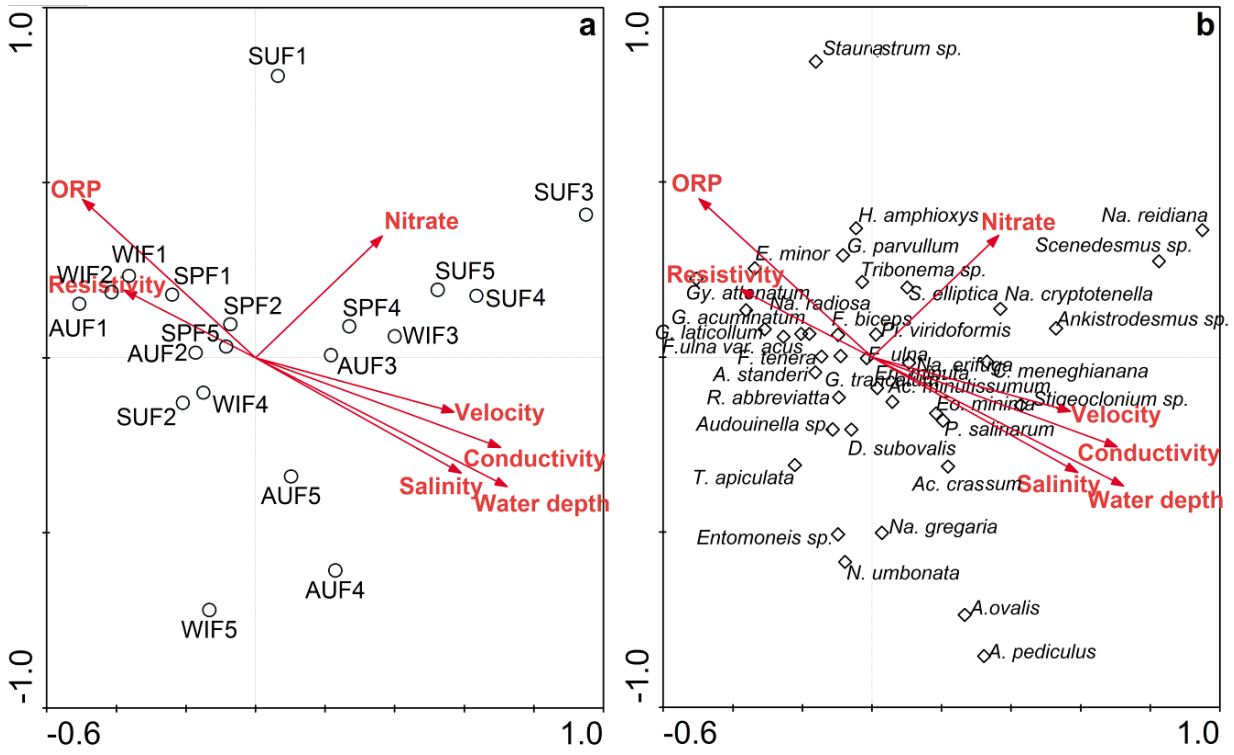

Figure 6.2. Canonical correspondence analysis plots showing effects of physico-chemical factors on (**a**) samples/sites and (**b**) species on the most frequently occurring periphyton taxa in the ordination space of the first and second axes. Abbreviations: sites F1–F5, AU – autumn, SP – spring, SU – summer, WI – winter and ORP – oxygen reduction potential.

Periphyton, floods and the river continuum concept

The periphyton biomass was generally high in spring, ranging between 2482 and 15551 mg m^{-2} (Figure 6.3). There was a drop in the periphyton biomass in summer due to the heavy rainfall (418 mm) which produced a strong flood (2.7×10^{8} m^3) along the Kowie River for 12 days in October and November. The discharge rate increased from a low (0.70 m^3 s^{-1}) to a record high (699.14 m^3 s^{-1}). The drop in biomass from upstream to downstream was strongly negatively correlated to canopy cover (r = -0.93, $p < 0.05$). The periphyton biomass observed in the upstream site (F1) in summer was associated with the floods, which washed away riparian vegetation and exposed new substrata for periphyton (Figure 6.4; Table 6.4). There was significant negative correlation (r = -0.96, p

< 0.05) between periphyton biomass and TDS while other variables were not significant ($p > 0.05$).

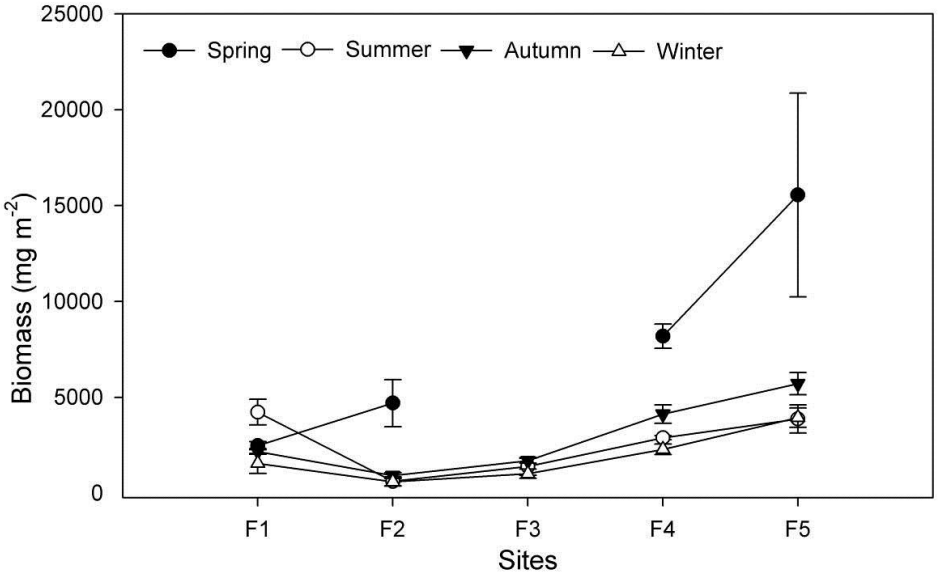

Figure 6.3. Periphyton biomass (±standard error) along the Kowie River continuum

Table 6.4. Morphometric features of the Kowie River. SE – standard error

Site	Stream order	River width ± SE (m)	Canopy cover range (%)
F1	2	1.3±0.3	55–90
F2	3	2.5±0.5	45–60
F3	4	12.3±1.7	28–35
F4	5	9.1±1.1	16–20
F5	5	9.5±1.3	5–7

Periphyton stable isotopes

Using $\delta^{13}C$ analysis, we identified that the periphyton communities during spring were significantly different from the post floods communities (Figure 6.6). The upstream periphyton communities were different from the downstream communities (Figure 6.2; Table 6.2). The upstream communities were dominated by *Fragilaria ulna, F. capucina*

var. *rumpens, F. ulna* var. *acus, F. tenera, Gomphonema truncatum, G. laticollum* and *Pinnularia viridiformis,* whereas the downstream sites were dominated by small sized periphyton taxa such as *A. minutissumum, S. elliptica, C. placentula, N. gregaria, Ankistrodesmus* sp. and *N. radiosa*.

Figure 6.4. Changes in river morphology along the Kowie River, before (*left*) and after (*right*) the floods: **(a)** F4 and **(b)** F1

Most of the upstream sites (F1 and F2) across all seasons grouped together, with low periphytic $\delta^{15}N$ values ranging between 4 ‰ and 8 ‰, whereas the periphytic $\delta^{13}C$ values ranged between -20 ‰ and -32 ‰ (Figure 6.5). The downstream sites (F3, F4 and F5) formed a second grouping, with high periphytic $\delta^{15}N$ values ranging between 10 ‰ and 16 ‰, whereas the periphytic $\delta^{13}C$ values were also high (-14 ‰ to -28 ‰).

Summer upstream site (F2) was grouped with downstream sites, whereas summer site (F4) was grouped with upstream sites (Figure 6.5). Canopy cover and water velocity was significantly negatively correlated (R = -0.76) with periphytic $\delta^{13}C$.

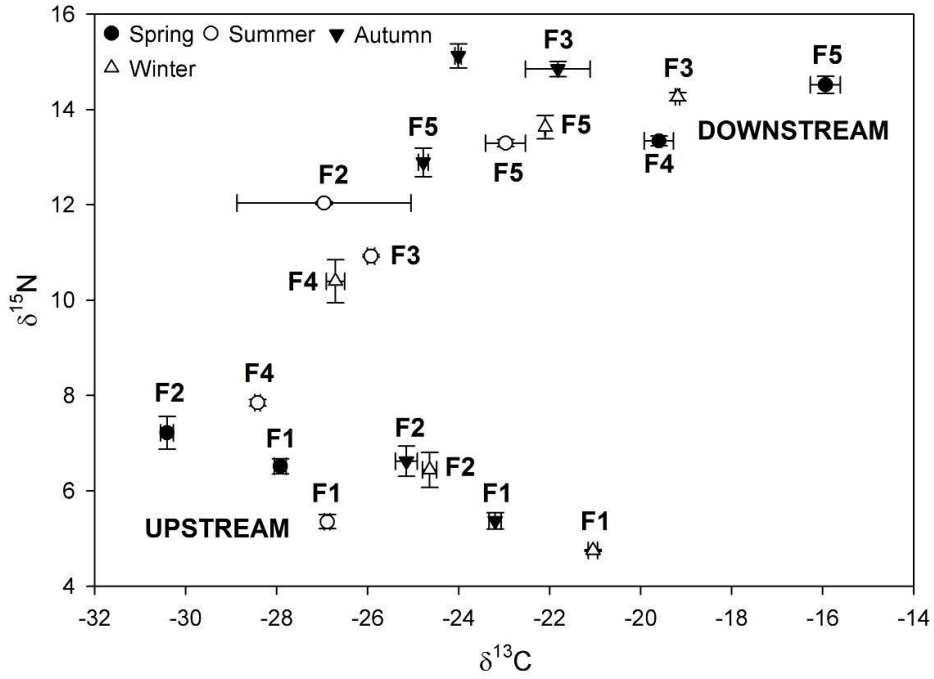

Figure 6.5. Mean $\delta^{15}N$ and $\delta^{13}C$ values (± standard error) plot of periphyton collected at different sites (F1–F5) and seasons

The periphytic $\delta^{15}N$ values were different between the upstream (F1 and F2) and downstream (F3–F5) sites (Figures 6.5 and 6.6b). The periphytic $\delta^{15}N$ values showed that the upstream sites were more ^{15}N-depleted than the more enriched downstream sites, suggesting that the upstream sites were associated with more pristine forests. The periphytic $\delta^{15}N$ values showed significant overlaps among the different sites (Figure 6.6a). The spring F1 and F2 periphytic samples had low $\delta^{15}N$ values upstream whereas winter site F3 and spring site F4 and F5 had high $\delta^{15}N$ values downstream. The autumn sites F1 and F2 had high periphyton $\delta^{15}N$ values upstream whereas site F5 (autumn) and F3 and F4 (summer) had low $\delta^{15}N$ values downstream (Figure 6.6a). We found no

significant differences among the study sites ($p > 0.05$), and seasons had similar stable isotope signatures ($p > 0.05$).

