The use of Periphyton and Macro-invertebrates and their susceptibility to changes in river flow characteristics and nutrient composition as an indicator of river health.

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by

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ABSTRACT

Freshwater systems are a valuable resource under increasing threat due to pollution from activities such as agriculture, industry, mining and domestic use which can pose a risk to human and animal health and may lead to eutrophication. In South Africa, river ecosystem management has shifted from the improvement of water quality to that of the creation of ecological reserves, ecological health and the improvement of biological integrity. This shift has allowed for the increased use of bio-indicators to determine ecosystem health. Macro-invertebrates, riparian vegetation and fish have been used in the suite of bio-monitoring mechanisms and the setting of environmental reserves. However, there is an increasing need to include periphyton as a tool in river ecosystem monitoring due to their absorptive nature and ability to indicate change environmental conditions.

In South Africa research suggesting algae as bio-indicators has been primary based on the use of diatoms as a bio- monitoring tool. This study aims to determine patterns and trends in periphyton communities in the summer rainfall region of KwaZulu-Natal, South Africa. This is achieved through five sampling sites aimed at determining the relationship between changes in nutrient and flow regimes on periphyton communities. Two sites on the Msunduzi River are comparable as they have similar flow but different nutrient levels, whilst on the Umgeni River the two sites are comparable as they have differences in flow regimes with similar nutrient conditions. The fifth site on the Hlatikhulu River, Kamberg, is used as a reference site. Sampling occurred over the period June 2014 to June 2015 on a monthly basis to collect algal and invertebrate samples and physico-chemical data.

Trends and relationships between physico-chemical and algal biomass were evident. Trends indicated the role rainfall played in increasing river depth and velocity which in turn influenced algal biomass growth and species composition and the effect that seasonality changes had on periphyton communities. Peaks in algal biomass was as a result of increases in nutrients within a particular system while decreases in algal biomass occurred due to an increase in invertebrate grazers. Sloughing events resulted due to increases in flow and velocity. General trends at all five sites showed peaks in algal biomass in early summer and a lesser algal biomass peak in early autumn. This research suggests that if better understanding of periphyton patterns and trends are established, periphyton can be used as an important bio-monitoring tool and aid in the creating and setting of ecological reserves.

Declaration

I ......**SAMIKSHA SINGH**.. declare that

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

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The harder the struggle, the more glorious the triumph

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CHAPTER ONE

Introduction

1.1 Introduction

In South Africa freshwater resources are scarce and difficult to manage and are threatened by changes in flow regimes and nutrient levels (Taylor *et al*., 2007). Large volumes of water are transferred in and out of the country to meet demands for domestic industrial and agricultural use, which results in increased amounts of effluent that are returned to rivers and streams (Taylor *et al*., 2007). The increase in drought conditions due to climate change has placed increasing pressure on our freshwater resources whilst attempting to fulfil the water demands of the population. This degrading ecosystem has associated negative effects on other sectors such as economic and social development, biodiversity and river ecosystem health.

The pollutants that contaminate river ecosystems are predominantly introduced into the system through anthropogenic dominance (Hart *et al*., 2013). The effects of anthropogenic pollution can result in changes in aquatic species density and composition (Rader *et al*., 2008). Therefore it is important to develop effective natural resource management strategies to combat and decrease the negative consequences of pollution in these fragile and vital river ecosystems (Venter *et al*., 2003; Molinos and Donohue, 2010).

Recent efforts have been made to effectively manage river ecosystems in a sustainable manner and have shifted from a general improvement of water quality to that of the creation of ecological reserves, ecological health and an improvement in biological integrity in South Africa (Kleynhans *et al*., 2005; Ewart-Smith and King, 2012). Ecological reserves refer to the amount of water needed to sustain aquatic health whilst ecological health is defined as the societal norms and goals identified which relate to sustained ecological conditions (Chessman *et al*., 1999) and biological integrity is defined as the ability to sustain and support a biological system that is balanced, integrated and adaptive taking into account the process and elements found within that system (Karr, 1995). Ecosystem management in South Africa is achieved through the use of bio-monitoring (Ewart-Smith and King, 2012).

These monitoring techniques include; gauging stations, water temperature loggers and water quality monitoring programmes providing physical and chemical data including measurements such as algal biomass, invertebrate abundance and chemical concentrations (Boulêtreau *et al*., 2008).

1.2 The use of periphyton in biological monitoring.

Bio-monitoring in South Africa is an integrated process. Methods such as whole effluent toxicity (WET), which is a tool used to evaluate effluent to ensure that it is not too toxic for discharge into rivers, can be used. The second approach to bio-monitoring is under the South African river health programme (RHP), which is used to assess river health in relation to anthropogenic factors making use of macro-invertebrates, fish and riparian vegetation as bio-indicators. Furthermore, the use of diatoms has recently been tested throughout South Africa as biological monitoring indices (De la Rey *et al*., 2004; Taylor *et al*., 2007).

Macro-invertebrates, riparian vegetation and fish have been used in the suite of bio-monitoring mechanism and the setting of ecological reserves in South Africa (Ewart-Smith and King, 2012). In recent years bio-monitoring has included benthic algae or periphyton in setting ecological reserves and being applied as bio-monitoring tools (Chessman *et al*., 1999; Grown and Growns, 2001; Downes *et al*., 2003; Chester and Norris, 2006). These periphyton communities are comprised of bacteria, fungi, algae including micro-algae and filamentous algae, meiofauna and protozoa, usually attached to substrates of cobbles or stones and are also known as biofilms (Stevenson, 1996; Villanueva and Modenutti, 2004; Ryder, 2004; Hodoki, 2005; Peters *et al*., 2007; Hillebrand, 2009; Dorigo *et al*., 2010; Larned, 2010). Benthic algae are usually the largest component in the periphyton community and as such the terms benthic algae and periphyton are often used interchangeably (Ewart-Smith and King 2012).

In unshaded, open canopied rivers periphyton in their role as primary producers convert dissolved nutrients into food for organisms at higher trophic levels (Biggs, 1996). These primary producers are good indicators of changing environmental conditions as a consequence of their rapid life cycles and wide geographic and temporal distributions (Matthaei *et al*., 2003; Higgens *et al*., 2008; Lear *et al*., 2008;Yang *et al*., 2009; Hart *et al*., 2013). These communities play an important role as the link between the physical, chemical and biotic components of a river ecosystem (Lowe and Pan, 1996). Due to this pivotal ecological role, changes in periphyton biomass and community structure especially due to changes in flow regimes and nutrient alterations have a profound effect on the river ecosystem (Biggs and Thomsen, 1995; Biggs and Smith, 2002; Uehlinger *et al*., 2003; Bergey and Resh, 2006). These factors suggest that periphyton communities are potentially an important monitoring tool which could lead to more efficient management of water resources. Thus, it is important to determine the factors that control periphyton dynamics in particular in terms of nutrient enrichment and flow regimes (Villeneuve *et al*., 2011).

Research in South Africa on the use of periphyton as a bio-monitoring tool has been limited, with river research in terms of primary producers being dominated by diatoms as indicators of pollution (De la Rey *et al.*, 2004; Taylor *et al.*, 2007). The use of periphyton as a bio-monitoring tool is still difficult as the relationship between biotic and abiotic factors, biotic responses to regulatory changes in ecosystems and associated patterns in spatial and temporal dynamics in river ecosystems have to be understood (Stevenson, 1997). Recent work by Ewart-Smith and King (2012) has focused primarily on determining and understanding patterns in periphyton community structure and biomass in the winter rainfall region of the south Western Cape. Factors that drive patterns of periphyton communities are important to identify because of the ecological importance of periphyton communities in river management and ecological monitoring (Ewart-Smith and King, 2012).This research project will serve as a comparison to the research conducted in the winter rainfall region of the Western Cape by Ewart- Smith and King (2012). This research was conducted in the summer rainfall region of KwaZulu-Natal using periphyton community composition and biomass to determine the ecological condition of rivers at different flow and nutrient levels.

1.3 Research aim and objectives

To determine the role of periphyton communities as indicators of the ecological condition of rivers by determining periphyton community structure and biomass, taking into consideration their links to environmental variables and relationship with macro-invertebrates.

The objectives are:

- To measure flow and water quality at selected sites to determine changes in these variables over a seasonal and temporal time scale to allow for patterns and trends in these variables to be observed.
- To collect, identify and determine biomass of periphyton communities in areas of altered flow and nutrient levels as these altered conditions will provide an indication of periphyton dynamics
- To collect and calculate abundance and biomass of macro-invertebrates to indicate the relationship between grazer density and periphyton biomass.

1.4 Hypotheses

- Periphyton biomass is related to the seasonal patterns in selected rivers of KwaZulu-Natal. Biomass levels will change according to seasons.
- Periphyton community composition will change over annual cycles with specific communities observed in winter, spring, summer and autumn.
- Peak periphyton biomass will be evident in areas of greater nutrient enrichment regardless of flow

- Periphyton community composition and biomass will differ significantly between rivers with different flow and nutrient composition.
- Grazing pressure as a result of aquatic invertebrates will be greatest with the highest invertebrate abundance.
- Differences in invertebrate grazer abundance, density and biomass will occur between rivers of different flow and nutrient conditions.

1.5 Structure of thesis

The thesis is presented as five chapters. A review of relevant literature is presented in chapter two including understanding the role of periphyton in ecosystems, the effects of flow and nutrient regimes on these periphyton communities, invertebrate grazer- periphyton biomass relationships, the spatial and temporal distribution of periphyton communities and the use of periphyton as biological monitoring indicators. Chapter three consists of the methods section including; site descriptions, data acquisition and data analysis methods. The analysis of results section is presented in chapter four determining the effect that nutrients, flow and invertebrate grazers have on periphyton communities. Chapter five includes the discussion and conclusion section of the thesis determining the key drivers that influence periphyton communities. This research project is integrated and adapted for a greater Water Research Commission project, WRC project K5/2351, KSA 2: Water-linked ecosystems thrust 2: Ecosystem management and utilization, The development and application of periphyton as indicators of flow and nutrient alterations for the management of water resources in South Africa.

CHAPTER TWO

Literature Review

2.1 Introduction

Increased pollution, drought conditions and the transfer of large volumes of water into and out of South Africa have placed our valuable freshwater resources in a state of jeopardy (Venter *et al*., 2003; Taylor *et al*., 2007). Accumulation of inorganic and organic pollution may result in eutrophication affecting the quality of a water source and posing a risk to human and animal health (Biggs, 2000; Venter *et al*., 2003; Lear *et al*., 2008; Cardinale, 2011). In South Africa a transformation has occurred from simply determining the quality of a water source to identifying the ecological health of a river, understanding biological integrity of the ecosystem and the creation of ecological reserves (Kleynhans *et al*., 2005; Ewart-Smith and King, 2012).

Ecosystem management in South Africa is predominantly concerned with the use of macro-invertebrates, fish and riparian vegetation in the suite of bio-monitoring mechanisms (Kleynhans *et al*., 2005; Ewart-Smith and King, 2012). The use of periphyton composed of algae, protazo, bacteria and fungi usually attached to a substrate (Stevenson, 1996; Villanueva and Modenutti, 2004; Ryder, 2004; Hodoki, 2005; Peters *et al*., 2007; Hillebrand, 2009; Dorigo *et al*., 2010; Larned, 2010), is limited to the use of diatoms as indicators of pollution (De la Rey *et al*., 2004; Taylor *et al*., 2007).

The use of diatoms has resulted in the identification of endemic diatom species indicating a need for a unique South African diatom index for water quality monitoring (Taylor *et al*., 2007b). In unshaded, open canopied rivers periphyton in their role as primary producers convert dissolved nutrients into food for organisms at higher trophic levels (Biggs, 1996; Larned, 2010). They are reliable indicators of changing environmental conditions because of their rapid life cycles and wide geographic and temporal distributions (Matthaei *et al*., 2003; Higgins et al., 2008; Lear et al., 2008; Yang *et al*., 2009; Hart *et al*., 2013), are good indicators of point source nutrients as they are attached to a substrate and act as nutrient recycling mechanisms (Montuelle *et al*., 2010; Murdock, 2013). Periphyton communities affect water chemistry, hydraulics, food availability and habitat conditions in the river ecosystem (Larned, 2010), indicating the need and importance of incorporating periphyton in the suite of biomonitoring tools especially in South Africa.

2.2 Periphyton

2.2.1 Introduction

Periphyton are the dominant primary producer in river systems, providing a key step or linkage between dissolved nutrients and organisms such as invertebrates and fish at higher trophic levels (Biggs, 1996; Villanueva and Modenutti, 2004; Peters *et al*., 2007; Hillebrand, 2009; Hart *et al*., 2013). Bacteria, fungi, algae including micro-algae and filamentous algae, meiofauna and protozoa make-up periphyton communities that are usually attached to substrates of cobbles or stones and are known as biofilms (Stevenson, 1996; Villanueva and Modenutti, 2004; Hodoki, 2005; Peters et al., 2007; Hillebrand, 2009; Dorigo *et al*., 2010; Larned, 2010). The bacteria within the periphyton community are important decomposers of algal material and organic matter such as leaf litter (Hodoki, 2005).

Algae, the largest component of periphyton communities, are characterised as diverse phototrophic organisms that contain chlorophyll *a* and have unicellular reproductive systems. Algal species either benthic or phytoplanktonic, are difficult to identify and are under-estimated due to their diversity of habitats (Stevenson, 1996). Benthic algae are usually attached to substratum and consist of blue-green algae (Cynophyta), green algae (Chlorophyta), diatoms (Bacillariophyta) and red algae (Rhodophyta). Each algae can be motile or non-motile depending on the stage of succession (Stevenson, 1996). Algae can further be described as epilithon, epiphyton and epipelon, which are algae that grow on cobbles and boulders, filaments of aquatic plants or on sand grains respectively (Biggs, 2000b).

Periphyton communities differ both physiologically and morphologically resulting in different abiotic resource requirements that vary between taxa (Larned, 2010). Short-term resource availability changes result in physiological changes while long-term resource availability changes result in biomass and taxonomic changes of periphyton communities. Benthic algae are found at different water depths, photic zones, in a river ecosystem (Stevenson, 1996). Due to the short generational timespan of periphyton communities they are more susceptible to changes in environmental conditions as compared to organisms with longer generational time spans (Dorigo *et al*., 2010).

2.2.2 Role of Periphyton in Ecosystems

It is important to quantify the relationship between periphyton abundance and biomass in relation to changes in flow regimes and nutrient composition to determine and manage the effects of water abstraction and eutrophication in rivers (Hoyle *et al*., 2013). Management of these effects and the study of algal ecology are important as water is necessary to sustain life, is used in agriculture, power generation, and industrialisation and positively effects our economy (Hoyle *et al*., 2013; Hoyle *et al*., 2015). Initial studies in algal ecology focused on the description of algal communities. This later advanced into studies of the effects that abiotic factors have on algal communities and the role of periphyton communities as an indicator of ecosystem health (Larned, 2010). Improvement in technology has resulted in better understanding of periphyton dynamics and has allowed for a deeper understanding of the interaction and relationship of anthropogenic influences on periphyton communities which can in turn be used as an indicator of ecosystem health (Larned, 2010).

Understanding factors that influence periphyton communities is crucial in understanding their importance as an indicator of ecosystem health. Moderate shifts in influencers such as flow conditions, biological factors and resource availability affect periphyton community structure and function (Larned, 2010), indicating the crucial role they play in predicting major ecosystem changes (Dorigo *et al*., 2010). These communities are reliable indicators of point source nutrients entering an ecosystem as they are attached to a substrate and act as nutrient cycling mechanisms (Fairchild *et al*., 1985; Montuelle *et al*., 2010; Murdock, 2013). Periphyton communities act as early warning systems to the introduction of water contaminants (Montuelle *et al*., 2010). For example, the introduction of sewage into a water course may have little to no identifiable effect on the composition of the water column but could impact on the periphyton community at the introduction point source (Fairchild *et al*., 1985). The conversion of inorganic nutrients to an organic form enables periphyton communities to be good chemical modulators (Stevenson, 1996; Matthaei *et al*., 2003). However, periphyton can affect the water chemistry, hydraulics, food availability and habitat conditions in a river ecosystem (Larned, 2010), together with the decrease of submerged aquatic plants as periphyton communities with a high biomass can decrease the amount of light, nutrient uptake and dissolved organic carbon that reach these macrophytes (Choudhury, 2014).

In lower order streams the increase in agricultural and domestic effluent in river ecosystems can result in algal blooms leading to eutrophication which can negatively affect river ecosystems and biodiversity (Biggs, 2000; Montuelle *et al*., 2010). For example, eutrophication occurs when blue green algae such as *Oscillatoria simplicissima* blooms extensively. This algal bloom can be attributed to the increase in phosphorus and nitrogen supply entering the river ecosystem. This excessive algal growth is filamentous, produces a white soapy scum on the surface of the water and causes bad tastes and foul odours in the final purified product. This blue-green algae is said to be non-toxic however, its appearance hinders recreational activity, it producers a foul odour and has associated negative economic impacts such as the high cost of algal removal from the river which is heightened by the fact that the filamentous nature of *Oscillatoria simplicissima* clogs pipes and filters used in the water purification process (Venter *et al*., 2003).

2.3 Factors affecting periphyton biomass accrual and loss in river systems.

Benthic algal growth is dependent on a number of factors including hydrological and biotic factors water quality, topography, slope, land use and vegetation. Differences in benthic algal growth differ across river catchments due to changes in climate, geology and human activity (Biggs, 1996; Godwin *et al*., 2009). Periphyton growth is controlled by factors that influence biomass accrual such as supply of nutrients, light and temperature that control metabolic rates (Biggs, 1996; DeNicola, 1996; Stevenson *et al*., 1996; Anderson *et al*., 1999; Dodds, 2003; Hill and Dimick, 2002) (Figure 2.1). Biomass loss occurs predominantly through disturbances which are defined as a discreet event that removes organisms at a rate faster than that of biomass accrual (Larned, 2010; Stanley *et al*., 2010). Disturbances are comprised of physical disturbances such as floods and desiccation that occur over large spatial-temporal scales together with disturbances such as grazing that occur over shorter scales usually at a habitat level (Biggs, 1996) (Figure 2.1). Other disturbances include substratum instability, sediment abrasion and water velocity (Biggs, 1996).

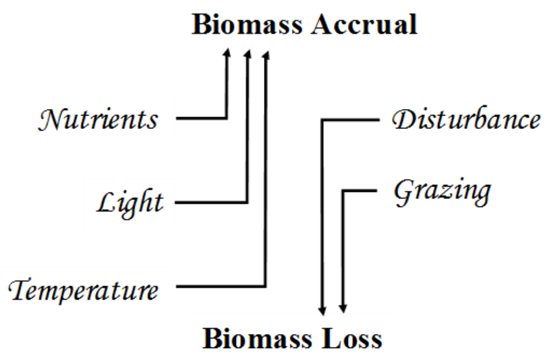

Figure 2.1 Factors effecting periphyton biomass accrual and loss in river ecosystems.

The interaction between the factors that cause biomass accrual and loss is a complex one (Larned *et al*., 2004). For example floods directly lead to biomass loss but nutrients are also moved during flood events which may result in algal growth. Flood events can remove grazers and therefore affect grazer-invertebrate relationships potentially leading to periphyton biomass accrual (Larned *et al*., 2004). This complex nature of interactions between biomass loss and accrual factors is iterated in a study by Rosemond *et al*. (2000) who indicate that when elevated nutrient levels, grazers and increased light intensities are present in an ecosystem there was no change to algal biomass however, changes in these factors such as the removal of grazers and an elevation in light intensities did increase algal biomass.

Biomass accrual can occur when a number of different factors interact. Ecosystems with low to medium flood disturbances and grazing pressure may lead to algal biomass accrual with the predominance of erect stalked diatoms or filamentous green algae. Likewise, algal biomass loss can be a result of a number of interacting factors which is particularly evident with the combined effect of medium to high flood frequencies and invertebrate grazing pressure, as a result of habitat stability being determined by flood regimes (Biggs, 1996). Other factors that affect periphyton ecology in terms of biomass and species composition include; thermal stress, ultraviolet radiation, pH and biological mechanisms such as grazing (Larned, 2010). These interactions are discussed below.

2.3.1 Nutrients

There is a link between the rate of algal growth and the concentration of nutrients in a water column (Borchardt, 1996). Within the water column nutrients are either taken up by free swimming phytoplankton or benthic algae that are attached to a substrate (Borchardt, 1996). The attached benthic algae create mats on the substrate that are many cells thick and differ physiologically and chemically according to the properties of the water column perpendicular to it (Borchardt, 1996). Nitrogen (N) as nitrates and ammonia and dissolved organic phosphorus (P) are considered to be the most important nutrients for algal production (Dodds and Welch, 2000; Dodds, 2003; Dodds, 2006; Mulholland and Webster, 2010). The varying quantities of these nutrients can result in either nutrient enrichment or nutrient limitation (Luttenton and Lowe, 2006).

Nutrient enrichment plays an important role in the increase of benthic algal biomass and taxonomic richness (Biggs and Smith, 2002). Algal growth is limited by the nutrient in least supply which can occur at all trophic levels. The growth of benthic algae is usually limited by N and P as the demand for these nutrients are often higher than availability (Borchardt, 1996; Larned, 2010; Ewart-Smith and King, 2012). Biggs and Smith (2002) showed that the greatest species richness was found in areas with the lowest nitrogen levels indicating a shift towards the dominance of nitrogen fixing taxa, which is the limiting factor. The pattern is dominant until the nitrogen fixing taxa become more abundant, causing phosphorus to become the limiting factor.

Even if there is more than one nutrient near limiting levels there is essentially only one nutrient that is limiting at a given time (Borchardt, 1996). Experiments to predict the effect that nutrients have on algal biomass has yielded varying results (Fairchild *et al*., 1985). However it was determined that the type of taxa present determines if an increase in certain nutrients will result in an increase in algal biomass. An experiment by Fairchild *et al*. (1985) showed that if a certain blue-green algal species *Rhopalodia gibba* was present in an aquatic system then algal biomass blooms could be attributed to an increase in phosphorus within that system. Other algal taxa such as *Achnanthes, Gomphonema* and *Cocconeis* are nitrogen limited.

The interaction between seasonality and nutrients in a river system can effect algal biomass. Experiments conducted by Rosemond *et al*., (2000) suggest that in spring algal growth was limited by the lack of nutrients however once nutrient levels increased algal growth became limited by macro-invertebrate grazers in the system. In summer, nutrients were not a limiting factor to algal growth. The experiments concluded that algal biomass increases are present regardless of the season but highest algal biomass estimates was experienced in spring and autumn as compared to summer in treatments with the absence of grazers (Rosemond *et al.*, 2000).

2.3.1.1 The interaction between nutrients and periphyton

Nutrient ratios in river systems have been calculated to indicate the interaction between nutrients and periphyton communities (Luttenton and Lowe, 2006). These ratios include the Resource Ratio Hypothesis (RRH), the ratio between dissolved inorganic nitrogen (DIN): Soluble reactive phosphorus (SRP) and the ratio between total nitrogen (TN): total phosphorus (TP). The RRH describes the level of displacement of neighbouring taxa by the dominant one. As the resource ratio increases there is greater displacement of neighbouring taxa (Luttenton and Lowe, 2006). However, if the amount of resources within a system is greater than the resource ratio the RRH becomes null and void. A critique of the RRH according to Luttenton and Lowe (2006) is that it cannot be used to explain population dynamics as there are no changes in the dominant algal community.

Luttenton and Lowe (2006) established that algal biomass levels decreased when there was lower nitrogen: phosphorus ratio. This could be attributed to the fact that elevated phosphorus levels result in elevated potassium levels which has growth inhibiting properties. This growth inhibition can decline with the introduction of nitrogen into the system. The DIN: SRP ratio indicates nutrient deficiency, trophic state and the interaction between nutrients and those that are limiting (Dodds, 2003). A limitation of this ratio is that the ratios are measured as unit mass/volume whereas nutrient supply is indicated as a change in unit mass/time (Dodds, 2003). TN: TP ratios estimate nutrient deficiency and trophic state and are used to indicate the nutrients used by the algal biomass and the nutrients available to be utilised by the algal biomass (Dodds, 2003). Nitrogen to phosphorus ratios (N: P) are used to determine which nutrient is limiting (Borchardt, 1996). The Redfield ratio indicates that N: P ratios greater than (20:1) are considered P-limited while N: P ratios that are less than 10:1 are N-limited (Dorigo *et al.*, 2010). Therefore, a low N: P ratio can lead to algal blooms (Venter *et al.*, 2003).

The limitation of these ratios are the ratios assumption that nutrient stock within a river system is indicative of supply, and the ratios cannot be used to determine turnover and demand rates for the nutrients. For example, nutrient content in a river system may be lower however supply is higher. The supply is said to be higher as it is utilised by biota in the system, causing an imbalance (Dodds, 2003). Dissolved inorganic nutrient levels are not a good indicator of trophic state as they cannot be correlated to algal biomass. On the other hand, the dissolved inorganic nitrogen ratios are beneficial when nutrient levels are high (Dodds, 2003).

2.3.1.2 Nutrient limitation and enrichment in river ecosystems

Nutrients such as nitrogen and phosphorus can be highly limiting as opposed to less limiting nutrients such as potassium and magnesium. A nutrient becomes limiting when the demand for that nutrient by the system is higher than supply (Larned, 2010). According to Larned (2010), periphyton communities can be limited by more than one nutrient at a time, this is in contrast to Borchardt (1996) who states that if there is more than one nutrient that is near limiting levels, it is essentially only one nutrient that is limiting at a time. Periphyton are adapted physiologically and morphologically for the uptake of nutrients. For example long filamentous algae are found in nutrient poor streams contrary to the belief that larger algae need more nutrients showing that morphological adaptations are important. Nutrient limitation occurs either under low light or high light intensities (Larned, 2010). An example from the Thames River indicates the concept of nutrient limitation. An increase in algal blooms still occurred even when phosphorus was added to the system by sewage treatment indicating that phosphorus was not a limiting nutrient (Bowes *et al*., 2012).

Aquatic foodweb structures are strongly determined by nutrient enrichment experienced in a particular system. Nutrient enrichment can occur from agricultural run-off (Montuelle *et al*., 2010), pesticides, herbicides, fungicides, insecticides and anthropogenic pollution (Dorigo *et al*., 2010). The introduction of pollutants, in particular inorganic nitrogen and phosphorus, can cause rapid periphyton community growth and can cause changes to the structure and function of river systems (Hill *et al*., 2010; Hoyle *et al*., 2013; Trochine *et al*., 2014). Not only does the introduction of a pollutant have detrimental effects of periphyton growth but the frequency, duration, and intensity of a pollutant will determine the effects the contaminant will have on the ecosystem. Long-term exposure to low pollution concentrations has a greater effect on periphyton communities than short duration, acute exposure to the pollutant (Dorigo *et al*., 2010). Godwin *et al* (2009) identified that in higher order streams the increase in pollution from fertilizer and contaminants increase the nitrogen levels in the river system due to run-off causing a growth in the algal community. Murdock (2013) suggests that improvements in fertilizer developments and the addition of atrazine in these fertilizers can inhibit periphyton growth.

Due to the absorptive nature of periphyton communities they have the ability to remove large quantities of contaminants from the water column (Godwin *et al*., 2009; Hill *et al*., 2010) indicating the importance for conserving the ecosystem for nutrient uptake, recycling and storage (Cardinale, 2011). On the other hand high levels of contaminants within periphyton communities can harm organism at higher trophic levels (Hill *et al*., 2010).

2.3.2 Flow

Hydrological patterns and flow regimes play an important role in community composition, structure and function of aquatic biodiversity (Bunn and Arthington, 2002; Choudhury, 2014) and can affect flow dynamics, water quality, sedimentation and substratum structure (Collier, 2002). Flow regimes in river ecosystems are developed through the interaction of variables such as seasonal patterns and timing and duration of floods and drought events (Olden and Poff, 2003). These interactions in particular with physical variables, can determine the type and abundance of aquatic species present in a particular system (Bunn and Arthington, 2002). Temporal variations in flow regimes influence differences in population dynamics, community structure and function (Biggs *et al*., 2005) and flow has an effect on algal resistance and resilience (Peterson and Stevenson, 1992).

Flow can either be measured as discharge or velocity (Olden and Poff, 2003). Flow velocity influences algal immigration, reproduction and post-disturbance recovery (Peterson and Stevenson, 1992; Matthaei *et al*., 2003), and has an effect on biomass accrual through nutrient mass transfer and biomass loss through sloughing (Biggs *et al*., 1998). Velocity can guide algal distribution and community structure (Hart *et al*., 2013). The effect that velocity has on an algal community depends on the algal growth forms within the community. For example, tightly woven mucilaginous diatoms and cyanobacteria are less likely to be affected by displacement and dislodgement by shear-stress at high velocity, indicating that these growth forms are more resistant to high velocities than filamentous algae. This results in biomass accrual through nutrient transfer at increased velocity. Filamentous algae on the other hand experience high rates of diffusion even at low velocity (Biggs *et al*., 1998; Schwendel *et al*., 2010). In contrast to the above however, Larned (2010) believes that velocity is not the major cause of biological change in river ecosystems. Abrasion, friction, drag effects, sloughing and scouring are as a result of increases in flow velocity (Growns and Growns, 2001; Schwendel *et al*., 2010). Increases in velocity according to Choudhury (2014) will result in increases in periphyton biomass. This could be attributed to increased nutrient renewal in fast currents (Peterson and Stevenson, 1992), however, nutrient uptake is limited through the viscous sub-layer surrounding each filamentous or protruding structure in the current (Larned, 2010).

Abrasion refers to the movement of sediments that are carried by high flows over the river bed, that can cause the scouring or spinning of small substratum in the river bed. At times, under extreme flow conditions, the entire river bed can be altered changing the habitat structure of the river ecosystem, displacing aquatic species (Larned, 2010; Schwendel *et al*., 2010). The effect of abrasion is iterated by studies conducted by Downes and Street (2005) who concluded that abraded rocks had a re-colonisation rate between 30% - 50% while overturned and abraded rocks could not re-colonise to a state that was as high as pre-disturbance states. Therefore, biomass loss due to scouring and abrasion is greater depending on the intensity of rainfall and flow velocity (Yang *et al*., 2009). Rocks that have been abraded and overturned have a greater influence on algal re-colonisation than rocks that have been abraded only (Downes and Street, 2005; Larned, 2010).

Sloughing refers to algal community loss when the attachment strength of algal mats to a substratum is exceeded by the weight of the developing algal mat above it. During high flow events the attachment of these mats to the substratum is broken resulting in algal loss (Biggs, 2000) and in free periphyton propagules within the water column (Larned, 2010). With the onset of disturbances periphyton communities may be reduced to a pioneer stage of succession (Biggs *et al*., 2005). Filamentous algae are more susceptible to sloughing because of their large surface areas (Biggs *et al*., 1998), while erect algal growth forms are susceptible not only to sloughing but also to abrasion and scouring (Yang *et al*., 2009).

Shear-stress is characterised as a hydraulic disturbance (Larned, 2010). These disturbances result in periphyton removal from substrates by the movement of bed materials which can lead to sedimentation. However, the effects of shear-stress have only been observed and not quantified with equations and theoretical observations. Due to this the observed effects of shear-stress are not suitable to make predictions (Schwendel *et al*., 2010).

Nutrient mass transfer refers to the uptake of nutrients by producers and by invertebrates through grazing and predation and the uptake of nutrients by fish through predation (Biggs *et al*., 2005). Nutrient mass transfer allows for the increase in algal photosynthetic, reproductive and specific growth rates. Nutrient mass transfer and biomass accrual is dependent on the periphyton community. For example, nutrient mass transfer will have the greatest effect on mucilaginous as compared to filamentous algal communities (Biggs *et al*., 1998).

During low flows there is no transportation of displaced sediment resulting in the patchy distribution of algal communities and invertebrates (Schwendel *et al*., 2010; Hart *et al.*, 2013). Experiments by Boulêteau *et al*. (2008) indicate that flood free, low flow periods result in greater periphyton biomass within that particular system due to low shear-stress. In contrast to this Biggs *et al*. (1998) believe that increased periphyton biomass is as a result of increased velocity as mucilaginous algae under periphyton mats are nutrient limited, until such time that velocity increases to levels at which shear-stress becomes a limiting factor. In systems with low flow velocity, light is a limiting factor (Peterson and Stevenson, 1992). Systems with natural changes in flow consist of periphyton communities that are in early to mid-successional stages as compared to stagnant water were high nutrient loads lead to biofilms in late successional stages (Ryder, 2004).

Flow regimes can be altered naturally or by anthropogenic factors. Natural flow can be altered along longitudinal gradients down a river course (Collier, 2002). Flood events can naturally alter flow regimes and can affect water chemistry, light intensity and temperature (Rader *et al*., 2008). Anthropogenically, flows can be altered through the creation of dams. The presence of dams have many associated effects such as flow regime changes, water quality degradation, base flow changes and water temperature changes (Collier, 2002; Rader *et al*., 2008) and slower natural flows, constant water temperature and a higher periphyton biomass downstream of the dam (Robinson *et al*., 2004; Collier, 2002).

Anthropogenic changes in flow regimes usually occur over a short-time scale thereby degrading the health of a river ecosystem (Hart *et al*., 2013). Alteration of flow regimes result in the introduction of non-indigenous aquatic plant species. For example, water hyacinth propagation, due to anthropogenic flow changes, floats on the surface of the water, hinders recreational activity, interferes with flow regimes and water transport and blocks light from penetrating through the water column. Inter-basin water transfer schemes are another example of anthropogenic flow alterations. For example the Orange-Vaal River and the Tugela-Vaal River transfer schemes in South Africa can propagate and spread alien species, alter the distribution of biota and aid in the spread of pests and disease (Bunn and Arthington, 2002). One of the major challenges with regard to anthropogenic flow alteration from factors such as transfer schemes or the creation of dams, is to meet the water demands of the population without causing major alterations to river ecosystems (Hart *et al.*, 2013).

2.3.2.1 Subsidy-stress response

The relationship between flow velocity and periphyton biomass can be determined through the subsidy-stress response. The model indicates that two direct, opposing factors relating to the effect of flow changes on biomass (Biggs and Thomsen, 1995). A change in flow from near zero levels will result in an increase in periphyton biomass due to nutrient mass transfer (Biggs and Stokseth, 1996; Biggs *et al.*, 1998; Larned *et al.*, 2004). As flow velocity increases there is an increase in sheer-stress and a decrease in algal immigration rates. Equilibrium is reached when the optimum flow velocity is reached and there is no difference between periphyton biomass accrual and loss. If the current velocity increases after this point of equilibrium there is a decrease in periphyton biomass (Biggs and Thomsen, 1995; Borchardt, 1996, Biggs *et al.*, 1998). The current velocity, periphyton biomass relationship is affected by the algal community growth form. For instance, filamentous algae are loosely woven and result in nutrient mass transfer at all velocity levels. For these filamentous growth forms the highest biomass was observed at the lowest velocity. Loosely woven stalked diatoms and short filamentous algae showed a peak biomass with increase nutrient transfer this was followed by a sharp decline. The third algal growth form, which are mucilaginous diatoms, had the lowest biomass at near zero velocity levels reaching highest biomass levels as velocity increases suggesting that diatoms are less vulnerable to sheer stress and sloughing than filamentous algae (Biggs *et al.*, 1998; Biggs, 2000b) (Figure 2.2).

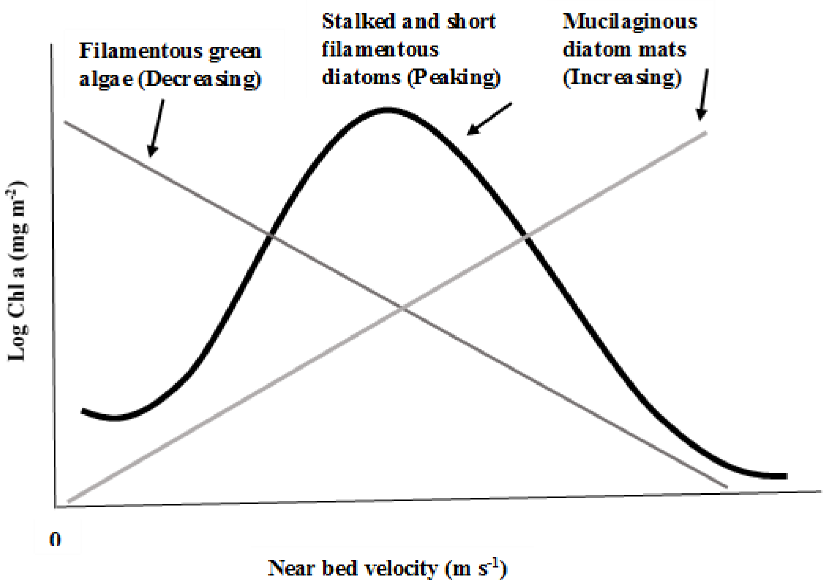

Figure 2.2: Interaction between biomass and flow velocity of three periphyton communities (adapted from Biggs, 2000b).

2.3.2.2 Floods events

Disturbances in terms of frequency, timing and intensity of floods play an important role in the dynamics and structure of river ecosystems affecting algal community structure, function and physiology (Peterson and Stevenson, 1992). Disturbances in slow current communities result in biomass loss, when discharge surpasses a threshold (Peterson and Stevenson, 1992; Schwendel *et al.*, 2010). During high flows, disturbances can result in algal communities retreating to a pioneer stage of succession (Biggs *et al.*, 2005). Disturbances resulting in patch dynamics can affect algal communities in different ways depending on the community attributes at the time of disturbance which may affect post-disturbance recovery (Matthaei *et al.*, 2003). The decrease of algal biomass could result from natural disasters (Godwin *et al.*, 2009) or result in an increase in algal biomass through scoring which removes the top layer of senescent algal cells leaving the more productive layer exposed to lights and nutrients in the water column (Molinos and Donohue, 2010).

Disturbance plays a role in species-coexistence by recolonization either through propagules from upstream refugia or from the resilient species left behind on substratum after disturbances. The type of species that will recolonize after a disturbance will depend on substratum size and geographic location of the disturbed patch (Steinman, 1996). The Intermediate Disturbance Hypothesis (IDH) is used to explain the role of species co-existence (Steinman, 1996; Downes and Street, 2005). The IDH indicates that the maximum species richness will occur at an intermediate stage of succession after a disturbance of average frequency (Steinman, 1996; Biggs and Smith, 2002). The dominant taxa is characterised as those that reproduce rapidly and are known as R-strategists. These organisms will exploit newly available, exposed habitats but will usually be outcompeted by more complex taxa with slower immigration rates known as C-strategists. This indicates that a community is in a transition from that of colonisation to that of a climatic state. The second prediction of the IDH is that species richness will peak in areas at which intermediate rates of disturbance is experienced (Biggs and Smith, 2002). However, the patterns of the IDH were not experienced in all experiments which could be attributed to ecosystem differences or differences in the type of disturbance experienced (Downes and Street, 2005). According to studies by Biggs and Smith (2002) those experiments that did not follow the predictions of the IDH, were as a result of fast colonisation of pioneer taxa and high variation in the immigration of climax taxa.

Other physical disturbances such as desiccation, freezing, substrate movement, increases in hydraulic processes, light and heat affect periphyton communities in manners that are difficult to predict (Larned, 2010). The longer the interval between disturbance events, the greater the effect of the disturbance. This is attributed to the fact that with a lack of disturbance algal mats grow in thickness resulting in senescent cells at the bottom of the mat near the substrate. Therefore, when a disturbance event occurs, even under low shear-stress, algal biomass loss is substantial due to the weak attachment of senescent cells to the substrate (Larned, 2010).

Disturbances from flood events can alter periphyton community composition, biomass and density (Uehlinger, 1991; Peterson, 1996; Biggs *et al.*, 2005; Francoeur and Biggs, 2006; Schwendel *et al.*, 2010). Disturbances are an important spatial and temporal control mechanism, in lotic ecosystems (Webb *et al.*, 2006; Schwendel *et al.*, 2010). Floods are accompanied by bed load transportation, increased sheer-stress and velocity and a change in species composition biomass and density of periphyton and invertebrate communities (Webb *et al.*, 2006; Schwendel *et al.*, 2010). Flooding events can result in patchy distribution of periphyton biomass, each of these patches have species of different ages and therefore a different species index (Robinson *et al.*, 2004; Downes and Street, 2005). Experiments conducted to determine the effect of floods on algal colonisation showed that there was no significant decrease in species richness due to propagules immigration (Biggs and Smith, 2002). Experiments in New Zealand streams showed that mean monthly taxonomic richness was higher after flood events however over a long period of time taxonomic richness was greater in years with less frequent flooding which can be attributed to the fact that there is a lower taxonomic richness in algal communities that are at early succession (Biggs and Smith, 2002).

The re-establishment of algal communities after a flood event occurs in two phases. The first, rapid phase, occurs a few days after the flood event and is composed of algae with high reproductive and immigration rates. The algae found in this early phase are highly tolerant to disturbance. The second phase of re-establishment can take up to a few months and is dominated by algae that are less resistant to disturbances and have slower immigration and growth rates (Biggs and Smith, 2002). Therefore, the relationship between flow velocities and disturbance events play a complex integrated role in the development of algal communities in terms of algal biomass, growth forms, community structure and function.

2.3.3 Light

Other factors such as light, temperature, pH and grazing affect biomass accrual and loss. Changes in light intensity play a role in algal community structure and growth (Hill, 1996). The effect that ultra-violet radiation (UVR) has on periphyton communities is influenced by the amount of shading, water depth and sedimentation of a particular system (Larned, 2010). Most periphyton are autotrophs and therefore use light in the process of photosynthesis to convert inorganic compounds into living matter (Hill, 1996; Larned, 2010). The rate of photosynthesis is effected by self-shading of the algal communities, shading due to riparian vegetation and can be affected by inorganic and organic dissolved particles in the water column (Hill, 1996).

The rate of photosynthesis is affected by self-shading especially in rivers were the overhead canopy shades the water body. Riparian vegetation does not form uniformly therefore certain areas receive more light than others (Hill, 1996). With this type of vegetation growth and patchy distribution of light, it has been observed that periphyton growth rates are higher in unshaded than shaded streams (Quinn *et al*., 1997; Ewart-Smith and King, 2012). This problems is not experienced in large rivers and lakes were riparian vegetation shading is not a concern (Hill, 1996).

Low light intensities in the Thames River reduced algal biomass levels by 50% (Bowes *et al*., 2012). Similarly studies by Han *et al*. (2000) using a dynamic model identified that high light intensities cause a decrease in periphyton biomass. The amount of light within a river system decreases further down the water column due to inorganic particles, phytoplankton and suspended sediments, and decreases with the increase in algal mat thickness (Dodds, 1992; Hill, 1996; Biggs *et al*., 1999; Higgins *et al*., 2008).

Seasonality changes in light intensity play a role in algal biomass levels. For example in spring and autumn when existing high levels of light intensity are increased there is less effect on algal biomass levels as compared to an increase in light intensity in summer when light intensities are low, resulting in the increase in algal biomass (Rosemond *et al*., 2000). The correlation between high biomass levels and increased light intensities allows for periphyton biomass indicators such as Ash Free Dry Mass (AFDM) and chlorophyll *a* estimations to be used as a parameter to measure light intensity. However, grazer density and abundance has to be taken into account in these situations (Hill, 1996).

2.3.4 Temperature

Water temperature has a significant impact on organism and water quality in lotic environments in terms of dissolved oxygen and suspended sediment concentrations (Webb *et al.*, 2008), influencing the establishment of aquatic plants and animals (Bunn and Arthington, 2002). Water temperature is susceptible to both natural and anthropogenic changes. (Webb *et al.*, 2008). Natural changes in water temperature can occur from solar radiation or from conduction and convection (DeNicola, 1996), while anthropogenic change can result from climate change, global warming, water abstraction, divergence and impoundment (Webb *et al.*, 2008; Rosa *et al.*, 2013).

These changes in water temperature can effect algal communities, macro-invertebrates and fish. For example cold, un-oxygenated water from dam releases can cause a decrease in macro-invertebrate populations as these communities synchronise their emergence patterns with water temperatures (Bunn and Arthington, 2002), in contrast to this, Collier (2002) believed that dam flow releases do not cause significant changes in water temperature.

Optimum growth temperatures range between $0^{o}C$ to $30^{o}C$ as heat stress can reduce the growth of periphyton communities (DeNicola, 1996; Larned, 2010). Species diversity is greatest between the temperatures of $0^{o}C$ to $25^{o}C$ and tend to decline at temperatures greater than $30^{o}C$ (DeNicola, 1996). Most algal species have their own optimum temperature for survival, for example diatoms usually dominate at temperature between $5^{o}C$- $20^{o}C$, chlorophytes between $15^{o}C$ and $30^{o}C$ and cyanobacteria at temperatures above $30^{o}C$ (DeNicola, 1996).

Some algal communities are resistant to changes in water temperature due to differences in spatial distribution (Larned, 2010) while lateral and vertical temperature changes can be attributed to spatial changes (DeNicola, 1996; Webb *et al.*, 2008). Changes in metabolic rates, community structure and function, biodiversity, latitude, altitude and groundwater can occur due to changes in water temperature (DeNicola, 1996; Rosa *et al.*, 2013). Temperature differences have different effects on algal taxa. An example of this is cyanobacteria that is more tolerant of higher temperature than diatoms or rhodophyta (Larned, 2010).

Seasonal changes in water temperature can affect the periphyton community. A study by Webb *et al.* (2008) determined that water temperature changes were more pronounced in summer. The effect that seasonal temperature changes has on periphyton communities is iterated by studies conducted by Rosa *et al.* (2013) who indicate that metabolic and respiration changes occurred in spring which was attributed to temperature changes, high temperature and high levels of light intensity. Highest algal biomass levels were observed in autumn due to nutrients leeched from leaf litter (Rosa *et al.*, 2013) which is in contrast to experiments conducted by De Nicola (1996) who observed highest periphyton biomass in spring and lowest biomass observations in winter depending on the driving factors within those particular seasons.

Temperature changes can affect periphyton differently at different ecological levels. At a physiological level there are changes in respiratory and photosynthetic enzymes of the algal species, at a population level temperature determines optimal growth rate, at a community level changes in temperature result in changes in geographic distribution and at a global level changes in temperature affect the paleo-climatic assemblages of periphyton communities (DeNicola, 1996). It is evident that temperature is a limiting factor under extreme conditions but can be counteracted in some cases due to temperature acclimation by algal species which will result in photosynthesis even in cold climates (Larned, 2010).

2.3.5 pH

Stream pH can influence the growth and taxonomic composition of periphyton communities. Environments that are highly acidic are comprised of algal species that are acidophilic and acidotolerant (Gross, 2000; Larned, 2010). Acidophilic species survive in these highly acidic environments through proton influx as they have to tolerate an environment that has lower amounts of carbon dioxide for photosynthesis (Gross, 2000).The most common component found in acidic environments is sulphuric acid (Gross, 2000). Acidic environments lead to low species diversity and usually have no higher order organisms except for macro-invertebrates and organism that only partially rely on photosynthesis (Gross, 2000). If there is a decrease in acidophilic species in an ecosystems it can be deduced that acid mitigation measures were successful (Larned, 2010).

2.4 Grazing

Grazing in aquatic ecology refers to the removal of periphyton biomass by aquatic macro-invertebrates, providing a top-down control of algal biomass, in particular in steams with low to moderate disturbances (Steinman, 1996; Hillebrand, 2009; Yang *et al*., 2009). The species abundance and richness of grazers are dependent on water velocity, depth, deposition, substratum size, location and the rate of biomass accrual within a particular system (Collier, 2002). The effects of invertebrate grazers on periphyton biomass cannot be generalised as many invertebrates are specially adapted to feed on certain algal growth forms (Steinman, 1996). Mature benthic algae usually consist of three layers. The lowest layer contains low profile algal species, the second layer consists of stalked or upright algal species and the last layer consists of filamentous species. Even though these layers are not always clearly defined they aid in determining algal-grazer interactions as grazers are separated into the zone they are most morphologically adapted too. For example, mayflies feed on loose portions of the periphyton mat or on the outer layers because of their gathering, collector feeding mouthpart structures, while, snails and caddisflies have scraping and rasping mouthparts that are better suited to feeding on lower parts of the algal community were the tightly attached algae are found (Steinman, 1996).

Food web composition is important in river systems as it determines grazer-algal relationships (Maasri *et al*., 2010). Grazers reduce periphyton biomass and can cause a change in periphyton community and physiognomy composition (Peters *et al*., 2007; Hillebrand, 2009). Invertebrates are usually unselective on the stage of succession at which the periphyton community is at. This results in the removal of entire algal mats (Hillebrand, 2009). Each invertebrate group can exert a different pressure on the periphyton community depending on mouth parts, foraging ability, motility, morphology and the successional stage of the periphyton community (Villanueva and Modenutti, 2004; Hillebrand, 2009). Therefore, invertebrates act as a control of periphyton biomass (Hillebrand, 2009). However, the resultant algal biomass loss due to invertebrate grazing is more apparent and detectable when initial periphyton biomass is high (Villanueva and Modenutti, 2004).

Periphyton biomass can increase with a decrease in grazers (Taylor *et al*., 2002) and some algal species can resist the effect of grazers and have no change to their biomass if there is an increase in nutrients in that particular system (Rosemond *et al*., 2000). In areas of uniform invertebrate abundance the effect on periphyton biomass may be different. This can be attributed to the interaction of other physical factors such as flow regimes (Peters *et al*., 2007). In rare cases periphyton biomass does not decline with the increase in invertebrate grazers or in some cases grazing may result in the increase in periphyton biomass (Steinman, 1996). Periphyton increases could occur when grazing removes senescent cells allowing healthy cells to gain resources and nutrients from the water column (Steinman, 1996).

The lack of decline in periphyton biomass with the increase in grazers could have occurred because algal decline is dependent on the abundance of invertebrates and in some cases there are not enough invertebrates to result in a substantial decline in periphyton biomass. This suggests that algal-invertebrate relationships can be density dependent. In other cases, invertebrate morphology and body parts may not be adapted to graze on a particular algal growth form (Steinman, 1996). Certain large grazers such as mayflies and caddisflies have a detrimental effect on algal abundance, growth and biomass. Small invertebrates however, do not have the ability to graze on a wide range of algal growth assemblages which makes them more selective in their foraging habitats, thereby having a greater effect on algal species composition rather than abundance (Maasri *et al*., 2010).

Algal-grazer relationships can be affected by external factors such as flow regimes and disturbances. Disturbances, especially large scale infrequent disturbances, have a drastic effect on invertebrate community composition, biomass and density (Biggs *et al*., 2005; Schwendel *et al*., 2010). After a disturbance event the distribution of invertebrates are usually patchy and are predominantly found on stable substratum (Matthaei *et al*., 2003). Through the processes of disturbances and scouring which removes algae, macro-invertebrates lose an important habitat and food source which can affect the re-colonisation of macro-invertebrates (Downes and Street, 2005). Other factors, such as increasing water temperatures, result in an increase in invertebrates due to an increase in metabolic activity (Hillebrand, 2009). The lack of floods have an effect on invertebrate abundance as there is decreased abundance of invertebrates below a dam (Rader *et al*., 2008). In contrast to this Robinson *et al*. (2004) believes that flooding causes a decline in some invertebrate families such as Chironomids and simuliidaes while other invertebrates flow downstream into the drift as a specific behavioural mechanism to decrease the effects that flood events have on invertebrate abundance. Increased sedimentation can result in a decrease in invertebrate abundance and species richness (Collier, 2002). Another concept of algal- invertebrate relationships in river ecosystems is the concept of efficiency increases with an increase in availability as a result of increased accessibility. This is iterated by the fact that invertebrate grazer abundance increases with an increase in periphyton biomass which in turn results in the increased removal of algae (Hillebrand, 2009).

Finally, seasonality and nutrients can effect grazer-algal relationships, for example, during summer and autumn, the negative grazing effects on periphyton biomass is heightened by an increase of nutrients in summer and light in autumn (Rosemond *et al*., 2000). Experiments by Rosemond *et al*. (2000) indicate that the effect of grazers is greater than the effect of resource availability on periphyton biomass. This could result in grazer resistant algal species are slower growers than the other more productive algal species which are consumed by grazers that are morphologically and physiologically adapted to graze on these species (Rosemond *et al*., 2000). Nutrient deficiency may be experienced by invertebrate grazers when nutrients are limiting. This can in turn result in a high grazing pressure on periphyton communities as they are rich in nutrients such as nitrogen and phosphorus (Dodds, 2003; Hillebrand, 2009). The effect of grazing is less severe in environments that are nutrient enriched due to high periphyton biomass in these systems (Hillebrand, 2009). Therefore, disturbances and external factors can impact algal invertebrate relationships. Comparative studies in rivers, lakes and marine systems suggest that the effect of periphyton removal was similar between all three habitats indicating that functional feeding groups and invertebrate-algal size relations are more important than differences in flow regimes and salinity (Hillebrand, 2009).

2.5 Temporal Patterns of benthic algal distribution in rivers

There is a complex interaction between abiotic and biotic factors in a river ecosystem that operate over a wide spatial and temporal scale, altering patterns in periphyton biomass and community composition. These alterations can occur at a fine scale, within micro-habitats over a few days or at broad scales, between catchments over years (Stevenson, 1997). Spatial and temporal ecology of a river is influenced by the way in which water moves across a landscape. Changes in algal assemblages are as a result of scouring, abrasion, substrate stability, desiccation, velocity and shear-stress due to flow regime changes. Physical disturbances such as droughts and floods influence spatial and temporal patterns in benthic algal communities (Bunn and Arthington, 2002).

Temporal variations in periphyton patterns such as biomass are influenced by seasonality changes. Studies by Yang *et al*. (2009) identified that there was greater algal biomass in the dry season than the wet season due to scouring and abrasion as a result of high flows in unpolluted rivers in Hong Kong. The effect that temporal variations have on periphyton biomass are often decreased in comparison to the effect that grazing invertebrate have on periphyton biomass. (Yang *et al*., 2009).

2.5.1. Short term temporal patterns- post disturbance recovery

After a flood event periphyton communities are usually in a stage of biomass accrual through the immigration and colonisation of propagules from upstream or from growth or reproduction of cells that survived the flood event (Poff *et al*., 1990; Biggs, 1996; Larned, 2010). Once a peak algal biomass is reached, algal loss occurs through death, immigration, grazing and sloughing (Poff *et al*., 1990; Biggs, 1996). These biomass accrual and loss patterns are influenced by a number of factors including the size and type of the propagules pool from upstream refugia which influences colonisation, near-bed velocity and substratum texture (Biggs, 1996).

These variations in algal accrual and loss patterns can result in differences in algal species composition. During the biomass accrual phase bacteria, fungi and a mucilage develop as an organic film over stable substrata. This development usually occurs a few hours after a disturbance. This is followed by the introduction of diatoms together with fungi bacteria and polysaccharides (Peterson *et al*., 1990; Peterson, 1996). With an increase in phosphorus levels in aquatic ecosystems, through the process of succession, there is a shift in dominance from macrophytes and periphyton to that of phytoplankton which is an indication of nitrogen limitation (Trochine *et al*., 2014).

The rate of succession and the recovery of algal communities can be dependent on the starting point of succession and regrowth or reproduction. For example, catastrophic disturbances can remove all living periphyton from a substratum while less severe disturbances may result in resilient taxa remaining on the substratum. During severe disturbance events refugia from upstream dominates recovery while less severe disturbances result in recovery from the growth of residual algal taxa after the vulnerable taxa was removed by the disturbance (Ewart-Smith and King, 2012). Therefore the initial phase of biomass accrual within the succession process can either be dominated by recovery of periphyton biomass from residual cells or the colonisation of periphyton from propagules from upstream refugia. The time taken for the periphyton community to reach peak biomass is depended on whether the initial phase was regrowth or re-colonisation (Stevenson, 1990). During low intensity floods, colonisation and growth is rapid and peak biomass is reached within two weeks, however during high intensity floods, regrowth is slow and peak periphyton biomass is only reached between 70 - 100 days after the disturbance event (Biggs, 1996). This illustrates that the severity of a disturbance is an important consideration when assessing periphyton community recovery (Ewart-Smith and King, 2012).

Morphological adaptations of algal taxa are important in terms of short-term recovery following a disturbance event. For example, taxa are more resistant to floods if they have a low profile, are tightly woven and have strong adhesion to substratum (Peterson, 1996). Examples of these low profile taxa include the diatoms *Achnanthes* and small *Navicula* species while examples of adhesive taxa include green algae such as *Stigeclonium*. Interwoven cyanobacteria are also resistant to floods (Biggs and Hickey, 1994). Taxa including filamentous green algae such as *Spirogyra* are loosely attached to substrata and are more susceptible to floods (Ewart- Smith and King, 2012).

Grazing can affect the succession trajectory during short-term recovery post-disturbance (Poff and Ward, 1995; Rutherford *et al*., 2000). For instance grazers can either inhibit or maintain succession rates but it essentially depends on the characteristics of both grazers and periphyton communities (Poff and Ward, 1995; Steinman, 1996; Rosemond *et al*., 2000). Grazers can become a limiting factor on periphyton growth over seasonal time scales. Grazers become a limiting factor in spring while nutrients become a limiting factor in summer (Rosemond *et al*., 2000).

The above demonstrates that the availability of resources such as light, temperature and nutrients have an effect on algal growth and reproduction (Biggs, 1996). Peak biomass is usually reached faster in nutrient enriched than in unenriched streams. Sometimes however, diffusion of nutrients into algal mats become limiting in nutrient poor systems resulting in sloughing early on in the algal biomass accrual cycle (Lohman *et al*., 1992; Biggs, 1996). Peak biomass is often greater in nutrient enriched environments in systems with similar velocity, temperature and light conditions (Biggs, 1995). Periphyton community structure affects the value of peak biomass. For example chlorophyll *a* estimates indicate that a higher peak biomass is experienced for filamentous green algae as compared to diatoms or cyanobacteria (Biggs, 1995). Algal loss is experienced after peak algal biomass is achieved by processes such as autogenic sloughing and factors such as age, parasitism and disease which affects the periphyton community (Ewart-Smith and King, 2012).

2.5.2 Long-term temporal patterns

Long-term temporal patterns are affected by flood frequency together with interactions between nutrients and light (Biggs, 1996). There are three main long-term temporal patterns, first, temporal patterns with a relatively constant biomass throughout the year. The second temporal pattern is found in rives with a moderate flood frequency and moderate nutrient and light levels, these patterns include that of cycles of accrual and sloughing. Finally, the third pattern comprises of seasonal growth with prevailing periods of moderate to low biomass (Biggs, 1996; Biggs, 2000b).

The temporal pattern of relatively constant low biomass occurs in systems with frequent flooding in which the periphyton communities are unable to reach a stage of biomass accrual due to the onset of the next flood. This frequent flooding devalues the positive effects of nutrients and light. In some cases these low biomass values are as a result of low nutrient and light supply (Biggs, 1995). However, these systems are usually oligotrophic so it is difficult to determine if low biomass levels are as a result of frequent flooding or low resource supply (Biggs, 1996). These systems are dominated by diatoms that are resistant to abrasion and turbulent flow (Francoeur and Biggs, 2006).

Other reasons for relatively low constant biomass in the absence of frequent flooding could be regulated by high densities of invertebrate grazers. This increased grazing prevents biomass accrual and algal composition present is comprised of grazer resistant species (Rosemond, 1994). Seasonal shifts are evident in these systems of low biomass. Nutrient light and grazers all play a limiting role on algal biomass accrual however, light availability was limiting in autumn and summer, while nutrient levels was the limiting factor in spring while grazing by snails for example can limit biomass accrual all year round in studies conducted in the Western Cape (Ewart- Smith and King, 2012).

In systems with seasonal flooding and moderate supplies of lights and nutrients, periphyton communities can develop and increase their biomass at different levels of succession (Uehlinger, 1991; Yang *et al*., 2009). Flood events do not remove all algal growth and the period between flood disturbances result in biomass accrual. During this time periphyton growth can be considerable in particular in the absence of invertebrate grazers. Invertebrate grazer absence is particularly evident after a flood event when regrowth and re-colonisation is slower than that of the algal community (Biggs, 1996). This increase in periphyton biomass occurs until such time as there is an increase in grazing pressure. This illustrates the shift from abiotic to biotic control of periphyton biomass after a flood event (Ewart-Smith and King, 2012).

Seasonality of disturbances, grazer density and light intensities affect periphyton growth and biomass at a seasonal time scale. Disturbances to the river ecosystems usually correlate with seasonal changes (Sabater and Sabater, 1992). In contrast to this, Hill and Dimick (2002) indicate that the availability of resources control periphyton growth over a seasonal time scale. Seasonal patterns of periphyton dynamics occur through the interaction of flood frequency, grazers and resource availability. For example in a Mediterranean climate, periods of flow stability in summer is followed by flood events in winter. These abiotic forces dominate in winter while biotic forces dominate in summer (Tornes and Sabater, 2010). This illustrates the importance of the relationship between resource availability, grazing and disturbance events.

2.6 Spatial patterns

Spatial variations are characterised by differences in biotype, flow type, substratum type, suspended material and water solutes (Lear *et al*., 2008). Interactions between hydrological, biological and geochemical factors can determine spatial patterns and community composition in a river ecosystem (Lutscher *et al*., 2007). This spatial variation results in the creation of micro-habitats that enhance patchy distribution and community variation at a number of different spatial scales (Biggs *et al*., 1998; Lear *et al*., 2008). This patchy distribution can occur due to the interaction of factors such as invertebrate grazer density, substratum type and the point of nutrient inflow (Biggs *et al*., 1998). In unpolluted systems spatial variation is driven by rainfall patterns, shading and grazing together with light intensity, nutrient supply, temperature and hydro-dynamics (Biggs *et al*., 1998; Peters *et al*., 2007; Yang *et al*., 2009). Spatial variations can occur at a micro-scale between biotypes, at a meso-scale occurring within catchments and finally at a broad scale were spatial variation occurs between catchments.

2.6.1 Micro-scale (biotypes)

Micro-scale variations refer to small changes in substratum patterns. For example algae can prefer sand grains of different mineralogy and topography. Stable flows can result in higher algal biomass on stable substrata. Even though most communities can grow on any substrata some communities can only mature when there are long periods of community stability (Biggs, 1996). During flood events abrasion by small particles occur on algae that are colonised on larger stable substrata. To overcome this, some algae colonise on protruding substratum resulting in a higher algal biomass than that of algae that are colonised on less stable smaller particles. These algal colonisations are at early successional stages however, without disturbances they can succeed to a cyanobacterial stage and even to the stage of filamentous green mats making the entire colony more stable (Biggs, 1996). According to Bunn and Arthington (2002) rivers with unstable substratum have low species diversity and exhibit similar signs to communities that are frequently disturbed.

2.6.2 Meso-scale (within catchment patterns)

Algal community composition can vary within pools, runs and riffles. These spatial differences reflect spatial differences in shear-stress, nutrient mass transfer and substratum type (Biggs, 1996). There is greater spatial heterogeneity between rocks within the same river system than between river systems (Lear *et al*., 2008). The differences in biotypes within a river system is determined by the relationship between the underlying geology and flow regimes (Bunn and Arthington, 2002). In nutrient enriched streams there is a higher biomass in low velocity areas such as runs and pools dominated by green filamentous algae. In streams with high velocity most biomass, consisting of diatoms, are found in the riffles due to movement of nutrients from higher velocity and turbulence (Biggs, 1996). At a meso-scale, over a river continuum, biomass increases from headwaters to mid-waters due to an increase in river width and a decrease in overhead riparian vegetation. At the lower regions, biomass decreases again due to increases in light attenuation, increased water depths and turbidity. However, this can vary due to differences in nutrients and flow conditions together with underlying geology and topography (Biggs, 1996).

2.6.3. Broad scale (inter-catchments patterns)

Broad scale inter-catchment patterns occur due to varying frequencies in flood events. Streams that experience frequent floods have a lower average biomass dominated by low growing shear-resistant taxa while, streams with less frequent disturbances, low velocity and grazing have an average or higher biomass with greater community complexity (Biggs, 1996). Differences in community composition can result due to differences in geology, land uses and associated enrichment. Low nutrient enrichment usually comprise of cyanobacteria, while enriched streams usually contain filamentous algae (Biggs, 1996). Therefore periphyton communities are important biological indicators and environmental management tool as they can be used to determine pressure that is exerted on trophic systems. To overcome the increase in contaminants, in river ecosystems, many benthic communities have managed to adapted to the increase in contaminants and function at these high levels of pollution by having shorter generation times (Montuelle *et al*., 2010).

2.7 Bio-monitoring

At present there is a shift in the use of bio-monitoring tools from that of water quality indicators to that of indicators of biological integrity which is the capacity of an ecosystems to support and maintain a balanced, integrated biological systems (Karr, 1995) and ecosystem health which is defined as a set of societal goals for ecosystem conditions (Chessman *et al*., 1999). The short generation time and sessile nature of periphyton makes them suitable for monitoring and detecting flow and nutrient changes (Burns and Ryder, 2001).

Bio-monitoring in South Africa is often an integrated process attempting to strive for the much debated ideal of ecosystem health which refers to the optimal functioning ecosystem with healthy flora and fauna. This is achieved in one of two ways, by the whole effluent toxicity (WET) which is a tool used to evaluate effluent to ensure that it is not too toxic for discharge into rivers while the second approach is bio-monitoring under the South African river health programme (RHP). This programme is used to assess river health in relation to anthropogenic factors. However, it has low sampling frequency and low resolution and only makes use of macro-invertebrates, fish and riparian vegetation as bio-indicators. The use of diatoms have recently been tested throughout South Africa as a biological monitoring indicia (De la Rey *et al*., 2004; Taylor *et al*., 2007).

Studies by Taylor *et al*. (2007), aim to include diatoms in the current suite of bio-monitoring tools used in South Africa as chemical monitoring of water quality is expensive and time consuming. Hence the call for a shift towards the use of biological monitoring. Studies along the Crocodile west and Marico management area saw the identification of many cosmopolitan diatoms and many endemic diatoms specific to South Africa indicating a need for a unique South African diatom index. Studies in the Vaal and Wilge Rivers used diatoms and developed indices to determine the correlation with chemical monitoring outcomes over a certain period of time.This suggested that diatom-based indices are a valuable addition to the suite of bio-monitoring tools (Taylor *et al*., 2007b). However, the use of periphyton as a bio-monitoring tool is still difficult as the relationship between biotic and abiotic factors, biotic responses to regulatory changes in ecosystems and associated patterns in spatial and temporal dynamics in river ecosystems have to be understood (Stevenson, 1997).

2.8 Climate change

Climate change, due to anthropogenic factors, can influence water quality, increase air temperature and changes in rainfall patterns and may influence flow conditions in rivers while increased water temperature may affect metabolic rates (Whitehead *et al*., 2009). Increased flow conditions may result in increased sedimentation and in turn affect the morphological characteristics of rivers. Droughts in some cases or alternatively flash floods may occur as a result of climate change. Low flow events and reduced velocity can eventually result in algal blooms (Whitehead *et al*., 2009). Laboratory experiments in the Netherlands to test the effects of climate change on four toxic and two non-toxic algal species yielded the following results. The two non- toxic species did not increase significantly with an increase in temperature. Two toxic species did not increase with the increase in temperature while the other two toxic species increased rapidly with the increase in temperature. This experiment reiterates the uncertainty of algal blooms associated with climate change (Peperzak, 2003)

Eutrophication through the increase of nutrients from pollution in river ecosystems has been linked to harmful algal blooms and the development of cyanobacteria so the monitoring and reduction of pollutant effluent into rivers can significantly decrease harmful algal blooms (Heisler *et al*., 2008). In South Africa, remote sensing and medium resolution satellite imagery was used in studies by Matthews (2014) to indicate the level at which eutrophication and the development of harmful algal blooms was affecting water resources. Fifty inland waterbodies were sampled and it was found that 62% of these waterbodies were at or near eutrophic levels while 23 showed signs of cyanobacterial presence. Cyanobacterial scum that poses health risks to humans and animals were observed at 26 of the sampled sites (Matthews, 2014). Nutrient levels play a highly influential role on algal blooms. For example at Albert Falls dam in KwaZulu-Natal an algal bloom of dinoflagellate *Ceratium hirundinella* caused major ecological shifts to the system indicating reduced water quality (Hart and Wragg, 2009).

2.9 Conclusion

Periphyton composed of algae, protozoa, bacteria and fungi play a crucial role in river ecosystems as chemical modulators and in their capacity to convert nutrients into food for organisms at higher trophic levels. However, excess nutrients in a river system may lead to eutrophication and an increase in algal blooms. There are a number of factors that influence algal biomass accrual and loss. For example nutrients, light and temperature may lead to algal biomass accrual while disturbances and grazing can lead to biomass loss. Nutrients can result in the increase in algal biomass and taxonomic richness. This algal growth is limited by the nutrient in least supply which can occur at all trophic levels. Aquatic food webs are strongly determined by the nutrient regimes in a particular system. The increase in anthropogenic pollution can influence and result in algal blooms leading to eutrophication and changes in river system composition and functioning.

Flow plays an important role in algal community biomass and composition. These flow events are influenced by seasons, disturbances, floods and droughts. Flow can either be a measure of discharge or velocity. Velocity can influence biomass accrual through nutrient mass transfer or biomass loss through disturbances, sloughing and sheer stress. Grazers provide a top-down control of algal biomass and are specifically physiologically and morphologically adapted to feed on algae. Periphyton patterns are influenced temporally and spatially at a number of scales. It is important to determine trends and patterns in periphyton communities as they have the potential to be an important bio-monitoring tool. These bio-monitoring tools are important as they allow for changes in ecosystems to be detected efficiently. Differences in outcomes, when using these tools. depend on a number of different factors such as the ecosystem conditions. These differences may yield different results making it difficult to create a generalised tool that can be used in all situations.

This research aims to develop an understanding of the temporal patterns of periphyton communities in a summer rainfall region. To date such research has only taken place in a winter rainfall region of South Africa but the idea is to create a generalised bio-monitoring tool using periphyton that is applicable to South Africa as a whole and not limited to specific regions.

CHAPTER THREE

Methods

3.1 Introduction

This chapter describes the sites selected and field sampling and laboratory procedures conducted on a monthly basis from June 2014 to June 2015 to inform the research aim and objectives. The KwaZulu-Natal region boasts a subtropical climate with summer rainfall together with cold, dry winters (Eeley *et al.*, 1999). The favourable atmospheric circulation in KwaZulu-Natal allows for a relatively balanced water budget with a warm coastal current supplying humidity to the area. This results in high rainfall, high soil moisture content and a dense vegetation cover. The mean annual rainfall in the area is 800 mm (Jury, 1998). The average temperatures ranges between 18-28.C in summer to 18- 23.C in winter.

Five sampling sites were selected. These site were situated along three river systems namely; the Msunduzi, the Umgeni and the Hlatikhulu river systems (Figure 3.1). The Msunduzi River flows through the city of Pietermaritzburg with a catchment size of 875 km^2 and a tributary length of 115 km. The river is heavily polluted by surrounding communities that utilise the river for domestic and recreational activities. The Msunduzi River is one of the main tributaries of the Umgeni River, joining the Umgeni River at the confluence between Nagel and Inanda dams. The Umgeni River has its source in the KwaZulu-Natal Drakensberg region, at an elevation of 1825 m and its mouth in Durban metropolitan (Dickens *et al.*, 2010). Midmar Dam is situated on the Umgeni River, built in 1965 near Howick, to provide drinking water to both the Pietermaritzburg and Durban regions. It is mandatory that 0.7 m^3/s of water is released at a constant flow from Midmar dam, is important for the downstream community users (Dickens *et al.*, 2010). The Hlatikhulu River originates in the Kamberg region of the Drakensberg and is relatively unimpacted by anthropogenic pollution apart from agricultural practices.

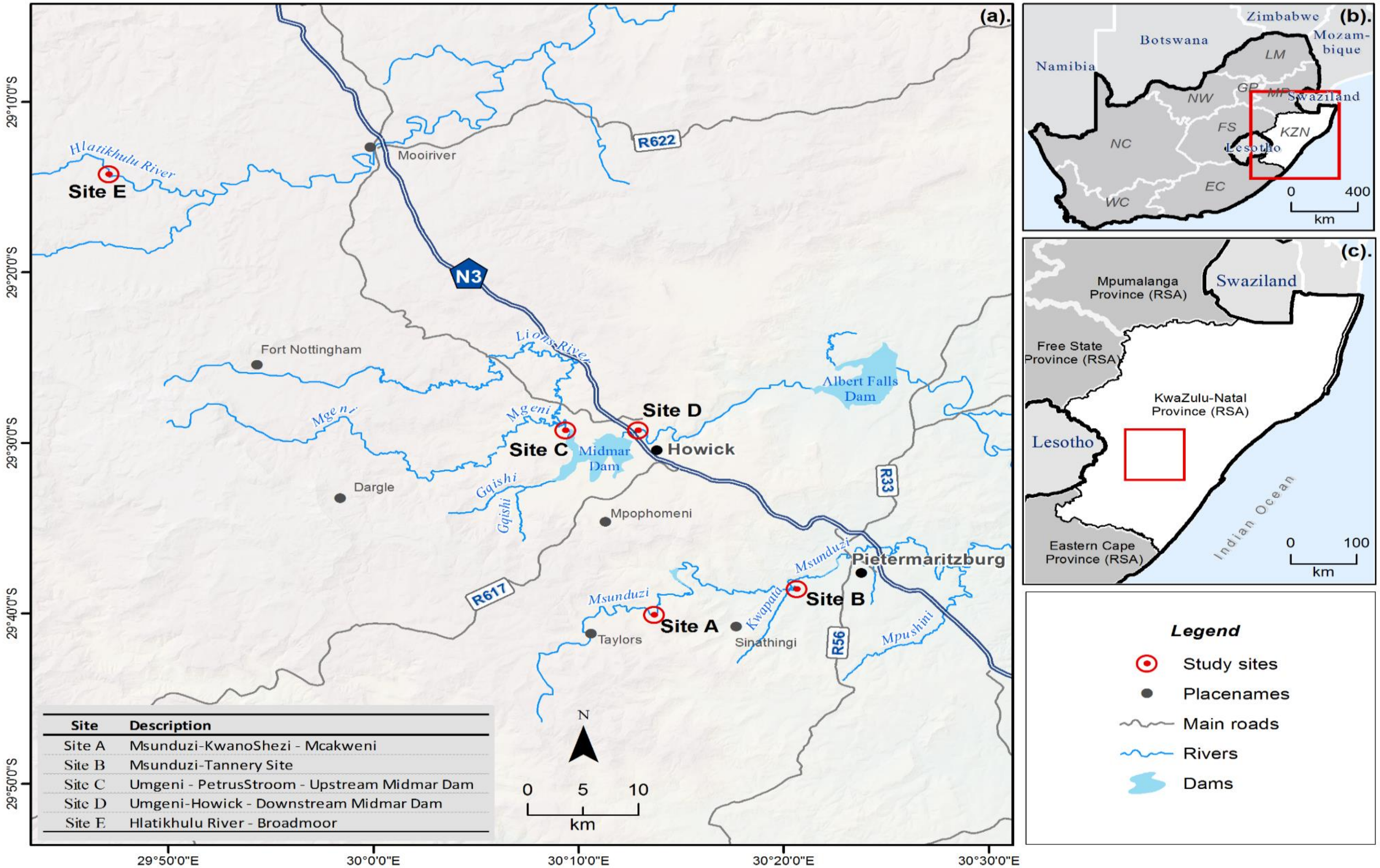

Figure 3.1: Site map of KwaZulu-Natal indicating sampling sites (a) and the location of the study sites within the KwaZulu-Natal province (b,c)

3.2 Site selection

Site selection involved identifying sites upstream and downstream of points where either natural flow regimes or nutrient statuses were altered to ensure that the research aim was fulfilled. This, together with the availability of flow and water quality data, flow and nutrient alterations and security and safety surrounding the sites, were taken into consideration. These factors led to the identification of the following sites:

Site A: Msunduzi- KwanoShezi- Mcakweni (29°40'5.83"S, 30°13'41.36"E) and **Site B:**Msunduzi Tannery Site (29°38'34.58"S, 30°20'37.44"E) are comparable as they have similar flow characteristics with differing nutrient loads. **Site A** is upstream of Henley Dam in the peri-urban surrounds north of Pietermaritzburg CBD. This site has a cobble bed substratum type and a safe, secure, easily accessible sampling habitat. This site was chosen as the nutrient unenriched site as it is not severely altered by anthropogenic pollution, has fairly natural flow conditions and unaltered water chemistry (Plate 3.1a). The gauging weir at Henley dam (U2H011) was used to obtain flow data from the Department of Water Affairs for site A. Changes in water depth and siltation between the seasons of winter (July 2014) and summer (December 2014) are indicated (Plate 3.1a). **Site B** has unaltered flow conditions but altered nutrients due to runoff from the communities living adjacent to the river system, utilising the river system and thereby increasing nutrient load, resulting in nutrient enrichment. Site accessibility is obtained through a local industry which is situated adjacent to the river. The substratum type at this site is cobble bed and site accessibility is challenging in particular during spring through to autumn when vegetation cover is dense and high. The gauging station at Mason Mill (U2H058) was used for water quality data at this site. Site condition changes in winter (June 2014) and in autumn (April 2015) were observed (Plate 3.1a)

Site C: Umgeni- Petrustroom- Upstream of Midmar Dam (29°29'15.69"S, 30° 9'21.92"E) and **Site D** Umgeni-Howick- Downstream of Midmar Dam (29°29'16.44"S, 30°12'53.39"E) are comparable as they have similar nutrient conditions but altered flow characteristics. **Site C**, Petrustroom, is located outside the town of Howick, upstream of the Midmar dam. The gauging weir (U2H013) at Petrustroom was used to obtain water quality data from the Department of Water Affairs. The site is safe, secure and easily accessible. This is a low impact site with no chemical introductions and no nutrient enrichment. Drastic changes in flow conditions in terms of water depth and velocity over the sampling season in winter (July 2014), spring (September 2015) and autumn (April 2015) are visible (Plate 3.1b). **Site D** is located downstream of Midmar dam and has altered flow conditions. This site is a low impact site that is suitable for sampling as it is secure and accessible. The site chosen is above the York Street pump station, this ensures that effluent from the pump will not affect the study. The gauging weir at Howick (U2H048) was used to obtain flow data for this site. Changes in flow conditions, namely water depth in winter (June 2014) and summer (February 2015) are indicated (Plate 3.1b).

Site E: Hlatikhulu River- Broadmoor (29°14'14.81"S, 29°47'5.35"E), is located in the Drakensberg Ukhahlamba Mountains. There is minimal flow modification or nutrient enrichment at this site. It is a safe, secure and easily accessible site that has a cobble bed substratum type. The lack of flow and nutrient modifications together with its close proximity to a gauging weir for flow data and water quality data from Umgeni water, who regularly monitor the site, made it a good reference. However, during the sampling period the land adjacent to the river banks, surrounding the site was planted with maize thereby potentially altering nutrient levels, flow regimes and other physico-chemical elements within the system. A new reference site was not identified due to time constraints and the lack of water quality and flow data at any other site upstream of this site. The gauging weir on the Hlatikhulu River (V2H007) was used to obtain flow data for the site. Changes in water depth over the sampling period in summer (January 2015) and June (2015) are visible. The maize plantations above the river bank can be viewed in the January 2015 photograph (Plate 3.1c).

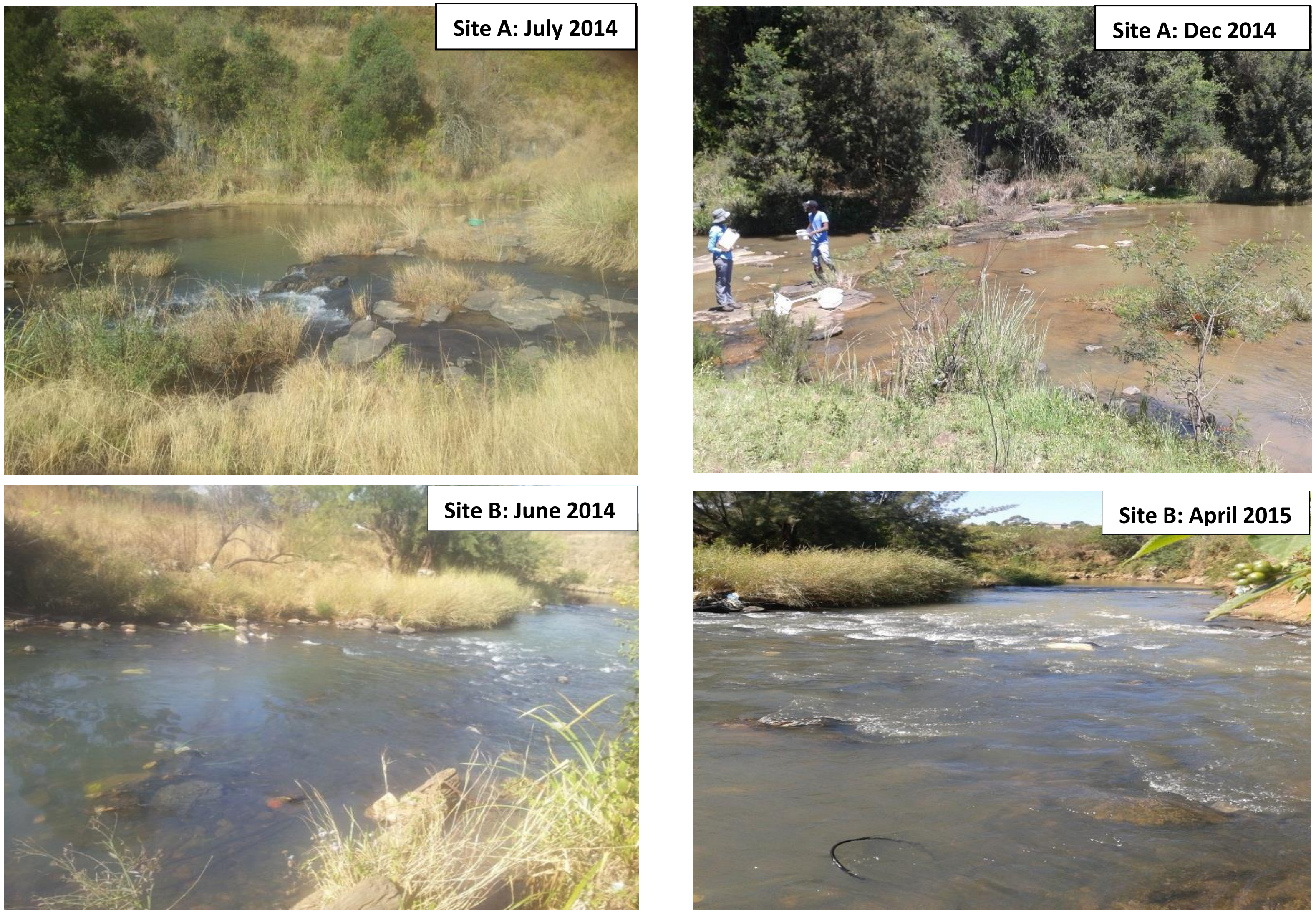

Plate 3.1a: Site A Msunduzi- KwanoShezi- Mcakweni photographed in winter (July 2014) and summer (December 2014) showing changes in depth and siltation which is comparable to site B Msunduzi Tannery site photographed in winter (June 2014) and autumn (April 2015) showing minimal changes in depth.