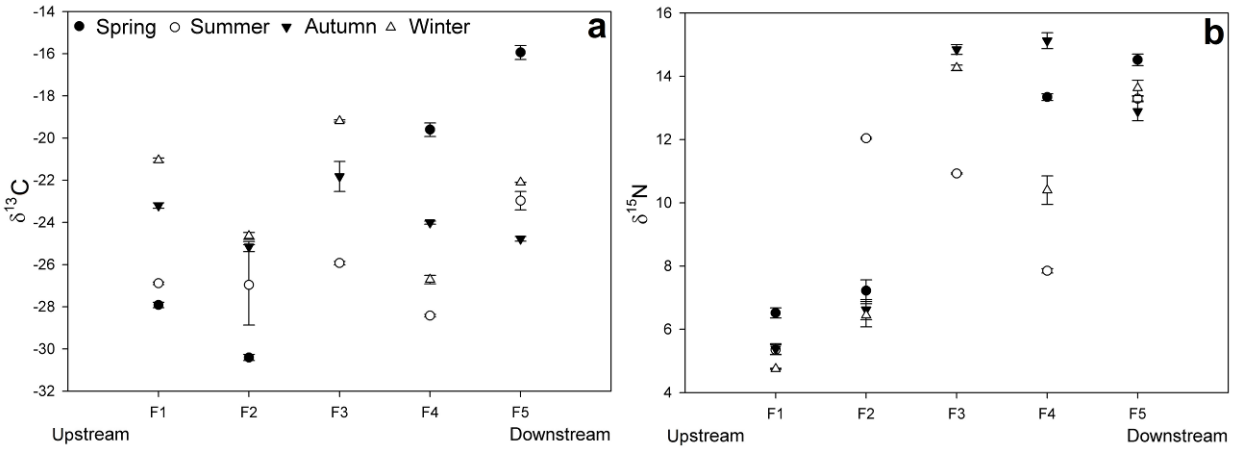

Figure 6.6. Mean (**a**) $\delta^{13}C$ and (**b**) $\delta^{15}N$ values (± standard error) of periphyton collected along a river continuum for different seasons

The upstream sites (F1 and F2) were significantly different from the downstream sites as indicated by the Tukey posthoc test (df = 4, F = 10.39, $p < 0.001$). Periphytic samples from summer had high $\delta^{15}N$ compared to the rest (Figure 6.6b). Site F4 showed a great variation in $\delta^{15}N$ values across the seasons, with summer periphytic $\delta^{15}N$ being low whereas autumn periphytic $\delta^{15}N$ values were high (Figure 6.6b). We found that the nitrates and ammonia were significantly positively correlated (r = 0.87 and 0.73, respectively) with periphytic $\delta^{15}N$.

Discussion

The current study managed to fill some missing research gaps and enhanced our knowledge on periphyton distribution, communities and as potential indicators of anthropogenic impacts and how they are structured in relation to the physico-chemical variables (Figures 6.1 and 6.2; Tables 6.2 and 6.3). The impact of the floods was found to have had a significant impact on the local periphyton community and biomass thereby

the system ended up not supporting some of the RCC principles (Vannote et al., 1982), with anthropogenic impacts being less significant on the how the periphyton communities relate to the RCC similar to Vis et al. (1998) study. The spatial and temporal characteristics of periphytic $\delta^{15}N$ and $\delta^{13}C$ values along the river varied significantly and stable nitrogen ($\delta^{15}N$) signatures were useful as potential indicators of sources of nitrogen pollution along the river system. The $\delta^{15}N$ was able to pick up trace changes in nitrogen which could be important and significant for water and environmental management and monitoring. Due to inadequate or unavailable data necessary for sound and informed decision on water and environmental monitoring in Africa, we the use of combination of classical periphyton taxonomy and stable isotopes will prove to be useful in determining the state or quality of a particular water system so as to provide sound water and environmental management advice.

Periphyton communities in relation to physico-chemical variables

Low periphyton taxa richness was observed upstream, where canopy cover was high due to water depth and high concentration of suspended solids in the Kowie River. Similarly, Welch et al. (1992) found that periphyton in upstream shaded sites (high canopy cover) had low periphyton biomass compared to downstream open sites (low canopy cover) which had higher periphyton taxa richness. Physico-chemical factors such as velocity, water depth, nutrients, substrata and canopy cover appear to be important in structuring periphyton communities within the Kowie River (Fig. 3). The low diversity indices observed in summer after the floods are similar to other studies (Biggs and Thomsen, 1995; Jowett and Biggs, 1997; Uehlinger et al., 2003; Murdock et al., 2004; Ewart-Smith and King, 2012).

In this study, similar to an earlier study on different substrata (Dalu et al., 2014a), we identified seven physico-chemical factors (Figure 6.2) that were associated with periphyton communities, with water depth and velocity being primary factors in explaining variations in periphyton community assemblages. These findings are

consistent with the results of Passy et al. (1999), Suren et al. (2003), Uehlinger et al. (2003), Murdock et al. (2004), Ewart-Smith and King (2012), Tan et al. (2014) and Dalu et al. (2014a). Velocity and water depth were identified as important, and we suggest that they played an important role with other closely related factors such as substrata embeddedness and land use or cover (Tan et al., 2014).

Periphyton, floods and RCC

We found that the October–November 2012 floods were important in structuring the periphyton communities in the Kowie River. In rivers in the south-west Cape, periphyton biomass and community structure were primarily controlled by flood disturbances (Ewart-Smith and King, 2012). In terms of biomass, the periphyton did not fully recover to spring levels (before floods) as the floods altered the hydromorphology of the Kowie River (Figure 6.4). The narrow upstream sites were the most affected by the floods as canopy cover was reduced to less than 20 % in some areas from about 90 %. This created more habitats for the periphyton communities upstream but the scouring of the floods washed away almost all the periphyton communities, hence the communities observed in summer can be considered as early succession communities. In two New Zealand rivers (Tongariro and West Kowai), Jowett and Biggs (1997) found that floods reduced the ash free dry mass of periphyton, mainly due to physical processes such as increased water velocity. In Spöl River (Swiss Alps), floods resulted in temporary periphyton biomass reduction, with the recovery patterns and disturbance impacts not uniform (Uehlinger et al., 2003). Biomass decrease could be attributed to the decrease in light attenuation due to the water depth and the levels of suspended solids.

Water velocity was important in structuring periphyton communities. Increased water velocities generally flush all unattached particles from the periphyton matrix, both organic and inorganic. During this process, water removes all attached periphyton by abrasion and may break off long strands of filamentous algae via shear stress (Biggs and Thomsen, 1995). Shear stress caused by a high discharge of up to 699.14 m^3 s^{-1}

appeared to have removed most, if not all, of the periphyton communities within the Kowie system. Jowett and Biggs (1997) found that a discharge of 154 m^3 s^{-1} swept away some substrata, and the abrasive nature of sediment scoured off waterproof ink markings. After a day of floods, periphyton was virtually non-existent on the substrata (Murdock et al., 2004). Murdock et al. (2004) highlighted that floods with a discharge of 61 m^3 s^{-1} in Carter Creek (Texas) were capable of scouring all visible periphyton from the stream. Despite these disturbances, periphyton biomass rapidly accumulated throughout the stream unlike in our study.

The RCC (Vannote et al., 1980) suggests that periphyton communities in river systems should have predictable changes along a river continuum. Specifically, phytobenthos concentrations, periphyton in our case, is predicted to increase from first to about sixth order stream reaches due to opening of the canopy cover (Vannote et al., 1980). This trend was observed in our dataset (Figure 6.3), where periphyton biomass general increased from upstream (2^{nd} order) to downstream (5^{th} order). In the Kowie River, we observed that the upstream was heterotrophic, dependent on allochthnous production, before and after the floods due to the close association of the river with the riparian vegetation as highlighted by the high riparian canopy cover. The close association of the river and riparian vegetation led to a canopy cover greater than fifty-five percent (>90 % before floods), resulting in reduced in-stream primary production (see Figure 6.3, which shows low biomass, a potential indicator of primary productivity, levels upstream; Murdock et al., 2004).

As the Kowie River expanded, light began to reach the stream bed, allowing more extensive periphyton growth and development and resulting in increased autochthonous primary production in the downstream. We found that midstream (site F3) was the deepest and most turbid due to high suspended solids. This decreased the light reaching the stream bed thereby affecting the development and growth of periphyton communities. The suspended solids observed at midstream were caused by the 3^{rd} order

polluted tributary (Bloukrans River, which drains Grahamstown) that joined the Kowie River channel. Highest periphyton biomass was observed in 5th order downstream sites, suggesting increased in-stream production. A similar observations was made by Cushing et al. (1983) in Middle Fork, were the standing crop of periphyton chlorophyll-*a* against stream order had a curve similar to photosynthesis to respiration (P/R) ratio curve in Vannote et al. (1980), with its peaks in the 5th order stream (Indian Creek). The impact of floods in October–November 2012, made the Kowie system not to follow most of the principles highlighted in the RCC. The periphyton biomass which was used as a determinant of primary production was found to be high upstream than midstream in summer.

Periphyton stable isotopes, its application to community structure

We found that periphyton $\delta^{13}C$ was negatively related to water velocity and canopy cover. These results were similar to Ishikawa et al. (2012), who studied a global dataset and attributed the relationship among periphyton $\delta^{13}C$ with water velocity and canopy cover to the influence of primary production. Increases in channel width, nutrient concentrations and light affected photosynthetic processes with the Kowie River, thereby causing an increase in carbon limitation effects on periphytic isotopic fractionation (Finlay 2011). This shift leads to an increase in periphyton $\delta^{13}C$ with change in river size. Changes in river morphology from upstream to downstream resulted in changes in canopy cover that produced smaller fractionation factors and higher $\delta^{13}C$ in the primary producers (Finlay, 2011; Ishikawa et al., 2012). Ishikawa et al. (2012) showed that periphyton $\delta^{13}C$ was low under high canopy cover and low-light conditions. The negative relationship between periphyton $\delta^{13}C$ values and water velocity suggests an increase in carbon dioxide supply to periphyton cells under high water velocity, which causes an increase in the isotopic fractionation between aqueous carbon dioxide and periphyton and hence an increase in $\delta^{13}C$ downstream (Finlay et al., 1999; Ishakawa et al., 2012). The low periphyton $\delta^{13}C$ observed upstream in the Kowie River could be a result of increased canopy cover which might indicate that there is considerable isotopic

discrimination between aqueous carbon dioxide and periphyton during photosynthesis, or possibly less hydrocarbonate ions use by periphyton in shaded habitats (Ishikawa et al., 2004; Finlay, 2011).

The downstream can be regarded as polluted, as indicated by the enriched periphytic $\delta^{15}N$ values. The biggest contributor to the more enriched $\delta^{15}N$ values could be attributed to the heavily polluted Bloukrans River which drains most of Grahamstown and the intensive agricultural farms (livestock farming, vegetable and pasture irrigation) along the Belmont Valley. In a study conducted on periphyton $\delta^{15}N$ values in the Bloukrans River, the $\delta^{15}N$ values ranged between -1.25 ‰ (sewage and livestock manure/dung) and 22.6 ‰ (inorganic fertilizers; Ning et al., 2013). The periphyton $\delta^{15}N$ values for the downstream site (sites F3–F5 = 7.9 ‰ to 15.2 ‰) did not account for all of the $\delta^{15}N$ variability, but we suggest that nitrogen transformations such as volatilization and denitrification that occur after nitrogen applications to fields or when nitrogen is transported through sewage systems could be the principal factor leading to these high $\delta^{15}N$ values (Figures 6.5 and 6.6b). Anderson and Cabana (2006) found that $\delta^{15}N$ values ranged from 1.8 ‰ to 15.1 ‰ in primary producers were mostly due to nitrogen transformations in Saint-Lawrence Lowlands in Québec (Canada). In the Kowie River, the downstream sites F3–F5 were associated high abundance of pollution tolerant species, such as *Gyrosigma attenuatum, N. erifuga, Gomphonema acuminatum, F. tenera* and *F. ulna*.