Plate 3.1b: Site C Umgeni- Petrustroom photographed during winter (July 2014), during spring (September 2014) and during autumn (April 2015) illustrating changes in water depth and velocity which is comparable to site D Umgeni-Howick photographed during winter (June 2014) and summer (February 2015).

Plate 3.1c: Hlatikhulu River- Broadmoor photographed in summer (January 2015) and winter (June 2015) showing changes in water depth and the maize plantation.

3.3 Data acquisition

Sampling occurred at each site for 13 months from June 2014 - June 2015 except at the reference site were sampling commenced in September 2014 - June 2015. At each of the five sites, five submerged stones were sampled. Each stone were processed on-site for biomass estimation and taxonomic composition of periphyton and macro-invertebrates which would be identified during laboratory analysis. These stones were collected from the run hydraulic biotype identified as least variable between the riffle, pool and run biotype according to a pilot study conducted by Ewart-Smith (2007). The run biotype is characterised by large and small cobbles on the river bed. This surface flow, which is usually unbroken, can either be rippled surface flow (RSF) or barely perceptible flow (BPF) and sometimes with insular standing waves during high flow events. These flow types are associated with the run biotype (King and Schael, 2001; Ewart-Smith, 2007). The five sampled stones were collected from hydrologically independent run biotopes.

3.3.1 Field sampling procedure

Stones with a width of between 100mm to 250mm along the longest axis were sampled to reduce the potential effects of stone size as indicated by the pilot study conducted by Ewart-Smith and King (2012). At each site physico- chemical data were collected using a YSI 556 MPS multiprobe system. These data were pH, water temperature, percentage dissolved oxygen, dissolved oxygen and conductivity. Water clarity was determined using a clarity tube. Further water quality data were obtained from Umgeni Water and flow data from the Department of Water Affairs. Before the removal of each stone, near bed velocity and depth were measured using a global water flow probe.

A invertebrate sampling net with an 80µm mesh size with an opening of 300 mm × 300 mm was placed below the selected stone in the current. The sourced stone was then lifted from the river bed.

Any invertebrates disturbed during this process were carried by the current into the net. Once the stone was lifted, it was placed in the current, in the invertebrate net and gently brushed to remove any invertebrates that still adhered to the stone. Once the invertebrate removal process was complete, each of the five selected stones were placed in separate sampling trays, removed from the river system and taken to the river bank for further sampling.

At the river bank chlorophyll *a* was determined for each stone using a *moldaenke bb*e BenthoTourch. This allows for *in-situ* real time determination of benthic algal concentrations. The BenthoTourch can be used to determine green algae, blue-green algae and diatoms. It is portable, reliable and there is no need for sample preparation, laboratory analysis or microscope work. Five readings on different sides of each stone were taken. This was carried out for each of the five stones at each site. After benthotorch chlorophyll *a* readings were taken any invertebrates that were still adhered to the stone were removed using forceps. Invertebrates from each stone, including those collected in the river and those removed using forceps were placed in jars and preserved with 99.9% ethanol.

After the removal of invertebrates, periphyton was scrubbed off the stones using a nail or tooth-brush until there was no change in the colour of the rinsing water. This scrubbed slurry was placed in dark containers to inhibit any further photosynthetic activity. Subsamples of 50ml of the scrubbed slurry was removed and preserved with Lugol's iodine solution for the preservation and later identification of algal species. The scrubbed sample was placed on ice and transported to the laboratory where it was frozen for biomass analysis.

Once the stones were scrubbed, the stone dimensions were measured as the longest axis (x), the longest horizontal axis, perpendicular to the x-axis (y) and the longest vertical axis (z). The area of each stone was calculated using the regression formula developed through a pilot study by Ewart-Smith (2007):

$$\textbf{Surface areas of stone (cm}^2\textbf{)} = 0.014(x)+33.819\ (xy+xz+yz)$$

Stone embeddedness that was estimated *in situ* was used to correct for stone area to ensure that stone surfaces on which periphyton could not grow were considered. This sampling procedure was followed for all five stones at all five sites for the duration of the sampling period. The entire sampling procedure is represented pictorially (Figure 3.2).

Temperature loggers were installed in December 2014. The Hobo U22 Water Temp Pro V2 temperature loggers were fixed to the river bed near the point of sampling at all five sites. Unfortunately data loggers at site A (Msunduzi-Kwanoshezi [nutrient unenriched]) and B (Msunduzi-Tannery [nutrient enriched]) were stolen and data could not be retrieved. To obtain missing values a regression, using r^2 values from scatter plots were used.

Figure 3.2: Pictorial representation of sampling procedures

3.3.2 Laboratory procedure

3.3.2.1 Periphyton biomass

Periphyton biomass was quantified using two procedures; the determination of ash free dry mass (AFDM) and chlorophyll *a* (Chl *a*). Frozen periphyton samples of the scrubbed slurry were defrosted overnight in dark cupboards, then processed for AFDM and Chl *a*. Laboratory procedure were adapted from Biggs and Kilroy (2000). AFDM was calculated by drying Whatmann GFF 4 glass fibre filters without algal samples in a furnace at 400^{o}C. The filter paper was removed from the furnace and placed in a Buchner filtration apparatus. A 15ml sample portion was filtered through the Buchner funnel onto filter paper. These samples were then dried in the oven overnight at 105 ^{o}C after which they were ashed at 400^{o}C for four hours. Filter paper and crucibles were weighed before and after the samples were placed in the furnace. The difference in dry and ashed weights were used to determine periphyton biomass (Equation) (Biggs and Kilroy, 2000) and represents the organic component of the periphyton.

AFDM (g/sample) =

[{(weight of crucible + filter + sample after drying)-(weight of crucible + filter + sample after ashing)} × sample volume/ [volume of filtered sample] **(1)**

AFDM (g/sample) =AFDM/ area of stone (m^2) **(2)**

Chlorophyll *a* was used as a measure of live algal biomass. This is effective as Chl *a* is present in most algae which form part of the periphyton community (Biggs and Kilroy, 2000). The Chl *a* extraction procedure was adapted from Biggs and Kilroy (2000). Algal sub-sample of 15ml from the scrubbed slurry was filtered onto Whatmann GFF 4 glass fibre filters through the Buchner filtration apparatus. The filter paper was boiled in 95% ethanol at 78^{o}C for 10 min to allow chlorophyll to be fixed by destroying the enzymes and to increase extraction efficiency. The filter paper was removed and the ethanol containing the chlorophyll was left to cool overnight at 4^{o} C in a fridge. Cooled samples were centrifuged at 3000rpm for 15min. If samples were concentrated they were diluted with 95% ethanol. Absorbance was measured at a wavelength of 665nm using a spectrophotometer while background absorbance was measured at 750nm. During spectrophotometry the live Chl*a* counts are interfered with by dead chlorophyll cells called phaeopigments. These dead cells occur through the natural degradation of chlorophyll communities as they age. To determine the amount of dead cells, the sample was acidified with 0.3M hydrochloric acid (HC*l*) to correct for phaeopigments. After two minutes absorbance reading were re-read at 655nm and 750nm. Chlorophyll *a* per sample was determined by the following equations adapted from Biggs and Kilroy (2000)

Chlorophyll *a* (mg per sample) =

[(absorbance$_{665before}$- absorbance$_{665after}$) × 28.66 × sample volume × extractant vol.]/ filtered sub-sample

Phaeopigments (mg per sample) =

[(1.72×absorbance$_{665after}$) - absorbance$_{665before}$] × 28.66 × sample volume × extractant volume]/ (filtered sub-sample)

Chl *a* (mg/m^2) = chlorophyll *a* (mg/sample)/ area of sample (m^2)

Where: absorbance$_{665before}$ and absorbance$_{665after}$ are absorbance readings at 665 nm wavelengths before and after acidifications and 28.66 is the absorption coefficient for chlorophyll (Biggs and Kilroy, 2000).

3.3.2.2 Taxonomic identification of algae

A 50ml sub-sample of scrubbed slurry preserved in Lugol's iodine solution was stored in a dark refrigerator for algal taxonomic identification. This analysis and sample preparation was conducted according to steps outlined in Ewart-Smith and King (2012). The 50ml sample was homogenised using a hand-held blender. Sub-sample of 5ml was centrifuged for 10 minutes at 3 000 rpm to concentrate the algal cells. Thereafter, 4.5ml of supernatant was discarded leaving behind 0.5ml of the pellet for identification purposes. (Ewart-Smith and King, 2012). Each sample was initially viewed using a compound microscope with a magnification of 1000 × using a glass slide and cover slip to view the entire sample and gain an impression of the types of algal species present. 0.1ml of sample was placed in the sample chamber of the haemocytometer which was used for algal species identification and abundance estimation. Algal taxon identification was obtained using the haemocytometer under a magnification of 400× using a compound microscope. The samples were concentrated to ensure approximately 30 to 50 algal cells per field of view and were counted within an area ranging between 1mm^2 to 9mm^2. Algal density per sample per site was determined as the number per m^2 of stone surface area.

3.3.2.3 Invertebrate grazer biomass

Invertebrates collected in-field and preserved within ethanol were identified to family level using a dissecting microscope. Invertebrates that were identified as grazers were split into five size categories.

Size categories were developed by measuring invertebrate body lengths and ranged from <0mm to >12mm. A sample of invertebrate grazers from each size category was air dried and weighed to an accuracy of ± 0.0001g. The number of invertebrates weighed per size category was recorded to establish length to weight relationship of invertebrate grazers within each size category to determine invertebrate biomass. These data enabled the conversion of individual number of invertebrates to grazer biomass per taxonomic group (Ewart-Smith and King, 2012).

The invertebrate grazers were split according to functional feeder groups namely; deposit feeders: that prefer to feed on larger algae or detritus, scrapers: that scrape thin films of algae of substratum and brushers: that feed predominately on large algae by brushing algal mats with the large scraping brushes on their bodies. Baetidae and leptophlebiidae are an example of deposit feeders, chironomidae and elmidae are examples of scrapers and heptageniidae are an example of brushers. These functional feeding groups, together with the feeding mode and dominant food type that belong to each of these functional feeding groups, were tabulated in the work by Ewart-Smith and King (2012). This was adapted from the functional feeding group definition by Schael (2005). Each of the functional feeder groups were divided into five size classes ([<0mm-3mm],[3mm-6mm], [6mm-9mm], [9mm-12mm], [>12mm]) used for invertebrate biomass estimation.

3.4 Data analysis

Analysis of physico-chemical factors, algal biomass and species identification and invertebrate identification was conducted to determine driving factors of periphyton dynamics. Physico-chemical factors collected in field are presented using trend graphs to suggest trends of each physico-chemical factors at each site and to indicate the interaction between these factors between each of the sampling sites. Independent sample t-tests were carried out to determine significant differences in physic-chemical factors between comparable sites. Independent sample t-tests between each physico-chemical factor between site A (Nutrient unenriched) and site B (nutrient enriched) and between Site C (unaltered flow) and site D (altered flow) were conducted. Trend graphs of water quality data namely Ammonia, nitrites and soluble reactive phosphorus, obtained from Umgeni Water were developed to illustrate the nutrient differences between sites.

Calculated algal biomass, AFDM and Chl *a* are presented as trend graphs for each site. Chl a values were also compared to benthotorch results. Two-way Analysis of Variance (ANOVA) in STATISTICA was used to indicate the differences in AFDM between comparable sites through the sampling period. Therefore, Two-way ANOVAs between differences in algal biomass at site A (nutrient unenriched) and site B (nutrient enriched) and between site C (unaltered flow) and site D (altered flow). An independent sample t-test to determine if significant differences in algal biomass were evident after the planting of maize at site E (reference) was conducted.

Trend graphs suggest the interaction between calculated AFDM and invertebrate biomass at each site. Trend graphs of invertebrate biomass, density and abundance were constructed between sites. Graphs indicating the specific invertebrate grazer density of each invertebrate family namely Baetidae, chironomidae, heptageniidae, Leptophlebiidae and elmidae-larvae at each site was conducted. Two-way ANOVAs to determine the differences in invertebrate density over the sampling period at comparable sites were calculated. Therefore, comparisons between site A (nutrient unenriched) and site B (nutrient enriched) and between site C (unaltered flow) and site D (altered flow) was conducted. An independent sample t-test to determine if any significant differences in invertebrate density at site E (reference) were conducted.

A Principal Component Analysis (PCA) was conducted to indicate the driving physico-chemical factors at comparable sites namely site A (Nutrient unenriched) and site B (Nutrient enriched) and site C (Unaltered flow) and site D (Altered flow). Another PCA to determine the relationship between algal biomass and invertebrate grazer families were conducted to determine the driving factors at comparable sites, between site A (Nutrient unenriched) and site B (Nutrient Enriched) and site C (Unaltered flow) and site D (Altered flow).

A Shannon Weiner index was conducted to determine the diversity of invertebrate families between comparable sites and trend graphs indicating surface water discharge obtained from the department of water affairs interactive website was used. Finally water temperature recorded hourly by the remaining loggers were downloaded and represented by a line graph. However once the graph was plotted a number of disparities and outliers were observed mainly at points when data loggers were found out of the water. To account for the daily moving averages were calculated together with a 95% confidence interval. All raw data that fell out of this range was removed as they were treated as outliers. The corrected data was then used to model temperature data for the period June 2014 to December 2014 which was the start of the project when the loggers had not been installed. The water temperature for this time period was modelled by calculating a simple linear regression between air temperature for Pietermaritzburg and water temperature for each site. An r^2 value of R^2=0.3373, R^2=0.4979 and R^2=0.6062 was achieved at sites C (unaltered flow), D (altered flow) and E (reference) respectively. The regressions calculated from the scatter plots were used to determine the missing water temperature data from June 2014 to December 2014 as air temperature data was available.

Table 3.1: Summary table of data analyses methods conducted

Data analysis method	**Outcome**
Trend graphs	To determine the changes in physico- chemical data at each site and between sites.
	To indicate changes in water quality variables at each site
	To indicate changes in algal biomass (AFDM and chlorophyll *a*) at each site over the sampling period
	To indicate the interaction between algal biomass and invertebrate biomass at each site over the sampling period.
	To show changes in invertebrate density and abundance between each site.
	To indicate density trends of invertebrate grazer density at each site.
	Indicating surface water discharge
	Indicating the abundance of identified algal species at each site over the spring and summer season.
Independent sample t-tests	To determine differences in physico-chemical factors between comparable sites
	To determine differences in invertebrate density between comparable sites over the sampling period
	To determine significant differences in invertebrate density before and after planting maize at the reference site
Two- way ANOVA	To indicate differences in biomass between comparable sites over the sampling period.
Shannon wiener index	To determine species diversity between comparable sites
Principal component analysis	To highlight similarities and differences between algal biomass between comparable sites

CHAPTER FOUR

Results

4.1 Introduction

The chapter presents the data collected from June 2014 to June 2015 at each of the five sampling sites. These sites are site A (Nutrient unenriched: Msunduzi-Kwanoshezi), site B (Nutrient enriched: Msunduzi-Tannery), site C (Unaltered flow: Umgeni- Petrustroom), site D (Altered flow: Umgeni-York road) and site E (Reference: Hlatikhulu). Physico chemical data collected at each site at each sampling occasion were analysed in terms of trends over the sampling period. Algal biomass was determined using AFDM and chlorophyll *a* at each site. Finally relationships between invertebrate grazer abundance, density and biomass, the differences between these indicators at each sites, and the relationship with algal biomass are explored.

4.2 Temporal physico-chemical patterns

Physico-chemical data were collected during monthly sampling events at each site using a YSI for temperature, conductivity, pH, % oxygen and dissolved oxygen and a flow meter for depth and velocity. Depth and velocity at each site were calculated as the average of five readings next to collected stones and clarity was determined using a clarity tube or flow meter. The mean monthly water temperature at each site was greater in summer than winter. Highest temperatures at each site was observed between October 2014 and February 2015, ranging from 19^0C to 24^0C whilst lowest temperatures were observed between June 2014 to October 2015 and March 2015 to May 2015 ranging from 7^0C to 14^0C (Figure 4.1).

Percentage dissolved oxygen levels varied considerably throughout the sampling period at each of the sites. A peak was experienced at site D (altered flow) in February 2015 (124.5%). Lowest % oxygen levels were experienced at site C (Unaltered flow) throughout the sampling period, with lowest % oxygen levels experienced at site C in December 2014 (80.6 %) (Figure 4.2).

A gradual decline in dissolved oxygen levels were experienced from September 2014 to December 2014 with lowest levels at 7.14mg/l experienced at site C (Unaltered flow) in December 2014, and a gradual increase from January 2015 to March 2015 at each of the sampling sites with the exception of site D were a sharp increase in oxygen levels were experienced in January 2015 from 8.24 mg/l in December 2014 to 10.92 mg/l in January 2015. The highest dissolved oxygen levels occurred in winter between May and June and lowest in summer in December at all five sampling sites (Figure 4.3).

Conductivity remained fairly constant at each of the five sites throughout the sampling period. Highest conductivity levels were experienced at site B ranging between 121 µS to 152 µS (Nutrient enriched). Lowest conductivity ranging between 30µS and 67µS occurred at site E 30µS to 67µS (Reference) (Figure 4.4). pH values varied considerably between the sampling sites. Lowest pH (6.11) was characteristic of site C (unaltered flow) in June 2015 while highest pH (8.06) was experienced at site B (nutrient enriched) in October 2014. A decline in pH was observed from June 2014 at a pH of 7.63 to July 2014 at a pH of 6.7 at site A (Nutrient unenriched) (Figure 4.5). Clarity remained constant at each of the sampling sites from June 2014 to October 2014. In general highest clarity occurred in winter while lowest clarity occurred in summer, which is the rainfall season (Figure 4.6).

A relationship between depth and velocity levels was evident at each of the sites. An increase in depth was followed by an increase in velocity either simultaneously of over a short lag time. Greater depths and velocity are experienced in summer, which is the rainfall season. Depth levels were constant at each site from June 2014 to October 2015. Peaks in depth were experienced in November 2014 and February 2015 at site A (nutrient unenriched). An increase in depth occurred from October 2014 to November 2014 at site B (nutrient enriched). Increases in depths occurred in December 2014 and March 2015 at site C (unaltered flow). Depth remained constant at site D (altered flow). Finally, at site E (reference) peaks in depth occurred in January 2015 and March 2015 (Figure 4.7). Peaks in velocity were experienced in December 2014 at site A (nutrient unenriched) and Site B (nutrient enriched) while peaks in velocity occurred in February at site E (reference) and March 2015 at site C (unaltered flow) and site D (altered flow) (Figure 4.8)

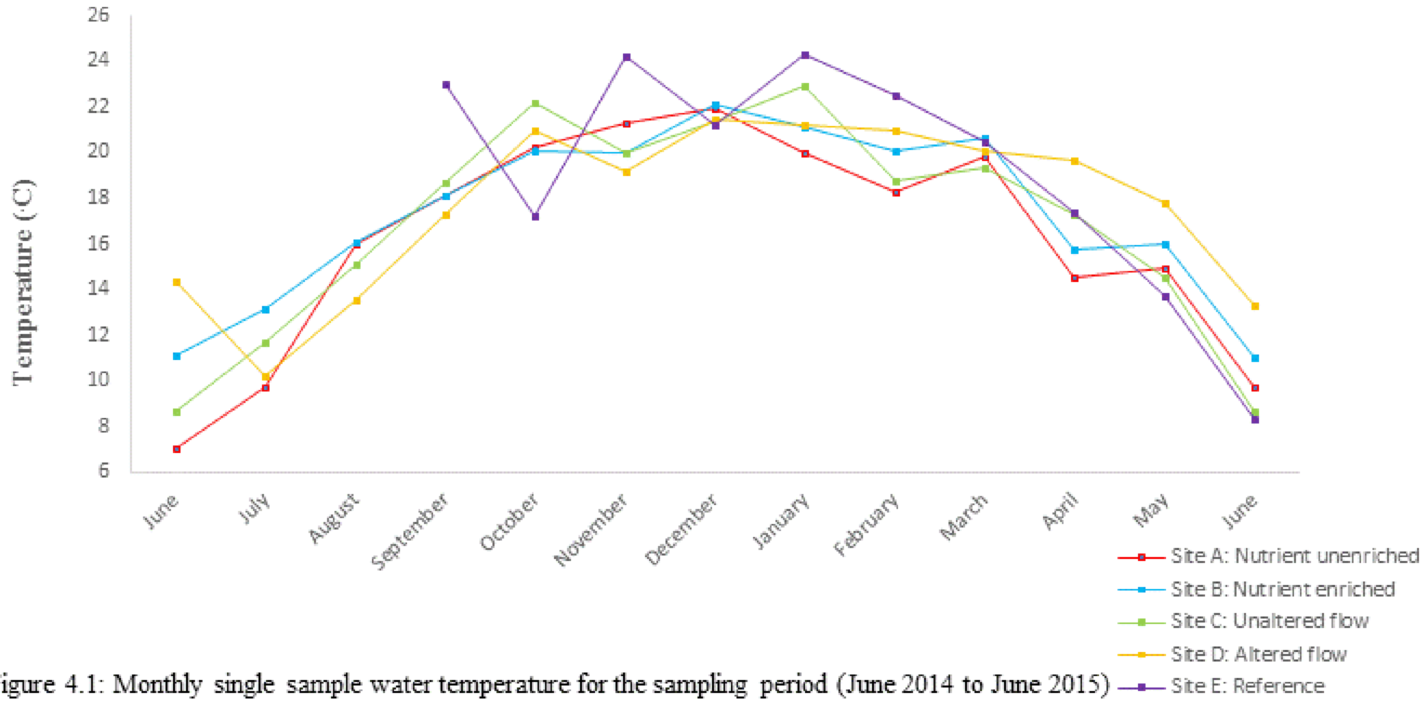

Figure 4.1: Monthly single sample water temperature for the sampling period (June 2014 to June 2015)

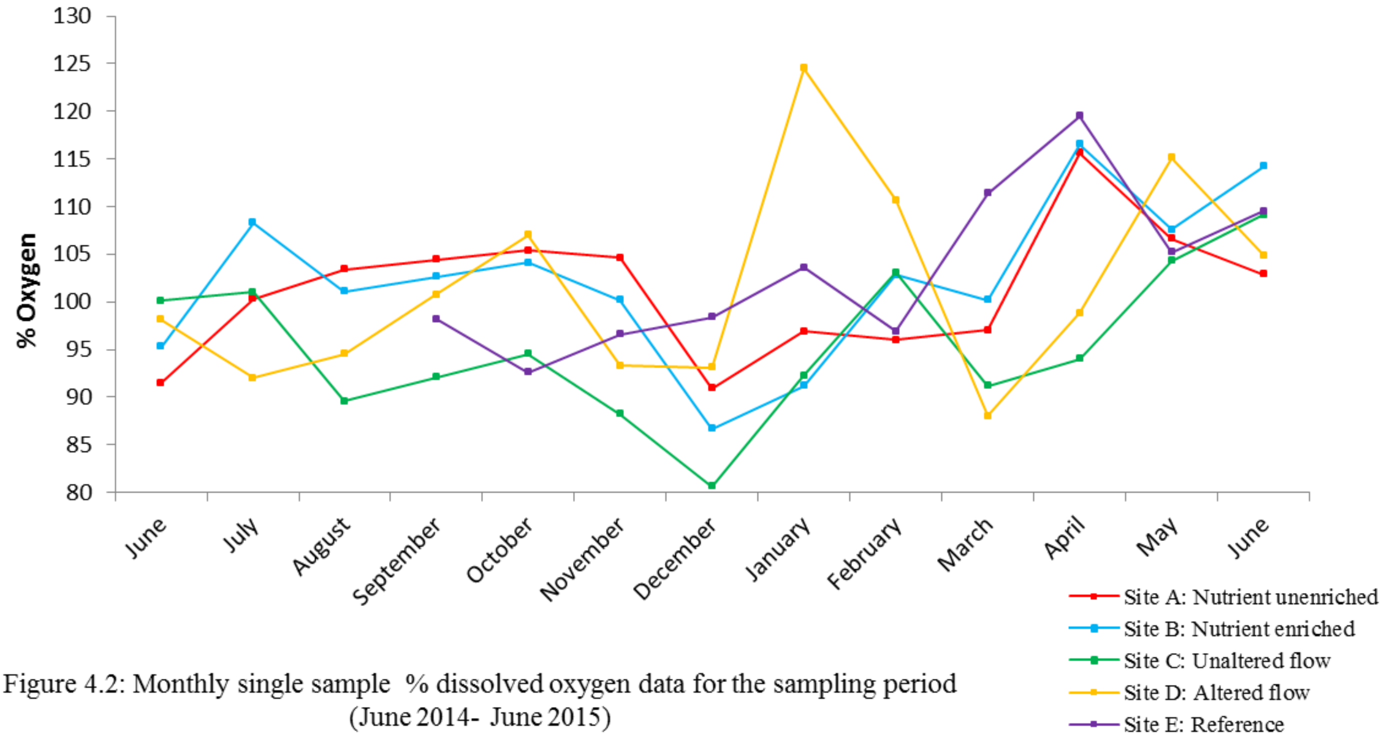

Figure 4.2: Monthly single sample % dissolved oxygen data for the sampling period (June 2014- June 2015)

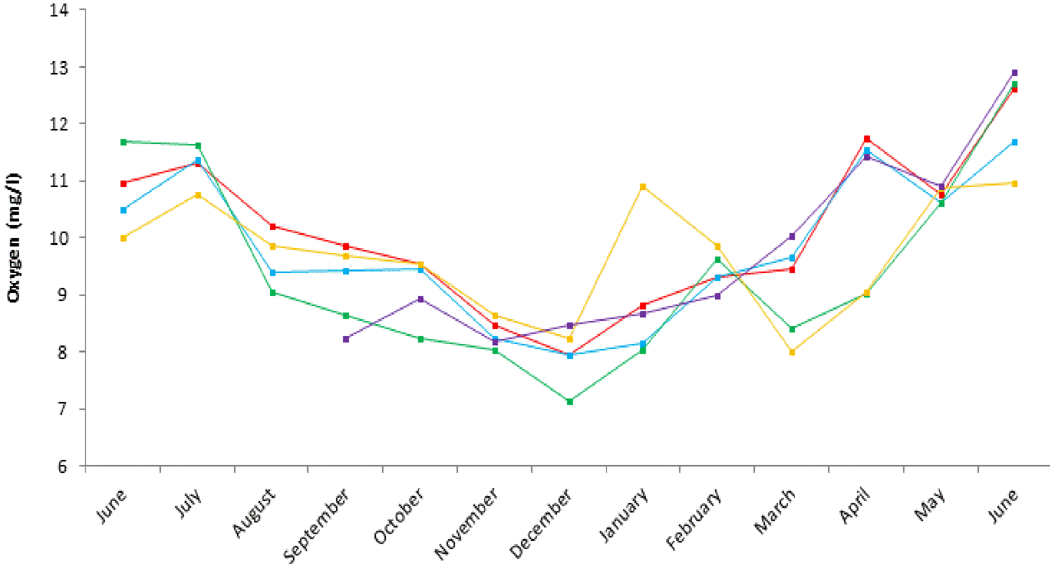

Figure 4.3: Monthly single sample dissolved oxygen data for the sampling period (June 2014- June 2015)

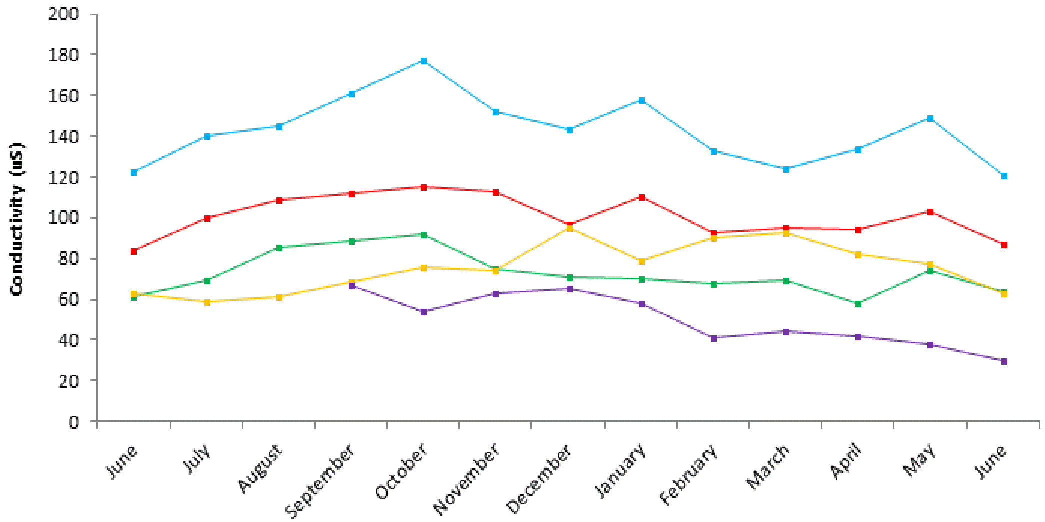

Figure 4.4: Monthly single sample conductivity data for the sampling period (June2014-June 2015)

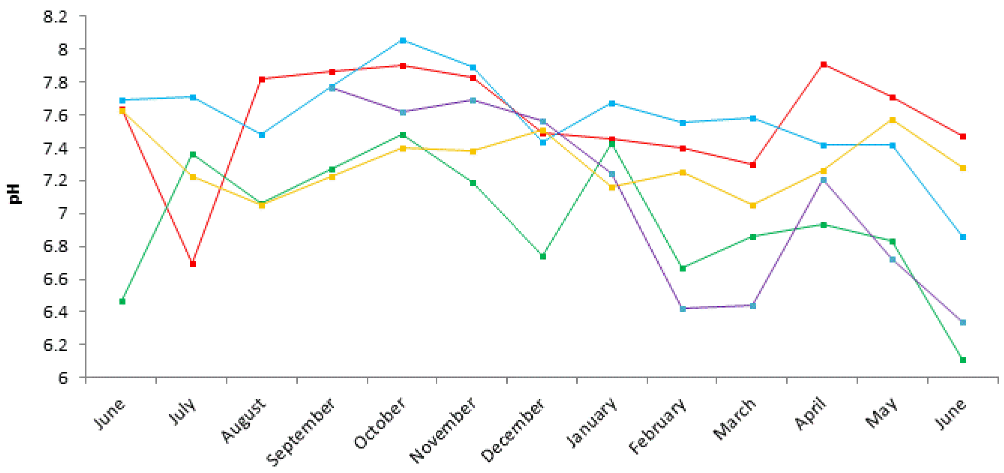

Figure 4.5: Monthly single sample pH data for the sampling period (June 2014- June 2015)

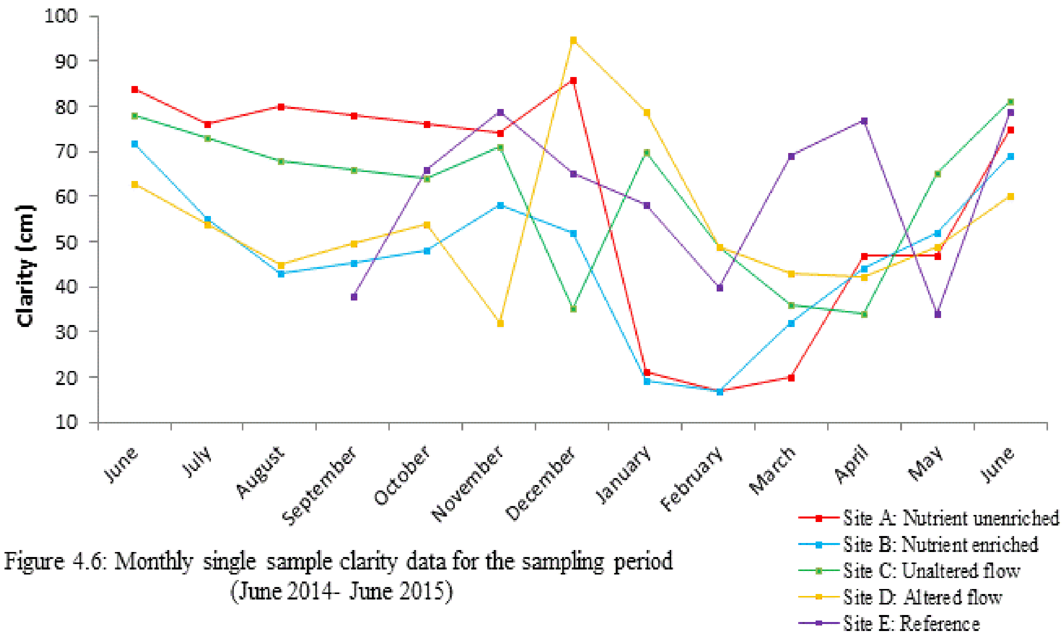

Figure 4.6: Monthly single sample clarity data for the sampling period (June 2014- June 2015)

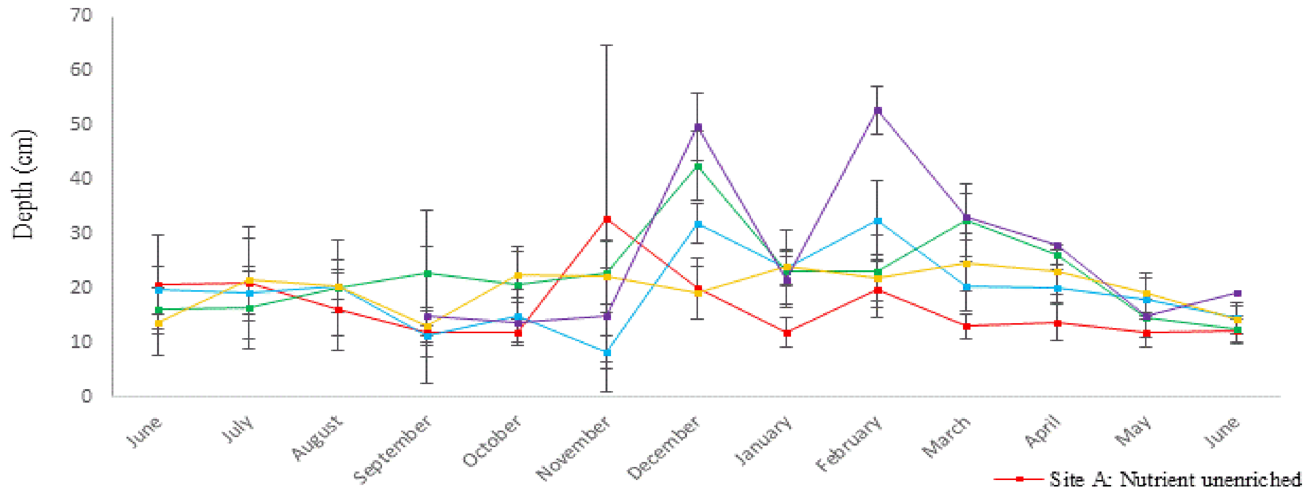

Figure 4.7: Average water depth data for the sampling period (June 2014- June 2015)
(error bars ±1SD about the mean)

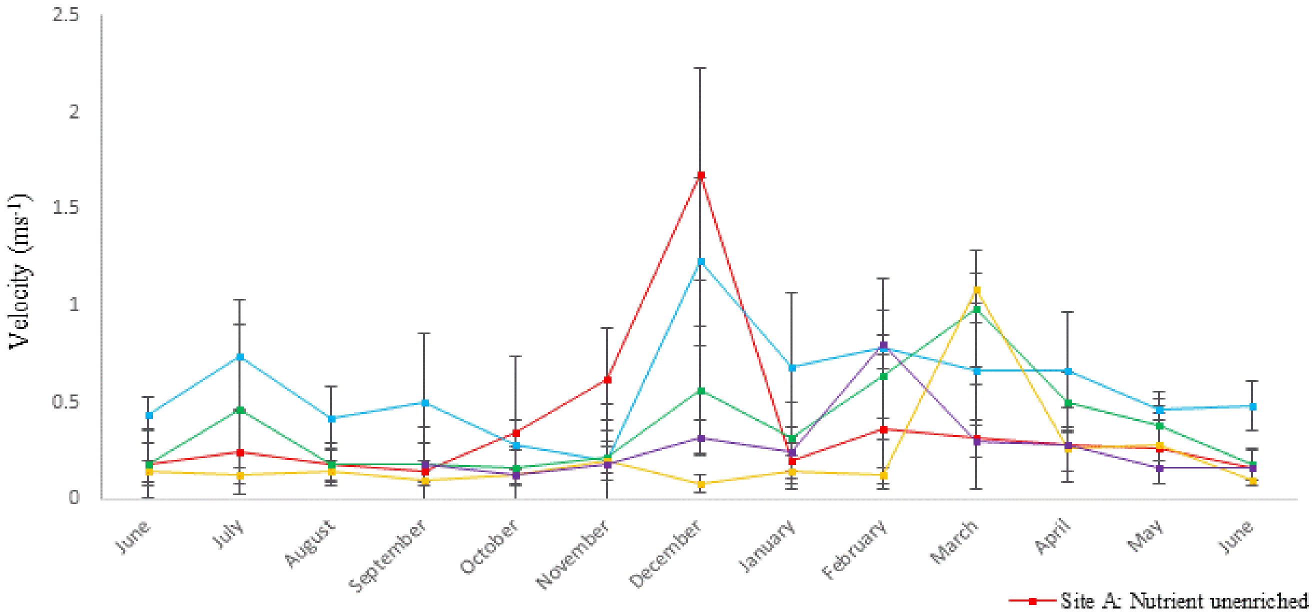

Figure 4.8: Average water velocity data for the sampling period (June 2014- June 2015)
(error bars ±1SD about the mean)

4.3 Water chemistry

Slight fluctuations in pH, dissolved oxygen, percentage dissolved oxygen and conductivity occurred at each site. Highest water temperature occurred in summer between November 2014 and February 2015 and lowest in winter between May to July. Clarity decreased in summer after rainfall events. The relationship between velocity and depth was evident were greater depths resulted in higher velocity. Greatest depth was observed in summer, in the rainfall season and lowest occurred in winter (Figure 4.9- 4.18).