In conclusion, the multivariate RDA analysis was a useful tool for determining how well each of the physico-chemical variables related to periphyton communities. Nonetheless, the RDA analysis has been shown to be insensitive to small changes in physico-chemical variables which exert dominance over periphyton communities in many streams/rivers over different time intervals (Biggs, 1990). Therefore, this study highlights the importance of using both multivariate analysis and stable isotope ($\delta^{15}N$) analysis. Stable isotope analysis is sensitive and is able to trace any small nitrogen changes that

occur in aquatic ecosystems as a result anthropogenic impacts. Both techniques could be useful, as RDA analysis will demonstrate the direction and magnitude of change which could be expected a result of anthropogenic impacts, while stable isotope analysis will pinpoint the potential sources of impacts. This will enable the development of sound water and environmental management policies to manage and monitor water systems.

Therefore, the immediate determinants of periphyton communities such as nutrients and salinity might act at smaller spatial scales (Ewart-Smith and King, 2012; Tan et al., 2014). Other factors such as land use, temperature, water depth and velocity probably contributed more to the periphyton temporal pattern. This study provides a platform for additional studies to look at relationships between environmental factors such as land use and adjacent periphyton communities. Future studies should focus on periphyton $\delta^{15}N$ values and what the riparian zone and watershed land use contribute to aquatic ecosystems in terms of nitrogen. Such data would allow precise management of nutrients in river systems.

CHAPTER 7

ASSESSING HABITAT CONNECTIVITY USING STABLE ISOTOPE RATIOS: CONTRIBUTIONS OF ALLOCHTHONOUS AND AUTOCHTHONOUS MATERIAL TO SUSPENDED PARTICULATE MATTER AND DETRITUS ALONG A RIVER-ESTUARINE CONTINUUM

Plate 7. Bridge view of the Kowie River channel along the Southwell Road, near site F1. Photo by Prof. Martin Villet

Abstract

Ecologists are interested in the factors that control, and the variability in, the contributions of different sources to mixed organic materials traveling through lotic systems. We hypothesized that the source matter fuelling mixed organic pools in a river-estuary continuum varies over space and time, with the upper reaches of a system characterized by allochthonous-dominated material and autochthonous contributions becoming more important in the lower reaches. Samples of the mixed organic pools and allochthonous and autochthonous source materials were collected along a small temperate river in southern Africa during four study periods: September (early spring) and November/December (late spring) 2012, and February (summer) and May/June (winter) 2013. The C:N ratios of suspended particulate matter (SPM) collected during summer and winter indicated that the lower reaches of the system had similar organic matter contributions from the freshwater and terrestrial sources. Stable isotope analysis in R revealed that the contributions of autochthonous organic matter were high in SPM along the entire continuum, and aquatic macrophytes were significant contributors to SPM specifically in the upper reaches. The terrestrial leaves made major contributions to the SPM in the middle regions of the system (i.e. downstream sites of the river, particularly in early and late spring). Bulk detritus had large allochthonous matter components in the lower reaches (estuary), and the contributions of aquatic macrophytes and benthic algae to bulk detritus were high (> 50 %) in the upper to middle reaches (river), but low (< 20 %) in the lower reaches (estuary). The evaluation of organic matter contributions to the SPM and detritus along the river–estuary continuum provided a baseline assessment of the nature and sources of potential food for consumers inhabiting different locations during different times of the year. Incorporating such spatio-temporal variations in SPM and detritus into food web studies will improve our understanding of the flow of carbon through aquatic systems.

This chapter is currently under review:

Dalu T, Richoux NB, Froneman PW. Assessing connectivity using stable isotope ratios: contributions of allochthonous and autochthonous material to suspended particulate matter and detritus along a river-estuarine continuum. *Science of the Total Environment.*

Part of the estuary results are published as:

Bergamino L, **Dalu T**, Richoux NB. 2014. Spatial and temporal patterns in sediment organic matter composition within an estuarine environment: stable isotope and fatty acid signatures. *Hydrobiologia*. 732(1):133–145.

Bergamino L, **Dalu T**, Whitfield AK, Carassou L, Richoux NB. 2014. Stable isotope evidence of food web connectivity by a top predator (*Argyrosomus japonicus*: Sciaenidae: Teleostei) in the Kowie Estuary, South Africa. *African Journal of Marine Sciences*. 36(2):207–213.

Introduction

Suspended particulate matter (SPM) serves as an important source of nutrition and energy to aquatic biota (Lu et al., 2014a). The SPM in lotic environments is composed of different organic matter sources such as autochthonously-produced phytoplankton, benthic algae and aquatic macrophytes, and allochthonous materials arising from marine, terrestrial and anthropogenic sources (Berto et al., 2013; Lu et al., 2014a, b). Research on the origin and nature of SPM composition can provide important insights about natural processes and anthropogenic pressures along any lotic continuum (Berto et al., 2013). In many aquatic ecosystems, terrestrial organic matter represents a significant portion of bulk particulate organic matter and carbon flow (Finlay et al., 1999; Carpenter et al., 2005; Marty and Planas, 2008).

The river continuum concept (RCC) suggests that allochthonous matter is the major energy source in shaded upper reaches of rivers, whereas the importance of autochthonous production increases in open and wider lower reaches (Vannote et al., 1980). Several studies from temperate lotic food webs generally support the RCC (Winterbourn et al., 1984; Thorp and Delong, 1994; Hall et al., 2001). The upper reaches of lotic systems can receive a significant supply of leaf litter, typically related to high riparian cover (Vannote et al., 1980; Lau et al., 2009). Thus, the upper reaches are expected to have low autochthonous production because of high percentage canopy cover (hence greater shading) and decreased canopy cover in lower reaches allows for greater productions of autochthonous sources.

Stable isotope analysis is a useful tool used by ecologists to address a wide range of questions related to the food sources of organisms, the length of food chains and the transfer of organic material or contaminants (Yoshii et al., 1999; Marty and Planas, 2008). Stable isotope ratios are highly varied in rivers and are influenced by multiple biogeochemical, physical and physiological processes that occur throughout watersheds (Finlay and Kendall, 2007). For example, environmental conditions like

rainfall, and organic matter type and abundance show substantial seasonal and spatial variability (Keller, 1999; Bade et al., 2006). Information on the flow and nature of organic carbon in river systems originates mostly from America (Barros et al., 2010; Lu et al., 2014a, b), Europe (Berto et al., 2013; Guerra et al., 2013) and Australasia (Bouillon et al., 2000; Dehairs et al., 2000; Bouillon et al., 2004; Pingram et al., 2014). Much of the evidence describing the contributions of different sources of organic matter in lotic systems is based on stable isotope analyses, as most of these sources have distinctive isotopic signatures and can thus be traced through food webs (Berto et al., 2013; Guerra et al., 2013). Generally, allochthonous leaf litter enters aquatic systems in seasonal pulses during summer to winter, and these inputs, along with those from agricultural runoff and sewage discharge, can result in a complex group of anthropogenic and natural factors that potentially interact to influence carbon availability and utilization. To begin characterising the structure and function of food webs along a highly variable system like a river or estuary, we must determine the variations in the contributions of allochthonous and autochthonous materials at the base of each food web. In determining the primary carbon sources potentially available to organisms, we gain information on the trophic ecology of different habitats, the relative connectivity between adjacent habitats, and how these connections vary through space and time (Richoux and Froneman, 2007; Bergamino et al., 2014).

The C:N elemental ratios and $\delta^{13}C$ and $\delta^{15}N$ signatures have been used to evaluate the sources and fate of organic matter in aquatic ecosystems (e.g. Thornton and McManus, 1994; Andrews et al., 1998; Graham et al., 2001; Antonio et al., 2012; Antonio and Richoux, 2014; Fu et al., 2014). The use of these tracers relies on the existence of gross differences in relative enrichment of ^{13}C and ^{15}N in organic matter pools relative to terrigenous matter (Thornton and McManus, 1994; Graham et al., 2001). Isotopic signatures in particulate organic carbon are typically conservative, and the C:N ratios depend upon the biological composition of the original source material combined with ratio increases upon degradation (Thornton and McManus, 1994; Andrews et al., 1998).

Thus, upon degradation C:N ratios in suspended particulates increase while $\delta^{13}C$ changes only slightly relative to the main source materials (Andrews et al., 1998).

Our aim was to determine the seasonal and spatial variability in the contributions of allochthonous and autochthonous organic matter to SPM and bulk detritus along a temperate river–estuary continuum. Drawing from the RCC (Vannote et al., 1982), we hypothesized that (i) the organic matter fuelling these suspended mixtures varies along the continuum, with the middle reaches of the system (i.e. the lower river) dominated by autochthonous algal material and the upper reaches by allochthonous plant material, and (ii) these relative contributions change over time based on shifting environmental parameters (e.g. rainfall), in that increased terrestrial inputs occur during periods of increased precipitation. To address these hypotheses, we measured $\delta^{13}C$ and $\delta^{15}N$ isotope signatures in SPM, bulk detritus, and potential allochthonous and autochthonous organic matter sources and incorporated them into stable isotope analysis in R (SIAR) models to determine the source contributions to SPM and detritus at different locations of a river system over time.

Materials and methods

Study area

A detailed description of the study site is provided in Chapter 2. Heavy rainfall in October–November 2012 produced a strong flooding of freshwater (2.7×10^8 m^3) along the Kowie system for 12 days, with total rainfall of 418 mm. The discharge rate increased from 0.70 m^3 s^{-1} on Oct 16 to a peak of 699.14 m^3 s^{-1} on Oct 21 (DWA, 2013) and full discharge and rainfall trends are reported in Dalu et al. (2014a). Suspended particulate matter (SPM), large detritus, epiphyton, epipelon, periphyton, aquatic macrophytes and terrestrial plants were collected concurrently (the terrestrial and aquatic plants were provided by S. Moyo, *unpublished data* and Bergamino et al. 2014) in four periods: September 2012 (early spring), November–December 2012 (late spring), February 2013 (summer) and May–June 2013 (winter). Eight sites were

selected along the river–estuary continuum: sites F1–F5 (freshwater) and E1–E3 (brackish water) (Figure 2.1; Table 7.1). The upper most reaches (sites F1 and F2) of the Kowie system were pristine, and the middle (sites F3 to F5) and lower reaches (E1 to E3) were affected by pollution from agricultural and sewage inputs (Figure 2.1).

Table 1. Morphometric features of the riverine-estuarine continuum of the Kowie system. Reproduced with permission from the Transactions of the Royal Society of South Africa (source, Dalu et al., 2014b)

Site name	Elevation (m)	Macrophyte species	Macrophyte cover range (%)	Channel width (m)	Aerial cover range (%)
F1	367	*Cyperus* sp.	5–15	1.3±0.3	55–90
		Lagarosiphon muscoides	0.5–2		
F2	237	*Cyperus eragrotis*	10–30	2.5±0.5	45–60
F3	112	*Cyperus eragrotis*	18–55	12.3±1.7	28–35
		Cyperus alopecuroides	2.5–3.5		
F4	53	*Cyperus eragrotis*	20–50	9.1±1.1	16–20
F5	27	*Phragmatis australis*	15–60	9.5±1.3	5–7
		Potamogeton pectinatus	45–85		
		Schoenoplectus brachycerus	1.5–4		
		Jancus sp.	2–6		
ES1	27	*Schoenoplectus brachycerus*	5–25	32.2±3.5	3.0–4.5
		Phragmatis australis	30–72		
ES2	8	*Schoenoplectus brachycerus*	30–45	46.8±3.2	0.5–1.2
		Phragmatis australis	3–12		
ES3	7	*Spartina maritima*	40–75	69.2±5.8	0

Sampling of autochthonous and allochthonous organic matter sources

At each site and during each period, aquatic macrophytes and benthic algae (epiphyton, epipelon and periphyton; which represented the main sources of autochthonous material), larger detritus, terrestrial plants (the main sources of allochthonous material) and SPM were collected from the river and estuary during day time. For epipelon, the top 1 cm of the sediment surface was collected from the littoral (river) or intertidal (estuary) zone, deposited in a tray (35×22 cm) and transported to the laboratory for further processing. The epiphyton was collected from *Cyperus* spp., *Potamogeton pectinatus*, *Schoenoplectus brachycerus* and *Spartina maritima*. At least 15 whole

stalks of each plant species comprising of stem and leaves were carefully cut and removed from the stream or estuary and the epiphyton removed with a toothbrush. Periphyton was collected by brushing with a toothbrush at least ten pebble (>2–64 mm) to cobble (>64–256 mm) sized rocks haphazardly collected at each sampling site. Dominant aquatic macrophytes (*Cyperus eragrostis, Cyperus* sp., *S. brachycerus, P. pectinatus* and *S. maritima*) were collected from the littoral zone and represented autochthonously-produced material in this study.

Epipelon samples were covered with a 64 µm mesh and a thin layer (5 mm) of pre-treated sand (i.e. washed with 1M hydrochloric acid and combusted at 500 °C for 5 hours). The epipelon samples covered with sand were incubated for 15 hours under artificial light to promote the movement of the microalgae towards the treated sand. Migrated microalgae were separated from the treated sand by pressure washing with distilled water and then concentrated by filtration onto pre-combusted Whatman GF/F filters (Couch, 1989; Antonio et al., 2010). All benthic algae samples were concentrated onto pre-combusted Whatman GF/F filters and visible non-periphytic and epiphytic particles were manually removed after filtration.

Twenty litres of water were collected from different parts of the river or estuary at each study site and period for SPM analysis. Surface water samples were filtered onto pre-combusted (500 °C; 5 hours) Whatman GF/F filters, after which all visible zooplankton were removed with forceps. We attempted to obtain phytoplankton samples by towing horizontal a 20 µm mesh net (32 cm in diameter) for 2 min periods and then passing the water through a 64 µm mesh to eliminate larger material (Dalu et al., 2014b), but there are practical difficulties in isolating adequate biomasses of phytoplankton from other suspended organic material (Finlay et al., 1999; Bouillon et al., 2000; Marty and Planas, 2008). Our data analysis later showed that the phytoplankton isotopic signatures were not statistically different from the SPM signatures ($P > 0.05$), so we combined these samples for subsequent analyses.

Terrestrial samples were represented by fresh riparian tree leaves (*Eucalyptus globulus, Eucalyptus* sp., *Olea* sp. and *Searsia* sp.) collected from the riparian zone. Bulk detritus was collected by picking settled and floating large organic material, sometimes including whole leaves and twigs, and also through collecting material falling into a 100 µm mesh net (50 cm diameter) left for 30 minute periods in the water at each site. All the material collected in the net and handpicked was combined together and placed in labeled ziplock bags. All samples were stored on ice and then at -20 ºC in the laboratory and processed within two days of collection. The detritus and terrestrial materials from each site and season were washed with distilled water and placed into pre-ignited aluminum foil envelopes.

Stable isotope sample processing and analysis

Triplicates of each sample type from each site and period were processed to obtain stable isotope ratio data. All samples in labelled pre-ignited (500 °C; 5 hours) foil envelopes were freeze-dried using a VirTis Benchtop 2K. Any bulk material (e.g. aquatic macrophytes, terrestrial matter and detritus) was ground to a fine homogeneous powder using a mortar and pestle and about 1 mg of each sample was placed in a tin capsule. For the filtered samples, about 1 mg of dried material was scraped off each filter and placed into tin capsules. Prior to encapsulation, all algae samples on filters were vortexed for two minutes in 2 M hydrochloric acid (to remove any calcified components), centrifuged for five minutes at 3600 rpm, washed twice in deionised water followed again by centrifugation, dried at 50 ºC and homogenised in a Retsch Mixer Mill. A detailed description of the stable isotope analysis procedure is highlighted in Chapter 4.

Data analysis

No data transformation was performed. Distance-based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) was conducted using PRIMER v6 add-on package PERMANOVA+ (Anderson et al., 2008).

To determine the stable isotope ($\delta^{13}C$ and $\delta^{15}N$) variation within benthic algae, SPM and detritus among the study sites and periods, we used site and period as the main factors in a PERMANOVA with Euclidean dissimilarities as distance measures. Each term in the analysis was tested using 9999 permutations of the correct relevant permutable units (Anderson and ter Braak, 2003), with significant terms investigated using PERMANOVA t statistic pair-wise comparisons (Anderson et al., 2008). Pearson correlations between chl-*a* concentration and stable carbon and nitrogen isotope values were carried out using SPSS 16.0 to test if chl–*a* concentration was correlated with stable carbon and/or nitrogen.

The Bayesian stable isotope analysis in R (SIAR, Parnell 2010) models were used to assess the relative contributions of 3 different organic matter sources, benthic algae (epipelon + epiphyton + periphyton), aquatic macrophytes and terrestrial leaves, to SPM and detritus (the latter two mixed materials considered as the 'consumers' in these models; see Bergamino et al., 2014). Prior to SIAR, no significant differences (P>0.05) in isotopic signatures were detected between the two benthic algae types (epipelon and epiphyton) in the estuary section, so we combined the benthic algae samples into one source group according to Bergamino et al. (2014). In the river section, we combined periphyton and epiphyton into a 'benthic algae' category for each season and site, except for sites F2 and F3 where we combined all 3 algal types (epipelon, epiphyton and periphyton), as no significant differences were observed among these (p > 0.05). The SIAR model was run using data from each river and estuary site during each season using only those sources collected from those locations and times. The Bayesian SIAR model incorporates uncertainty and variation in parameters (Parnell, 2010), and site- and season-specific models were run so that we could assess the spatial and seasonal variability in the composition of SPM and detritus. We used 0.5 ‰ as the fractionation factor from source to mixed pool for both $\delta^{13}C$ and $\delta^{15}N$ based on short-term decomposition experiments (Keough et al. 1988, Schweizer et al., 1999; Dehairs et al., 2000). Pearson correlations were performed using transformed [log (x +

1)] SIAR model proportions of aquatic macrophytes, benthic algae and terrestrial material, in detritus and SPM against mean monthly rainfall for the sampling period using SPSS 16.0.

Results

Benthic algae, SPM and detritus isotopic values

Significant spatial and temporal variations were detected in stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures ($p < 0.05$) in benthic algae and SPM and $\delta^{15}N$ for detritus, while no significant variation was observed for detritus $\delta^{13}C$ ($p > 0.05$) (Table 7.2). There were significant interactions between site and study period ($p < 0.05$) for benthic algae and SPM, whereas no significant interactions occurred in $\delta^{13}C$ values in detritus. Pairwise comparisons of detritus $\delta^{13}C$ values indicated significant differences between sites F2 and F4 ($t = 2.28$, p(Monte–Carlo (MC)) = 0.0279) and between late spring and summer ($t = 5.54$, p(MC) = 0.0114). Benthic algae had high $\delta^{13}C$ values (-10 to -25 ‰) throughout the study, while SPM had low $\delta^{13}C$ values (-24 to -32 ‰) compared to other organic matter sources. The SPM $\delta^{13}C$ values were high in the brackish water section (sites E1–E3;-22 to -26 ‰) and lower in the middle reaches (F3–F5;-28 to -32 ‰). The $\delta^{13}C$ values of all organic matter sources generally decreased from the upper to the middle reaches of the entire system before increasing again in the lower reaches (Figure 7.1). In benthic algae and detritus, the $\delta^{13}C$ values increased from the upper to lower parts of the system. The $\delta^{15}N$ values in all sample types increased from the upper reaches to the middle reaches before decreasing slightly in the lower reaches during all study periods (Figure 7.1). All of the isotopic data for the aquatic macrophytes and terrestrial leaves were provided by S. Moyo, *unpublished data* and Bergamino et al. (2014).

Table 7.2. Permutational ANOVA results of the effects of site and season on the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of several organic materials in the Kowie system. Tests significant at $p < 0.05$ are indicated in bold. *Abbreviations: perm – permutation, MC – Monte Carlo, df – degrees of freedom*

Material		df	MS	F	*P* (perm)	*P* (MC)
Benthic algae						
$\delta^{13}C$	Site	7	34.55	49.47	**0.0005**	**0.0007**
	Season	3	10.03	24.29	**0.0044**	**0.0053**
	Site×season	20	53.89	22.76	**0.0001**	**0.0002**
	Residual	250	13.62			
$\delta^{15}N$	Site	7	24.72	36.82	**0.0006**	**0.0005**
	Season	3	13.23	20.29	**0.0268**	**0.0381**
	Site×season	20	11.28	21.71	**0.0153**	**0.0311**
	Residual	250	6.58			
Detritus						
$\delta^{13}C$	Site	7	43.63	1.12	0.3763	0.3564
	Season	3	36.85	0.95	0.4242	0.4216
	Site×season	16	64.8	1.67	0.1525	0.0697
	Residual	78	38.83			
$\delta^{15}N$	Site	7	68.28	107.81	**0.0001**	**0.0001**
	Season	3	17.62	27.83	**0.0001**	**0.0001**
	Site×season	16	18.88	29.81	**0.0001**	**0.0001**
	Residual	78	0.63			
SPM						
$\delta^{13}C$	Site	7	64.67	116.06	**0.0001**	**0.0001**
	Season	3	7.67	13.76	**0.0001**	**0.0001**
	Site×season	20	10.3	18.49	**0.0001**	**0.0001**
	Residual	56	0.56			
$\delta^{15}N$	Site	7	85.25	226.58	**0.0001**	**0.0001**
	Season	3	17.83	47.39	**0.0001**	**0.0001**
	Site×season	20	4.19	11.14	**0.0001**	**0.0001**
	Residual	56	0.38			

The SPM had a wider range of $\delta^{13}C$ values (-22.5 to -32.1 ‰) throughout the study compared to that of the detritus (-25.5 to -31.1 ‰). The $\delta^{15}N$ values of detritus (0.8 to 13.3 ‰) had a wide range (Figures 7.1 and 7.2). The $\delta^{13}C$ values of the benthic algae (-

12.9 to -28 ‰) varied significantly over time and across sites ($p < 0.05$; Figure 7.1). The benthic algae (4.1 to 19.2 ‰) had low $\delta^{15}N$ values relative to other sources throughout the study. The C:N ratios of different organic matter sources varied significantly ($p < 0.01$) among the study periods and sites. High C:N ratios in benthic algae (8.9 to 19.2) and low ratios in SPM (7 to 9.3) occurred in early spring. In late spring, the C:N ratios of SPM were low before increasing in summer. C:N ratios of SPM in early spring ranged between 7.5 and 8.6, late spring (7.8 to 8.4), summer (8.1 to 9.7) and winter (7.4 to 10.6).

Relative contributions of aquatic macrophytes, benthic algae and terrestrial leaves to SPM and detritus

The estimated contributions of plants and benthic algae to SPM and detritus during different study periods along the river–estuary continuum are summarized in Figure 7.3. The SIAR models for detritus indicated spatial variation in the contributions from terrestrial leaves, which contributed >70 % in the estuarine section of the continuum (Figure 7.3; Table 7.3). The contributions of aquatic macrophytes and benthic algae to detritus were more variable (Table 7.3), with these autochthonous components more dominant in the upper reaches than the lower reaches. In general, the contributions of aquatic macrophytes and benthic algae to detritus decreased from the upper to the lower reach sites, while terrestrial leaves increased from upstream to downstream (Figure 7.3; Table 7.3). The SIAR mixing models estimated that the benthic algae was an important component in the SPM in the middle to lower reach sites, whereas contributions from the aquatic macrophytes were most significant in the upper reach sites and tended to decrease in the downstream direction (Figure 7.3; Table 7.3). Substantial but highly variable (over space and time) contributions of terrestrial leaves to the SPM were apparent from the SIAR models (Figure 7.3; Table 7.3).