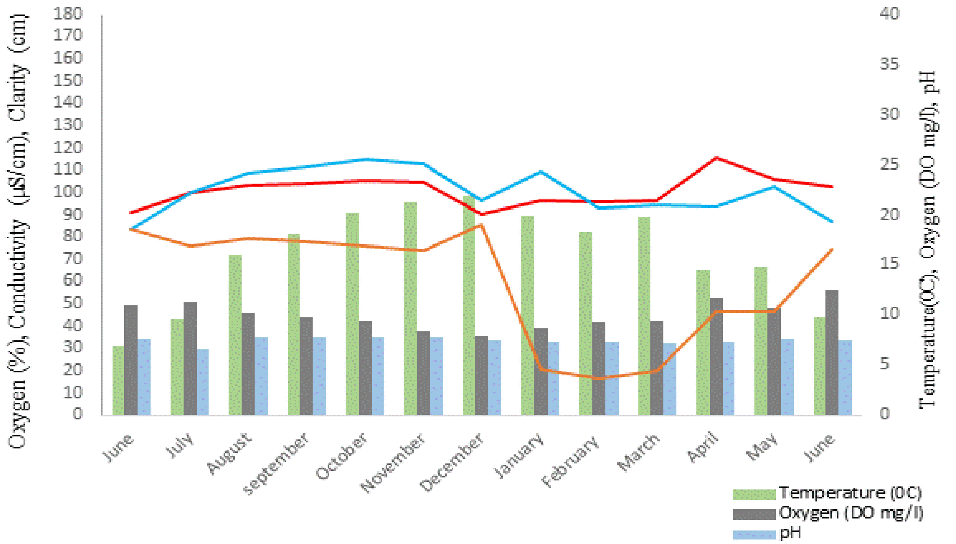

Figure 4.9 Monthly Physico-chemical changes (June 2014- June 2015) collected at Site A (nutrient unenriched: Msunduzi-Kwanoshezi)

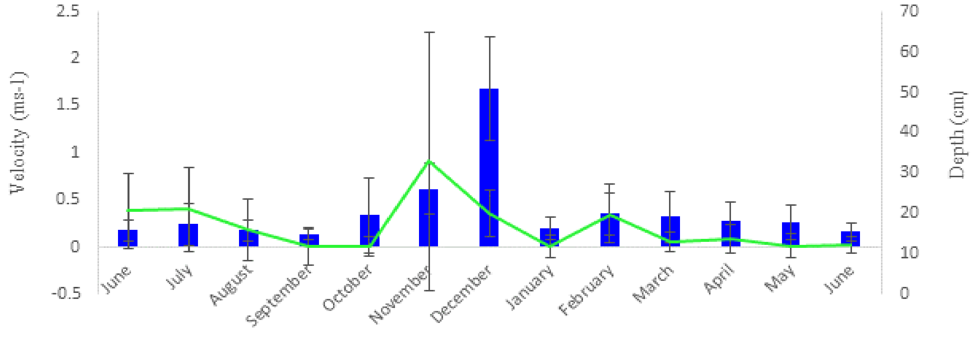

Figure 4.10: Monthly depth and velocity changes (June 2014- June 2015) at site A (Nutrient unenriched- Msunduzi Kwanoshezi)

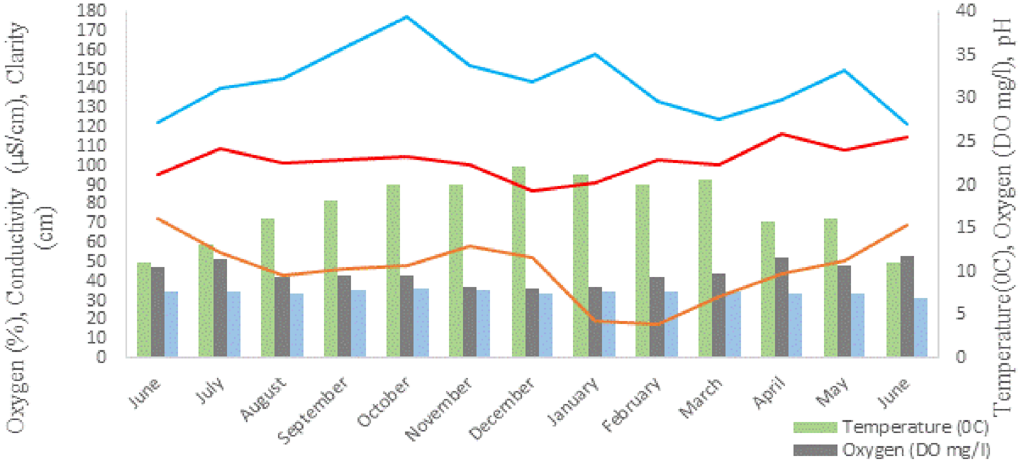

Figure 4.11 Monthly Physico-chemical changes (June 2014- June 2015) at Site B (nutrient enriched: Msunduzi- Tannery)

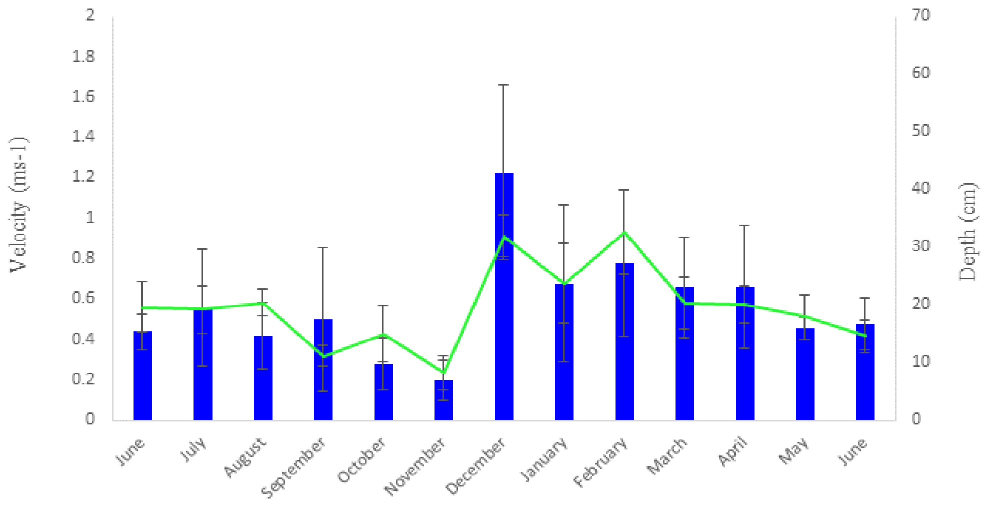

Figure 4.12: Monthly depth and velocity changes (June 2014- June 2015) at site B (Nutrient enriched- Msunduzi Tannery)

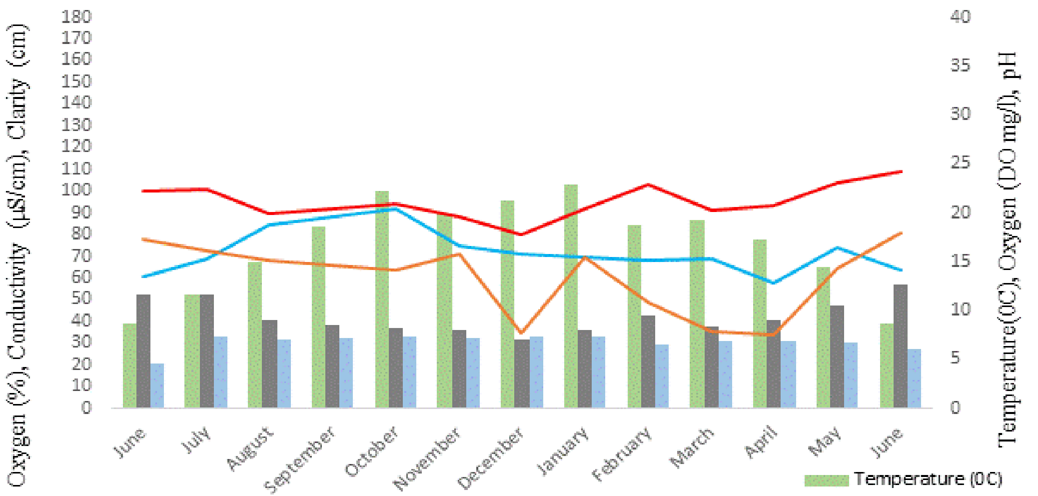

Figure 4.13 Monthly Physico-chemical changes (June 2014- June 2015) collected at Site C (unaltered flow: Umgeni Petrustroom)

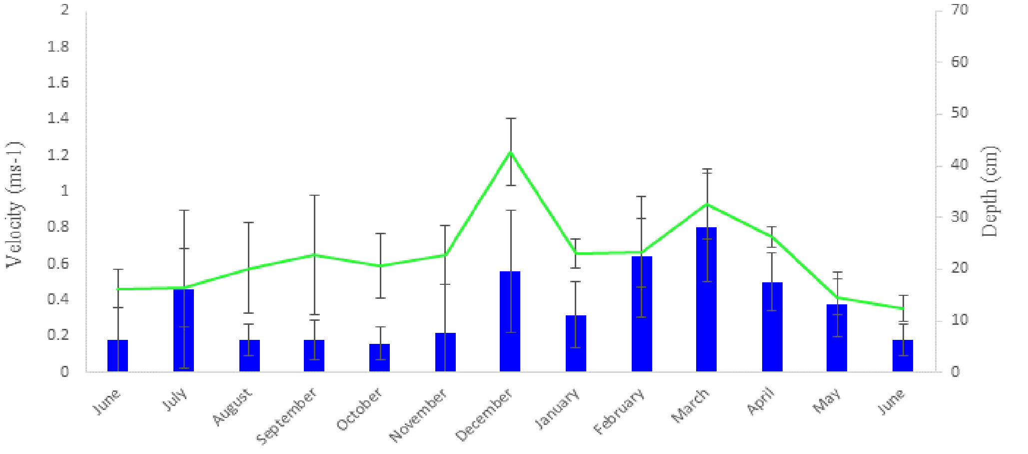

Figure 4.14: Monthly depth and velocity changes (June 2014- June 2015) at site C (Unaltered flow- Umgeni petrustroom)

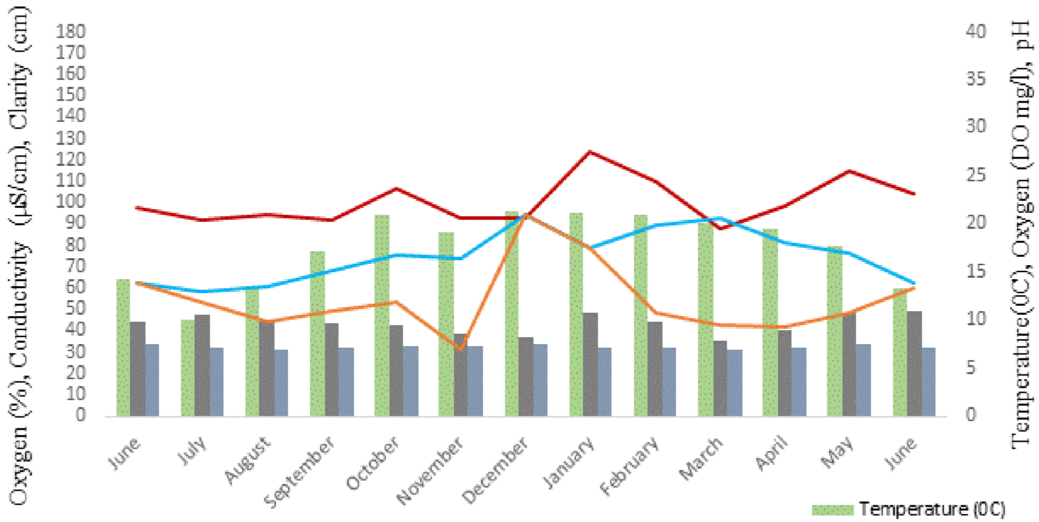

Figure 4.15 Monthly Physico-chemical changes (June 2014- June 2015) collected at Site D (altered flow: Umgeni York road)

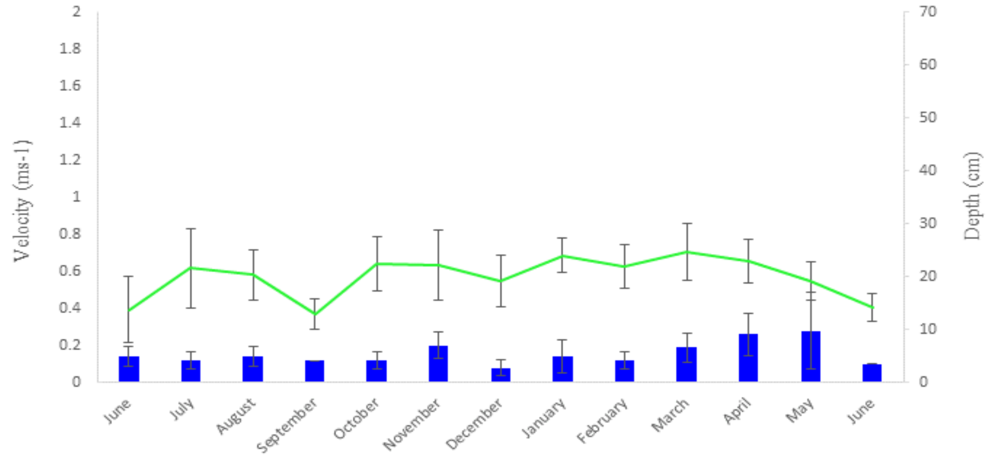

Figure 4.16: Monthly depth and velocity changes (June 2014- June 2015) at site D (Altered flow- Umgeni York Road)

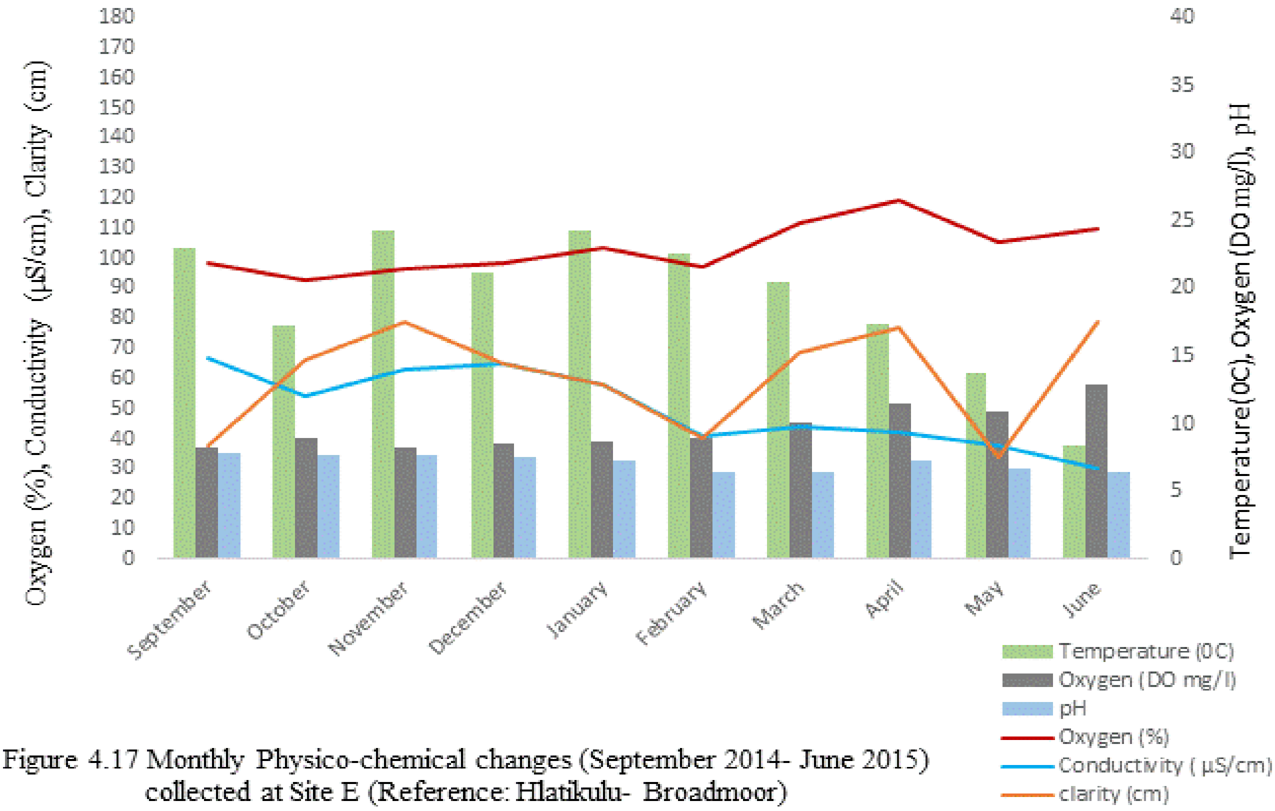

Figure 4.17 Monthly Physico-chemical changes (September 2014- June 2015) collected at Site E (Reference: Hlatikulu- Broadmoor)

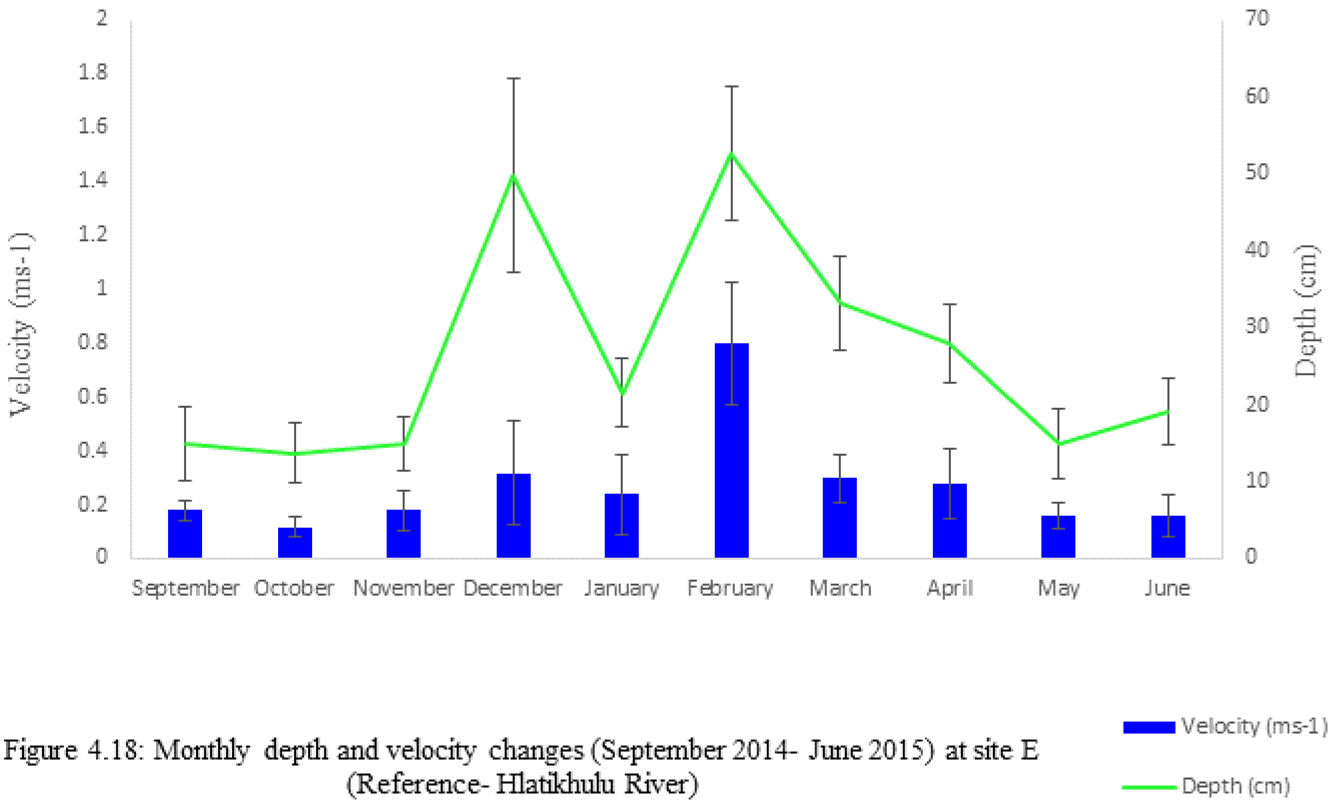

Figure 4.18: Monthly depth and velocity changes (September 2014- June 2015) at site E (Reference- Hlatikhulu River)

4.4 Water quality

Ammonia (NH_3) levels peaked at site A (nutrient unenriched) (0.53mg N/L) in November 2014, at site B (nutrient enriched) (0.32mg N/L) in October 2014 and December 2014 (0.58mg N/L) and at site D (altered flow) (0.5mg N/L) in April 2014. At site C (unaltered flow) the ammonia levels remained low and fairly constant with no peaks. Highest ammonia levels occurred at site B at 0.58 mg N/L. During months that no peaks in ammonia were observed levels remained between 0 and 0.2 mg N/L (Figure 4.19). Ammonia data for site E (reference) was unavailable.

The largest fluctuations in soluble reactive phosphorus (SRP) occurred at site A (Nutrient unenriched) and site B (nutrient enriched). Peaks in SRP at site A occurred in September 2014 (9.44 ug P/L) and December 2014 (8.24 ug P/L). SRP levels between these two peaks were still high. A decrease in levels of SRP at site A occurred in August 2014 (3 ug P/L) and January 2015 (3.53 ug P/L). Peaks in SRP at site B occurred in October 2014 (7.36 ug p/L) and March 2015 (9.41 ug P/L), while lowest SRP levels at site B were observed from December 2014 to January 2015 (3 ug P/L). At site C (unaltered flow) SRP remained constant with a 0.5 ug P/L increase from December 2014 to January 2015 after which levels remained constant. At site D (altered flow) a peak in SRP was observed in August 2014 (6.47 ug P/L) after which levels remained constant throughout the sampling period (Figure 4.20). SRP water quality data was unavailable for site E (reference).

Nitrite (NO_2) levels remained low at site A (nutrient unenriched) with a peak from 0.04mg N/L to 0.53 mg N/L in November 2014 after which levels return to being consistently low. Peaks in nitrate levels at site B (nutrient enriched) occurred in October 2014 and December 2014 peaking to 0.32 mg N/L and 0.58 mg N/L respectively. Nitrite levels at site C (unaltered flow) were overall higher than at site A and B. Levels remained between 0.1 mg N/L and 0.45 mg N/L. highest nitrite levels occurred at site D (altered flow) remaining between 0.37 mg N/L and 0.49 mg N/L from July 2014 to January 2015 after which nitrate levels declined to between 0.21mg N/L and 0.22 mg N/L until the end of the sampling period (Figure 4.21). There was no nitrite data available for site E (reference).

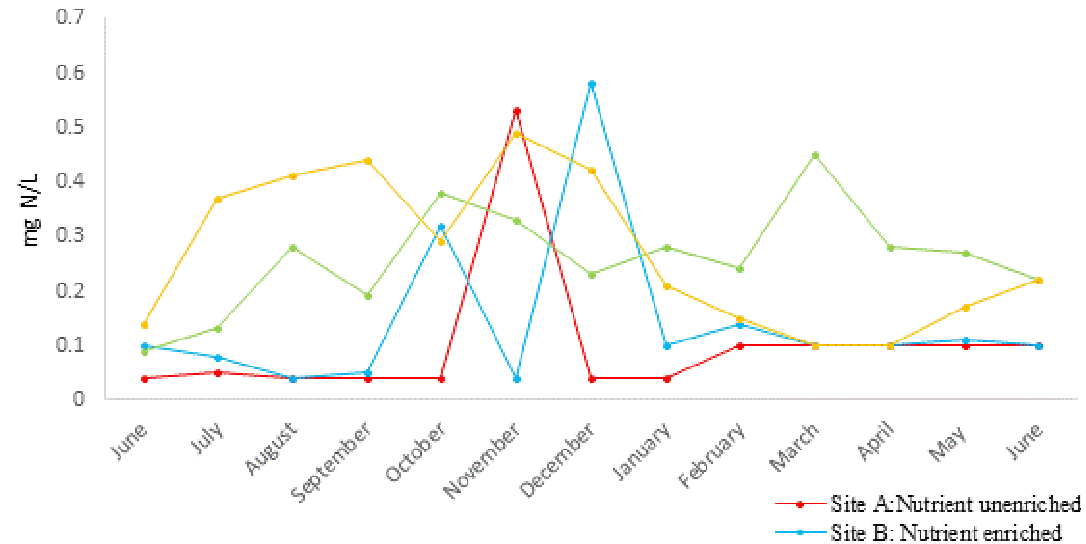

Figure 4.21: Nitrite levels recorded for the sampling period (June 2014- June 2015)

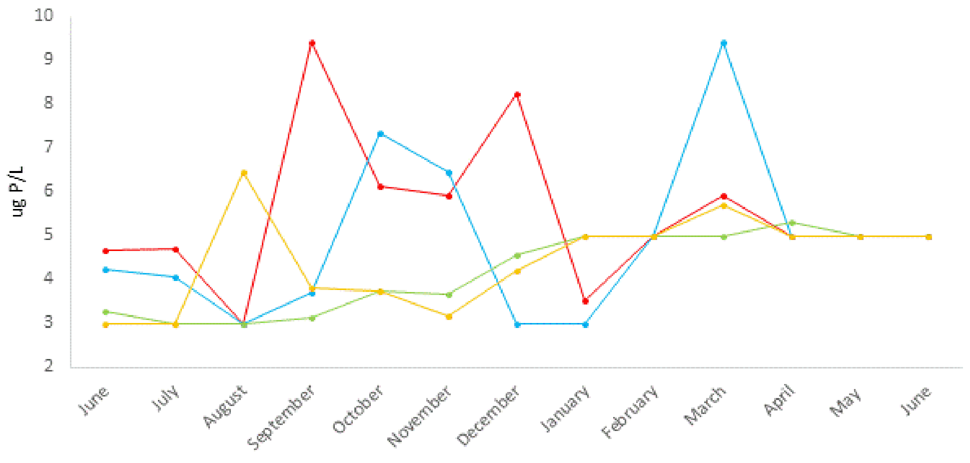

Figure 4.20: Monthly soluable reactive phosporus (SRP) levels recorded for sampling period (June 2014- June 2015)

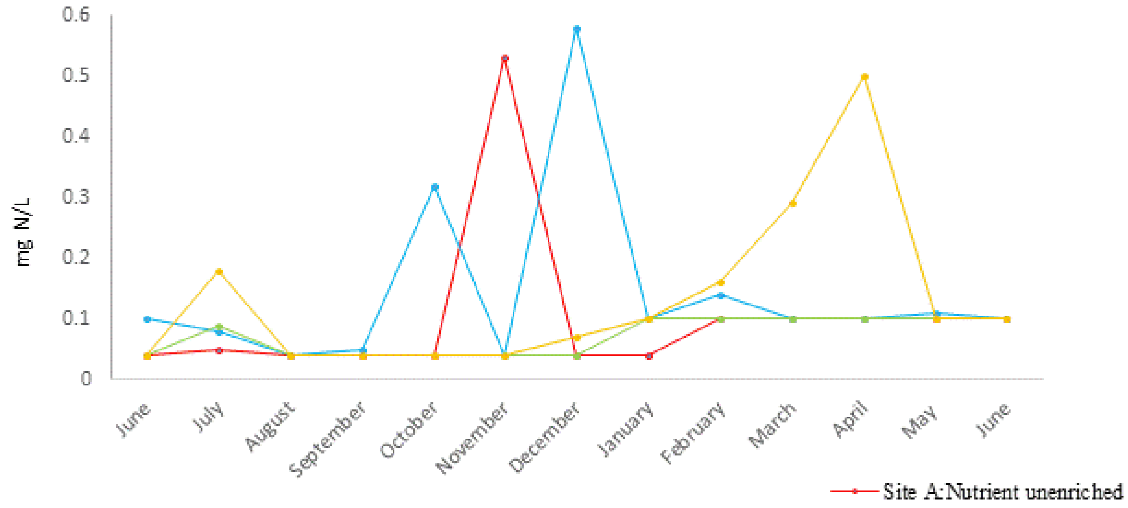

Figure 4.19: Monthly ammonia levels recorded for the sampling period (June 2014- June 2015)

4.5 Driving physico-chemical factors

Independent sample t-tests were conducted between physico-chemical factors at comparable sites, between sites of nutrient changes and sites of flow alteration. An independent sample t-test was conducted to compare field physico-chemical factors; monthly water temperature, % oxygen, oxygen, conductivity, pH, clarity, velocity and depth for sites that were nutrient unenriched (site A) and nutrient enriched (site B). By testing the assumptions of normality of distribution and equality of variances it was determined that non-parametric tests for clarity, pH, velocity and depth had to be conducted. The Mann-Whitney U test indicated a significant difference between velocity at sites that were nutrient unenriched (site A) and nutrient enriched (site B), (U= 26.5, p= 0.00) and no significant difference in pH (U=85.50, p=0.84), clarity (U=48.50, p=0.06) and depth (U=71.5, p=0.50) at sites that were nutrient unenriched (site A) and nutrient enriched (site B) (Table 4.1).

Independent sample t-tests indicated that conductivity differences were significant between sites that were nutrient unenriched (site A), (M=100.92, SD= 10.25) and sites that were nutrient enriched (site B) (M= 143.00, SD= 16.59), p= 0.00. Independent sample t-test showed no significant difference in temperature at nutrient unenriched (site A), (M=16.28, SD= 4.87) and nutrient enriched (site B), (M= 17.33, SD= 3.80) sites, p= 0.54. Percentage oxygen was not significantly different at nutrient unenriched (site A), (M= 101.19, SD= 6.76) and nutrient enriched (site B) sites, (M= 102.37, SD= 8.35) sites, p= 0.70. There were no significant differences in oxygen levels at the nutrient unenriched (M= 10.08, SD= 1.36) and nutrient enriched (site B), (M= 9.79, SD= 1.27) sites, p= 0.58 (Table 4.1)

An independent sample t-test was conducted to compare field physico-chemical factors including monthly temperature, % oxygen, oxygen, conductivity, pH, clarity, velocity and depth for sites of unaltered (site C) and altered flow (site D). By testing the assumptions of the test it was determined that non-parametric tests for the factors temperature, clarity, pH and velocity had to be conducted. The Mann-Whitney U test indicated a significant difference in pH at sites of unaltered (site C) and altered flow (site D), (U=40.00, p= 0.02) and in velocity between sites of unaltered and altered flow (U= 29.00, p= 0.00). The Mann-Whitney U tests for temperature and clarity showed no significant difference between sites of unaltered and altered flow, temperature (U=79.00, p=0.78) and clarity (U=61.00, p=0.23) (Table 4.1)

Independent sample t-tests suggest no significant difference between % oxygen at sites of unaltered (site C), (M=95.39, SD=4.81) and altered (site D), (M=101.59, SD= 10.48) flow, p= 0.10 and between sites of unaltered (M=9.45, SD=1.70) and altered (M=9.72, SD=1.02) flow, p=0.62. No significant differences in conductivity were experienced between sites of unaltered (M=72.65, SD= 10.28) and altered (M= 75.42, SD= 12.25) flow, p= 0.54. Finally depth was not significantly different between sites of unaltered (M= 22.58, SD=8.01) and altered (M= 19.98, SD= 3.96) flow, P= 0.31 (Table 4.1)

Table 4.1: Comparisons between physico-chemical factors between Site A (Nutrient unenriched-Msunduzi- Kwanoshezi) and site B (Nutrient enriched-Msunduzi-Tannery) and between Site C (Unaltered flow- Umgeni Petrustroom) and Site D (Altered flow- Umgeni- York Road)

	Site A: Nutrient unenriched vs Site B: Nutrient enriched		**Site C: Unaltered flow vs Site D: Altered flow**	
Physico- chemical factors	**Statistical test**	**p-value**	**Statistical test**	**p-value**
Temperature	Independent sample t-test	0.54	Mann-Whitney U test	0.78
% Oxygen	Independent sample t-test	0.7	Independent sample t-test	0.10
Oxygen	Independent sample t-test	0.58	Independent sample t-test	0.62
Conductivity	Independent sample t-test	0.00*	Independent sample t-test	0.54
pH	Mann-Whitney U test	0.84	Mann-Whitney U test	0.02*
Clarity	Mann-Whitney U test	0.06	Mann-Whitney U test	0.23
Velocity	Mann-Whitney U test	0.00*	Mann-Whitney U test	0.00*
Depth	Mann-Whitney U test	0.5	Independent sample t-test	0.31

*significant difference

4.6 Algal biomass

The trend between Ash Free Dry Mass (AFDM) and chlorophyll *a* as an indicator of biomass is similar at site A (nutrient unenriched) Peaks in biomass were experienced between December 2014 and January 2015 for both AFDM and chlorophyll *a*. A decrease in both biomass indicators was observed in February 2015 and a second peak in both AFDM and chlorophyll *a* was experienced in April 2015 (Figure 4.22 (a)). At site B (nutrient enriched) AFDM and chlorophyll *a* followed a similar trend throughout the sampling period. Peaks in both biomass indicators were obtained between December 2014 to February 2015. From June 2014 to October 2014 AFDM and chlorophyll *a* levels were fairly low. A second peak in both AFDM and chlorophyll *a* was experienced between March 2015 and April 2015 (Figure 4.22 (b)).

Chlorophyll *a* and AFDM measurements peaked in October 2014 after being fairly constant at the beginning of the sampling period at site C (unaltered flow). A second peak occurred in January 2015 for AFDM. Finally another peak in both AFDM and chlorophyll *a* was reached in April 2015 after which a decline in biomass was experienced until the end of the sampling period in June 2015 (Figure 4.22 (c)). At site D (altered flow) peaks in chlorophyll *a* and AFDM were experienced in October 2014. AFDM peaked again in January 2015 while chlorophyll *a* peaked in February 2015. A last peak was experienced in April for AFDM and May for chlorophyll *a* (Figure 4.22 (d)) At site E (reference site) both AFDM and chlorophyll *a* declined in October 2014. Peaks for both indicators were experienced in December 2014 and March 2015 (Figure 4.22 (e)).

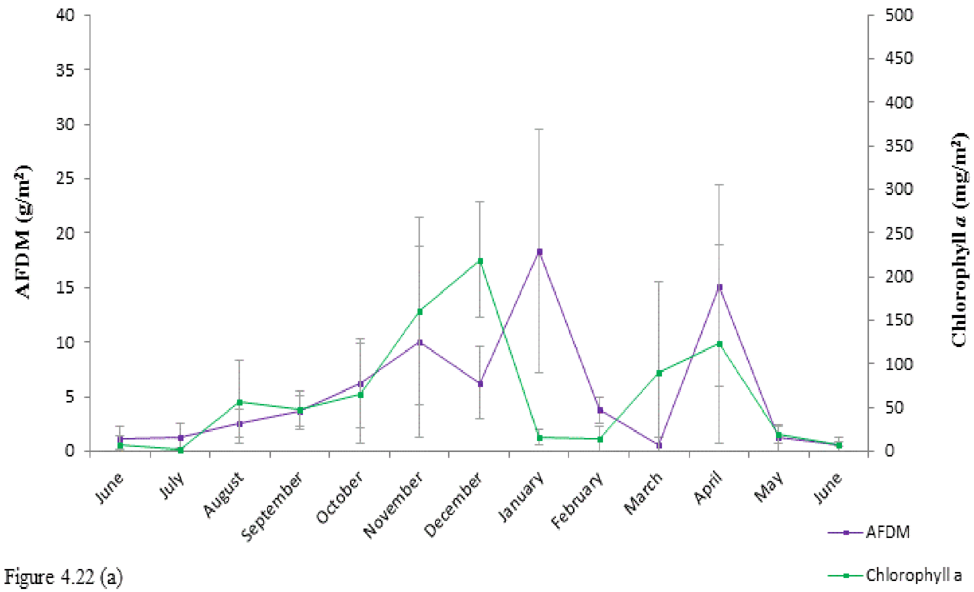

Figure 4.22 (a)

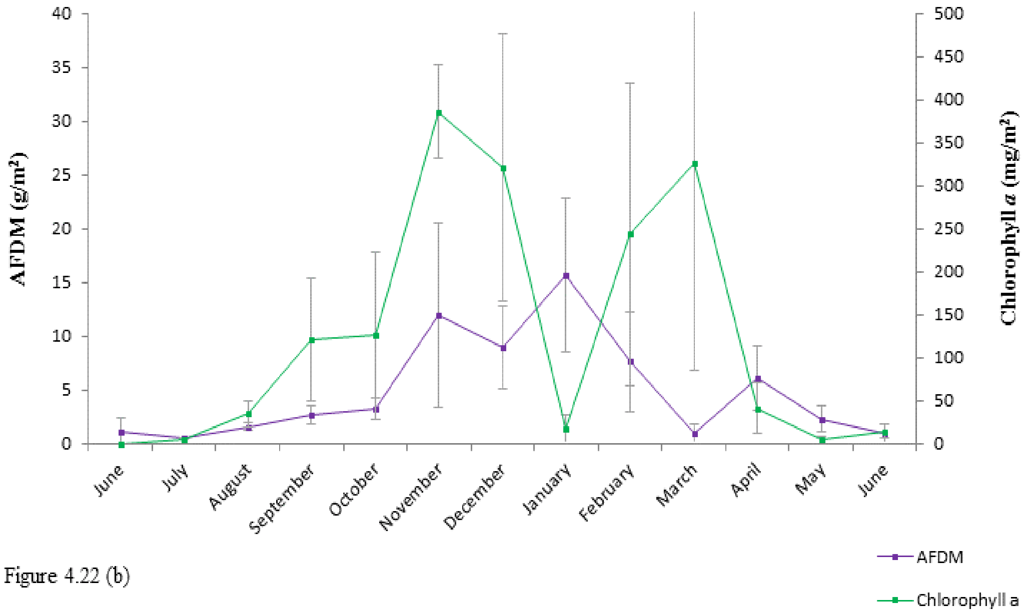

Figure 4.22 (b)

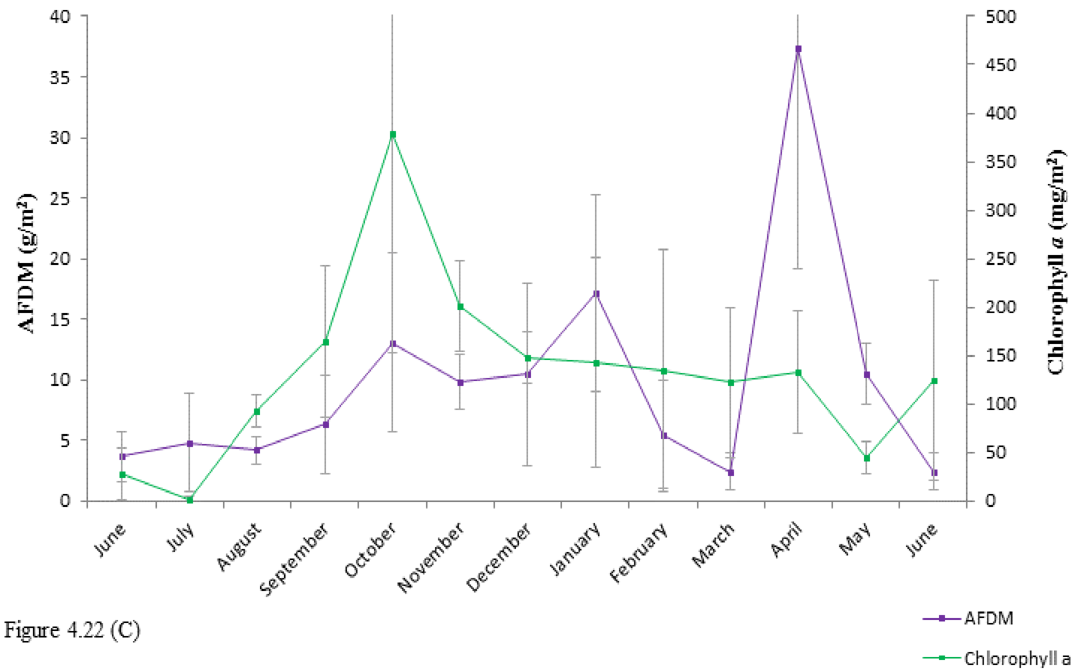

Figure 4.22 (C)

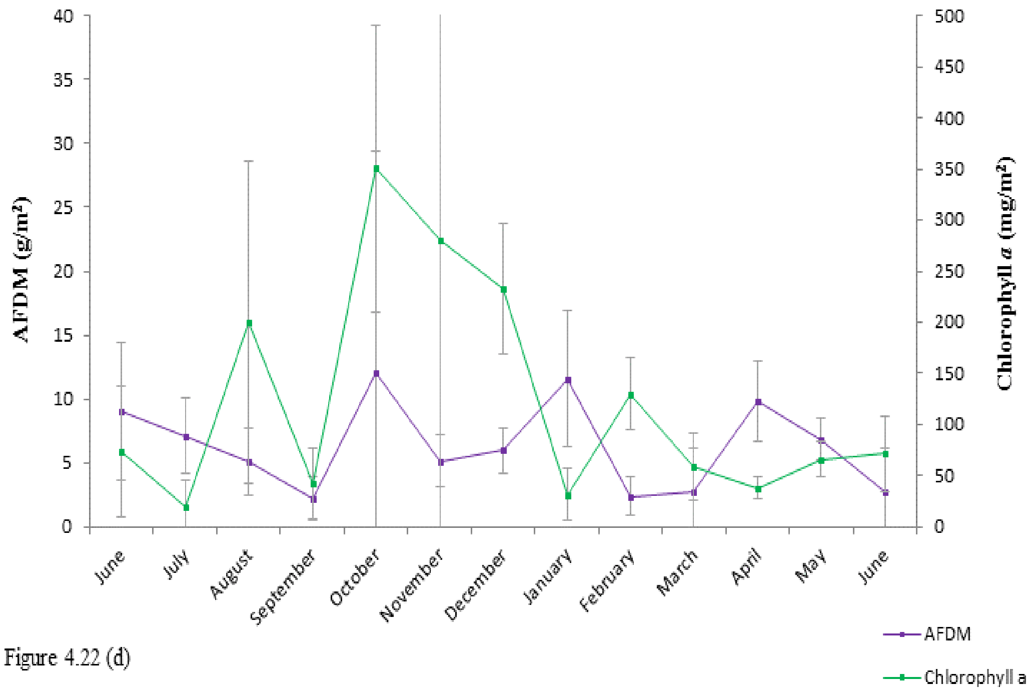

Figure 4.22 (d)

Figure 4.22: Algal biomass represented by AFDM and Chlorophyll *a* at (a)- site A (Nutrient unenriched- Msunduzi-Kwanoshezi), (b)- Site B(Nutrient enriched- Msunduzi-tannery), (c)- Site C (Unaltered flow- Umgeni-Petrustroom) and (d)- Site D (Altered flow- Umgeni-York Road) for the sampling period June 2014 to June 2015

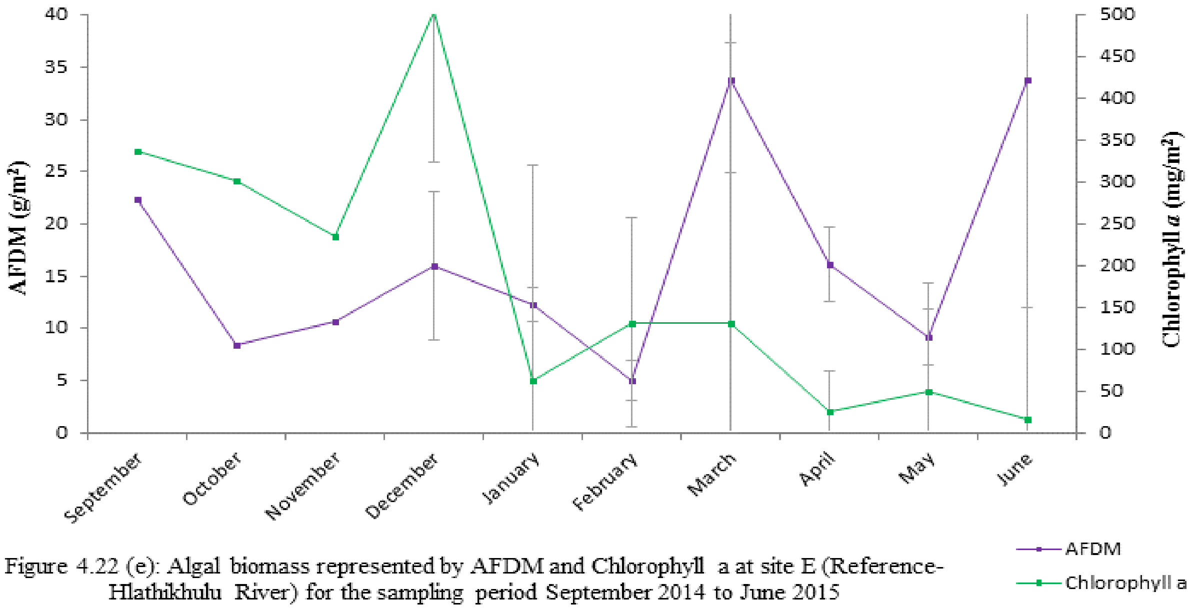

Figure 4.22 (e): Algal biomass represented by AFDM and Chlorophyll a at site E (Reference- Hlathikhulu River) for the sampling period September 2014 to June 2015

4.6.1 Algal biomass between sites

A Two-way analysis of variance on the influence of nutrient regimes and seasons on algal biomass was conducted. Nutrient differences included two levels (nutrient unenriched (site A) and nutrient enrichment (site B)) while seasons included four levels (winter, spring, summer, autumn). The effects of seasons on algal biomass at sites of nutrient enriched versus unenriched sites was statistically significant at a .05 significance level. The main effect of seasons, $p = 0.00$, $P<0.05$ indicated a significant difference in biomass over seasons at each site, winter ($M= 1.22$, $SD= 0.95$), spring ($M=6.31$, $SD= 1.10$), summer ($M=10.12$, $SD= 1.10$) and autumn ($M=4.39$, $SD= 1.10$). The main effect of site, $P= 0.584$, $P>0.05$ showed no significant difference in biomass between sites of different levels of nutrient enrichment. The interaction effect between sites of nutrient unenriched and nutrient enriched over seasons, $p = 0.67$, $P>0.05$ showed no significant effect (Table 4.2).