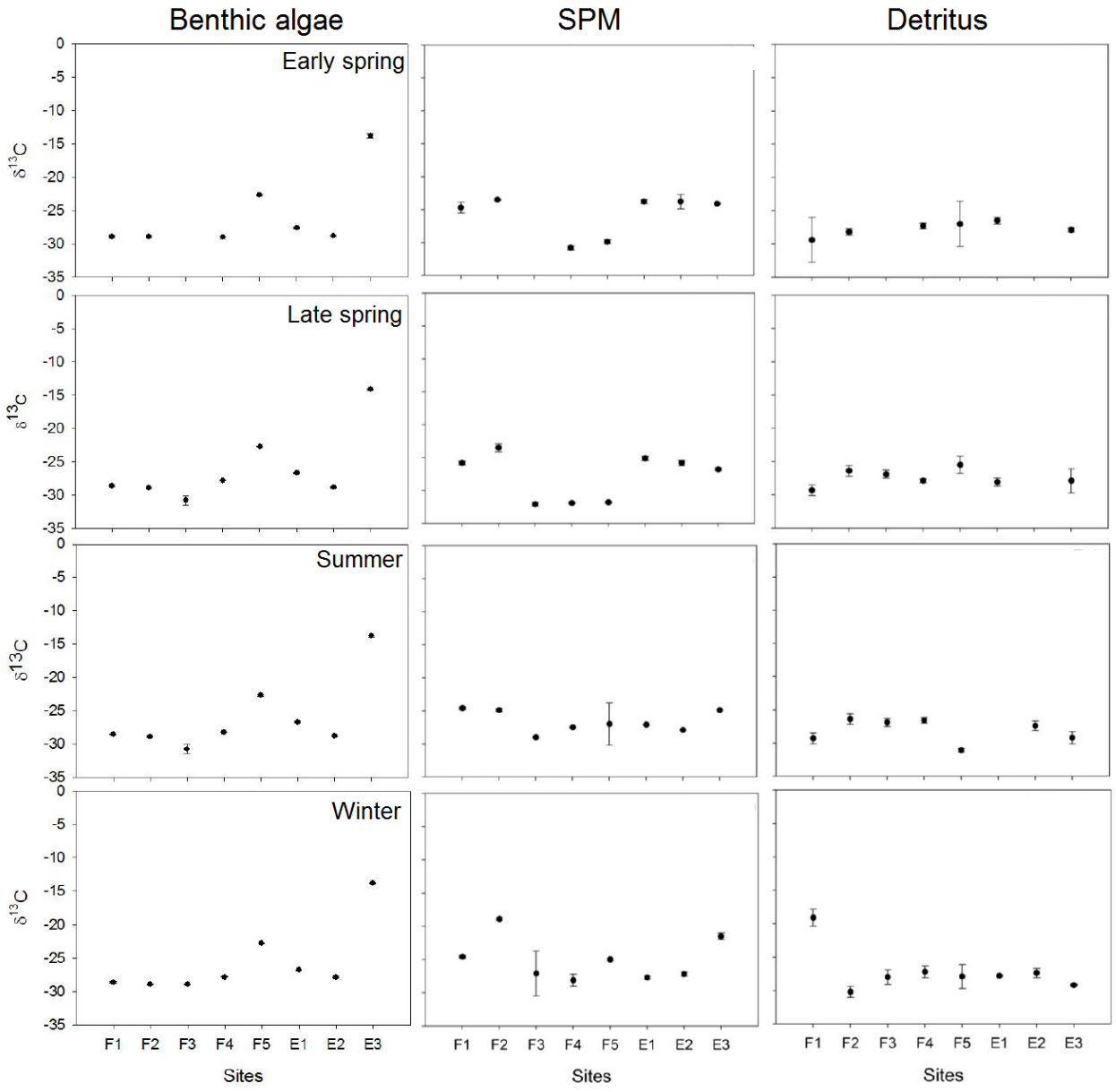

Figure 7.1. Mean $\delta^{13}C$ values (± standard deviation) for organic matter sources in Kowie system in September 2012 (early spring) to May–June 2013 (winter): (**a**) early spring, (**b**) late spring, (**c**) summer and (**d**) winter.

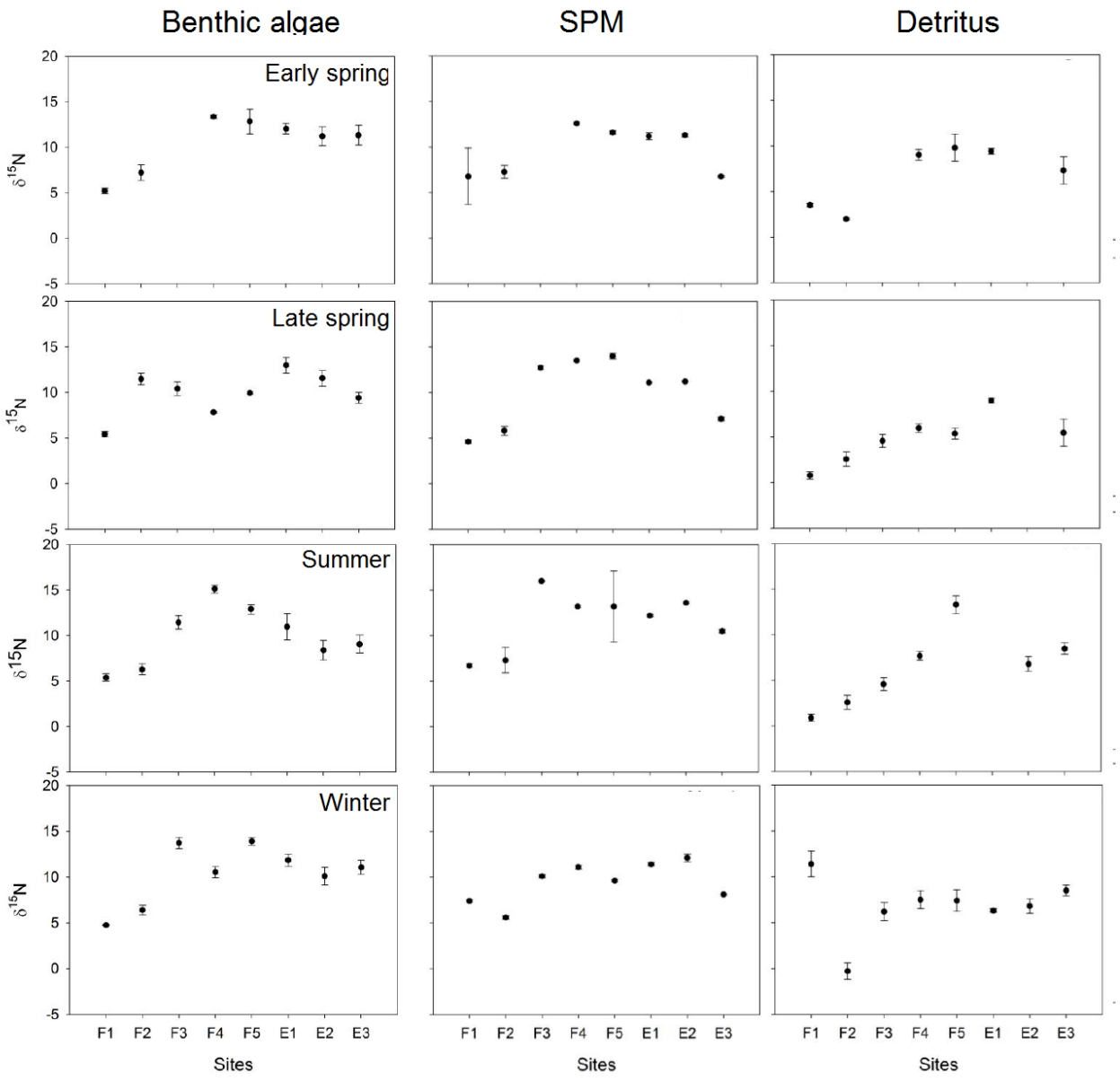

Figure 7.2. Mean δ^{15}N values (± standard deviation) of organic material in the Kowie system in September 2012 (early spring) to May–June 2013 (winter): (**a**) early spring, (**b**) late spring, (**c**) summer and (**d**) winter.

Pearson correlations showed that benthic algae (r = -0.1, p = 0.48), macrophytes (r = -0.1, p = 0.72) and terrestrial leaves (r = 0.2, p = 0.27) contributions to SPM were not significantly correlated with rainfall. Similarly, benthic algae (r = 0.1, p = 0.57),

macrophytes (r = -0.05, p = 0.81) and terrestrial leaves (r = -0.01, p = 0.97) contributions to detritus were also not significantly correlated with seasonal rainfall.

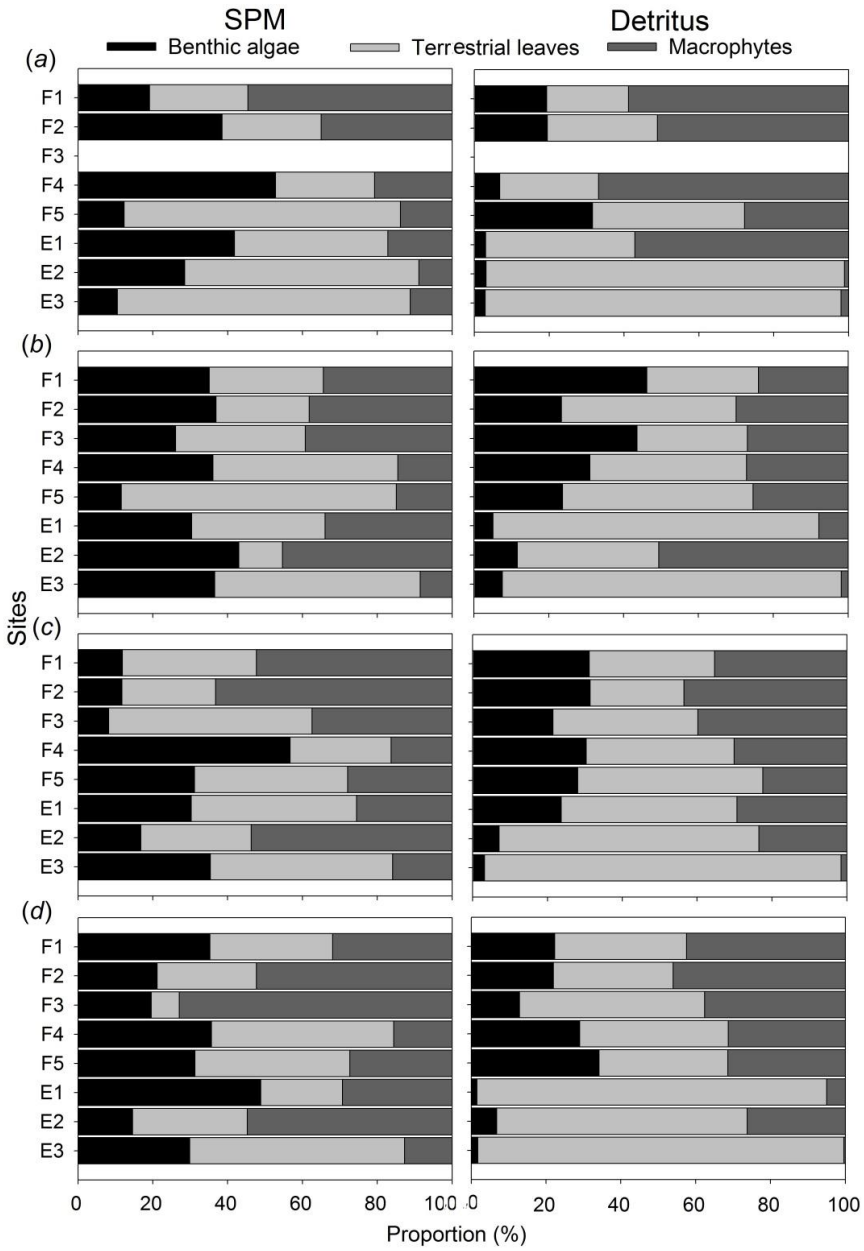

Figure 7.3. Relative mean percentage contributions, as estimated by SIAR, of benthic algae and aquatic and terrestrial plants to suspended particulate matter (SPM) and detritus in (**a**) early spring, (**b**) late spring, (**c**) summer, and (**d**) winter. *The 95% credibility intervals are provided in Table 7.3*