Table 4.2: Two-way ANOVA of algal biomass between nutrient unenriched (Site A- Msunduzi Kwanoshezi) and nutrient enriched (Site B- Msunduzi tannery) sites over the sampling period (June 2014 to June 2015)

	SS	df	MS	F	P
Site	11.20	1	11.20	0.31	0.58
Season	1415.77	3	471.92	12.95	0.00*
Site×Season	56.69	3	18.90	1.52	0.67
Error	4447.39	122	36.45		

*significant difference

A Two-way analysis of variance on the influence of flow type and seasons on algal biomass was conducted. Flow type included two levels (unaltered flow (site C) and altered flow (site D)) while seasons included four levels (winter, spring, summer, autumn). All effects were statistically significant at a .05 significance. The main effect for sites p= 0.014, P< 0.05, indicating that the effect of flow changes on algal biomass was significant, unaltered flow (M=10.33, SD=1.12) and altered flow (M= 6.41, SD= 1.12). The interaction effect between sites of unaltered and altered flow over seasons, p = 0.038, P<0.05 showed a significant effect. Site C and winter, site C and spring, site C and summer and site C and autumn and between Site D and winter site D and spring, site D and summer and site D and autumn. The main effect of seasons p = 0.025, P<0.05 indicated a significant difference in biomass over seasons at each site, winter (M= 4.89, SD= 1.40), spring (M=8.12, SD= 1.62), summer (M=8.84, SD= 1.62) and autumn (11.64, SD= 1.62). (Table 4.3).

Table 4.3: Two-way ANOVA of algal biomass between sites of altered (Site C- Umgeni Petrustroom) and unaltered (Site D- York Road) flow over the sampling period (June 2014 to June 2015)

	SS	df	MS	F	P
Site	491.67	1	491.67	6.27	0.014*
Season	802.42	3	267.47	3.40	0.020*
Site×Season	681.50	3	227.16	2.90	0.038*
Error	9579.79	122	78.46		

*Significant difference

An independent sample t-test was conducted to compare algal biomass levels before and after the plantation of maize at site E (reference). The independent sample t-tests showed no significant difference between algal biomass prior the introduction of maize (site E), (M=13.06, SD=7.99) and post planting (site E), (M=15.89, SD= 32.34), p= 0.71. However average biomass values do suggest that there was a greater algal biomass post planting

4.6.3. Algal biomass comparisons

A comparison between laboratory calculated chlorophyll a and in-field benthotourch readings was conducted. The trend graphs show similar trends especially at site C, site D and site E. For example peaks in both chlorophyll a laboratory and benthotourch readings indicated an increase in March 2014 flowed by a dip in April 2014 at site C (unaltered flow) (Figure 4.23 (c)). Dips and peaks in both benthotorch and laboratory calculated chlorophyll a were similar at site D (alerted flow) and site E (reference) (Figure 4.23 (d) - Figure 4.23 (e)). Clear trends were unable to be established at site A (figure 4.23 (a)) and (figure 4.23 (b)).

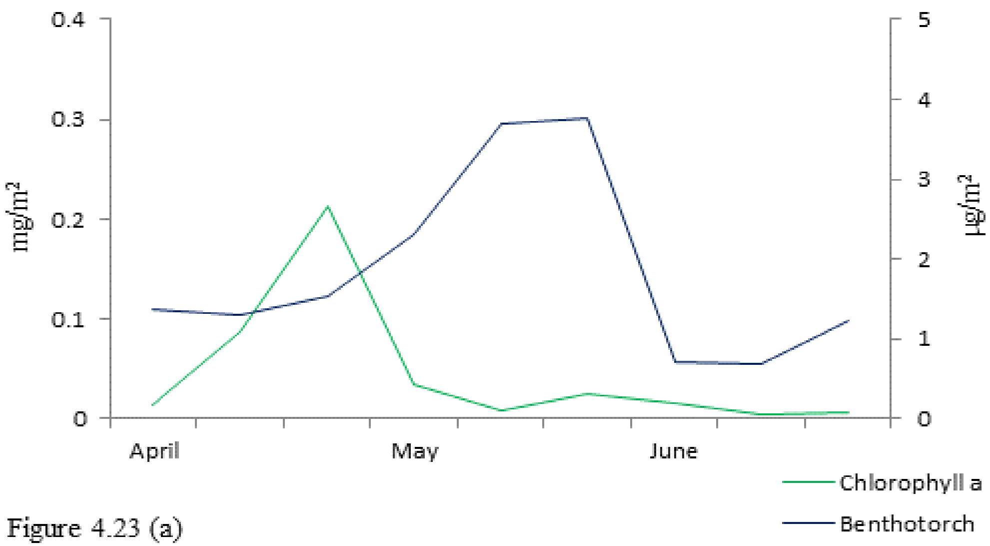

Figure 4.23 (a)

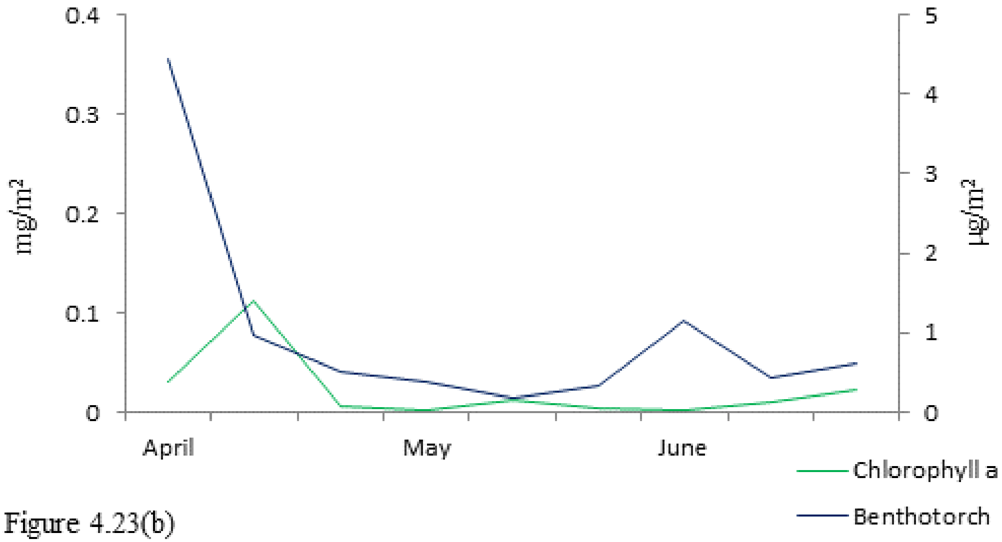

Figure 4.23(b)

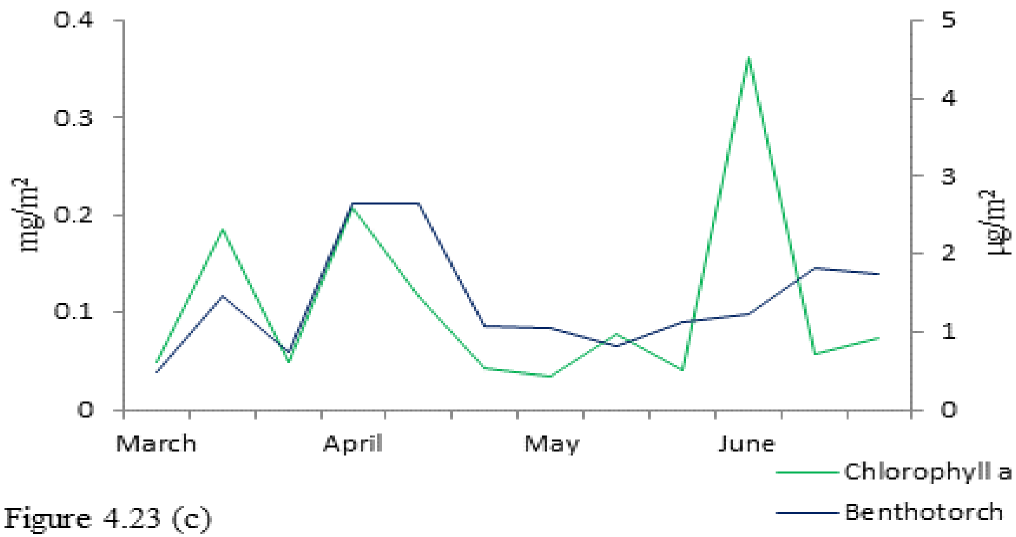

Figure 4.23 (c)

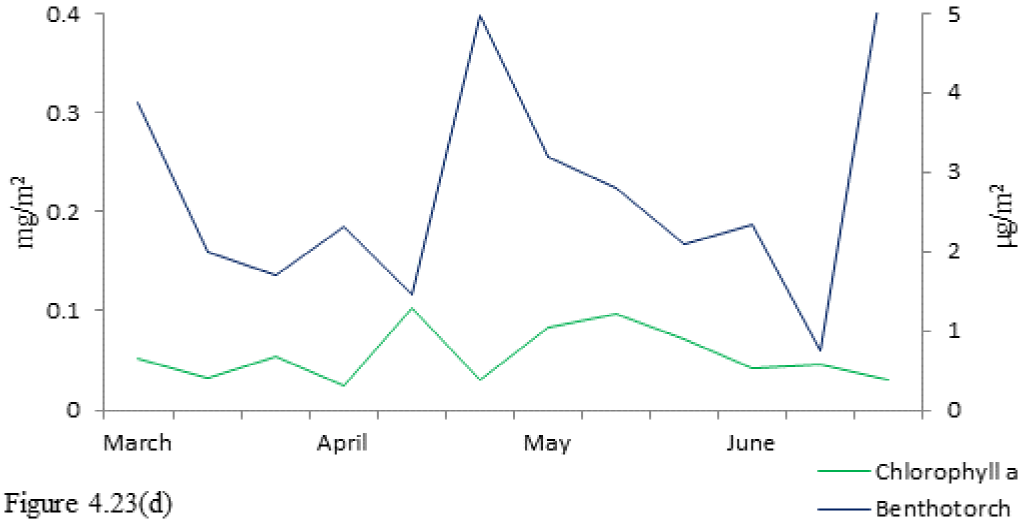

Figure 4.23(d)

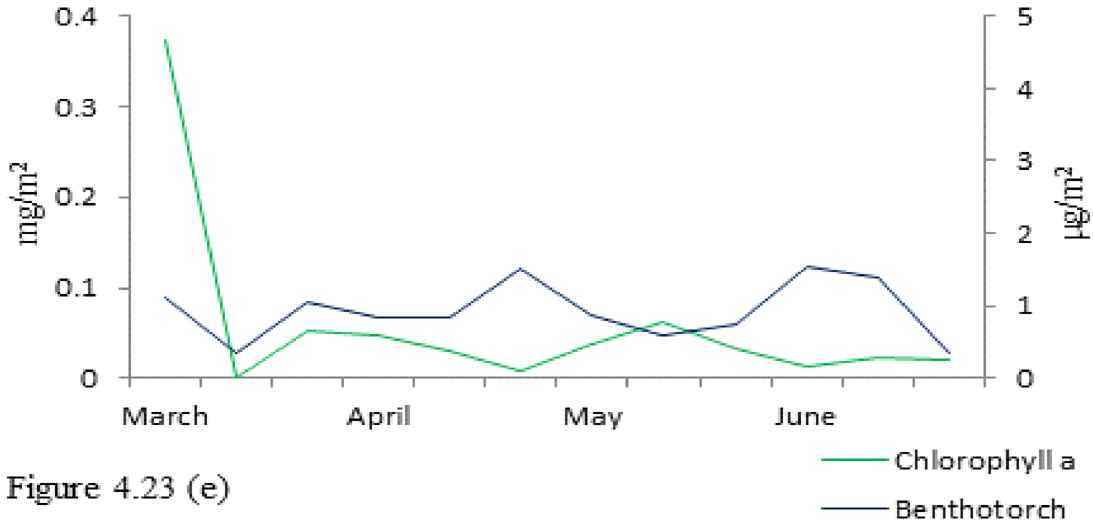

Figure 4.23: Chlorophyll *a* and BenthoTorch comparisons for the sampling period April 2015 to June 2015 at (a)- site A (Nutrient unenriched- Msunduzi-Kwanoshezi) and (b)- site B (Nutrient enriched- Msunduzi Tannery) and from March 2015 to June 2015 at (c)- site C (Unaltered flow- Umgeni-petrustroom), (d)- site D (Altered flow- Umgeni-York Road) and (e)- site E (Reference- Hlatikhulu River)

4.7 Invertebrate grazer and algal biomass relationships

A clear relationship between invertebrate biomass and algal biomass (AFDM) is observed at all five sampling sites. This relationship suggests that when grazer biomass is high, algal biomass is low as grazers provide a top down control of algal biomass by feeding on these communities. At site A this relationship is clearly evident. For example, between June 2014 to September 2014, invertebrate biomass is greater than algal biomass. From October 2014 to January 2015 the opposite is true where algal biomass is greater than invertebrate biomass. (Figure 4.24 (a)). At site B (nutrient enriched), low invertebrate biomass resulted in high algal biomass between October 2014 to February 2015. From February 2015 to June 2015 invertebrate biomass is greater than algal biomass (Figure 4.24 (b)).

At site C (unaltered flow), invertebrate biomass is higher from June 2014 to August 2014 than algal biomass. September 2014 to January 2015 has higher algal biomass due to low inveterate biomass. February 2015 has a peak in invertebrate biomass and a decrease in algal biomass. Lower invertebrate biomass levels from March 2015 to June 2015 was coupled with higher algal biomass (Figure 4.24 (c))

Algal biomass remained greater than invertebrate biomass throughout the sampling period except for June 2014 and June 2015, together with November 2014 and between February 2015 and March 2015 were the opposite is true at site D (altered flow) (Figure 4.24 (d)). At site E (reference site) algal biomass was greater than invertebrate biomass between September 2014 and December 2014 and between February 2015 and May 2015 (Figure 4.24 (e)).

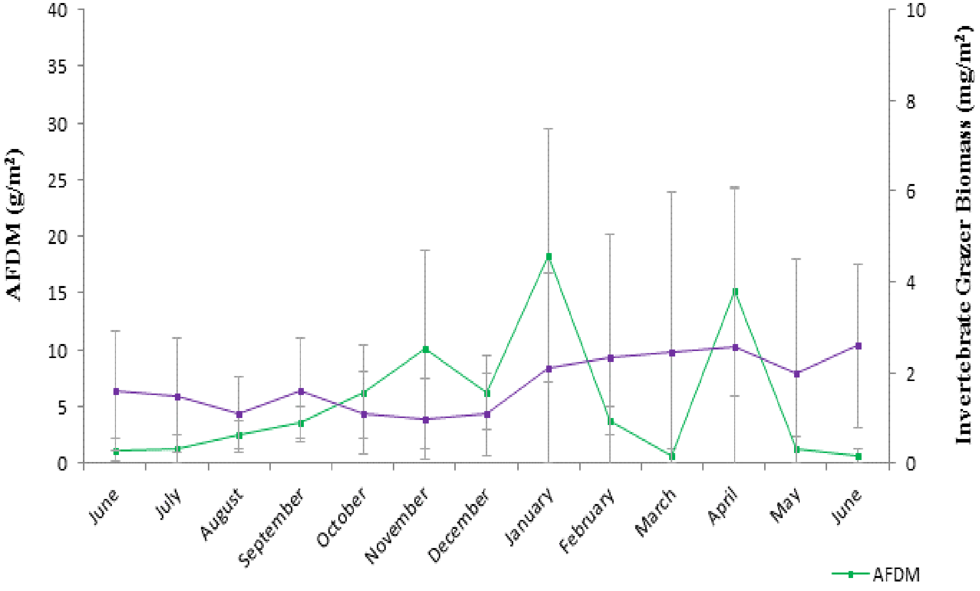

Figure 4.24 (a)

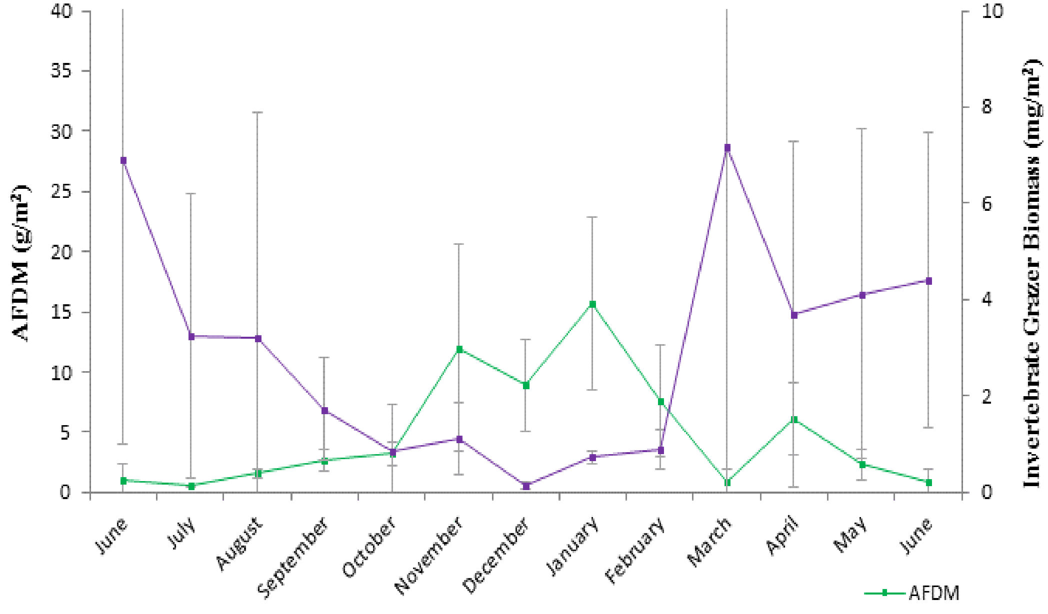

Figure 4.24 (b)

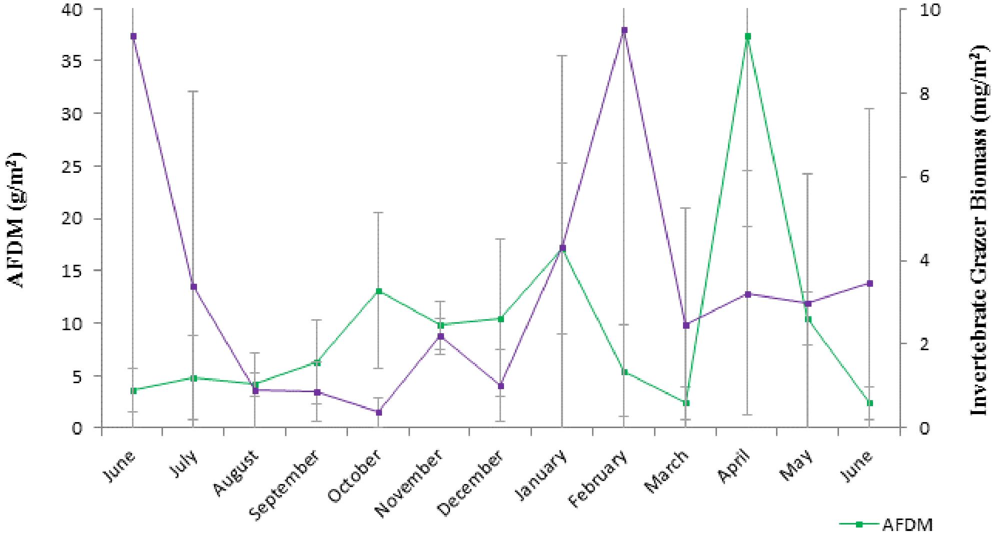

Figure 4.24 (c)

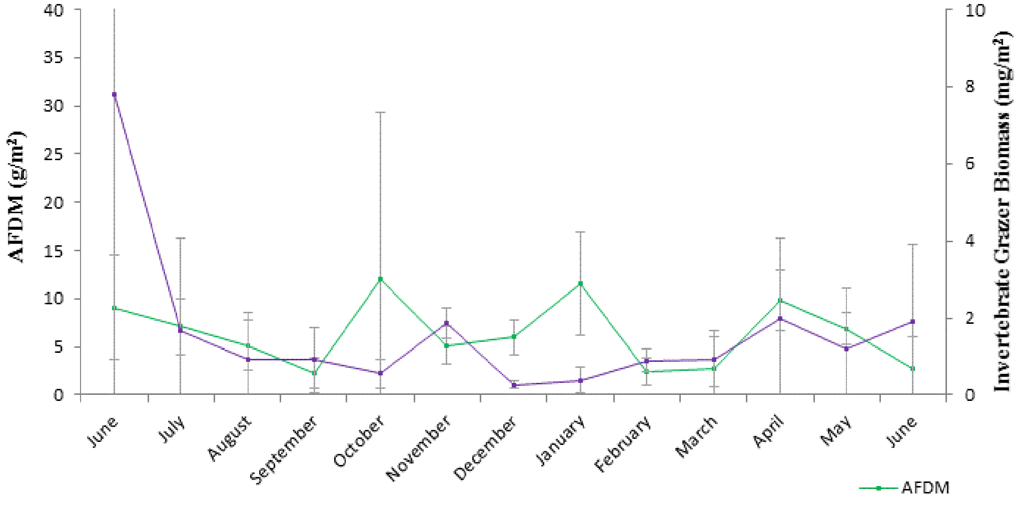

Figure 4.24 (d)

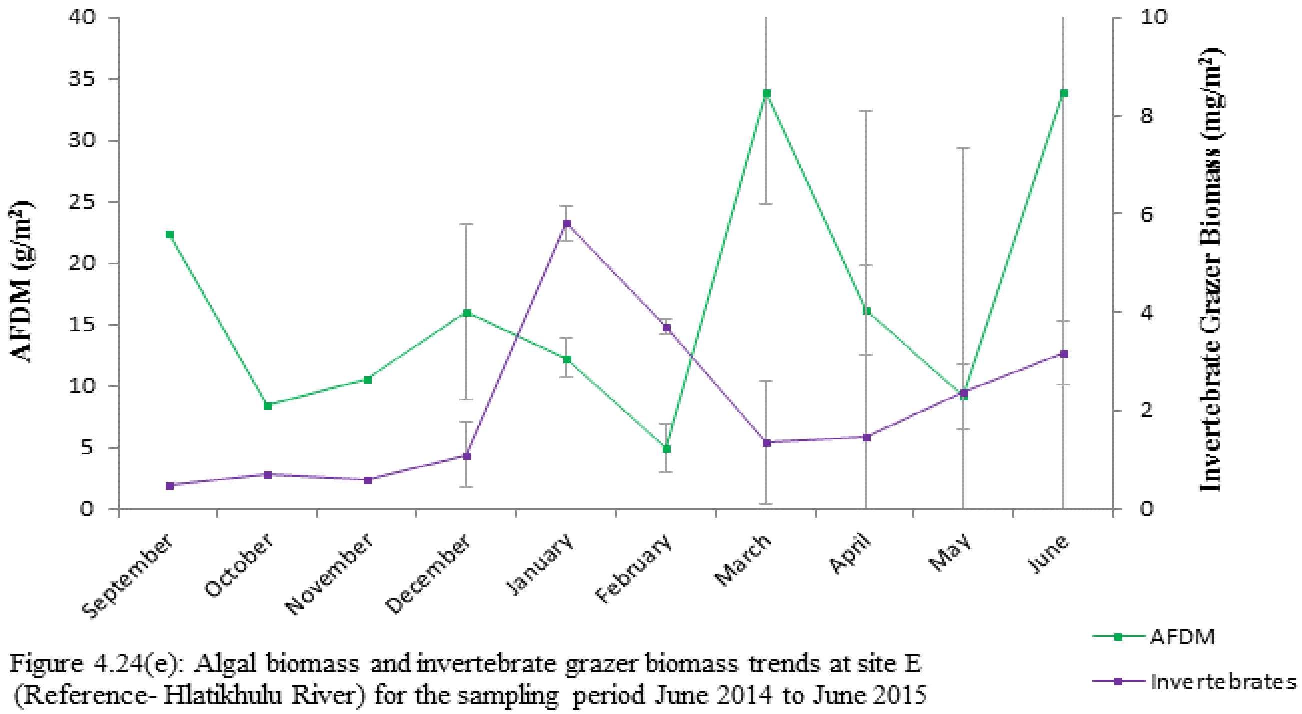

Figure 4.24(e): Algal biomass and invertebrate grazer biomass trends at site E (Reference- Hlatikhulu River) for the sampling period June 2014 to June 2015

4.8 Invertebrate abundance, density and biomass trends

Invertebrate abundance decreased at all sites from September 2014 to December 2014. Greatest invertebrate abundance was experienced at site B (nutrient enriched) from June 2014 to August 2014 and June 2015 with abundance levels over 500 individuals per site. Lowest invertebrate abundance levels were experienced at site D (altered flow) from December 2014 to April 2015 with number of individuals below twenty. Highest invertebrate abundances were observed in February 2015 and April 2015 at site A (nutrient unenriched) while lowest levels occurred in November 2014. Highest invertebrate abundances at site C (unaltered flow) was observed in June 2014 while lowest abundances occurred in October 2014. Highest invertebrate abundances at site E (reference) occurred from January 2015 to February 2015. At this point highest invertebrate abundances between all the sites was experienced at site E. Lowest abundance of invertebrates were observed in September 2014 (Figure 4.25). The invertebrate density trends at each site followed similar trends as the invertebrate abundance trends (Figure 4.26).

Invertebrate biomass per unit at each of the five sites were low between September 2014 and December 2014 as was similar to results in the invertebrate density graphs (Figure 4.27). Highest invertebrate biomass occurred in February 2015 at site C (unaltered flow) at 66.78mg/m^2. Biomass at site D (altered flow) remained constant throughout the sampling period with only a difference of 22.59g/m^2 between the highest biomass in June 2014 and the lowest biomass in December 2014. Highest invertebrate biomass was experienced from February 2015 to April 2015 at site A (nutrient unenriched). Highest invertebrate biomass occurred in January 2015 and February 2015 at site E (reference) (Figure 4.27). The trends between invertebrate density in terms of peaks and decreases was similar to that of invertebrate biomass at each of the five sampling sites.

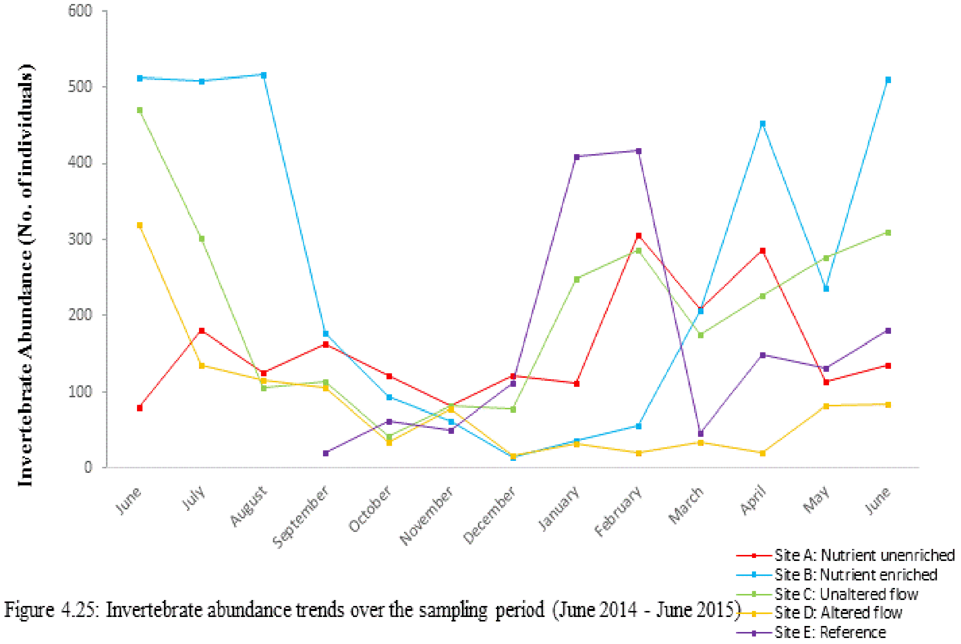

Figure 4.25: Invertebrate abundance trends over the sampling period (June 2014 - June 2015)

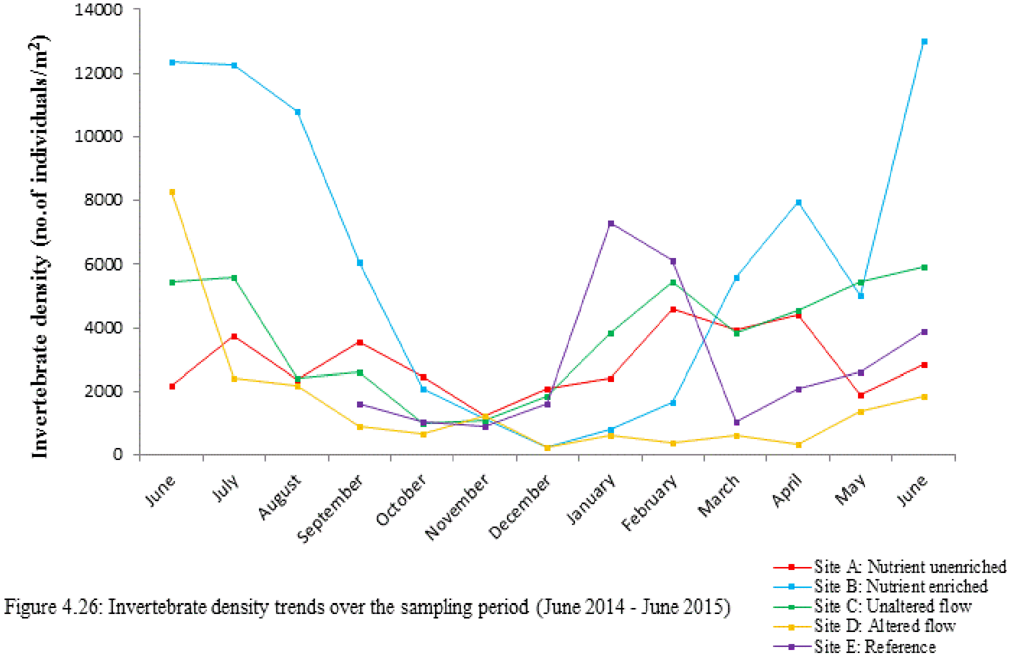

Figure 4.26: Invertebrate density trends over the sampling period (June 2014 - June 2015)

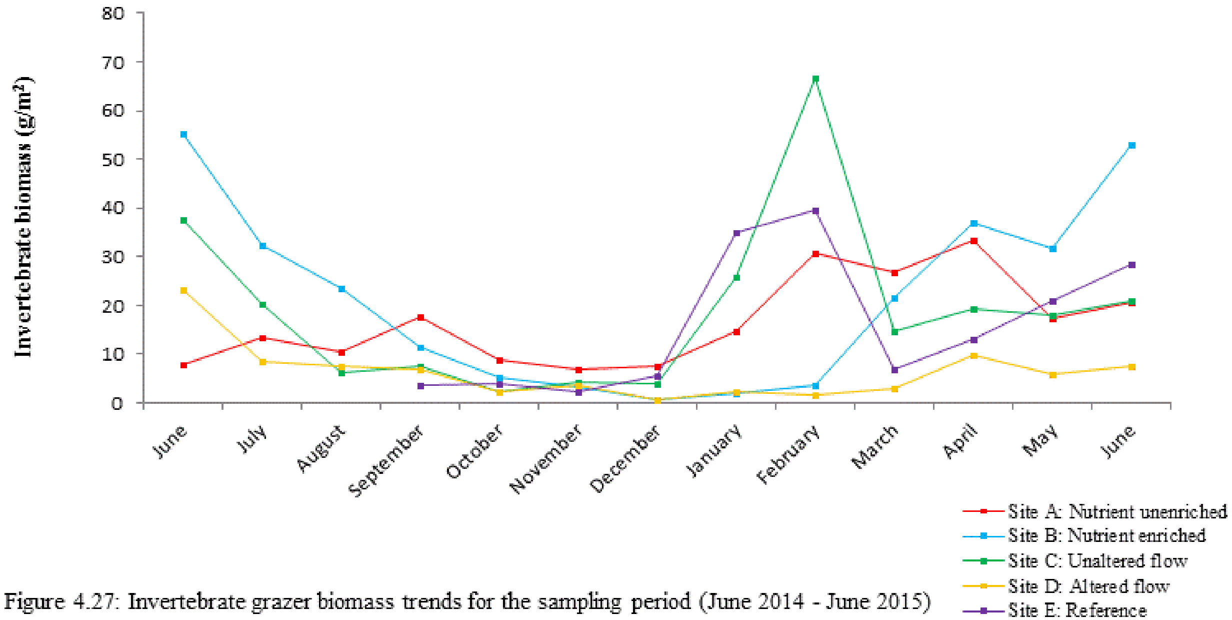

Figure 4.27: Invertebrate grazer biomass trends for the sampling period (June 2014 - June 2015)

4.9 Invertebrate grazer density

The density of Baetidae remained higher than all other invertebrate grazers except between January 2015 to April 2015 at site A (Nutrient unenriched), when Chironomidae densities were higher. Lowest Chironomidae density occurred in January 2014 when no Chironomidae were observed while highest density occurred in February 2015 when Chironomidae density was 3086.56 no. of individuals/m^2 (Figure 4.28 (a)).

At site B (Nutrient enriched) variations in Chironomidae and Baetidae density were observed. Heptageniidae and Elmidae larvae remained low with highest density was 229.54 no. of individuals/m^2 and 103.22 no. of individuals/m^2 respectively. Baetidae density was higher than the other invertebrate grazers for the entire sampling period except between February 2014 to April 2014 when Chironomidae density was higher. (Figure 4.28 (b)).

Density levels of Leptophlebiidae, Elmidae larvae and Heptageniidae remained low at site C (Unaltered flow). Chironomidae density was higher than Baetidae density from June to July 2014 and between December 2014 and May 2015. The range in density of 4848.65 no. of individuals/m^2 was observed in the Chironomidae family were highest Chironomidae density was observed in June 2014 (4848.65 no. of individuals/m^2) while lowest density was observed in November 2014 (0 no.of individuals/m^2). A range in density of 919 no. of individuals/m^2 was observed in the Baetidae fairly were highest density occurred in June 2015 while lowest density was observed in January 2015 (Figure 4.28 (C)).

Invertebrate density at site D (altered flow) was lower than at the other sampling sites. Heptageniidae, Leptophlebiidae and Elmidae larvae densities were very low. Baetidae densities were higher than Chironomidae levels from September 2014 to January 2015 and at March 2015. Highest Baetidae density was observed at 1217 no. of individuals/m^2 in November 2014 and lowest Baetidae density occurred at 158 no. of individuals/m^2 in February 2015. Highest Chironomidae density was observed at 6712 /m^2 in June 2014 while no Chironomidae were observed in November 2015 and December 2015 (Figure 4.28 (d)).

At site E (reference), increased levels of Leptophlebiidae density were observed from October 2014 to November 2014 and from April 2015 to June 2015. Baetidae density levels had a range of 1078 no. of individuals/m^2 and were higher than other invertebrate grazer families throughout the sampling period except between December 2014 and March 2015 when Chironomidae levels were higher. Highest Chironomidae density was observed in January 2015 at 5968 no. of individuals/m^2 and lowest density was observed in September 2014 at 85 no. of individuals/m^2 (Figure 4.28 (e)).

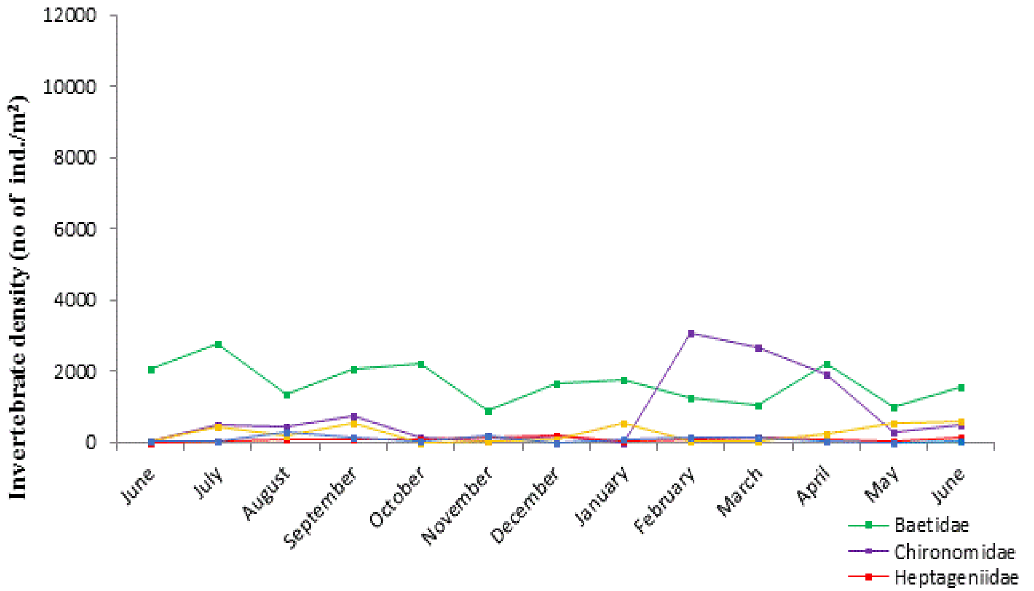

Figure 4.28 (a)

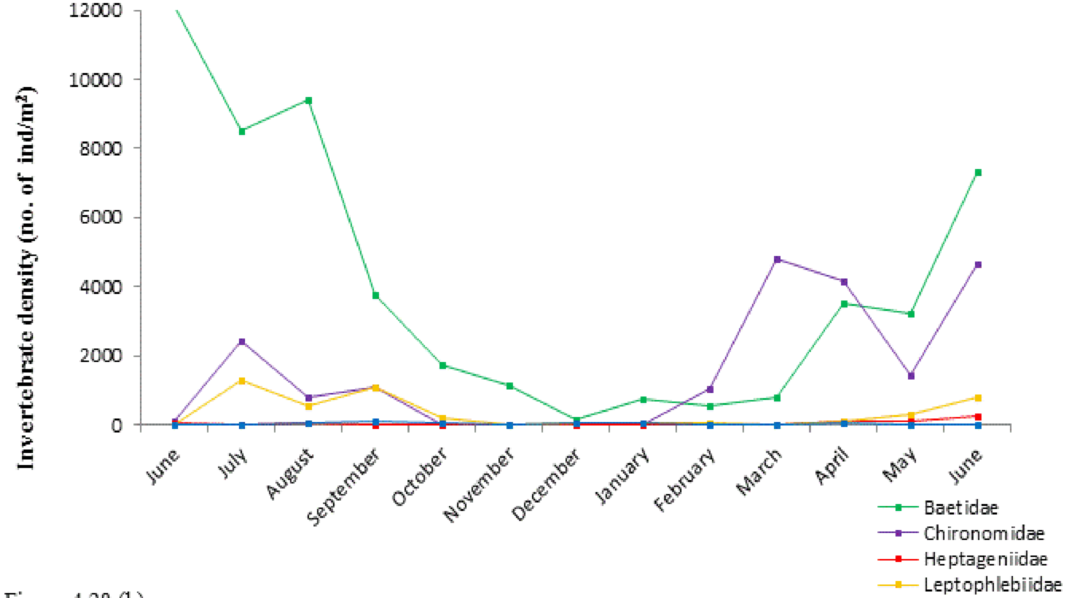

Figure 4.28 (b)

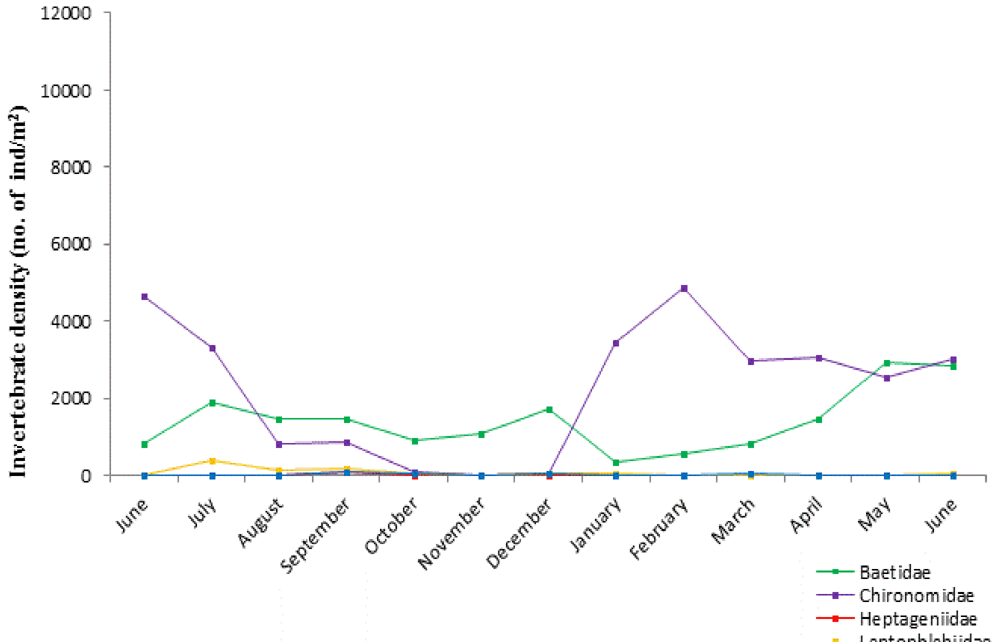

Figure 4.28 (c)

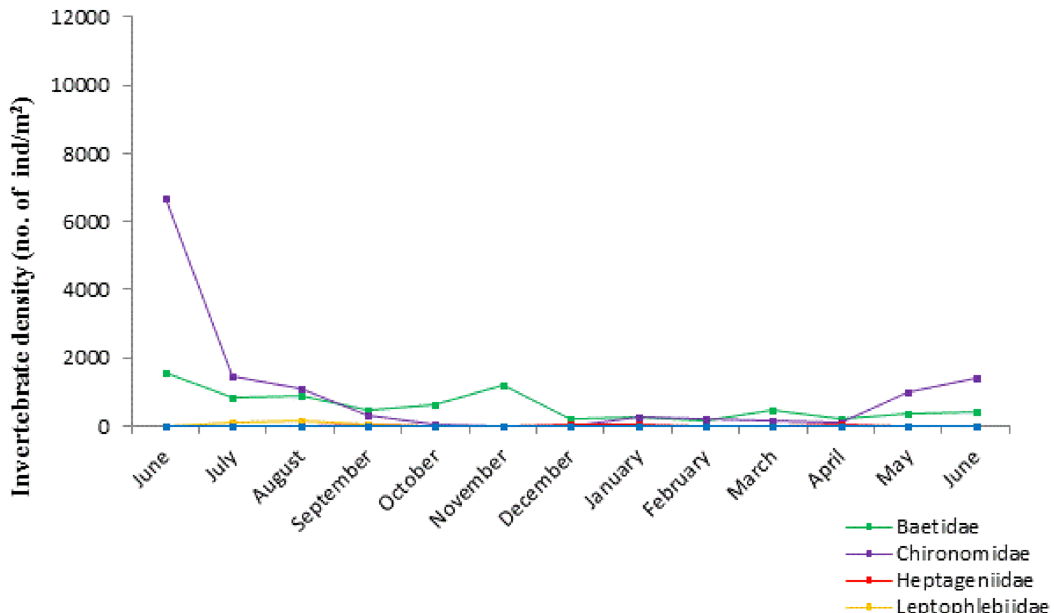

Figure 4.28 (d)

Figure 4.28: Invertebrate grazer density at (a)- site A (Nutrient unenriched- Msunduzi-Kwanoshezi), (b)- Site B (Nutrient enriched- Msunduzi-tannery), (c)- Site C (Unaltered flow- Umgeni-Petrustroom) and (d)- Site D (Altered flow- Umgeni-York Road) for the sampling period June 2014 to June 2015

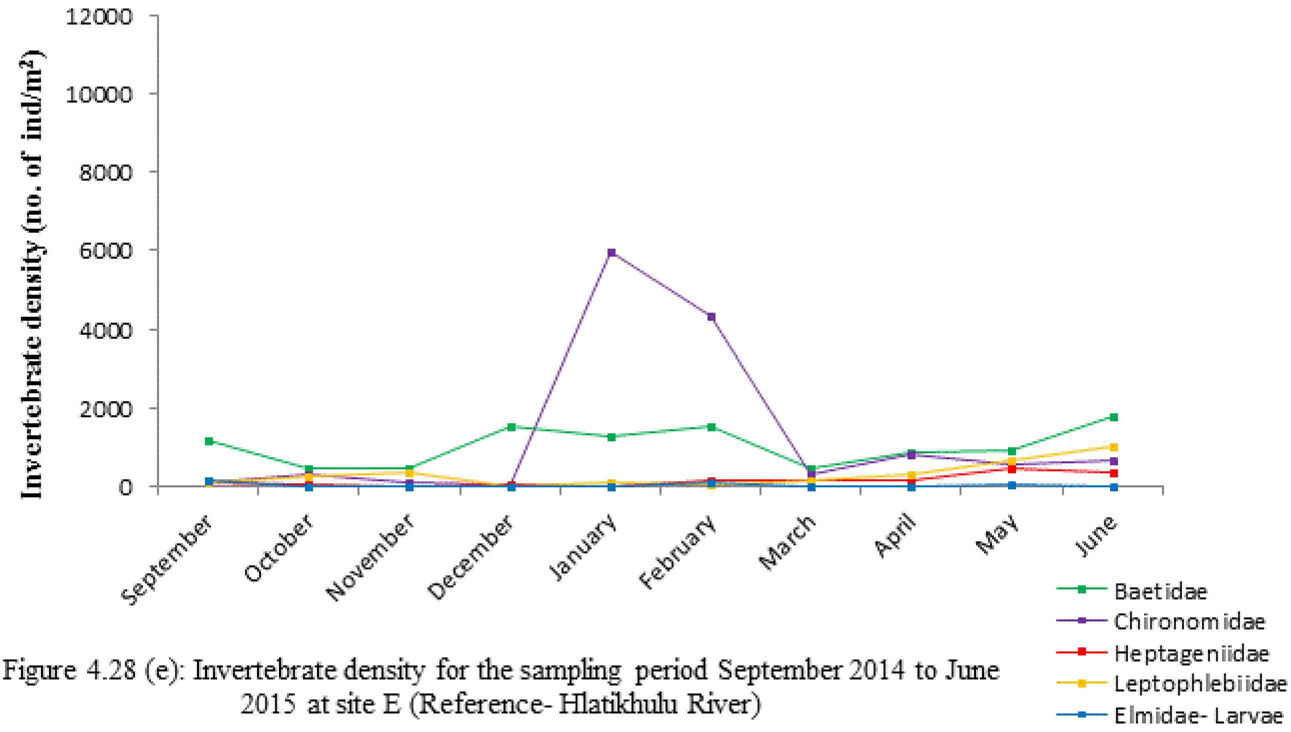

Figure 4.28 (e): Invertebrate density for the sampling period September 2014 to June 2015 at site E (Reference- Hlatikhulu River)

4.10 Invertebrate biomass

An independent sample t-test was conducted to compare invertebrate biomass pre- and post- planting of maize at site E (reference). The independent sample t-tests showed a significant difference between invertebrate biomass before the planting of maize (site E), (M=0.0023, SD=0.0038) and after the introduction of maize (site E), (M=1.72, SD= 0.45) p= 0.00

A Two-way analysis of variance on the influence of two independent variables (nutrient enrichment and seasons) on invertebrate biomass was conducted. Nutrient alteration included two levels (nutrient unenriched (site A), nutrient enrichment (site B)) while seasons included four levels (winter, spring, summer, autumn) p= 0.02, P<0.05, indicating that the effect of nutrient enrichment on invertebrate biomass was significant, nutrient unenriched sites (M=1.77, SD=0.29) and nutrient enriched sites (M= 2.82, SD= 0.29). The main effect of seasons p = 0.00, P<0.05 indicated a significant difference in invertebrate biomass over seasons at each site, winter (M= 3.07, SD= 0.37), spring (M=1.24, SD= 0.43), summer (M=1.22, SD= 0.43) and autumn (M=3.66, SD= 0.43). The interaction effect between sites of nutrient unenriched and nutrient enriched over seasons, p = 0.00, P<0.05 showed a significant effect (Table 4.4).

Table 4.4: Two-way ANOVA of invertebrate biomass between nutrient unenriched (Site A- Msunduzi Kwanoshezi) and nutrient enriched (Site B- Msunduzi tannery) sites over the sampling period (June 2014 to June 2015)

	SS	df	MS	F	P
Site	7.01	1	7.01	6.45	0.02*
Season	29.60	3	9.87	9.08	0.00*
Site × Season	19.11	3	6.37	5.87	0.00*
Error	19.55	18	1.09		

*Significant difference

A Two-way analysis of variance on the influence of two independent variables (flow type, seasons) on invertebrate biomass was conducted. Flow type included two levels (unaltered flow (site C), altered flow (site D)) while seasons included four levels (winter, spring, summer, autumn). All effects were not statistically significant at a .05 significance level except for the main effect of sites. The main effect for sites, $p= 0.00$, $P< 0.05$, indicating that the effect of flow alteration had a significant effect on invertebrate biomass, unaltered flow ($M=3.31$, $SD=0.69$) and altered flow ($M=1.52$, $SD=0.69$). The main effect of seasons, $p = 0.08$, $P>0.05$ indicated no significant difference in invertebrate biomass over seasons at each site, winter ($M= 3.67$, $SD=0.87$), spring ($M=1.13$, $SD=1.01$), summer ($M=2.73$, $SD=1.01$) and autumn ($M=2.12$, $SD=1.01$). The interaction effect between sites of unaltered and altered flow over seasons, $p = 0.47$, $P>0.05$ showed no significant change in invertebrate biomass over the sampling period at both sites (Table 4.5)

Table 4.5: Two-way ANOVA of invertebrate density between unaltered flow (Site A- Msunduzi Kwanoshezi) and altered flow (Site B- Msunduzi tannery) sites over the sampling period (June 2014 to June 2015)

	SS	df	MS	F	P
Site	88.24	1	88.24	12.27	0.00*
Season	81.69	3	27.23	4.09	0.08
Site × Season	26.94	3	8.91	1.35	0.47
Error	119.71	18	6.65		

*Significant difference

4.11 Species diversity index

A Shannon Weiner index was conducted to determine the diversity of invertebrate families between comparable sites. Site A (Nutrient unenriched) had a higher species diversity than site B (Nutrient enriched), with an index of 2.137 and 1.881 respectively. Site D (Altered flow) had an index of 2.125 which was higher than the species diversity at site C (unaltered flow). There was a higher species diversity at site E (reference) after the maize plantation, 1.712 as compared to the species diversity before the maize plantation, 1.480 (Table 4.6)

Table 4.6: Shannon Winer diversity index using macro- invertebrate families at comparable sites.

	Sampling Site	**Shannon Weiner index**
Nutrient alteration	**Site A- Nutrient unenriched**	2.137
	Site B- Nutrient enriched	1.881
Flow alteration	**Site C- Unaltered flow**	1.806
	Site D- Altered flow	2.125
Maize plantation	**Site E- Before Maize**	1.480
	Site E- After Maize	1.712

4.12. Temporal patterns summary

Fluctuation in each physico-chemical and biomass factors throughout the sampling period at each sampling site were indicated. An inverse relationships between temperature and dissolved oxygen was identified were higher water temperatures were associated with lower dissolve oxygen levels. Limited data was obtained for water quality indicators. An inverse relationship between algal biomass and invertebrate biomass was identified were periods of higher invertebrate biomass were the same periods of low algal biomass levels. A summary table of the above analysis is indicated below. (+) indicated an increase in the values of a particular factor from the previous sampling month, (-) represents a decrease in the value of the factor from the previous sampling month and (0) indicated no change in the value of a particular factor from the previous sampling month (Table 4.7)

Table 4.7: Physico-chemical and biomass fluctuations indicated at each site throughout the sampling period

Factor	Site	June 2014	July 2014	Aug-2014	Sep-2014	Oct-2014	Nov-2014	Dec-1204	Jan-2015	Feb-2015	Mar-2015	Apr-2015	May-2015	Jun-2015
Temperature (°C)	nutrient unenriched	General trend of higher temperatures in summer and lower temperatures in winter												
	nutrient enriched	General trend of higher temperatures in summer and lower temperatures in winter												
	unaltered flow	General trend of higher temperatures in summer and lower temperatures in winter												
	altered flow	General trend of higher temperatures in summer and lower temperatures in winter												
	reference	General trend of higher temperatures in summer and lower temperatures in winter												
% Dissolved oxygen	nutrient unenriched	+	+	+	+	+	_	+	_	+	+	+	_	_
	nutrient enriched	+	_	+	+	_	_	+	+	_	+	_	+	+
	unaltered flow	+	_	+	+	_	_	+	+	_	+	+	+	+
	altered flow	_	+	+	+	_	_	+	_	_	+	+	_	_
	reference				_	+	+	+	_	+	+	_	+	+
Dissolved oxygen (mg/L)	nutrient unenriched	General trend of higher oxygen levels in winter than in summer												
	nutrient enriched	General trend of higher oxygen levels in winter than in summer												
	unaltered flow	General trend of higher oxygen levels in winter than in summer												
	altered flow	General trend of higher oxygen levels in winter than in summer except for a peak in January												
	reference	General trend of higher oxygen levels in winter than in summer												
Conductivity (µs/cm)	nutrient unenriched	minimal fluctuation throughout sampling period												
	nutrient enriched	minimal fluctuation throughout sampling period (Highest conductivity)												
	unaltered flow	minimal fluctuation throughout sampling period												
	altered flow	minimal fluctuation throughout sampling period												
	reference	minimal fluctuation throughout sampling period (Lowest conductivity)												
pH	nutrient unenriched	+	_	+	+	+	+	_	_	_	_	+	_	_
	nutrient enriched	+	+	_	+	+	_	_	+	_	+	_	+	_

	unaltered flow	_	+	_	+	+	_	_	+	_	+	+	+	_
	altered flow	+	_	_	+	+	_	+	_	+	_	+	+	_
	reference				+	_	+	_	_	_	+	+	_	_
clarity (cm)	nutrient unenriched	+	_	+	_	_	_	+	_	_	+	+	_	+
	nutrient enriched	+	_	_	+	+	+	_	_	_	+	+	+	+
	unaltered flow	+	_	_	_	_	+	_	+	_	_	_	+	+
	altered flow	+	_	_	+	+	_	+	_	_	_	+	+	+
	reference				+	+	_	_	_	+	+	+	_	+
Depth (cm)	nutrient unenriched	_	+	_	_	_	+	_	_	+	_	+	_	+
	nutrient enriched	+	_	+	_	+	_	+	_	+	_	_	_	_
	unaltered flow	_	+	+	+	_	+	+	_	_	+	_	_	_
	altered flow	_	+	_	_	+	_	_	+	_	+	_	_	_
	reference				+	_	+	+	_	+	_	_	_	+
Velocity(ms^{-1})	nutrient unenriched	_	+	_	_	+	+	+	_	+	_	_	_	_
	nutrient enriched	_	+	_	+	_	_	+	_	+	_	+	_	+
	unaltered flow	_	+	_	_	_	+	+	_	+	+	_	_	_
	altered flow	+	_	+	_	+	+	_	+	_	+	_	_	_
	reference				+	_	+	+	_	+	_	+	_	+
Ammonia (mg N/L)	nutrient unenriched	_	0	0	0	0	+	_	0	+	0	0	0	0
	nutrient enriched	+	_	_	_	+	_	+	_	+	_	_	+	_
	unaltered flow	_	+	_	0	0	0	0	+	0	0	0	0	0
	altered flow	_	+	_	0	0	0	+	+	+	+	+	_	_
	reference						No data available for this site							
phosphorus (SRP) (ug P/L)	nutrient unenriched	_	_	_	+	_	_	+	_	+	+	_	0	0
	nutrient enriched	+	_	_	+	+	_	_	0	+	+	_	0	0
	unaltered flow	+	_	+	+	+	0	+	+	0	0	+	_	0
	altered flow	_	0	+	_	_	_	+	+	0	+	_	0	0
	reference						No data available for this site							

Nitrite (mg N/L)	nutrient unenriched	_	+	0	0	0	+	_	0	+	0	0	0	0
	nutrient enriched	+	_	_	+	+	_	+	_	+	0	0	0	0
	unaltered flow	_	+	+	+	_	+	_	_	_	_	0	+	+
	altered flow	_	+	+	_	+	_	_	+	_	+	_	_	_
	reference	No data available for this site												
AFDM (g/m^2)	nutrient unenriched	_	+	+	+	+	+	+	+	_	+	+	_	+
	nutrient enriched	_	+	+	+	+	+	_	+	_	+	_	_	_
	unaltered flow	+	_	+	+	+	+	_	+	_	+	+	_	_
	altered flow	+	_	_	_	+	_	+	+	_	+	+	_	_
	reference				+	_	+	_	+	_	+	_	_	_
Chlorophyll *a* (g/m^2)	nutrient unenriched	+	_	+	+	_	+	+	_	_	+	_	_	+
	nutrient enriched	+	_	+	+	+	+	_	_	+	+	_	_	+
	unaltered flow	_	+	+	_	+	+	_	_	+	+	_	_	_
	altered flow	+	_	+	_	_	+	+	_	+	_	+	_	_
	reference				+	_	+	+	_	+		_	_	_
Invertebrate grazer biomass (g/m^2)	nutrient unenriched	_	+	_	+	_	_	+	+	+	_	+	_	+
	nutrient enriched	+	_	_	_	_	_	_	_	_	+	+	_	+
	unaltered flow	+	_	_	+	_	+	+	+	+	_	+	+	+
	altered flow	+	_	_	+	_	+	_	+	+	+	+	_	+
	reference				_		_	+	+	+	_	+	+	+
Invertebrate grazer abundance (no. of ind)	nutrient unenriched	_	+	_	+	_	_	+	_	+	_	+	_	+
	nutrient enriched	+	_	+	_	_	_	_	+	+	+	+	_	+
	unaltered flow	+	_	_	+	_	+	_	+	+	_	+	+	+
	altered flow	+	_	_	_	_	+	_	+	_	+	_	+	+
	reference				_	+	_	+	+	+	_	+	_	+

Invertebrate grazer density (g/m^3)	nutrient unenriched	_	+	_	+	_	_	+	_	+	_	+	_	+
	nutrient enriched	+	_	+	_	_	_	_	+	+	+	+	_	+
	unaltered flow	+	_	_	+	_	+	_	+	+	_	+	+	+
	altered flow	+	_	_	_	_	+	_	+	_	+	_	+	+
	Reference				_	+	_	+	+	+	_	+	_	+

+ increase in factor

_ decrease in factor

0 no change in factor

4.13 Principal component analysis

Two principal component analyses (PCA) were conducted. One between the physico-chemical factors at site A (Nutrient unenriched) and site B (Nutrient enriched) and the second between physico-chemical factors at site C (Unaltered flow) and site D (Altered flow). The outcomes indicate the distribution of driving physico-chemical factors over sampled months. The distribution is indicated between comparable sites using different colours and the sampling month and year. Temperature, conductivity and pH are the driving factors at site B (Nutrient enriched), while depth and velocity play a more secondary role (Figure 4.29a and Figure 4.29b). Depth and velocity play a driving role when comparing site C (Unaltered flow) and site D (Altered flow) (Figure 4.30a and Figure 4.30b).

A second set of PCAs was conducted to determine the interaction and driving factors between algal biomass and invertebrate grazer families. Invertebrate grazers played a greater role in winter at site B (Nutrient enriched) (Figure 4.31a and Figure 4.31b) Comparisons between site C (unaltered flow) and site D (altered flow) did not indicate any major driving factors influencing distributions between algal biomass and invertebrate grazer families (Figure 4.32a and Figure 4.32b)

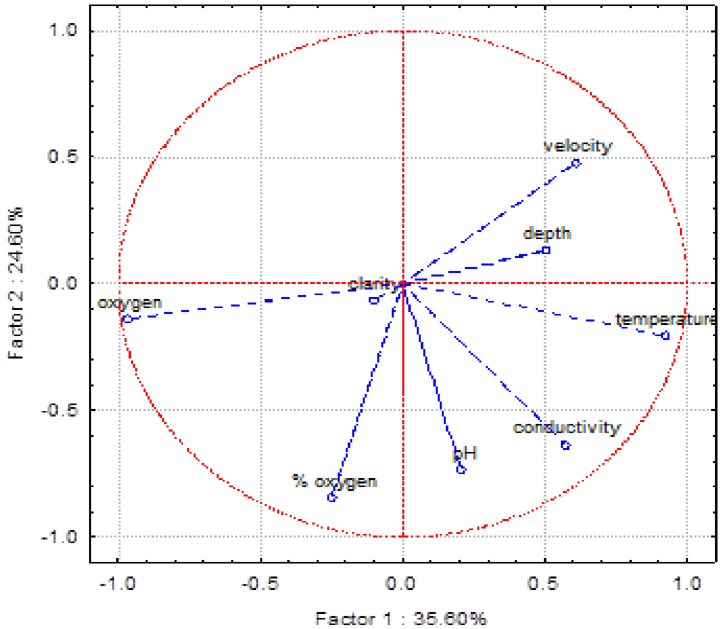

Figure 29a: Physico-chemical factors at Site A (Nutrient unenriched) and Site B (Nutrient enriched)

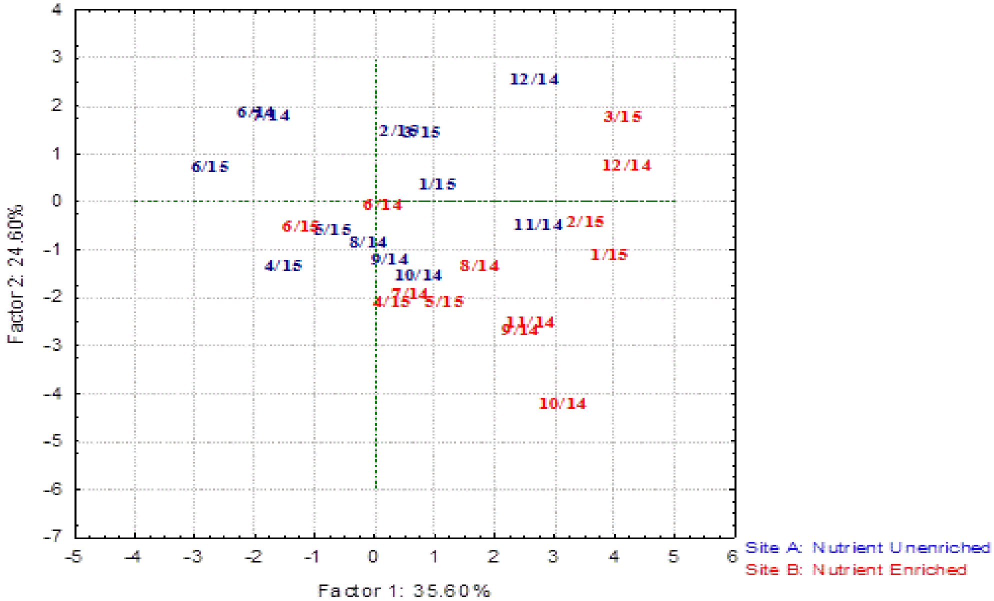

Figure 29b Distribution of sampling sites over the sampling period (June 2014- June 2015) in relation to physico-chemical factors

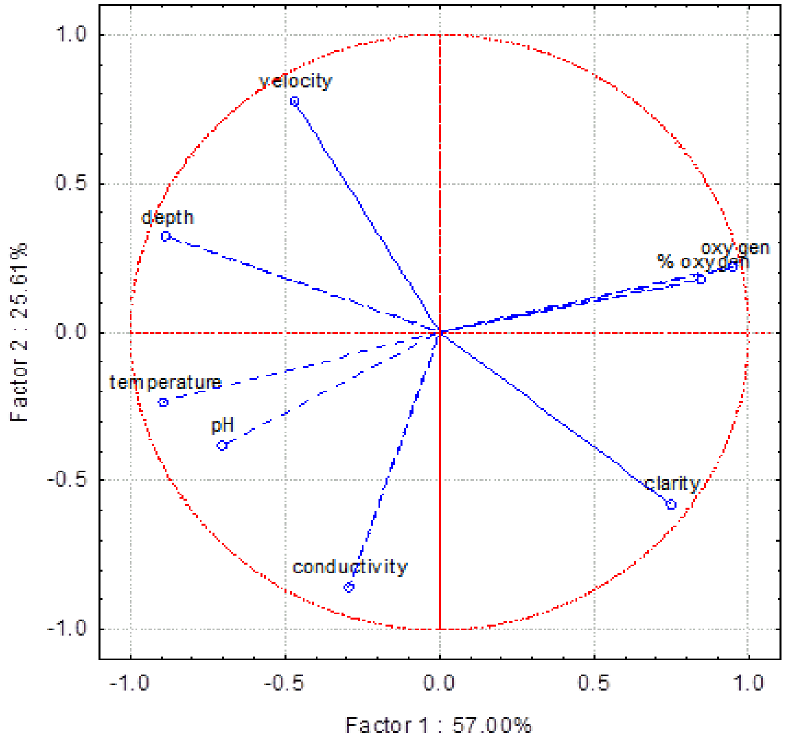

Figure 30a: Physico-chemical factors at Site C (Unaltered flow) and Site D (Altered flow)

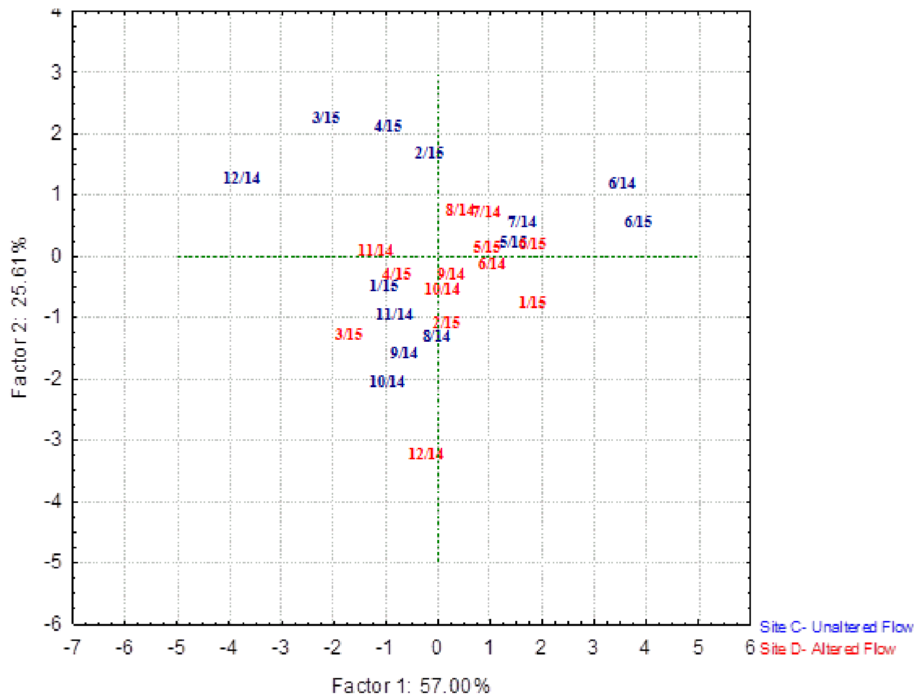

Figure 30b Distribution of sampling sites over the sampling period (June 2014- June 2015) in relation to physico-chemical factors

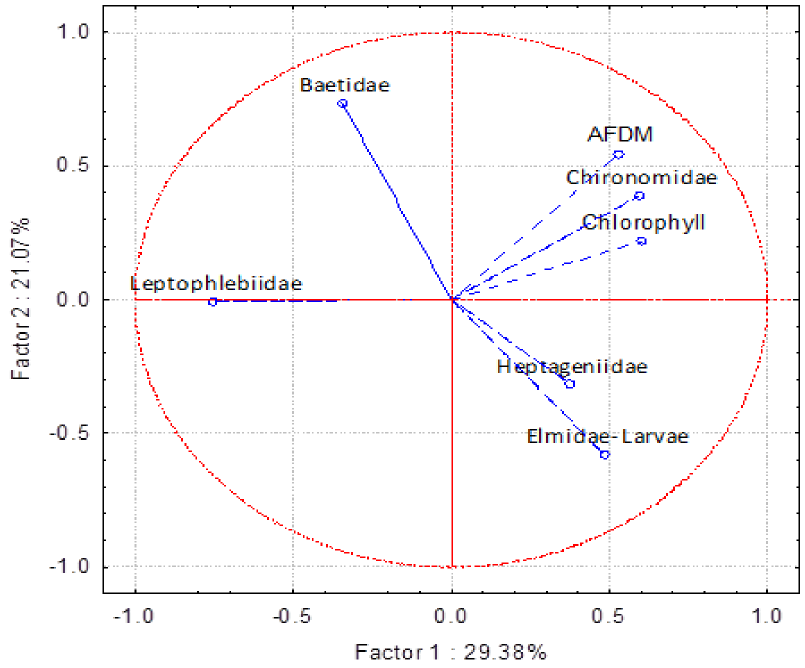

Figure 4.31a: Algal biomass and invertebrate grazer families

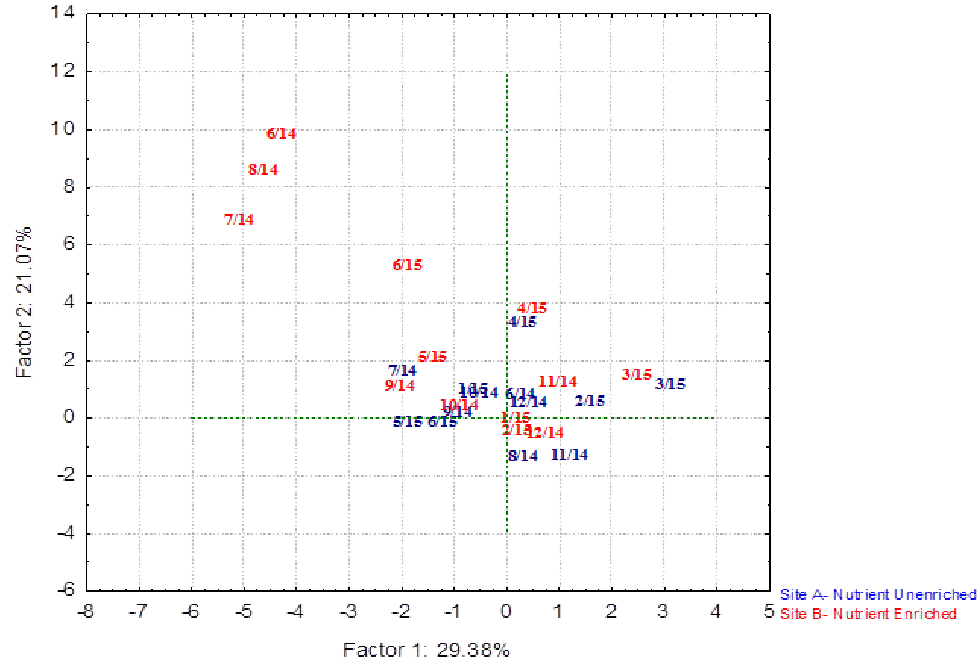

Figure 4.31b: Distribution of sampling sites of the sampling period (June 2014- June 2015) in relation to algal biomass and invertebrate grazers)

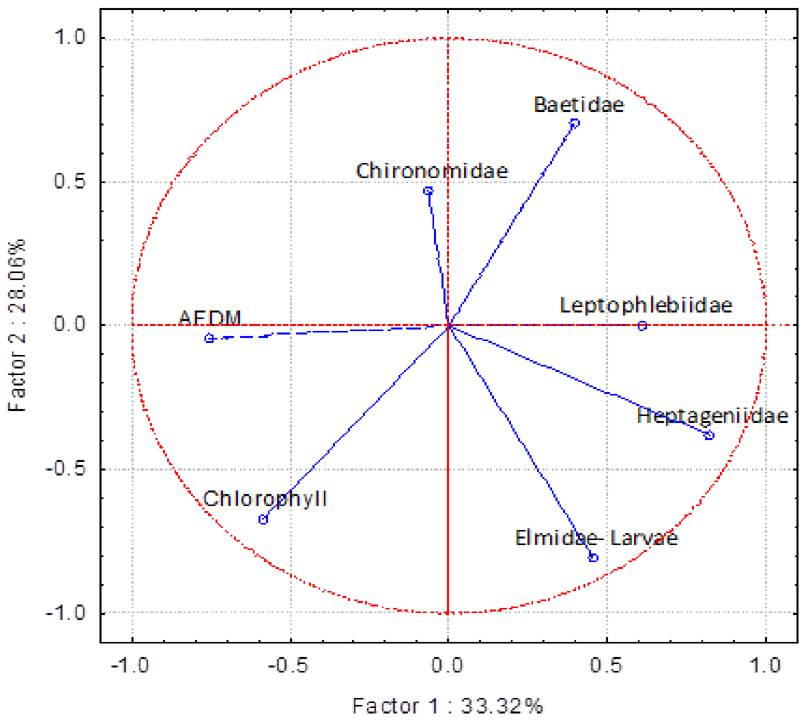

Figure 4.32a: Algal biomass and invertebrate grazer families

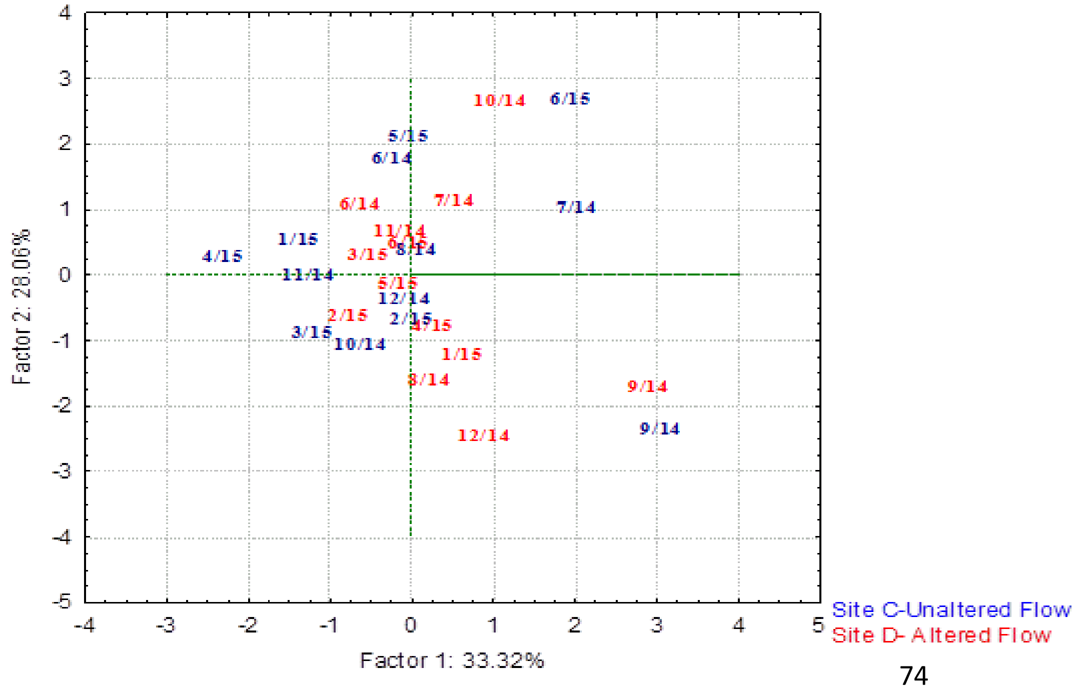

Figure 4.32b: Distribution of sampling sites of the sampling period (June 2014- June 2015) in relation to algal biomass and invertebrate grazers)

4.14 Discharge and water temperature

Surface water discharge data for sites A (nutrient unenriched), B (nutrient enriched), C (unaltered flow), site D (altered flow) and E (reference) showed that highest discharge was experienced in summer between January 2015 and April 2015. Peaks in discharge at site B (nutrient enriched) occurred in September 2014 and November 2014. Lowest discharge was observed in winter between June 2014 and November 2014 and between May 2015 and June 2015. Surface water discharge for site D (altered flow) indicated very low flows as these flows are regulated by Midmar dam releases. Slight fluctuations were evident between June and July 2014 (Figure 4.33).