Table 7.3. The 95% credibility intervals as determined by SIAR mixing models of three organic matter sources to SPM and detritus composition at eight sites of the Kowie system during four study periods. *Abbreviations: BA – benthic algae, Macro – macrophytes, and TL – terrestrial leaves and the values in bold indicates the main contributor.*

Season/Site	SPM			Detritus		
	BA range	TL range	Macro range	BA range	TL range	Macro range
EARLY SPRING						
F1	0.0-48.5	0.0-57.4	**15.8-97.5**	0.0-50.1	0.0-53.7	**15.9-96.9**
F2	**0.4-74.5**	0.0-56.3	0.0-67.6	0.0-48.2	0.0-64.8	**11.6-89.3**
F3						
F4	**17.0-75.2**	0.0-51.4	0.0-46.7	0.0-24.6	0.0-59.4	**29.2-98.9**
F5	0.5-21.6	**54-90.1**	0.0-37.9	0.0-62.6	**1.1-78.3**	0.0-57.8
E1	**10.9-75.5**	5.5-75.8	0.0-47.9	0.0-9.8	24.9-52.2	**48.1-66.4**
E2	11-54.2	0.0-55.6	**12.1-76.1**	0.0-5	**18.0-100.0**	0.0-80.6
E3	0.0-30.9	**50.2-70.6**	0.5-44.5	0.0-7.3	**89.4-94.3**	0.0-8.8
LATE SPRING						
F1	0.0-67.2	0.0-61	**0.0-66.2**	9.2-80.8	0.0-58.7	**0-51.1**
F2	0.0-75.1	0.0-56.5	**0.3-72.8**	1.2--43.2	19.4-68.1	**0.4-55.8**
F3	0.0-57.5	**0.0-67.4**	0.5-75.5	20.1-63.7	0.2-55.6	**0.2-50.8**
F4	**0.0-69.4**	10.1-92.7	0.0-43.2	0.0-61.7	**2.2-79.3**	0.0-57.2
F5	0.0-21.1	**55.2-88.5**	0.0-35.7	0.0-54	**12.5-95.4**	0.0-55.7
E1	1.4-54.7	**6.2-60.5**	1.2-62	0-15.5	**66.1-100**	0.0-24.5
E2	24.0-64.0	0.0-33.5	**16.2-70.9**	0.0-28.5	**27.1-49.4**	27.5-70.9
E3	0.0-57.7	**27.3-69.2**	0.5-38.9	0.0-24.1	**67.3-100**	0.0-13.8
SUMMER						
F1	0.0-37.2	1.6-61.6	**22.1-82.6**	0.0-62	0.0-64.6	**0.0-67.8**
F2	0.0-25.7	0.0-49.3	**39.7-84.1**	4.4-54.9	0.0-50	**20.9-64.5**
F3	3.6-13.4	**17.5-94.1**	0.2-71.5	10.0-32.9	2.4-72.2	**5.1-72.2**
F4	**37.9-69.3**	0.3-46.8	0.0-36.8	3.7-52.3	**2.3-73**	0.0-57.6
F5	0.0-63.8	**4.6-73.4**	0.0-53.3	0.0-59.1	**10.2-93.6**	0.0-51.7
E1	0.5-56.8	**7.4-80.1**	0.0-54.7	0.4-43.1	**12.4-87.2**	1.0-53.4
E2	0.0-48.9	0.0-61.1	**12.2-94.4**	0.0-21.2	**32.6-99.9**	0.0-55.9
E3	2.2-63.5	**27.1-69.9**	0.3-29.9	0.0-9.8	**86.7-100.0**	0-4.8
WINTER						
F1	**14.9-53.8**	0.6-63.2	3.3-56.4	0.0-51.2	0.0-67.6	**3.1-79.6**
F2	0.0-52.8	0.0-57.8	**11.2-95.8**	0.0-46.7	3.4-56.6	**22.1-70.6**
F3	9.9-35.3	0.0-20.6	**44.9-47.2**	0.0-28.3	**28.7-71**	5.1-62.5
F4	1.0-60.9	**10.9-93.7**	0.0-43	0.0-59.4	**1.0-75.9**	0.0-62.5
F5	0.6-55.9	**3.5-48**	0.1-54.3	0.0-64.6	**0.1-58.5**	0.0-59.8
E1	**1.5-16.1**	0.0-58.9	0.0-59.5	0.0-4.6	**82.5-100.0**	0.0-15.5

E2	0.0-47.9	0.0-64.3	**14.8-95.5**	0.0-22.5	**29.8-100.0**	0.0-61.9
E3	0.0-51.3	**18.4-59.1**	12.8-55.7	0.0-4.6	**94.2-99.5**	0.0-2.4

Discussion

Carbon and nitrogen stable isotope values in organic matter sources (benthic algae, macrophytes and terrestrial leaves) and the mixed category "consumers" (SPM and detritus) indicated that there were shifts in the contributions of the sources to the pools across periods and sites (Figures 7.1 to 7.3). The relative influence of allochthonous and autochthonous sources in the SPM and detritus pools varied significantly (Figure 7.3), with allochthonous materials more substantial in the detritus and autochthonous materials generally more substantial in the SPM. The relative contributions of allochthonous and autochthonous matter into the organic pools of the river-estuary continuum were spatially and temporally variable because the physical conditions of the system change over space and time. Studies by Bouillon et al. (2000, 2004) and Abrantes and Sheaves (2008) have highlighted that, for the majority of aquatic systems, hydro-geomorphological factors, e.g. riparian vegetation type, water velocity and channel width, influence the balance between inputs from the terrestrial environment *versus* the water column. In the Kowie system, the channel width and relative biomass of aquatic macrophytes increase from the upper reaches to the lower reaches, whereas the riparian vegetation cover decreases in the downstream direction (Dalu et al., 2014b). These factors combined contribute towards changes in the proportions of aquatic macrophytes, benthic algae and terrestrial leaves in the mixed organic pools in the system.

Spatio-temporal variations in organic matter sources and pools

The $\delta^{13}C$ values of SPM decreased from the upper to middle reaches (sites F3 to F5) before increasing again in the lower reaches (sites E1–E3; Figure 2.1). This trend can likely be attributed to the differential uptake by photosynthetic organisms of isotopically light dissolved inorganic carbon resulting from the bacterial respiration as it breaks

down organic matter (Bouillon et al., 2000). Another factor affecting $\delta^{13}C$ and $\delta^{15}N$ values longitudinally along the system could be the differential rates of decomposition of terrestrial leaves and other organic material such as aquatic macrophytes at different locations, which could lead to high quantities of organic material having low $\delta^{13}C$ values to be released into the water at the upper and middle reaches (see for example Ishikawa et al., 2012).

We also found a pattern of high $\delta^{15}N$ values in organic materials from the middle to lower reaches characterised by high mean nitrate concentrations (range of 0.5 to 2 mg L^{-1}; Dalu et al., 2014b). The $\delta^{15}N$ values of all organic matter sources became higher towards the lower reaches (Figure 7.2), with the middle reach sources having high $\delta^{15}N$ values as a result of large volumes of partially-treated sewage and agriculturally-derived sediments and nutrients being washed into the middle and lower reaches of the Kowie River. Thus, the initial carbon pool available for benthic algae from the middle to lower reaches included respiratory ^{13}C-depleted carbon dioxide of terrestrial (and/or sewage; see Abrantes and Sheaves, 2008; Sarma et al., 2012) origin, further explaining why $\delta^{13}C$ values of benthic algae were consistently more negative in the middle reaches. Bacterial respiration would be high in the middle reaches of the system, as water polluted with discharged sewage and inorganic fertilisers from the polluted tributary joins the Kowie above site F3, thereby affecting mostly the phytoplankton and SPM which absorb the high nutrients, causing an increase in $\delta^{15}N$ (Figures 7.1 and 7.2). A study by Diebel and Vander Zanden (2009) in Wisconsin, USA, showed that for a given nitrogen application rate, streams with high $\delta^{15}N$ had high nitrate concentrations, a pattern that is consistent with nitrogen loss by denitrification.

Benthic algae $\delta^{13}C$ values had an increasing trend from the upper to the lower reaches throughout the study sites and periods (Figure 7.1). The high $\delta^{13}C$ values found in the benthic algae downstream (site E3) could probably be attributed to the influence of $\delta^{13}C$ sources of marine origin, marsh grass detritus and dissolved inorganic carbon in the

Kowie Estuary (Antonio and Richoux, 2014; Bergamino et al., 2014). Ishikawa et al. (2012) showed that phytobenthos $\delta^{13}C$ in freshwater systems were high under low canopy cover and high-light conditions as it improved growth and this might explain the differences observed in benthic algae $\delta^{13}C$ values within the freshwater sections of our study.

The $\delta^{13}C$ values of detritus throughout the sites and study periods exhibited a wide range (Figure 7.2), mostly due to variable terrestrial leaf contributions. The relatively low to moderate detritus $\delta^{13}C$ values upstream (sites F1 and F2) indicated large contributions of terrestrial leaves (Figures 7.1 and 7.3). The low $\delta^{13}C$ values of organic matter sources in the upstream regions could be due to increased input and uptake of ^{13}C-depleted organic matter from terrestrial plants (Usui et al., 2006; Jha and Masao, 2013).

According to Antonio et al. (2012) and Fu et al. (2014), estuarine SPM with low C:N ratios (<8) indicate phytoplankton of marine origin, whereas elevated C:N (>8) indicate increased freshwater and/or terrestrial leaf contributions. The C:N ratios and $\delta^{13}C$ values for SPM in the Kowie system showed an increase of marine and a decrease of riverine and terrestrial origin organic matter from the upper to the lower reaches of the system, a pattern typical of a transitional environment (e.g. Usui et al., 2006; Barros et al., 2010; Berto et al., 2013; Jha and Masao, 2013; Antonio and Richoux, 2014; Bergamino et al., 2014; Fu et al., 2014). The Kowie Estuary was marine dominated in early and late spring, as evident from the predominance of marine algae (Dalu et al., 2014b). In summer and winter, the low $\delta^{15}N$ values of SPM in the system, particularly the estuary, compared to the high C:N ratios in SPM suggested an increase in terrestrial–riverine organic matter inputs within the estuary compared to other seasons (Usui et al., 2006; Berto et al., 2013; Jha and Masao, 2013). These findings were similar to those of Antonio and Richoux (2014), who showed that the Kowie Estuary was generally riverine and/or terrestrial dominated in winter and marine dominated from

spring to summer in 2011 to 2012. In 2012 to 2013, higher values of $\delta^{13}C$ in SPM in the Kowie system during summer compared to winter, and an increase in SPM C:N ratios in the same period, could likely be explained by the sedimentary re–suspension induced by strong events of winds and high water velocity (e.g. Jha and Masao, 2013). These findings are supported by the high $\delta^{13}C$ values determined in the sediment organic matter in the Kowie Estuary in 2012 and 2013 (Bergamino et al., 2014). The mean $\delta^{13}C$ signatures of benthic algae in each site were low during periods of high flow (i.e. late spring), that is after the October–November floods (Figure 7.1; Dalu et al., 2014a, b). Discharge increased from 0.14 m^3 s^{-1} before the floods to a peak of 699.14 m^3 s^{-1} (flood period) on October 21 and decreased to 1.98 m^3 s^{-1} in early spring after the floods (Dalu et al., 2014a). In the river section of the Kowie system, the SPM from the upper reach sites F1 and F2 had high C:N ratios, which suggested that the system was dominated by organic matter of terrestrial origin. Seasonal shifts of SPM C:N ratios and $\delta^{15}N$ values in the Kowie system were within the range of data derived in a study by Jha and Masao (2013) in Ishikari River, who observed relatively high C:N ratios (10 to 16) and low $\delta^{15}N$ values (-1 to 4 ‰) in SPM during winter, autumn and spring compared to low C:N ratios (6 to 9). The large variations in SPM C:N (7 to 10.6) and $\delta^{13}C$ (-21.3 to -29.3 ‰) along the salinity gradient (lower reaches sites E1 to E3) within the Kowie Estuary indicated the replacement of terrestrial particulate organic matter in the plume by *in situ* production of marine phytoplankton.