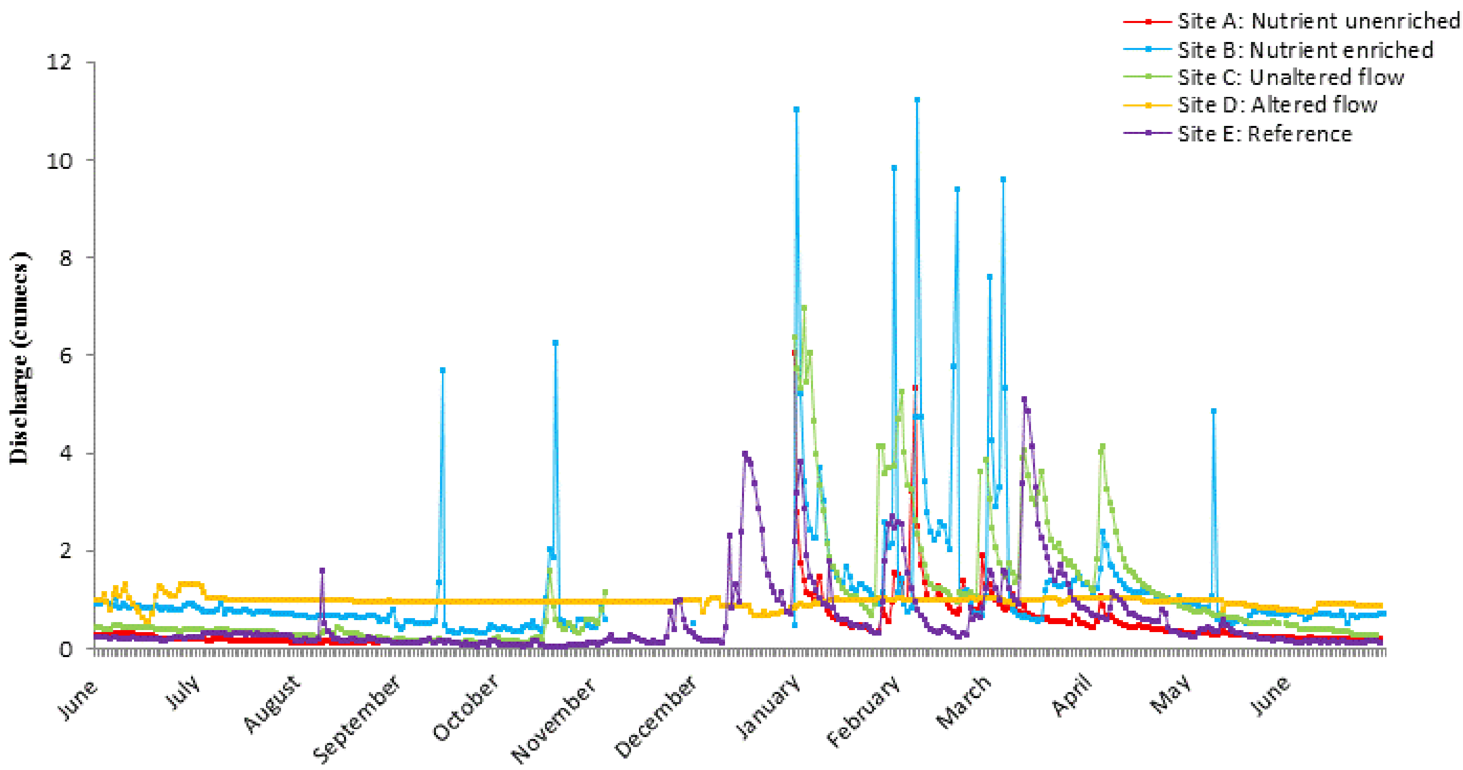

Figure 4.33: Surface water discharge at sites A, B, C, D and E for the period June 2014 to June 2015

Water temperature data collected hourly using a hobo temperature logger was installed at all five sampling sites in December 2014, six months into the project. Unfortunately loggers installed twice at both site A (Nutrient unenriched) and site B (Nutrient enriched) were stolen. Data indicated highest water temperatures in summer while lowest water temperatures in winter at site C (Unaltered flow). A trend could not be established at site D (Altered flow) due to extended periods when the temperature loggers were found out of the water. Site E (reference) saw a trend of higher water temperatures in summer and gradual decreasing from December 2014 to June 2016 (Figure 4.45). Modelled water temperature with outliers removed showed fluctuations in temperature at all sites. However calculated values indicated a water temperature in summer and colder water temperatures in winter (Figure 4.35)

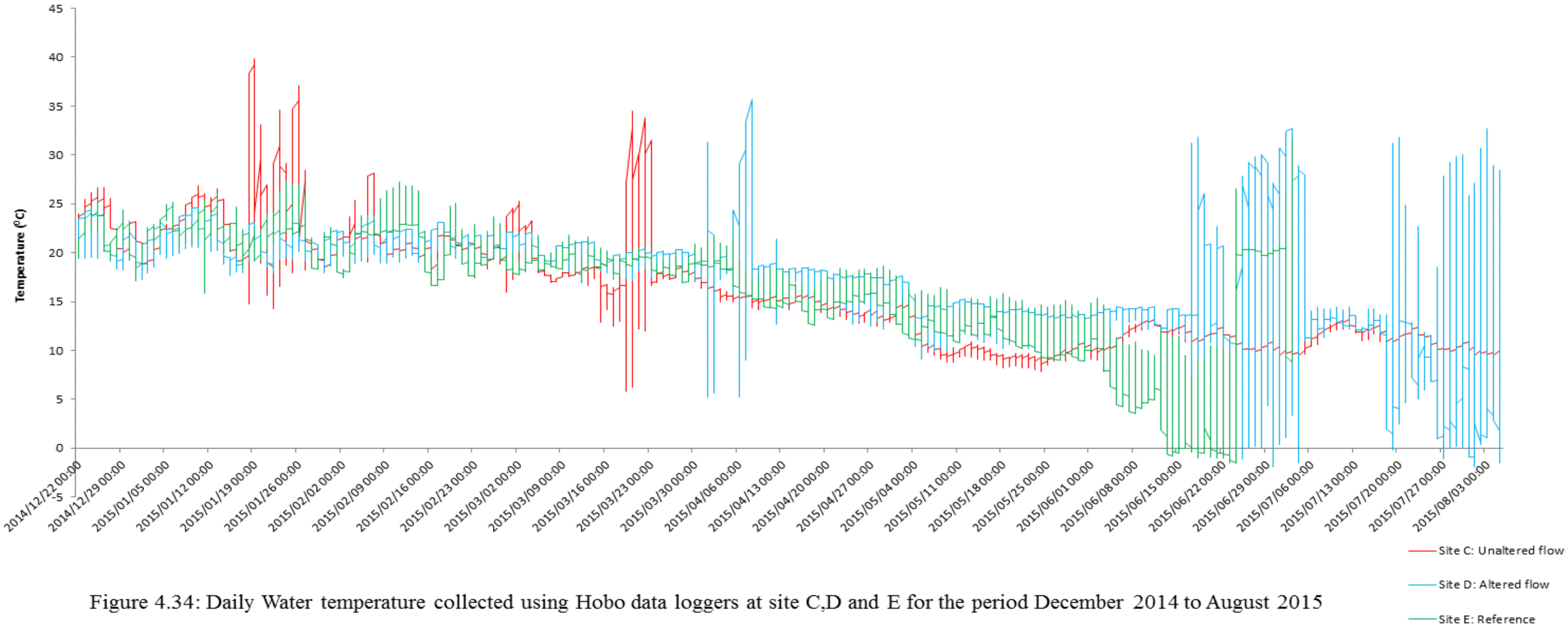

Figure 4.34: Daily Water temperature collected using Hobo data loggers at site C,D and E for the period December 2014 to August 2015

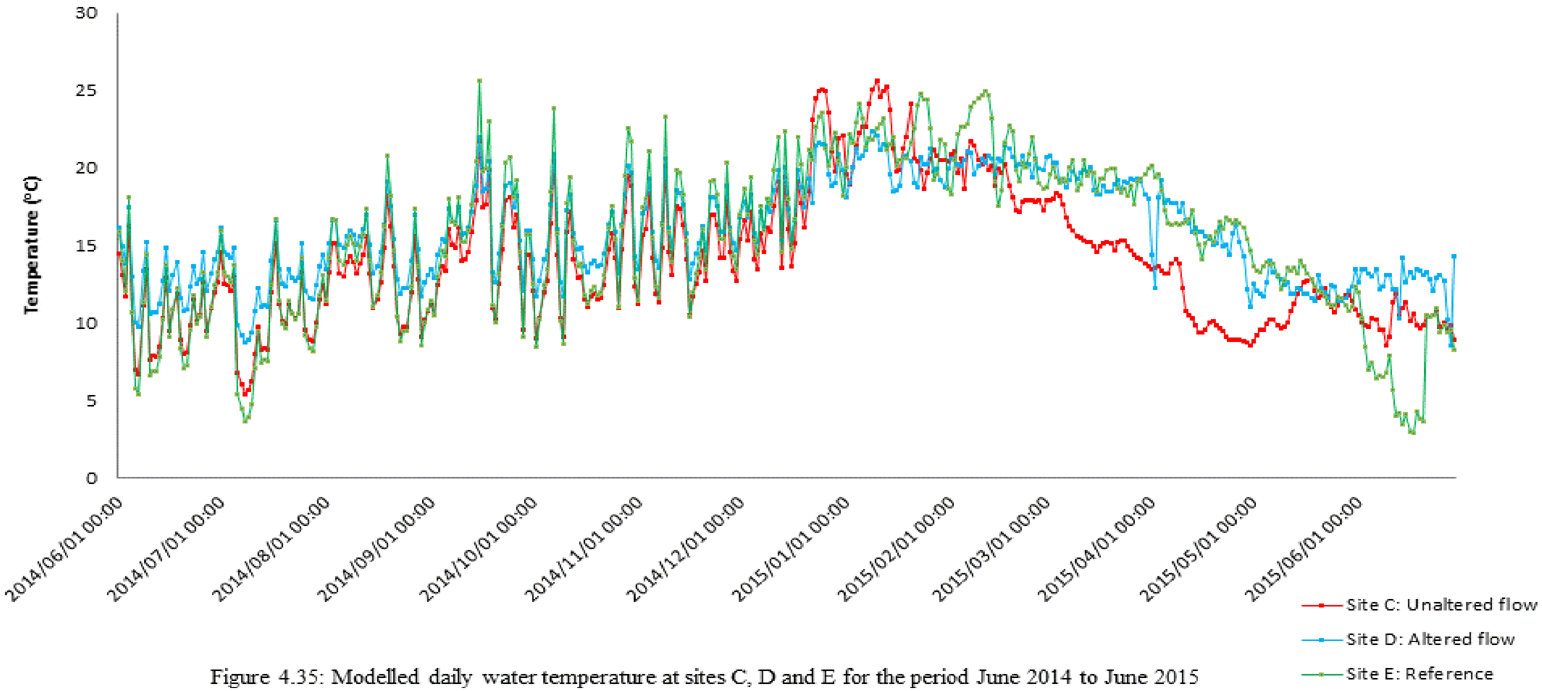

Figure 4.35: Modelled daily water temperature at sites C, D and E for the period June 2014 to June 2015

4.15 Algal abundance

Algal density over all five sampling sites indicated that diatoms were the most dominant class division in terms of density at all sites except site D (altered flow) were cyanobacteria was the dominant algal division over the sampling period June 2014 to June 2015. High densities of green algae were present at site C (Unaltered flow), site D (altered flow) and site E (reference) as compared with site A (nutrient unenriched) and site B (nutrient enriched). Highest diatom densities were experienced at site E (reference) while highest cyanobacteria densities at site D (altered flow) (Figure 4.46). Figure 4.47 to Figure 4.51 indicate the algal class divisions in terms of algal cell density at each of the sampling sites individually over the sampling period. At site A (nutrient unenriched) diatoms were dominant with lower peaks in cyanobacteria throughout the sampling period. Green algae was little to none during the sampling period (Figure 4.47). Site B (nutrient enriched) followed similar trends to site A nutrient unenriched (Figure 4.48). Cyanobacteria and green algae densities were very low at site C (unaltered flow) and were dominated by diatoms (Figure 4.49). Cyanobacteria were the dominant algal growth form at site D (altered flor) (Figure 4.50). Highest diatom levels were experienced at site E (reference) were they were the most dominant growth form (Figure 4.51).

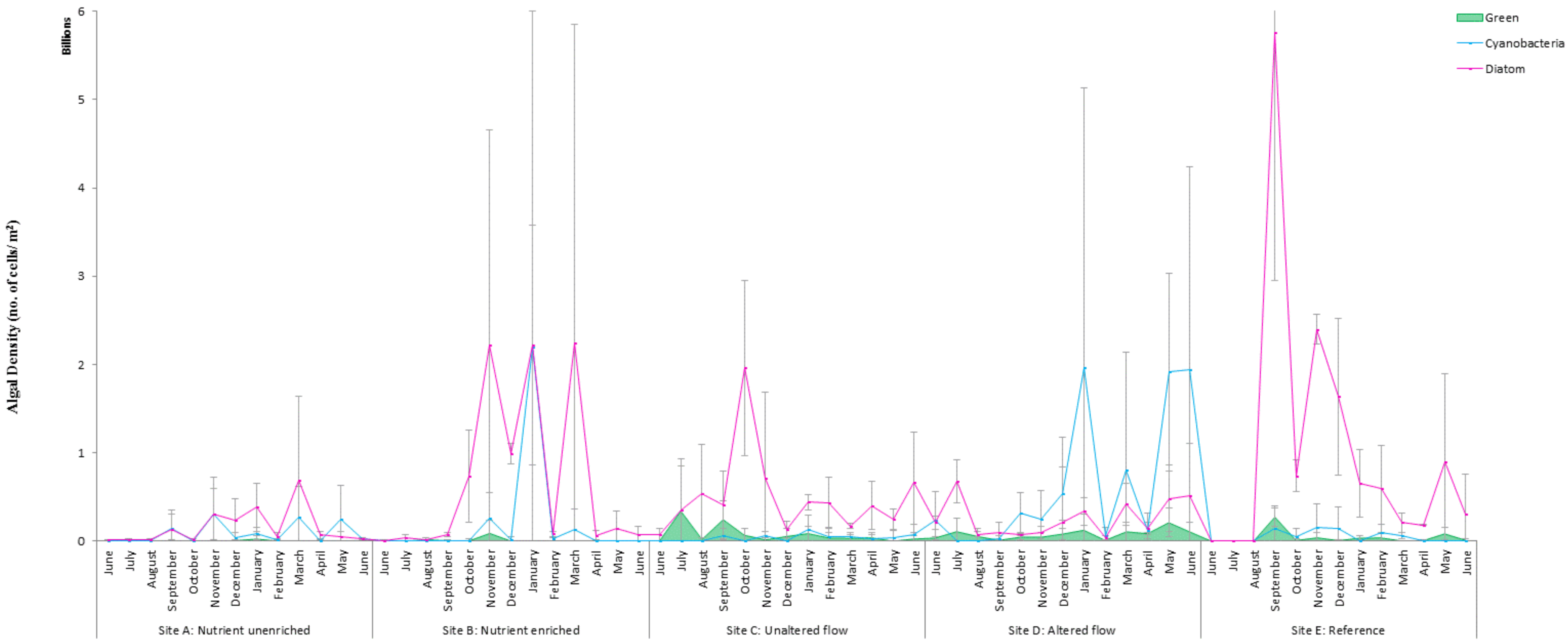

Figure 4.36: Algal class divisions at each site over the entire sampling period (June 2014 to June 2015)

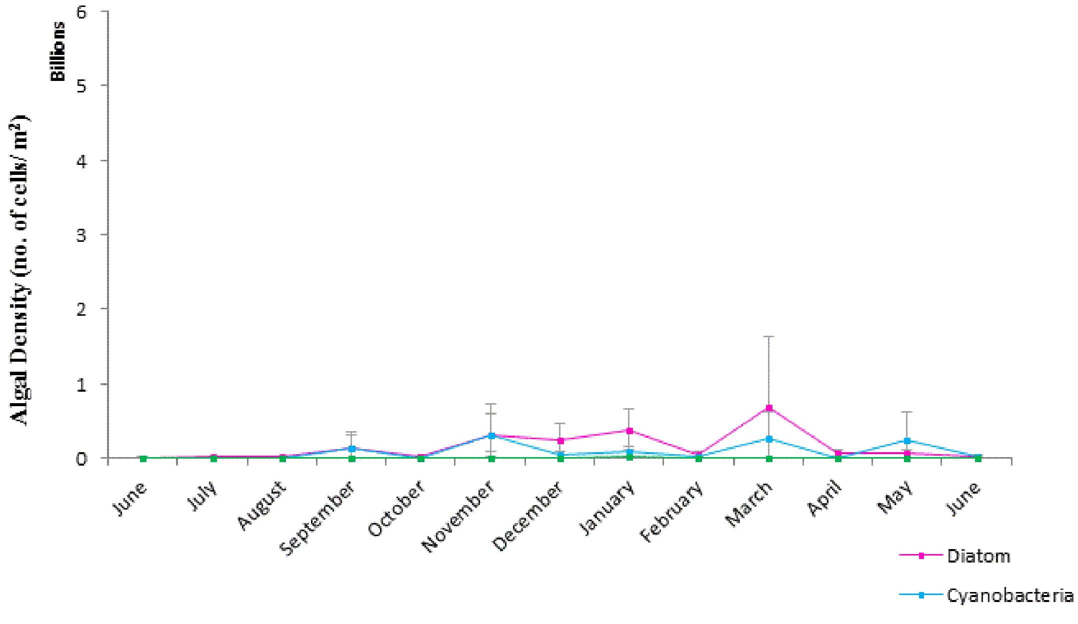

Figure 4.37 (a)

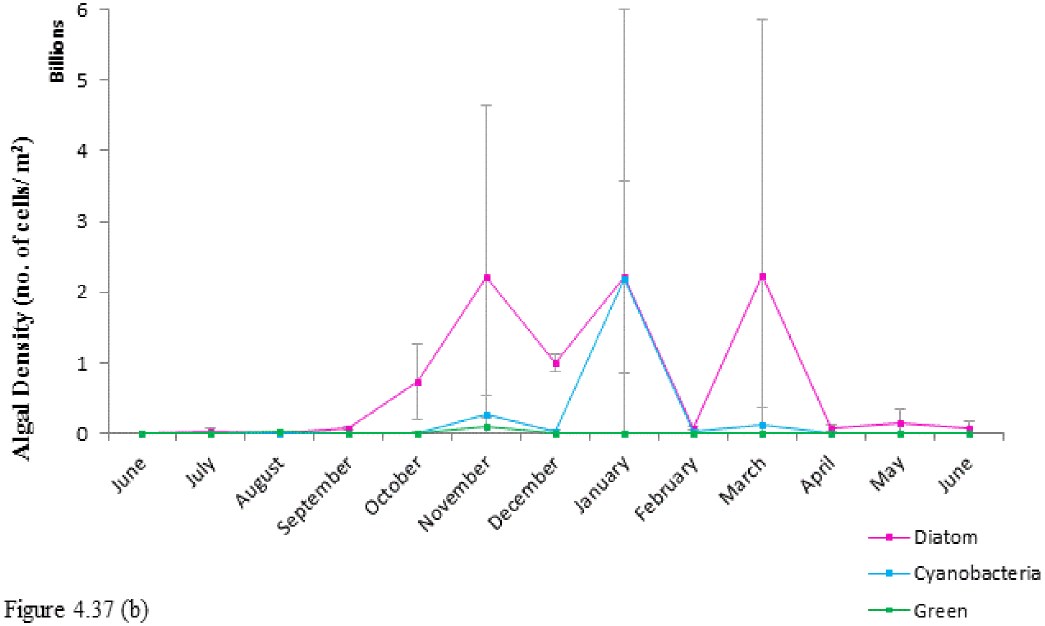

Figure 4.37 (b)

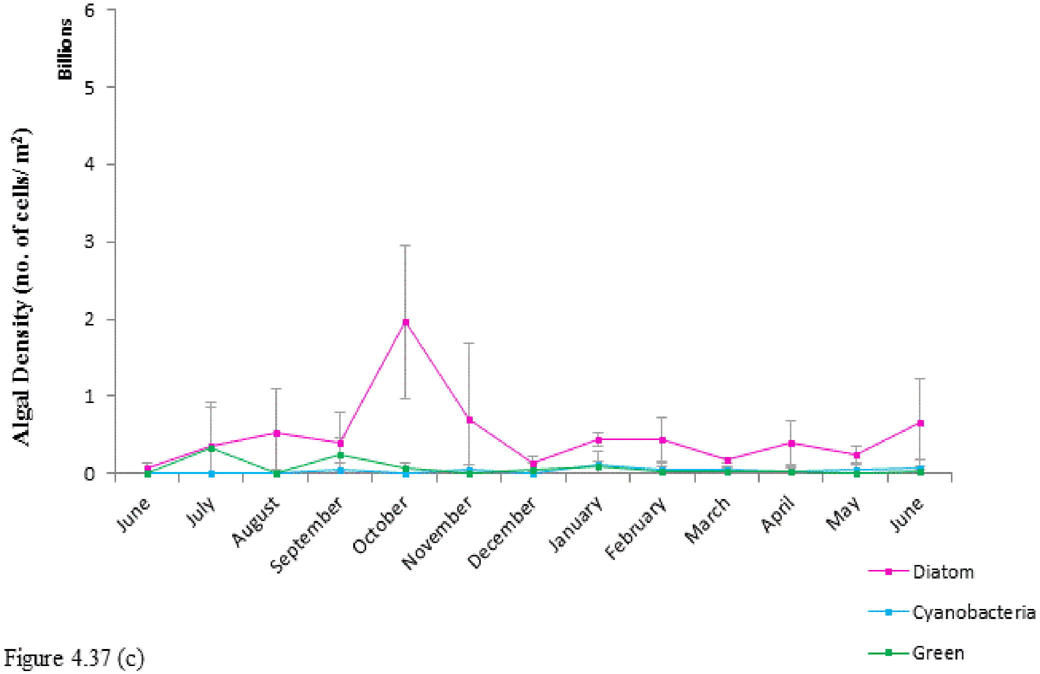

Figure 4.37 (c)

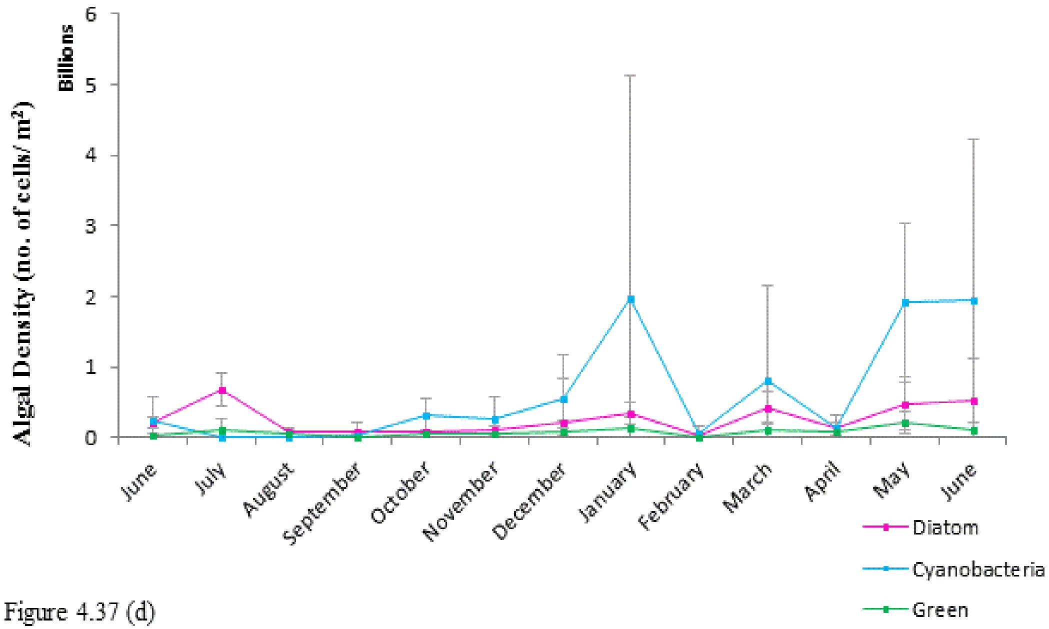

Figure 4.37 (d)

Figure 4.37: Algal class divisions at (a)- site A (Nutrient unenriched- Msunduzi-Kwanoshezi), (b)- Site B (Nutrient enriched- Msunduzi-tannery), (c)- Site C (Unaltered flow- Umgeni-Petrustroom) and (d)- Site D (Altered flow- Umgeni-York Road) for the sampling period June 2014 to June 2015

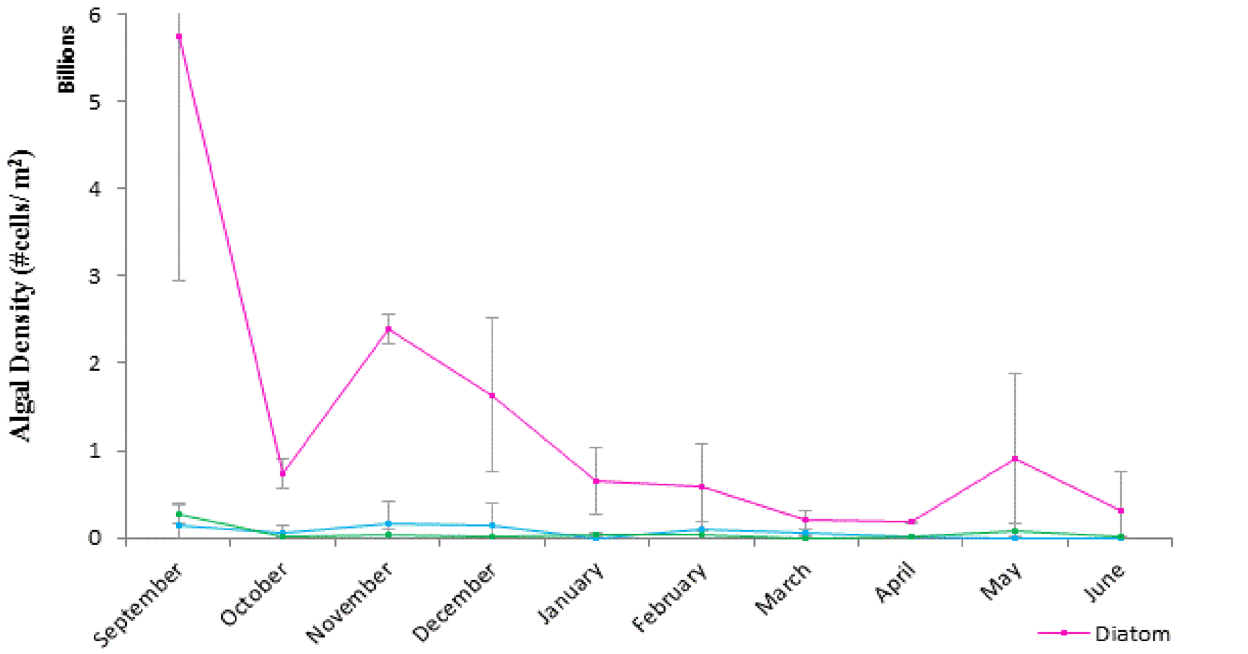

Figure 4.37(e): Algal class divisions at site E (Reference) for the sampling period September 2014 to June 2015

4.16 Conclusion

A summary graph indicating relationships between periphyton, nutrients, invertebrates and flow dynamics show that grazers provide a top down control of algal biomass while nutrients play a bottom up control resulting in the increase in algal biomass. Flow death and velocity play a role in sloughing of algal biomass. The summary graph will be discussed further in Chapter five, section 5.8.

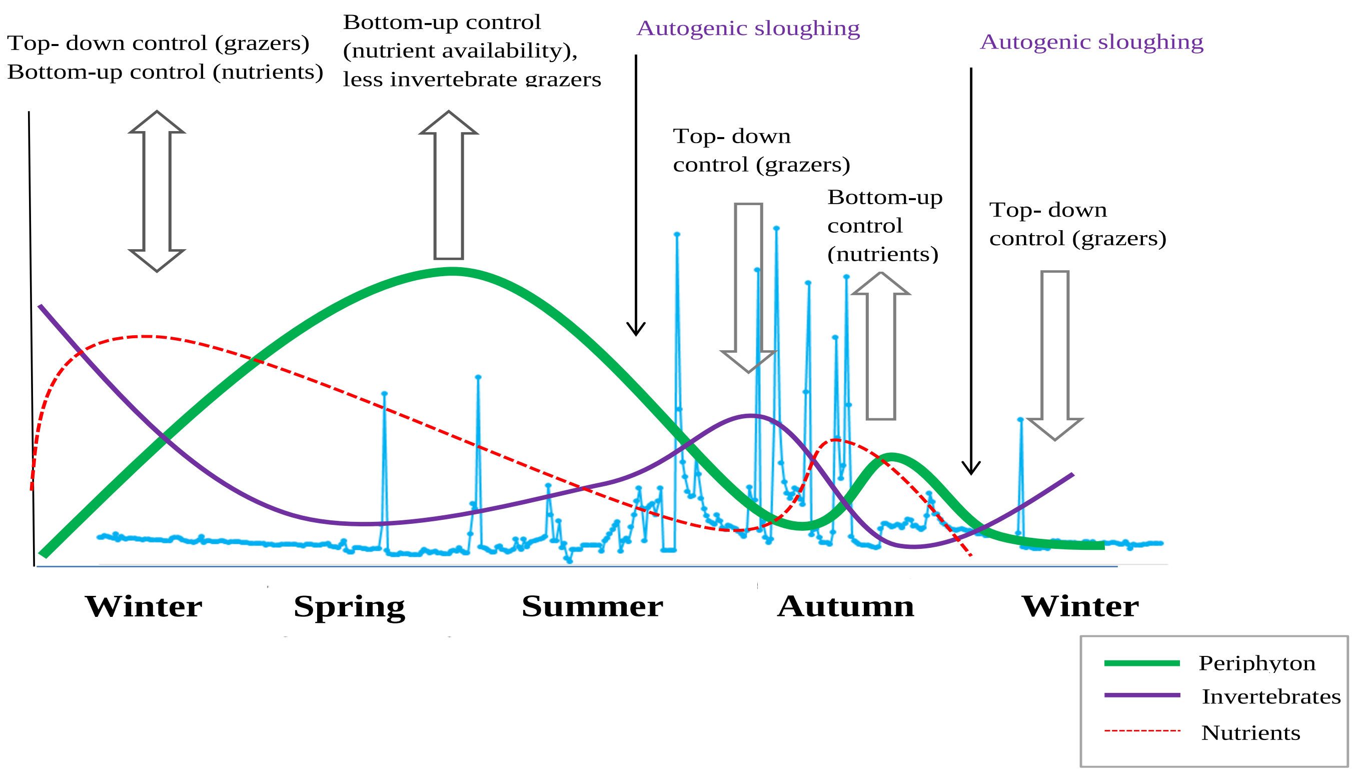

Figure 4.38: Summary graph indicating relationships between periphyton biomass, invertebrates, nutrient levels and flow dynamics within a system

CHAPTER FIVE

Discussion

5.1 Introduction

The research aim was to understand the role of periphyton communities in determining the ecological condition of rivers by taking periphyton community composition, algal biomass and their interaction with environmental variables and invertebrates into consideration. These periphyton patterns were determined under nutrient unenriched and natural flow conditions and to compare these patterns under conditions of nutrient enrichment and altered flow. This was achieved by comparing conditions at site A (nutrient unenriched-Msunduzi-Kwanoshezi) and site B (nutrient enriched- Msunduzi-Tannery) and by comparing site C (unaltered flow-Umgeni- Petrustroom) and site D (altered flow- Umgeni- York road). These four sites were compared to a control site E (reference- Hlatikhulu- Broadmoor).

5.2 Physico-chemical variations

Algal biomass levels were highest when temperatures were higher, therefore, highest algal biomass was observed in summer. However, it was evident (figures 4.22(a) to 4.22 (e)) that in February 2015 a decrease in algal biomass were observed at all five sampling sites. This could be attributed to sloughing as algal mats become too heavy for attachment to the substrate and consequently are removed, in particular if there is an increase in flow. These trends were experienced in studies by Biggs (2000) and Higgens *et al*. (2008) who indicate that seasonal variations in temperature can affect biomass accrual rates and species diversity (Rader *et al*., 2008). High temperature can result in mid-summer sloughing (Biggs, 2000; Higgens *et al*., 2008). This research shows that there was higher algal species diversity in summer when temperatures were higher than in spring when temperatures were lower. Water temperature at each site was highest between October 2014 and February 2015. Temperature was lowest in winter, June 2014 and May 2014 and June 2015. Optimum growth temperatures for periphyton communities range between 0^{o}C to 30^{o}C as heat stress can reduce the growth of periphyton communities (DeNicola, 1996; Larned, 2010). Water temperature at each of the sites remained above 0^{0}C and below 30^{0}C.

There were large variations in % oxygen levels within and between sampling sites, with highest levels in winter and lowest levels in summer. Dissolved oxygen levels are usually lower in stagnant water such as in pools (Growns and Growns, 2001), however this relationship was not visible as sampling occurred in the runs biotype. A relationship was observed between temperature and dissolved oxygen levels. When water temperature levels were lower, dissolved oxygen levels were higher and vice versa. Therefore, highest oxygen levels were experienced in winter. Conductivity remained constant throughout the sampling period at each site. Highest conductivity levels were experienced at site B

(Nutrient enriched) while lowest conductivity values were experienced at site E (Reference). Conductivity is linked to water temperature (Goodwin *et al.*, 2009), so even though the conductivity levels remained fairly constant, increases in water temperature will increase conductivity levels.

pH varied between the sampling sites with lowest pH characteristic of site C (unaltered flow) and highest pH was experienced at site B (nutrient enriched). However, even with variations, pH did not fluctuate drastically from that of a neutral pH of 7. Decreases in pH could be attributed to an increase in nitrogen levels in the water column (Venter *et al*., 2003). This trend was evident as pH levels decreased between January 2015 and February 2015 while nitrite levels peaked between October 2014 to December 2014. One of the most evident declines in pH occurred between December 2014 and February 2015 at site E (reference). This decline coincides with the maize plantation on the banks of the river. With the lack of water quality data for this particular site it can be assumed, in accordance with the idea presented by Venter *et al*. (2003), that an increase in nutrients such as nitrogen can result in a decrease in pH levels. Therefore as a result of the maize plantation and the use of fertilizers, with an associated increase in nitrogen levels, a decrease in pH was observed at site E (reference).

Although fluctuations in clarity within each site made it difficult to observe a trend, declines in clarity occurred after the onset of the first rainfall event with increases in both water depth and velocity. Thus, increase rainfall resulting in increased depth and velocity, resulted in a decrease in clarity. This decrease could have resulted from increased run-off of embankment sediments that enter the river when there is a rainfall event.

5.3 Water quality

Ammonia (NH_3) levels peaked between October 2014 and January 2015, coinciding with rainfall. These high levels of ammonia could be as a result on fertilizer runoff into the river system. However, ammonia is most often converted to nitrates by bacteria within the water column. Peaks in ammonia coincided with peaks in nitrites indicating a relationship between the two. According to the South African water quality guidelines it is believed that ammonia levels lower than 0.2mg/L are not contaminated by organic waste. Ammonia levels above 10mg/L are found in raw untreated sewage (DWAF, 1996). Peaks in ammonia levels at sampling sites did not exceed 10mg/L mark. There is also a strong relationship between ammonia and pH levels however as pH levels did not fluctuate around neutral it can be deduced that non-toxic ammonia was present. The South African water quality guidelines suggest that ammonia has fairly low toxicity but ammonia levels above 1.5mg/L may cause a decrease in the quality of taste and odour in a water source. Ammonia levels in this research remained between 0 and 1.0mg/L indicating no associated health or aesthetic effects (DWAF, 1996). The largest fluctuations in soluble reactive phosphorus (SRP) occurred at site A (Nutrient unenriched) and site B (nutrient enriched).

SRP is the phosphorous available to and taken up by plant cells. During times of high algal biomass low levels of SRP were identified. (NO_2) and ammonia followed a similar trend. Nitrites are particularly harmful to infants if they enter the blood stream and attach to haemoglobin cells. In some cases nitrites can be found in food compounds and may have a carcinogenic effect when the food is consumed. However nitrite levels at the sampling sites remained below 6mg/L indicating that there are no adverse health effects (DWAF, 1996). It was observed that even during times when nutrient levels were low there was still biomass accrual, suggesting that factors other than nutrient levels could be necessary for algal biomass accrual.

According to Biggs (2000) flow regimes are more important for biomass accrual than nutrient regimes. However, in areas of consistently low flow such as those areas below dams, nutrient regimes become important (Biggs, 2000). High nutrient levels in spring and summer resulted in high levels of diatoms. This is reiterated by Biggs (2000) who notes that diatom mats increased with an increase in nutrients. According to Rosemond *et al*. (2000) periphyton biomass accrual, due to an increase in nutrients was greatest in seasons when light levels were higher. Therefore the positive effects of the increase in nutrients on algal biomass were experienced across all seasons but the magnitude was greatest in spring and autumn. The relationship between light and nutrients are interdependent and is greatest when there are no other limiting factors (Rosemond *et al*., 2000; Hill *et al*., 2010). The ratio between nitrogen and phosphorus are particularly important when determining the role that these nutrients have on blue-green algal blooms (Venter *et al*., 2003).

Highest levels of blue-green algae were at site B (Nutrient enriched) suggesting that nutrient levels played a role in the onset of blue-green algae. Biomass is higher in nutrient rich environments and there is a decrease in biomass when there are lower nitrogen to phosphorus ratios (Luttenton and Lowe, 2006; Zhang *et al*., 2013). However, studies have suggested that biomass levels are not influenced only by nutrient levels. Bowes *et al*. (2012) noted that an increase in phosphorus levels had no effect on algal biomass accrual. Therefore, even though nutrients have the potential for high productivity this potential can only be realised when there is sufficient light to reach the river bed. Therefore, high levels of both light and nutrients are required for periphyton to reach high rates of productivity. High nutrient levels do still encourage high rate of primary productivity which in turn affects benthic macro invertebrate assemblages (Hill *et al*., 2010). Trochine *et al*. (2014) indicated that periphyton responded differently to nutrients depending on nutrient status and temperature regimes which were seasonal and time sensitive.

5.4 The implications of flow regimes on periphyton communities.

Periphyton communities that are attached to substrates usually require high levels of shear-stress to be detached from the substratum and are therefore driven by hydrodynamics (Lutscher *et al*., 2007 Bouletreau *et al*., 2008). When velocity and depth were high, algal biomass levels were low followed by periods of high algal biomass. The decrease in algal biomass could be attributed to sloughing due to high depths and velocity. This was followed by an increase in algal biomass due to the niche and habitable environment created through the process of sloughing (Boulêtreau *et al*., 2008). Another possible scenario for this occurrence was that higher velocity results in greater macro-invertebrate abundances due to greater surface areas for grazers to feed on contributing to the decline in periphyton biomass (Choudhury *et al*., 2014).

High velocity can result in greater periphyton biomass due to nutrient mass transfer (Biggs *et al*., 1998). The age of the periphyton community could play a role in biomass loss that occurs with an increase in flow velocity (Villeneuve *et al*., 2009). Alternately, in areas of low flow, biofilms can grow on lose assemblages and are not removed by shear stress (Boulêtreau, *et al*., 2008). At site D (altered flow), the site below the dam, had fairly constant flow velocity and depth conditions however there were fluctuations in algal biomass indicating that in these environments shear-stress does not occur but the low flows do allow for the accumulation of algal biomass (Boulêtreau, *et al*., 2008).

Surface discharge is an important indicator of flow events and the arrival of propagules (Rader *et al*., 2008). Discharge data within this research indicated that highest surface discharge was experienced in summer and during this high discharge periods there was high algal and low invertebrate biomass. Rader *et al*. (2008) recorded that disturbances such as floods do not restore macro-invertebrate diversity, in particular downstream from a deep dam release. High discharge or flood events can results in a decrease in algal biomass. The magnitude of the disturbance will determine the time it takes for the periphyton community to recover. Recovery after a disturbance can occur either from disturbance resistant algae that remain after the disturbance event has occurred or from propagules upstream that were moved by the disturbance event (Robinson *et al*., 2004). The frequency and duration of a flood or disturbance event can affect periphyton biomass and metabolic rates. Therefore floods are important as they bring about scouring and sloughing which limit sediment accumulation and reset biofilm development (Ryder, 2004). All sites reflected a correlation between AFDM and chlorophyll *a*. The trend was also observed at site D (altered flow) below Midmar dam which was a site of low disturbance and regulated flow. According to Ryder (2004) there is a high correlation in terms of algal biomass between chlorophyll *a* and AFDM values in areas of low disturbance and regulated flow.

Periphyton such as *achnanthes* and *fragilaria* occurred predominately in summer at the sampling sites or in much greater abundances in summer than in spring. This could be attributed to the fact that some periphyton species can withstand and survive at higher velocities associated with the summer rainfall period. This idea was reiterated by Hart *et al*. (2013) who found that certain periphyton species are able to withstand higher velocity and increased drag force. Diatoms had the highest density at site E (reference) site while green algae was present at site C (unaltered flow), site D (altered flow) and site E (reference). Cyanobacteria were predominate at site B (nutrient enriched) and site D (altered flow).

The effect of flow on periphyton communities can be explained through the subsidy-stress response (Biggs and Thomsen, 1995). For example, at site D with consistently low flows, periphyton, especially the filamentous types was allowed to develop. The increase in periphyton as flow increased between December 2014 to April 2015 when velocity increases from near zero levels will result in an increase in periphyton biomass through nutrient mass transfer as iterated by the subsidy- stress model (Biggs and Stokseth, 1996; Biggs *et al*., 1998; Larned *et al*., 2004). However once an equilibrium between increasing velocity and algal biomass has occurred, any further increases in velocity will result in a decrease in algal biomass (Biggs and Thomsen, 1995; Borchardt, 1996; Biggs *et al*., 1998).

5.5 Periphyton-macro invertebrate relationships.

There was an evident relationship between periphyton biomass and macro-invertebrate density. For example, during periods of high invertebrate density there were periods of lower algal biomass (Figure 4.24 (a)-(e)). Invertebrate grazers provide a top-down control of algal biomass resulting in the decrease of algal biomass with an increase in invertebrate grazers (Taylor *et al*., 2002; Hillebrand, 2009; Peters *et al*., 2007; Trochine *et al*., 2014).Grazer effect on algal biomass levels are dependent on grazer foraging mouthparts, foraging ability and spatial variations (Peters *et al*., 2007). Periphyton is susceptible to invertebrate grazing as they are good quality food that are high in nitrogen and phosphorus. Due to increased grazing pressure, with an increase in grazer biomass, large proportions of algal biomass can be removed. Grazing pressure exerted by invertebrates is fairly constant throughout a range of habitats (Hillebrand, 2009), however, there were significant differences in invertebrate biomass across the sampled sites.

In contrast to this, Villanueva and Modenutti (2004) did not observe a reduction in periphyton biomass with an increase in invertebrate grazers which could have been attributed to fast algal replacement. The effect that invertebrates have on algal communities is dependent on the age and level of succession of the periphyton community (Villanueva and Modenutti, 2004). Invertebrate effects are dependent on specific adaptations of invertebrates to spatial variations and ecosystem changes (Peters *et al*., 2007).

Flow velocity can affect grazer efficiency (Peters *et al*., 2007), this effect is predominantly indirect due to velocity effects on periphyton biomass (Hart *et al*., 2013). During flood events or events of increased flow, there is usually a decline in invertebrate diversity and density in streams that are unregulated (Rader *et al*., 2008). This was observed in this research. However, in some cases, this trend was not observed as there was an increase in invertebrate grazer density during periods of higher velocity and surface discharge. This could be attributed to the high proportion of Chironomids at each of the sampling sites. According to Rader *et al*. (2008) Chironomids are usually resistant to disturbances increasing their abundances during this time to make use of the habitat available as a result of the displacement of other sensitive, sometimes dominant invertebrate species.

Families such as Chironomids have specific behavioural traits to combat disturbance events. These traits include entering into the drift during a flood period. Certain taxa are found in regulated rivers however, these taxa show little recovery after a disturbance event and therefore decrease in abundance. Some invertebrate families such as simuliidaes and Chironomids show fast recovery after flood events and Baetidae have a high dispersal range and can resist flood events (Robinson *et al*., 2004). Even after disturbance events Baetidae and Chironomids had the highest abundance and biomass at each sampled site.

At site D (altered flow) low abundances of Leptophlebiidae was observed from June 2014 to September 2014 and were not observed for the rest of the sampling period while Baetidae abundances were low except for slight peaks in November 2014. According to Growns and Growns (2001), Baetidae and Leptophlebiidae are affected by flow regulation. High abundances of chironomidae at site B (nutrient enriched) and a drastic increase in chironomidae abundances were observed at site E (reference) after the planting of maize. This suggests that chironomidae have a range of responses to pollution (Growns and Growns, 2001) whilst Hansson (1992) showed a reduction in the effect of grazers in nutrient enriched environments. However, this could be attributed to the fact that invertebrates were able to maintain periphyton at low levels and therefore did not have to increase drastically or increase grazer efficiency. This idea is evident in this research were even though levels of nutrients were high, grazer densities were low. This could be attributed to, and resulted in, the increase in algal biomass levels or that grazers were able to maintain the algal biomass at low levels. Therefore, it is important to determine if algae are resource limited or if invertebrates are food limited (Rosemond *et al*., 2000).

Catastrophic disturbance events can result in the reduction of macro-invertebrates in natural rivers (Robinson *et al*., 2004). After a disturbance event the recovery of grazers along a temporal gradient is limited by the food source available. Initial colonisation of invertebrates, such as browsers and filter feeders, occur simultaneously with the introduction of bacteria and biofilms in a river ecosystem. This is followed by a late colonisation of leaf feeders occurring with the slower accumulation of leaf litter (Lepori and Malmqvist, 2007). According to Lepori and Malmqvist (2007) all invertebrates, including their spatial variations, are controlled by resource availability. It was observed through this research that there were lower invertebrate abundances and biomass in spring than abundances and biomass in winter and summer (Figure 4.25). These research findings are in contrast to that of Lepori and Malmqvist (2007) who found that due to harsh winters invertebrate abundances were low with a rapid growth rate in spring. In summer food availability is high which is an important factor as it coincides with the emergence patterns of the invertebrates (Lepori and Malmqvist, 2007). It is important to identify whether grazers have a greater effect than physical factors on algal biomass even when conditions for growth are favourable and to determine whether food limitation had an effect or influenced the results (Yang *et al*., 2009).