In the middle reaches of Kowie system (sites F3–F5), the C:N ratios of all organic matter sources were low (5 to 11) possibly due to pollution, leaching, autolysis and microbial decomposition (see Thornton and McManus, 1994; Andrews et al., 1998). The C:N ratios of SPM were variable within the estuary section, with site E2 having high C:N values (>8) throughout the study periods. The high $\delta^{15}N$ values (2 to 7 ‰) observed by Jha and Masao (2013) in summer indicated a seasonal shift in the particulate organic matter source contribution in Ishikari River, Japan. Inland or offshore transports of

organic material during the summer (wet season) were expected to be controlled by freshwater discharge.

SIAR models, chl-*a* concentration and the riverine continuum concept

SIAR models indicated that terrestrial leaf contributions to bulk detritus generally increased in the downstream direction (upper to lower reaches), whereas aquatic macrophyte contributions decreased from the upper to the lower reaches (Figure 7.3). Overall, the detritus was dominated by aquatic macrophytes and benthic algae in the upper reaches in all seasons but there were differences in the aquatic macrophyte and benthic algae contributions to the detritus in the different seasons, with detritus in the lower reaches dominated by terrestrial plants. The seasonal differences observed for aquatic macrophytes and benthic algae could be related to seasonal and site changes in patterns of physico-chemical factors within the Kowie system (Dalu et al., 2014b, c). These trends contrast with the RCC, which predicts that terrestrial inputs are greater in headwater regions and autochthonous production and hence autochthonous inputs into the mixed organic pools are increasingly important in downstream regions where the canopy cover is lower. As such, the RCC predictions are not applicable to bulk detritus in the small temperate system that we have studied. A Pearson correlation indicated no relationship ($p > 0.5$) between terrestrial leaf contributions to detritus and rainfall, thereby questioning the importance of rainfall in linking the land and water regarding the detrital pool. The absence of any significant correlations between rainfall data and the spm/detritus may reflect the resilience of the system in that although rainfall may contribute to changes in the spm/detritus; this effect is very limited temporally. A study conducted in parallel to this investigation demonstrated that flow rates within the river returned to pre-rain fall rates within one week (see Bergamino *et al.,* 2014 and Dalu *et al.,* 2014a (Chapter 3) for rainfall trends). The absence in any correlations likely reflects the lag phase between the rainfall events and the actual sampling. We sampled at the beginning of early spring (1 to 7 September) and late spring (27 November to 12 December), with the floods occurring in mid-spring (16 to 21 October).

The large influence of benthic algae and aquatic macrophytes in the bulk detritus in the upstream direction indicated that the growth of aquatic macrophytes and benthic algae colonizing the detritus was not curtailed by the canopy cover in the headwater regions of the system particularly in late spring and summer when ambient light levels were relatively high. We expect that as the stream width widened in the downstream direction, the inputs of aquatic macrophytes and benthic algae were increasingly 'diluted' by high terrestrial leaf inputs along the entire system (Table 7.1). In turn, differential decomposition rates of these different organic components, with terrestrial leaves likely having the slowest decomposition rates overall, affected their relative proportions in the bulk detritus. Furthermore, fast-growing bacteria could feasibly be outcompeting slower-growing benthic algae colonizing the detritus as the material moves downstream and experiences rapid changes in the physical environment, particularly salinity once the material reaches the first estuarine site E1.

SIAR models indicated that the contributions of aquatic macrophytes to the SPM were greatest in the upper and lower reaches and smaller in the middle reaches (particularly site F4) where benthic algae showed the greatest influence (Figure 7.3). The contributions of terrestrial plants to the SPM increased in the downstream direction during early and late spring, but were relatively equivalent among sites during summer and winter. Unlike the bulk detritus, the enhanced contributions of benthic algae to the SPM in the middle reaches supported the RCC, which presumes that the mid reaches of a system receive the largest variety of energy inputs and host the most biological diversity (Vannote et al., 1980). Research on streams has indicated that organisms in the middle reaches are more dependent on in-stream primary production, whereas fine particulate organic matter of terrestrial origin is the principal carbon source in downstream reaches, with much of it derived from upstream processing (Vannote et al., 1980; Statzner and Higler, 1985; Carpenter et al., 2005). The midstream of the Kowie system showed the greatest phytoplankton biodiversity and highest chl-*a* concentration

(Dalu et al., 2014b), thereby supporting the RCC, but we were unable to include a pure phytoplankton signature in the SIAR models so the influence of this source on the suspended particulate pools remains to be investigated. Studies by Dalu et al. (2014a, b) have indicated that the benthic algal communities in the middle reaches made up large proportions of the phytoplankton community due to re-suspension processes, so phytoplankton contributions to the SPM may mirror the benthic algal contributions.

The contributions of terrestrial plants to the SPM were quite different from those to the bulk detritus, and they showed different longitudinal trends (Figure 7.3). The large influence of terrestrial leaves in the bulk detritus but not the SPM in the most downstream sites likely reflected the limitations of micro-organisms in breaking down that bulk material to smaller suspended particles. Habitat connectivity between water and land is particularly interesting to consider in light of the contrasting contributions of terrestrial plants to the detritus and SPM categories. If one considers the bulk detritus alone, the two habitats can be considered highly coupled owing to the very large proportions of terrestrial plants to the mixed pool particularly in the lower reaches of the system. However, if one considers the SPM alone, the terrestrial contributions are much smaller, hence reflecting a decreased level of connectivity between the habitats. The next step in examining degree of connectivity in such a system is to evaluate the assimilation of different components by the consumers in the river. Such a study may reveal completely different levels of connectivity again as a result of food selectivity by consumers that are variability abundant at different locations throughout the year.

In conclusion, our first hypothesis that the organic matter fuelling the suspended mixtures varies along the continuum, with the middle reaches of the system (i.e. the lower river) dominated by autochthonous benthic algae material and the upper reaches by allochthonous plant material was partially supported. Essentially, the influence of benthic algae in SPM followed our predictions, but terrestrial plants did not dominate the SPM (or the bulk detritus) in the upper river. The second hypothesis that the relative

contributions of different sources change over time was supported by the SIAR output, although these changes were complex owing to the dynamic nature of a river from head to mouth and over time. The results from the present study suggest that a combination of land use (anthropogenic) patterns, hydro- geomorphology structures and riparian vegetation changes may be contributing factors of the variations in the river–estuary continuum in organic carbon parameters. Organic matter pools can vary greatly from one site to the next within an aquatic system, and also over time, and further research will help to illuminate the factors that affect these changes and, in turn, how these changes can affect food webs. Our study provides a basis for investigating potential food web variability at a regional level based on shifts in the sources of organic materials available at any given time or place.

CHAPTER 8

GENERAL CONCLUSIONS AND SYNTHESIS

Plate 8. View of the main channel site F5 showing aquatic and terrestrial (riparian) vegetation interaction within the Kowie system. Photo by Tatenda Dalu

General introduction

A large part of South Africa has a semi-arid climate, characterised by relatively low average rainfall (497 mm per year compared to 860 mm in the rest of the word). Currently approximately 98 % of all the available freshwater resources in South Africa are allocated for use and consumption (Blignaut et al., 2009). This has contributed to the majority of river catchments within southern African being highly modified and ecologically threatened (Blignaut et al., 2009; Nel and Driver, 2012). The reduction in freshwater flow in rivers has been associated with changes in the hydrodynamics and physico-chemical variables within these systems. From a biological perspective, reduced freshwater inflow contributes to a loss of habitat and a decrease in the macronutrient concentrations necessary to sustain primary producers' growth (Blignaut et al., 2009; Nel and Driver, 2012; Dallas and Rivers–Moore, 2014). Changes at the base of the food web directly impact the ecosystem functioning in terms of both species composition or and trophic interactions up to the top predators. The limited freshwater outflow from rivers has also affected near-shore marine ecosystem structure. Studies suggest that freshwater outflow into the marine environment represents a significant source of macronutrients and carbon and plays an important role in determining the plankton community structure and trophic interactions in the marine environment adjacent to estuaries (Vorwerk and Froneman, 2009).

The production of pollutants, insufficient or poorly maintained sewage infrastructure and poor land use management practices and biological invasions have largely contributed to the decline in water quality in southern African riverine and estuarine systems (Mead et al., 2013). In addition to representing a potential threat to human health, the presence of these human derived compounds may significantly alter ecosystem structure and functioning (Mead et al., 2013). Aquatic ecosystems provide a number of invaluable ecosystems services for humans. The sustainability of these ecosystems is invariability linked to ecosystem health and integrity. The majority of previous studies conducted on the biology of South Africa aquatic ecosystems have been descriptive in nature. Thus,

little is known about the factors controlling ecosystem functioning and the responses of these ecosystems to environmental change or perturbations. Predicting the response of aquatic ecosystems to perturbations (e.g. freshwater abstraction, eutrophication, over-exploitation, loss of habitat and biological invasions) and global climate change is critically dependent on understanding the various physical and biological factors that structure these systems. Importantly, the functioning of these systems is often linked to the interconnectivity between freshwater, estuarine and marine ecosystems.

General conclusions

The Kowie system is largely considered to be ecologically pristine and in a good ecological condition (DWA, 2013). Like many other systems within the same geographic region, however, the Kowie River is increasingly becoming impacted by different land use patterns, water pollution, flow modifications, destruction or degradation of habitats and invasion by exotic species (Dallas and Rivers–Moore, 2014). A detailed assessment of spatio-temporal variation and development of phytoplankton and phytobenthos community structure along the river-estuary continuum is presented. The importance of the various physico-chemical factors in structuring the phytobenthos is discussed in detail in Chapters 3 to 6. One of the key findings of this study is that of the effect of flood disturbances in the structuring phytoplankton and phytobenthos communities within the Kowie River system. The thesis provided a better understanding and contributed significantly to the body of literature on the community structure and development of phytoplankton and phytobenthos communities along a river–estuary continuum using a combination of microscopic and stable isotope analyses. A solid foundation for future studies and how phytoplankton and phytobenthos communities might affect food web structure is provided in Chapter 7.

To author's knowledge, this is the first comprehensive study on the phytobenthos and phytoplankton to be carried in the Kowie system since the initial study by Giffen (1970). Although several other studies have e.g. Kruger and Strydom (2011), Antonio and

Richoux (2014) and Bergamino et al. (2014) been conducted within the system, these studies have largely been restricted to the estuarine region of the Kowie River, specifically the chl–*a* concentrations. Giffen (1970) identified 286 species within the Kowie River system which is substantially more than the 178 recorded during the present investigation. The higher number of species recorded by Giffen (1970) may result from the fact that the latter author also sampled lagoons and small reservoirs along the channel which have been documented to have distinct phytoplankton and phytobenthos species (Wu et al., 2011). Phytoplankton and phytobenthos do not predominate in lotic systems since they can easily be swept away into areas unfavourable for growth (Song, 2007; Wu et al., 2011). Attached algae may also suffer since moving water can carry soil particles in suspension, blocking out light (Bere, 2011; Song, 2007). Alternatively, it is possible that the reduced number of diatom species recorded during the present study may reflect increased freshwater abstraction and subsequent habitat loss since the 1970's within the Kowie River system.

In agreement with studies conducted elsewhere in the northern (e.g. Phlips et al., 2000; Claquin et al., 2006; Wu et al., 2011; Pirsoo et al., 2008) and southern hemisphere (e.g. Pieterse and van Zyl, 1988; Ezekiel et al., 2011; Bere, 2011; Bere and Tundisi, 2012; Fonge et al., 2013), physico–chemical factors such as water flow, pH, ammonium, conductivity, salinity, phosphates, nitrates, channel width, water depth and canopy cover were identified as important variables in structuring phytoplankton and phytobenthos communities within the Kowie River system (Chapter 3–6). The nutrient enriched water derived from the tributaries JP de Wet Steyn and Bloukrans Rivers, resulted in increased phytoplankton abundances and Chl-*a* concentrations in the middle reach of the Kowie River system. This result is again consistent with the published literature. For example, Ewart-Smith (2012) observed a peak in phytoplankton biomass at those sites which were enriched in macronutrient concentrations in the Western Cape, South Africa. It is important to note, however, that elevated dissolved nutrient concentrations measured at the time of sampling, did not always correspond to an

increase in phytoplankton biomass. Dissolved nutrient concentrations in the water column reflect the residual concentration after removal by periphyton or growth state (Biggs, 2000; Ewart-Smith, 2012). Therefore, the ambient nutrient concentrations do not necessarily reflect the trophic potential of the system, but instead reflect what is left after the periphyton has removed what it needs for growth and production (Biggs, 2000). These findings therefore, suggest that phytoplankton and phytobenthos peak biomass may provide a better indicator of trophic status in lotic systems than water column nutrient concentrations.

One of the objectives of this study was to better understand the growth and development of phytobenthos along the river-estuary continuum. A key finding of the current investigation was that the phytobenthos communities preferentially grow and develop in high abundances on specific substrates (Chapter 4). Barbiero (2000), Lane (2003) and Bere and Tundisi (2012) found high taxa richness and diversity in natural substrates compared to artificial substrates. These findings suggest that future studies assessing the spatial and temporal patterns in phytobenthos communities within rivers need to consider the substrate type when establishing their monitoring protocols. The study further highlighted that the phytoplankton communities were largely comprised of re-suspended phytobenthos diatom (Chapter 5) as highlighted in other studies by Wu et al. (2011) in lowland Germany. Moreover, the phytoplankton and phytobenthos communities were highly adapted to the specific region of the river system as indicated by the high abundances of the relatively large *Fragilaria* sp., *Pinnularia* sp. and *Gomphonema* sp. upstream and the small sized species downstream such as *Achnanthes* sp., *Achnanthidium* sp., *Nitzschia* sp. and *Navicula* sp. mostly likely due to water flow (Chapters 2 to 6). Large sized phytobenthos species grow and develop better at low water flow while the small sized phytobenthos seem to be more adapted to fast water flow as they can withstand the water current (Devercelli, 2006; Pirsoo et al., 2008). Additionally, the change in season was important in triggering shifts in phytoplankton species composition (Chapters 4–6; Devercelli, 2006; Pirsoo et al., 2008;

Wu et al., 2011; Fonge et al., 2013). The flooding events that occurred in October-November 2012 contributed to a reduction in nutrient inputs and increased turbidity within the Kowie which likely coincided with a the significant reduction phytobenthos growth. The flood event significantly scoured the channel further contributing to changes in the phytobenthos and loss of biomass (Chapter 6).

The use of tracer techniques, such as stable isotopes enabled the differentiation of different phytobenthos communities along the river-estuary continuum (Chapter 4). The enriched δ^{15}N isotope values of phytobenthos provided first evidence that the upper reaches of the Kowie River were nutrient enriched most likely derived from the agricultural activities and poorly maintained sewage systems within the catchment area. Toda et al. (2002) showed that the periphyton δ^{15}N values can be excellent indicators of nitrogen pollution derived from anthropogenic activities including livestock waste, synthetic fertilisers and sewage containing more enriched δ^{15}N values (Anderson and Cabana, 2006; Ning et al., 2013). From a South African perspective this study provides a platform for additional studies to examine the relationships between land use patterns, nutrient enrichment of inland waters and phytobenthos communities. With little information available on the use of phytobenthos and stable isotopes, an understanding of how useful this method will be important for water and environmental management purposes is required. Future studies should focus on periphyton δ^{15}N values and what the riparian zone and watershed land use contribute to aquatic ecosystems in terms of nitrogen. Such data would allow precise management of nutrients in river systems.

The occurrence and magnitude of flood events within a given period is relatively unpredictable (Ewart-Smith, 2012). Larned (2009) suggested that in contrast to mobile organisms, the effects of flooding on the periphyton communities are independent of their predictability. The October–November floods in 2012 contributed to a significant shift in the phytoplankton and phytobenthos communities' community structure and composition (Chapters 3–6). The episodic flood event resulted in the entire removal of

all the periphyton communities (Chapters 3 and 6) at a rate in excess of the accrual or recruitment rate (Larned, 2010; Ewart-Smith, 2012). This shift also coincided with a decrease in total chl-*a* concentrations within the system. Cardinale et al. (2006), Awade et al. (2012) and Villard and Metzger (2014) highlighted that habitats are species-specific concepts, with species occurrence and abundance increasing with habitat volume, each fragment becoming more accessible as habitat becomes increasingly connected. Although the flood event coincided with a dramatic change in the channel morphology of likely resulting in more habitats opening in the Kowie system, (Chapter 6), the periphyton communities did not recover to pre-floods biomass levels. This result is in in contrast with studies conducted elsewhere in the world (e.g. Ewart-Smith, 2012; Hadley and Betts, 2012) that showed that the immigrants' pool increases with an increase habitat availability. The indirect effects of habitat availability on functional connectivity and matrix and/or edge effects still require detailed scientific investigations, particularly in freshwater ecosystems (Villard and Metzger, 2014). Cardinale et al. (2006) showed that in streams where the frequency of floods is low relative to the rate of biomass production, assemblages were dominated by stalked algal species that are especially prone to scour and have characteristics that imply low resilience such as slow reproduction and/or poor dispersal, but which tend to be competitively superior because they grow upright above the periphyton canopy to sequester nutrients and light. This was the case with the Kowie system where, *Fragilaria* spp. dominated with other stalked algal species and there were easily scoured by the October–November 2012 floods thereby after the system productivity.

The δ^{15}N and δ^{13}C isotope values increased along the continuum from the upper to the lower reaches. The relative organic matter contribution calculated using a SIAR (Stable isotope analysis in R) mixing model of two different organic matter sources (SPM and detritus) found that phytobenthos and terrestrial organic matter were the most important contributors to the SPM and detritus, respectively. The C:N ratios also revealed that that SPM in the Kowie Estuary was made up of organic matter derived from a variety

of sources including riverine, marine and terrestrial systems (Antonio and Richoux, 2014; Bergamino et al., 2014). The spatio-temporal variation in the allochthonous organic matter sources composition within the system was linked to hydrodynamics (flow) and physical chemical factors. This result is in agreement with studies conducted elsewhere in the world (e.g. Liu et al., 2007; Sarma et al., 2012; Jha and Masao, 2013, Lu et al., 2014a, b; Pingram et al., 2014) and highlights both the complexity and interconnectivity of these river-estuary systems. Additionally, this study provides baseline information on how the aquatic organic matter carbon pools in riverine systems may change over spatio-temporal scales which is critical when evaluating the organic carbon cycles in lotic aquatic systems (Sarma et al., 2012; Jha and Masao, 2013; Pingram et al., 2014). At national and regional level, this is the first study to provide a first comprehensive assessment of possible food web changes at basal (primary) sources along a river-estuary continuum.

Global climate change

Over the past century, global temperatures have increased by a mean of ~0.6 ºC and climate change models depending on emission scenarios, predict a further increase in temperature of 1.4–5.8 ºC by the year 2100 (Houghton et al., 2001). The increase in global temperatures is likely to be associated with disruptions in global earth weather systems including changes in rainfall patterns and increased temperature extremes (Houghton et al., 2001; Mead et al. 2013). Such changes will have far reaching effects on biotic interactions, diversity and ecosystem functioning of aquatic ecosystems (Domis et al., 2007). Global climate models suggest that global warming along the south-eastern seaboard of South Africa is likely to be associated with a rise in water temperatures an increased frequency of occurrence of extreme weather events (e.g. coastal storms and flooding events) and sea level rises (Mead et al., 2013). Results of the current study suggest that the increased frequency of occurrence of extreme flood events is likely to have significant impacts on the phytobenthos and phytoplankton community structure within the system. The expected changes in the flow rates within

the river following extreme rainfall events is also likely to have far reaching consequences for the sources and composition of the SPM and detritus within the river-estuary system. The impact of these changes on the biology of the system remains unclear. Moreover, inter-annual variations in phytoplankton and phytobenthos composition and biomass within the Kowie system are anticipated to reflect changes water temperature (Domis et al., 2007; Ndebele-Murisa et al., 2014). Thus, we will expect to observe increased abundances and biomass of cyanobacteria which are favored by climate change as they respond more strongly compared to diatoms and to lesser extent green algae (Weyhenmeyer, 2001; Domis et al., 2007; Ndebele-Murisa et al., 2014). The implications of these changes on the ecosystem structure and functioning in response to global climate change are currently unknown.

Future studies

The present study has clearly demonstrated the importance of physico-chemical variables and hydrology (i.e. flood event) in determining the spatial and temporal patterns in the phytobenthos and phytoplankton community structure and the composition of the SPM within the Kowie River system. The observed changes were consistent with the River Continuum Concept as proposed by Vannote et al. (1980) and broadly in agreement with the published literature for both northern and southern hemisphere systems (Winterbourn et al., 1984; Thorp and Delong, 1994; Hall et al., 2001, Lau et al., 2009). These data have provided valuable insights into the various factors that structure phytoplankton communities within temperate southern African rivers. There is an urgent need for further such studies in other regions of South Africa, particularly in the Western Cape region where up to 85 % of all river systems are considered as threatened or endangered (Nel and Driver, 2012).

The effects of predation/herbivory, the so called “top down effects”, on lower trophic levels have received much attention, highlighting the often far reaching consequences of top-down pressure under specific conditions (Dye and Lasiak, 1986; Strong, 1992;

Fey et al. 2009; Pillay et al., 2009; Alaxender et al., 2012; Dick et al., 2014). To the author's knowledge, no such studies have been undertaken in southern African riverine systems. Future studies should begin to assess the role of biological processes such as grazing by invertebrates or the role of ecosystem engineers in accounting for some of the observed spatial and temporal patterns in the phytoplankton and phytobenthos community structure within the river system. This could likely be achieved by employing inclusion/exclusion experiments which would allow for the manipulation of predator numbers/guilds within different treatments.

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Plate 9. Grey clay tile substrate structure after its removal from the Kowie River following a 28 day incubation period. Photo by Tatenda Dalu

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