The species diversity index indicated higher invertebrate diversity at site A (Nutrient unenriched) at site D (Altered flow) and at site E (Reference) after the plantation of maize. Species diversity may have been greater at site D (altered flow) as opposed to site C (unaltered flow) as site D experiences infrequent disturbances as compared to site C. This idea was expressed by both Biggs *et al*. (2005) and Schwendel *et al*. (2010) who found that large infrequent disturbances resulted in the mosaic distribution of invertebrate species which were mostly found on stable substrata. At sites with high velocities, scouring may remove algal communities and in turn remove an important feeding habitat for invertebrate grazers resulting in the decrease in invertebrate species diversity which may have been the case at site C (unaltered flow). The increase in periphyton biomass at site E (reference) as a result of the increase in nutrients from run-off of fertilizer from the maize planting, may have been the reason for the increase in invertebrate diversity. The higher invertebrate species diversity index experienced at site A as compared to site B (Nutrient enriched) does not follow the trend that one expects were higher invertebrate species will be experienced in the nutrient enriched environment. This could be as a result that site B (nutrient enriched) is highly polluted and therefore only certain invertebrate species can survive in such conditions thereby reducing the species diversity of that particular site. Due to this it is evident that grazing pressure and the effect of grazers plays a more important role on periphyton biomass than resources availability Rosemond *et al*. (2000).

5.6. Seasonal cycles and periphyton growth.

Seasonal cycles affect algal biomass more significantly than just temperature alone (Lutscher et al., 2007). Yang *et al*. (2009) found that seasonal variations effect algal biomass. From this research the changes in seasons did have significant effects on biomass levels at all sampling sites. Algal blooms occur due to growth conducive environmental factors (Venter *et al*., 2003) and are restricted by the interaction of grazers and available resources (Rosemond *et al*., 2000). Algal species composition is usually as a result of past environmental factors (Venter *et al*., 2003). At the sites that were nutrient enriched there was an increase in algal biomass in spring attributed to increased nutrients and light. Rosemond *et al*., (2000) suggest that these factors can sometimes be limiting in summer while higher biomass levels in spring compared to summer was evident in studies by Villanueva and Modenutti (2004). In summer at sites of altered flow (site D), there were high biomass values in summer. This was in contrast to the idea expressed by Yang *et al*. (2009) who indicated that higher biomass occurred in winter than in summer due to high mortality from scouring and abrasion in the wet season due to high rainfall. However, the trends from this research could have been as a result of the lack of rainfall at that particular time. Therefore once a rainfall event did occur a decrease in algal biomass was observed. The differences in algal biomass throughout the seasons could be as a result of water quality factors. For example nitrogen limitation occurs in the warmer seasons while phosphorus limitation occurs in spring and early summer (Trochine *et al*., 2014). Interactions between factors such as light intensity and flow regimes are important factors in understanding differences in biomass across the seasons (Villeneuve *et al*., 2009)

5.7 Key findings

The paucity of periphyton research in South Africa has resulted in the need for an understanding of the key temporal factors that drive these communities in order to use of periphyton as biological monitoring tools. The thesis identified those interactions between physico-chemical, water quality, flow data and grazing pressure that affects the periphyton community biomass and composition in the summer rainfall region of KwaZulu-Natal, South Africa.

Key findings suggest that there are constant shifts in periphyton communities in particular in terms of biomass. These shifts are strongly influenced by flow regimes and macro-invertebrate grazing, and to a lesser extent by seasonality. The effect of flow is predominantly greater during the wet season, summer, in KwaZulu-Natal. During periods of low rainfall and therefore low flow, periphyton communities are able to grow and succeed from that of diatoms to that of filamentous algae. Downstream of Midmar dam at site D (altered flow), the flow regimes were regulated this and remained low and constant allowing for biomass accrual.

5.7.1 Nutrient implications

Nutrient levels played a role in periphyton biomass accrual cycles between peaks in flow. For example, peaks in ammonia levels at site B (Nutrient enriched) coincided with peaks in biomass levels. Algal species indicated that at sites with natural unregulated flow, algal taxa remained at a pioneer stage of succession due to flooding events that reset the system. However, in areas of regulated low flow, the algal community was able to succeed into a later stage of succession which was evident through presents of filamentous algal. The end of the wet season usually indicated the start of the periphyton accrual cycle which continued into the dry season which is controlled by nutrient, light and temperature.

5.7.2 The relationship between invertebrate and periphyton

There was a relationship between periphyton biomass and invertebrate density At each site, periods of high invertebrate density witnessed periods of low periphyton biomass suggesting that one of the drivers of periphyton dynamics are invertebrate communities. There were significant differences in invertebrate density across seasons and sites indicating that macro-invertebrates can be indicators of both changing seasonal conditions together with differences in flow and nutrient regimes. The types of invertebrates found at the sites reflecting site characteristics and relationships between periphyton dynamics. For example, lower invertebrate densities were experienced between November 2014 and January 2014 while increases in invertebrate density occurred during winter (Figure 4.26)

5.7.3 Driving Factors at comparable sites

Principal component analyses were conducted to determine the primary physico-chemical factors at comparable sites. It was determined that pH, conductivity and temperature drove conditions at site B (Nutrient unenriched) particularly in summer. According to Webb *et al*. (2008) temperature changes can affect water quality, effecting dissolved oxygen and suspended sediment concentrations. This indicates that as a result of different levels of nutrient enrichment between site A (Nutrient unenriched) and site B (Nutrient enriched) temperature is an important physico-chemical driver. Depth and velocity were major driving factors in summer between site C (unaltered flow) and site D (altered flow). This result iterates that the dam plays a major role in altering river systems. Depth and velocity drove changes in summer at site C (Unaltered flow) and according to Olden and Poff (2003) flow regimes are developed through seasonal interactions These flow regimes play an important role in altering community composition and distribution of aquatic flora and fauna (Bunn and Arthington, 2002; Choudhury, 2014). Flow velocity can drive river ecosystems by affecting algal immigration, reproduction and post-disturbance recovery (Peterson and Stevenson, 1992; Matthaei *et al*., 2003). Temperature was a driving physico-chemical factor in summer which could be attributed to the fact that dams alter base flows, resulting in water quality degradation and water temperature changes (Collier, 2002; Rader *et al*., 2008).

Together with driving physico-chemical factors, it was identified that algal biomass was a driving factor at site C (Unaltered flow) and site D (Altered flow) regardless of season as compared to invertebrate grazer biomass. This could be attributed to the link between algal biomass and nutrients within the water column (Borchardt, 1996) as nutrient enrichment plays a crucial role in aquatic food web structures (Montuelle *et al*., 2010).

At site A (Nutrient unenriched) in particular during winter, invertebrate grazers, namely baetidae and leptophlebiidae, are driving factors. This could be attributed to the fact that during winter low rainfall and therefore low flows allow for algal biomass accrual providing a large food source for invertebrate grazers. Both baetidae and leptophlebiidae are deposit feeders and prefer to feed on larger algae (Schael, 2005). Larger algae will be present when no disturbance has occurred, such as in winter when there are no intense rainfall events.

5.8 Comparison between summer and winter rainfall areas.

Similar research was conducted in the winter rainfall region of the Western Cape, South Africa (Ewart-Smith and King, 2012). A number of key findings and trends were indicated in this work (Ewart-Smith and King, 2012). During this present research conducted it was indicated that shifts in periphyton biomass was as a result of influence by both flow regimes and invertebrate grazers. This trend was also observed in the outcome of research conducted in the Western Cape were it was evident that invertebrate grazers provided a top-down control of periphyton biomass (Ewart- Smith and King, 2012). In the summer rainfall region highest invertebrate density was experienced during late summer while in the winter rainfall region highest invertebrate densities were experienced during spring or early summer, during the dry season (Figure 5.1). Algal biomass peaks in the winter rainfall season were experienced in summer and early spring while peaks in periphyton biomass were experienced in summer and autumn in the summer rainfall region of KwaZulu-Natal (Figure 5.1 and Figure 5.2). Figure 5.1 and Figure 5.2 are schematic diagrams of periphyton biomass peaks over a seasonal time scale together with their interaction between invertebrate grazers and nutrients.

This research recognises the complex relationship between periphyton biomass, invertebrate grazers, flow regimes and nutrient levels. Each of these factors plays a different role on periphyton biomass accrual or loss depending on the season. The peaks in periphyton biomass were as a result of in an increase in nutrient levels and low flow velocity. Autogenic sloughing due to increased velocity resulted in the decrease of algal biomass, occurring when periphyton biomass was high resulting in a weak attachment between the substratum and the algal mat. In many instances, even if low flows and high nutrient levels are present, periphyton biomass levels are low usually due to the top down control that invertebrate grazers have on periphyton biomass (Figure 5.2).

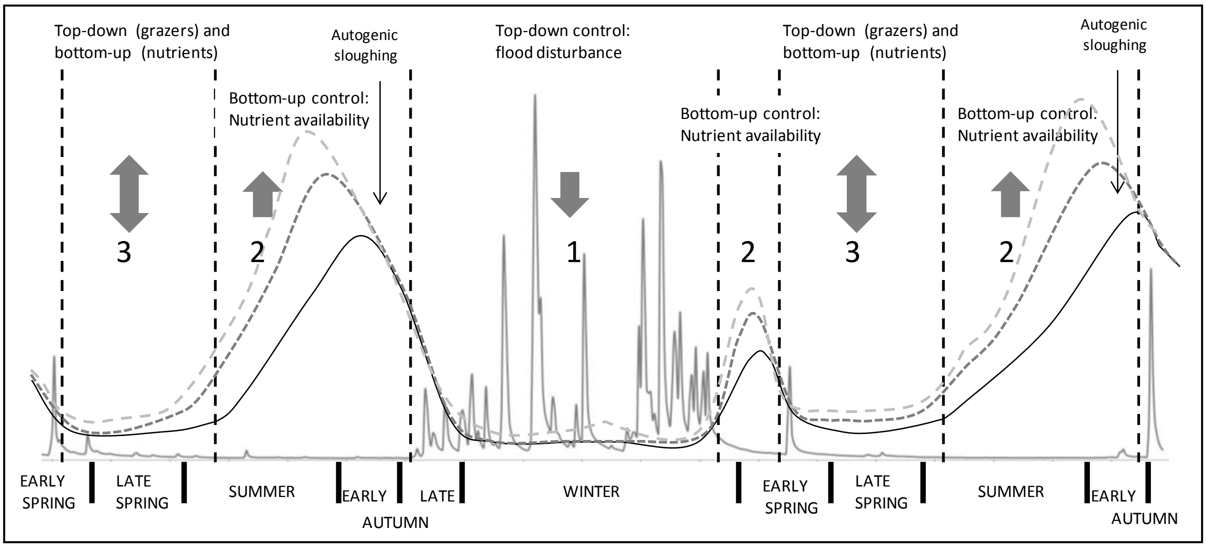

Figure 5.1: Schematic diagram of algal biomass loss and accrual and interaction between invertebrate grazers and nutrients in the winter rainfall region of the Western Cape (after Ewart-Smith and King, 2012)

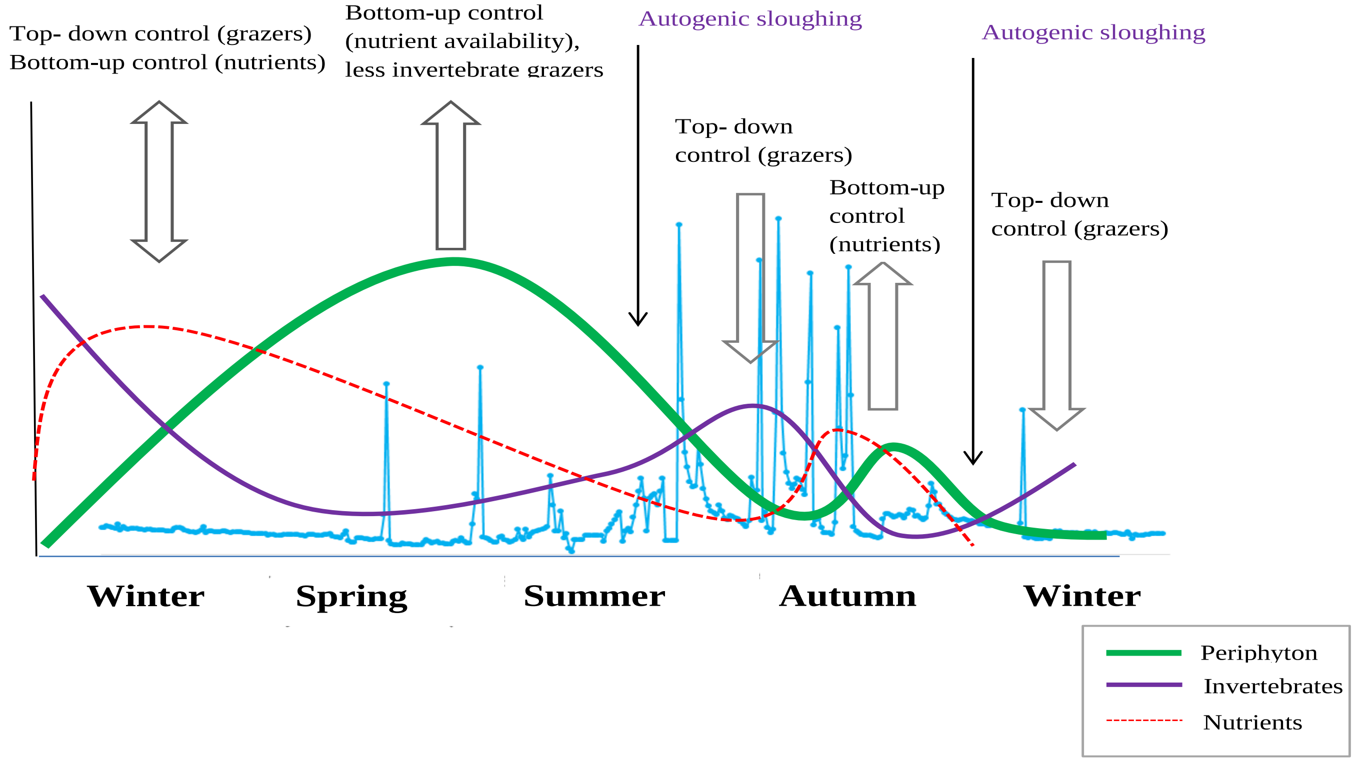

Figure 5.2: Schematic diagram of algal biomass accrual and loss and the interaction between invertebrates and nutrients in the summer rainfall region of KwaZulu-Natal.

5.9 Reflections for ongoing research

Once-off sampling fails to establish trends and drivers within these river systems. Monthly sampling allowed for the establishment of depth and velocity trends which influenced flow together with changes in algal biomass and invertebrate density. Monthly sampling played an important role in determining seasonal changes of environmental variables. Even though this thesis was important in establishing these drivers and their links to periphyton communities there is still analysis and sampling that can be done to solidify and improve our understanding.

Future work to develop general models to indicate responses of periphyton to changes in flow and environmental drivers which can be transferred from one river system to the other will be important in the initiation of using periphyton as a bio-monitoring tool. This can be achieved through greater and more rigorous sampling and statistical analysis. However, the field methods utilised through this project are important as it complements other more rigorous methods of empirically evaluating aquatic ecology. Further long term field sampling and observational studies together with studies of an entire river will be an important tool in determining these periphyton dynamics. Overall the project fulfilled its aim and objectives to collect, identify and determine periphyton and invertebrate dynamics together with their relationships with environmental drivers to determine the ecological condition of rivers in KwaZulu-Natal.

5.10 Conclusion

The research provides an account of periphyton community dynamics in terms of biomass in the rivers of the summer rainfall region of KwaZulu-Natal, South Africa. The thesis provides information on temporal periphyton community dynamics together with the environmental factors that drive them. Flow changes due to rainfall patterns are one of the overarching controllers of periphyton community dynamics. Nutrients and temperature influence periphyton community dynamics and invertebrates provide a top down control on periphyton communities and is another major factor that control and drive these periphyton communities.

CHAPTER SIX

Conclusion

6.1 introduction

The aim of this research was to determine the role of periphyton communities as indicators of the ecological condition of rivers by determining periphyton community structure and biomass, taking into consideration their links to environmental variables and relationship with macro-invertebrates.

6.2 Objectives

6.2.1 To measure flow and water quality at selected sites to determine changes in these variables over a seasonal and temporal time scale to allow for patterns and trends in these variables to be observed.

Monthly field sampling was conducted for a period of 13 months from June 2014 to June 2015. During this time physico-chemical data was collected. This data included monthly water temperature data collected during site visits at all five sites using a YSI and hourly water temperature collected using HOBO data loggers at site C, site D and Site E. Dissolved oxygen, conductivity, pH, clarity depth and velocity were measured during each sampling occasion. Water quality data was provided by Umgeni water. Data was used to establish trends in these physico-chemical factors which was used to explain periphyton dynamics in relation to these factors.

6.2.2 To collect, identify and determine biomass of periphyton communities in areas of altered flow and nutrient levels as these altered conditions will provide an indication of periphyton dynamics

To fulfil the second objective, periphyton was collected by scrubbing algae from five rocks at each site for the duration of the 13 month sampling period. The collected algae was used to determine periphyton biomass using two lab procedures namely Ash Free Dry Mass (AFDM) and chlorophyll *a* analysis. In situ algal biomass was determined using a benthotorch. Algal species were identified in most cases to genus level under a compound microscope and counts of these algal cells were conducted using a haemocytometer.

6.2.3 To collect and calculate abundance and biomass of macro-invertebrates to indicate the relationship between grazer density and periphyton biomass.

The last objective was fulfilled by collecting invertebrates on and around each sampled stone in a net at each of the five sites for the duration of the sampling period. The collected invertebrates were identified to family level under a dissecting microscope. Counts of invertebrates within each family identified were recorded in order to calculate abundance. Biomass of invertebrate grazers were determined by weighing the invertebrates to calculate the weights per individual. A relationship was evident between invertebrates and periphyton. The research indicated the clear top-down control of invertebrate on periphyton

6.3 Hypotheses

The research fulfilled all the hypotheses set out. The research found that Periphyton biomass is related to the seasonal patterns in selected rivers of KwaZulu-Natal. Biomass levels will change according to seasons. For example periphyton biomass peaks were experienced in summer and autumn. Periphyton community composition did change over annual cycles as was predicted in the hypothesis. The community composition of periphyton also differed between rivers of alter flow and nutrient conditions. For example diatoms were most abundant at the reference site while green algal were observed more in the sites that had flow alterations. The effect that grazing had on periphyton in terms of providing a top down control was more evident during periods when invertebrate abundance was highest. Finally the research indicated that factors such as nutrient regimes and flow conditions do play a role in differences in invertebrate grazer abundance.

6.4 Conclusion

This research fulfilled all the aims and objectives. The hypothesis set out at the start of the project were also accepted. The research indicated complex interactions between physico-chemical factors such as temperature, river flow and velocity and nutrient regimes on periphyton biomass. The relationship becomes further complexed with the interaction between invertebrates and periphyton and the effect that external factors such as nutrients and flow have on invertebrates as well. Understanding these relationships is the key step in the process of using periphyton as a bio-monitoring tool and to utilise periphyton as a tool to assist in the setting of ecological reserves. Therefore, the aim to determine the role of periphyton communities as indicators of the ecological condition of rivers by determining periphyton community structure and biomass, taking into consideration their links to environmental variables and relationship with macro-invertebrates was fulfilled.

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Appendix 1

AFDM calculations for each month at each sampling site

Sample	Tot vol	Filtered	Pre-heat bare weight(g)			Post heat bare weight(g)		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	Total matter
Sample	ml	ml	Foil	Foil+Filter	Filter	Foil+Filter	filter	Foil+Filter	Filter	Foil+ Filter	filter	AFDM (g)	Inorganic(g)	Matter (g)	x	y	z	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1JUN14	126	15	1.2761	1.3639	0.0878	1.3679	0.0918	1.375	**0.0989**	1.3777	0.1016	-0.02268	0.08232	0.05964	115	85	25	14775	0.0240669	0%	0.0240669	dry<ash	3.4204655	2.478092318
ED2JUN14	191	15	1.2693	1.3587	0.0894	1.3608	0.0915	1.3663	0.097	1.3652	0.0959	0.0140067	0.05602667	0.0700333	140	110	55	29150	0.0441919	50%	0.022096	0.63390199	2.535608	3.169509948
ED3JUN14	246	15	1.2568	1.3462	0.0894	1.341	0.0842	1.3494	0.0926	1.3473	0.0905	0.03444	0.10332	0.13776	180	100	55	33400	0.0501419	40%	0.0300851	1.144751196	3.4342536	4.579004784
ED4JUN14	347	15	1.2548	1.3439	0.0891	1.3391	0.0843	1.3423	0.0875	1.3403	0.0855	0.0462667	0.02776	0.0740267	110	65	45	15025	0.0244169	40%	0.0146501	3.158104064	1.8948624	5.052966502
ED5JUN14	355	15	1.2437	1.335	0.0913	1.3312	0.0875	1.3373	0.0936	1.3358	0.0921	0.0355	0.10886667	0.1443667	190	95	35	28025	0.0426169	20%	0.0340935	1.041253587	3.1931777	4.234431255
TAN1JUN14	133	15	1.2596	1.3497	0.0901	1.3448	0.0852	1.3514	0.0918	1.3503	0.0907	0.0097533	0.04876667	0.05852	120	85	30	16350	0.0262719	30%	0.0183903	0.530351186	2.6517559	3.182107118
TAN2JUN14	165	15	1.2559	1.3446	0.0887	1.341	0.0851	1.3449	0.089	1.343	0.0871	0.0209	0.022	0.0429	140	130	5	19550	0.0307519	5%	0.0292143	0.715402951	0.7530557	1.468458688
TAN3JUN14	131	15	1.2574	1.3452	0.0878	1.3414	0.084	1.3515	0.0941	1.3492	0.0918	0.0200867	0.06812	0.0882067	190	160	90	61900	0.0900419	50%	0.045021	0.446162657	1.5130734	1.959236015
TAN4JUN14	319	15	1.2699	1.3608	0.0909	1.3565	0.0866	1.3624	**0.0925**	1.3739	0.104	-0.2445667	0.37004	0.1254733	130	115	10	17400	0.0277419	0%	0.0277419	dry<ash	13.338668	4.522881754
TAN5JUN14	326	15	1.262	1.3499	0.0879	1.3463	0.0843	1.3554	0.0934	1.351	0.089	0.0956267	0.10214667	0.1977733	165	95	20	20875	0.0326069	20%	0.0260855	3.665890757	3.9158379	7.581728612
PET1JUN14	157	15	1.2685	1.356	0.0875	1.3531	0.0846	1.4422	0.1737	1.419	0.1505	0.2428267	0.68975333	0.93258	135	110	40	24650	0.0378919	0%	0.0378919	6.408405666	18.203187	24.61159245
PET2JUN14	177	15	1.2474	1.3349	0.0875	1.3549	0.1075	1.3907	0.1433	1.3796	0.1322	0.13098	0.29146	0.42244	156	95	550	152870	0.2173999	0%	0.2173999	0.602484178	1.340663	1.943147168
PET3JUN14	309	15	1.2723	1.3593	0.087	1.3329	0.0606	1.3792	0.1069	1.367	0.0947	0.25132	0.70246	0.95378	195	120	60	42300	0.0626019	0%	0.0626019	4.014574638	11.221065	15.23563981
PET4JUN14	207	15	1.2668	1.3544	0.0876	1.3529	0.0861	1.419	0.1522	1.3973	0.1305	0.29946	0.61272	0.91218	185	110	65	39525	0.0587169	0%	0.0587169	5.100064888	10.435156	15.53522069
PET5JUN14	289	15	1.2649	1.353	0.0881	1.3491	0.0842	1.3662	0.1013	1.3606	0.0957	0.1078933	0.22156667	0.32946	170	150	35	36700	0.0547619	10%	0.0492857	2.189140287	4.4955559	6.684696233
YO1JUN14	148	15	1.2597	1.348	0.0883	1.3442	0.0845	1.3722	0.1125	1.37	0.1103	0.0217067	0.25456	0.2762667	140	125	50	30750	0.0464319	5%	0.0441103	0.492099673	5.7709871	6.263086747
YO2JUN14	171	15	1.2424	1.3294	0.087	1.3265	0.0841	1.4028	0.1604	1.3656	0.1232	0.42408	0.44574	0.86982	130	90	50	22700	0.0351619	0%	0.0351619	12.0607817	12.676789	24.73757106
YO3JUN14	292	15	1.2464	1.3354	0.089	1.3316	0.0852	1.3954	0.149	1.3516	0.1052	0.85264	0.38933333	1.2419733	165	110	60	34650	0.0518919	0%	0.0518919	16.43108077	7.5027766	23.93385737
YO4JUN14	284	15	1.2574	1.345	0.0876	1.3436	0.0862	1.3719	0.1145	1.3591	0.1017	0.2423467	0.29346667	0.5358133	125	60	45	15825	0.0255369	5%	0.0242601	9.989534923	12.096702	22.08623737
YO5JUN14	313	15	1.272	1.365	0.093	1.3617	0.0897	1.3787	0.1067	1.3715	0.0995	0.15024	0.20449333	0.3547333	155	80	40	21800	0.0339019	30%	0.0237313	6.330871468	8.6170195	14.94789097

			Pre-heat bare weight(g)			Post heat bare weight(g)		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	Total matter
Sample	Tot vol	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	filter	Foil+Filter	filter	Foil+ Filter	filter	AFDM (g)	Inorganic(g	Matter (g)	x	y	z	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1JUL14	359	15	1.2674	1.3567	0.0893	1.356	0.0886	1.3772	0.1098	1.3756	0.1082	0.0382933	0.4690933	0.5073867	185	120	32	31960	0.0481259	20%	0.0385007	0.994613434	12.18401457	13.178628
ED2JUL14	295	15	1.268	1.3568	0.0888	1.3555	0.0875	1.366	0.098	1.3652	0.0972	0.0157333	0.1907667	0.2065	185	110	45	33625	0.0504569	70%	0.0151371	1.039390935	12.60261508	13.64200602
ED3JUL14	330	15	1.2473	1.3377	0.0904	1.3364	0.0891	1.3499	0.1026	1.349	0.1017	0.0198	0.2772	0.297	135	115	75	34275	0.0513669	40%	0.0308201	0.642437056	8.994118781	9.636555837
ED4JUL14	379	15	1.2513	1.3429	0.0916	1.342	0.0907	1.3456	0.0943	1.3455	0.0942	0.0025267	0.0884333	0.09096	155	114	50	31120	0.0469499	30%	0.0328649	0.07688033	2.690811553	2.767691883
ED5JUL14	368	15	1.2654	1.354	0.0886	1.3529	0.0875	1.3637	0.0983	1.3603	0.0949	0.0834133	0.1815467	0.26496	140	110	55	29150	0.0441919	50%	0.022096	3.775050782	8.216286997	11.99133778
TAN1JUL1	378	15	1.2446	1.333	0.0884	1.332	0.0874	1.3361	0.0915	1.3352	0.0906	0.02268	0.08064	0.10332	175	120	45	34275	0.0513669	20%	0.0410935	0.551911834	1.962353189	2.514265023
TAN2JUL1	384	15	1.2684	1.3574	0.089	1.3561	0.0877	1.3608	0.0924	1.3602	0.0918	0.01536	0.10496	0.12032	150	95	20	19150	0.0301919	10%	0.0271727	0.565273026	3.862699009	4.427972035
TAN3JUL1	361	15	1.2662	1.3532	0.087	1.3521	0.0859	1.3656	0.0994	1.3649	0.0987	0.0168467	0.3080533	0.3249	130	85	67	25455	0.0390189	0%	0.0390189	0.431756576	7.894977391	8.326733967
TAN4JUL1	362	15	1.2598	1.3501	0.0903	1.349	0.0892	1.3541	0.0943	1.3534	0.0936	0.0168933	0.1061867	0.12308	140	110	30	22900	0.0354419	0%	0.0354419	0.476648637	2.996077148	3.472725785
TAN5JUL1	350	15	1.2635	1.3522	0.0887	1.3508	0.0873	1.3628	0.0993	1.362	0.0985	0.0186667	0.2613333	0.28	150	120	60	34200	0.0512619	30%	0.0358833	0.520204414	7.282861801	7.803066215
PET1JUL14	259	15	1.2746	1.3616	0.087	1.3608	0.0862	1.385	0.1104	1.3779	0.1033	0.1225933	0.29526	0.4178533	130	70	50	19100	0.0301219	10%	0.0271097	4.52211895	10.89130057	15.41341952
PET2JUL14	325	15	1.2658	1.3549	0.0891	1.3543	0.0885	1.3851	0.1193	1.3755	0.1097	0.208	0.4593333	0.6673333	215	150	30	43200	0.0638619	40%	0.0383171	5.428380093	11.98767271	17.4160528
PET3JUL14	335	15	1.2625	1.3502	0.0877	1.3495	0.087	1.365	0.1025	1.3664	0.1039	-0.0312667	0.3774333	0.3461667	150	90	10	15900	0.0256419	0%	0.0256419	dry<ash	14.71939807	13.50003965
PET4JUL14	330	15	1.2628	1.3525	0.0897	1.3516	0.0888	1.3627	0.0999	1.3576	0.0948	0.1122	0.132	0.2442	200	140	65	50100	0.0735219	30%	0.0514653	2.180108434	2.564833452	4.744941886
PET5JUL14	311	15	1.2475	1.3351	0.0876	1.3335	0.086	1.371	0.1235	1.3622	0.1147	0.1824533	0.5950467	0.7775	210	160	50	52100	0.0763219	80%	0.0152644	11.95288203	38.98269479	50.93557681
YO1JUL14	361	15	1.2462	1.3333	0.0871	1.3325	0.0863	1.3548	0.1086	1.3456	0.0994	0.2214133	0.3152733	0.5366867	110	80	60	20200	0.0316619	0%	0.0316619	6.993052638	9.957498866	16.9505515
YO2JUL14	198	15	1.2612	1.3499	0.0887	1.349	0.0878	1.4022	0.141	1.3877	0.1265	0.1914	0.51084	0.70224	240	115	40	41800	0.0619019	10%	0.0557117	3.435543443	9.169346983	12.60489043
YO3JUL14	317	15	1.2664	1.354	0.0876	1.3531	0.0867	1.3704	0.104	1.3619	0.0955	0.1796333	0.1859733	0.3656067	175	70	100	36750	0.0548319	40%	0.0328991	5.460122463	5.652832668	11.11295513
YO4JUL14	320	15	1.2652	1.3526	0.0874	1.3515	0.0863	1.3931	0.1279	1.3741	0.1089	0.4053333	0.4821333	0.8874667	160	90	50	26900	0.0410419	20%	0.0328335	12.34510748	14.68418048	27.02928796
YO5JUL14	311	15	1.263	1.3523	0.0893	1.3505	0.0875	1.3981	0.1351	1.3826	0.1196	0.3213667	0.66554	0.9869067	210	120	110	61500	0.0894819	50%	0.044741	7.182830643	14.87541056	22.0582412

			Pre-heat bare weight(g)			ost heat bare weight(g		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	Total matter
Sample	Volume	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filter	Filter	AFDM (g)	Inorganic(g	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	Stone area	g.m2	g.m2	g.m2
ED1EDUG1	325	15	1.2676	1.3575	**0.0899**	1.3555	**0.0879**	1.3659	**0.0983**	1.3628	**0.0952**	0.0671667	0.1581667	0.2253333	170	130	70	43100	0.0637219	40%	0.0382331	1.75676564	4.136899733	5.893665373
ED2EDUG1	515	15	1.2684	1.3572	**0.0888**	1.3564	**0.088**	1.3625	**0.0941**	1.3592	**0.0908**	0.1133	0.0961333	0.2094333	195	130	60	44850	0.0661719	10%	0.0595547	1.902452384	1.614202023	3.516654406
ED3EDUG1	411	15	1.2507	1.3411	**0.0904**	1.3377	**0.087**	1.346	**0.0953**	1.3441	**0.0934**	0.05206	0.17536	0.22742	145	95	90	35375	0.0529069	50%	0.0264535	1.967985272	6.629003022	8.596988295
ED4EDUG1	395	15	1.2545	1.3437	**0.0892**	1.3393	**0.0848**	1.3474	**0.0929**	1.3425	**0.088**	0.1290333	0.0842667	0.2133	175	115	50	34625	0.0518569	50%	0.0259285	4.976515501	3.249969307	8.226484807
ED5EDUG1	421	15	1.2695	1.3597	**0.0902**	1.3549	**0.0854**	1.3585	**0.089**	1.3561	**0.0866**	0.06736	0.03368	0.10104	130	80	45	19850	0.0311719	0%	0.0311719	2.160920573	1.080460286	3.241380859
TAN1AUG	313	15	1.2482	1.3362	**0.088**	1.3313	**0.0831**	1.3361	**0.0879**	1.3342	**0.086**	0.0396467	0.0605133	0.10016	110	105	75	27675	0.0421269	0%	0.0421269	0.941124713	1.436453509	2.377578222
TAN2AUG	312	15	1.2719	1.4506	**0.1787**	1.4451	**0.1732**	1.4516	**0.1797**	1.4481	**0.1762**	0.0728	0.0624	0.1352	145	125	35	27575	0.0419869	0%	0.0419869	1.733874137	1.486177832	3.220051969
TAN3AUG	474	15	1.2691	1.3589	**0.0898**	1.3543	**0.0852**	1.3657	**0.0966**	1.3618	**0.0927**	0.12324	0.237	0.36024	250	160	50	60500	0.0880819	10%	0.0792737	1.554613755	2.989641837	4.544255592
TAN4AUG	377	15	1.2626	1.3524	**0.0898**	1.3472	**0.0846**	1.3526	**0.09**	1.3499	**0.0873**	0.06786	0.06786	0.13572	180	100	45	30600	0.0462219	0%	0.0462219	1.468135235	1.468135235	2.936270469
TAN5AUG	583	15	1.2665	1.3548	**0.0883**	1.3498	**0.0833**	1.3529	**0.0864**	1.352	**0.0855**	0.03498	0.0855067	0.1204867	110	75	20	11950	0.0201119	20%	0.0160895	2.174085989	5.314432417	7.488518406
PET1AUG1	509	15	1.2776	1.4545	**0.1769**	1.4489	**0.1713**	1.4647	**0.1871**	1.4595	**0.1819**	0.1764533	0.3596933	0.5361467	165	101	55	31295	0.0471949	10%	0.0424754	4.154246736	8.468272192	12.62251893
PET2AUG1	440	15	1.2693	1.358	**0.0887**	1.3531	**0.0838**	1.3742	**0.1049**	1.3651	**0.0958**	0.2669333	0.352	0.6189333	160	120	78	41040	0.0608379	0%	0.0608379	4.387615834	5.785867034	10.17348287
PET3AUG1	473	15	1.2652	1.3536	**0.0884**	1.3492	**0.084**	1.3664	**0.1012**	1.3621	**0.0969**	0.1355933	0.40678	0.5423733	120	95	26	16990	0.0271679	0%	0.0271679	4.990939062	14.97281718	19.96375625
PET4AUG1	428	15	1.264	1.3508	**0.0868**	1.3467	**0.0827**	1.3558	**0.0918**	1.3518	**0.0878**	0.1141333	0.14552	0.2596533	130	95	50	23600	0.0364219	40%	0.0218531	5.222742971	6.658997288	11.88174026
PET5AUG1	386	15	1.2495	1.4285	**0.179**	1.4237	**0.1742**	1.4376	**0.1881**	1.4336	**0.1841**	0.1029333	0.25476	0.3576933	175	85	65	31775	0.0478669	0%	0.0478669	2.150407345	5.322258178	7.472665523
YO1AUG14	500	15	1.248	1.4227	**0.1747**	1.4193	**0.1713**	1.4288	**0.1808**	1.4229	**0.1749**	0.1966667	0.12	0.3166667	205	75	55	30775	0.0464669	0%	0.0464669	4.232403424	2.582483445	6.814886869
YO2AUG14	559	15	1.2635	1.4416	**0.1781**	1.4364	**0.1729**	1.4578	**0.1943**	1.4482	**0.1847**	0.35776	0.4397467	0.7975067	178	135	83	50009	0.0733945	10%	0.0660551	5.41608855	6.65727551	12.07336406
YO3AUG14	531	15	1.2694	1.3576	**0.0882**	1.3531	**0.0837**	1.3635	**0.0941**	1.3589	**0.0895**	0.16284	0.20532	0.36816	150	135	60	37350	0.0556719	0%	0.0556719	2.924994477	3.688036514	6.61303099
YO4AUG14	429	15	1.2673	1.3553	**0.088**	1.3529	**0.0856**	1.3604	**0.0931**	1.3551	**0.0878**	0.15158	0.06292	0.2145	135	93	90	33075	0.0496869	0%	0.0496869	3.050703505	1.266329757	4.317033262
YO5AUG14	581	15	1.2618	1.3515	**0.0897**	1.3473	**0.0855**	1.37	**0.1082**	1.3627	**0.1009**	0.2827533	0.5964933	0.8792467	123	92	69	26151	0.0399933	30%	0.0279953	10.10002509	21.30690224	31.40692733

			Pre-heat bare weight(g)			ost heat bare weight(		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Tot vol	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filter	Filter	AFDM (g)	Inorganic(g)	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1SEP14	466	15	1.2671	1.3556	0.0885	1.3552	0.0881	1.3725	0.1054	1.3665	0.0994	0.1864	0.351053333	0.537453	220	120	65	48500	0.0712819	10%	0.0641537	2.905522	5.472066	8.377588
ED2SEP14	466	15	1.2677	1.3562	0.0885	1.3553	0.0876	1.36	0.0923	1.3574	0.0897	0.080773	0.06524	0.146013	170	35	30	12100	0.0203219	20%	0.0162575	4.968367	4.012912	8.98128
ED3SEP14	414	15	1.2468	1.3357	0.0889	1.3353	0.0885	1.3402	0.0934	1.3382	0.0914	0.0552	0.08004	0.13524	145	90	47	24095	0.0371149	0%	0.0371149	1.487273	2.156546	3.64382
ED4SEP14	442	15	1.2507	1.3421	0.0914	1.3417	0.091	1.3516	0.1009	1.3462	0.0955	0.15912	0.1326	0.29172	190	110	40	32900	0.0494419	40%	0.0296651	5.363872	4.469893	9.833764
ED5SEP14	421	15	1.2655	1.3534	0.0879	1.35	0.0845	1.3662	0.1007	1.3624	0.0969	0.106653	0.348026667	0.45468	135	110	75	33225	0.0498969	40%	0.0299381	3.562457	11.62486	15.18732
TAN1SEP1	380	15	1.2444	1.3343	0.0899	1.3338	0.0894	1.3403	0.0959	1.3389	0.0945	0.035467	0.1292	0.164667	140	110	10	17900	0.0284419	10%	0.0255977	1.385541	5.047326	6.432867
TAN2SEP1	381	15	1.2675	1.356	0.0885	1.3561	0.0886	1.3603	0.0928	1.3576	0.0901	0.06858	0.0381	0.10668	110	85	35	16175	0.0260269	20%	0.0208215	3.293708	1.829838	5.123545
TAN3SEP1	378	15	1.2652	1.3545	0.0893	1.3556	0.0904	1.3583	0.0931	1.3561	0.0909	0.05544	0.0126	0.06804	130	55	40	14550	0.0237519	40%	0.0142511	3.890215	0.88414	4.774355
TAN4SEP1	470	15	1.2595	1.3496	0.0901	1.3488	0.0893	1.3536	0.0941	1.3522	0.0927	0.043867	0.106533333	0.1504	160	75	30	19050	0.0300519	30%	0.0210363	2.085281	5.064255	7.149536
TAN5SEP1	373	15	1.2629	1.35	0.0871	1.3346	0.0717	1.3686	0.1057	1.3652	0.1023	0.084547	0.76092	0.845467	130	80	65	24050	0.0370519	20%	0.0296415	2.852305	25.67075	28.52305
PET1SEP14	431	15	1.274	1.3622	0.0882	1.3611	0.0871	1.3738	0.0998	1.3704	0.0964	0.097693	0.26722	0.364913	125	110	50	25500	0.0390819	10%	0.0351737	2.777453	7.597151	10.3746
PET2SEP14	515	15	1.2655	1.3546	0.0891	1.3538	0.0883	1.38	0.1145	1.3736	0.1081	0.219733	0.6798	0.899533	100	75	35	13625	0.0224569	30%	0.0157198	13.9781	43.24474	57.22284
PET3SEP14	425	15	1.2631	1.3526	0.0895	1.3513	0.0882	1.3706	0.1075	1.364	0.1009	0.187	0.359833333	0.546833	155	150	50	38500	0.0572819	20%	0.0458255	4.080696	7.852248	11.93294
PET4SEP14	405	15	1.2628	1.3535	0.0907	1.3528	0.09	1.3759	0.1131	1.37	0.1072	0.1593	0.4644	0.6237	90	75	60	16650	0.0266919	10%	0.0240227	6.631225	19.33171	25.96293
PET5SEP14	445	15	1.2473	1.3353	0.088	1.3527	0.1054	1.364	0.1167	1.354	0.1067	0.296667	0.038566667	0.335233	180	145	70	48850	0.0717719	0%	0.0717719	4.133465	0.537351	4.670816
YO1SEP14	565	15	1.2455	1.3332	0.0877	1.3329	0.0874	1.3479	0.1024	1.342	0.0965	0.222233	0.342766667	0.565	155	150	40	35450	0.0530119	0%	0.0530119	4.192141	6.465844	10.65798
YO2SEP14	475	15	1.26	1.3496	0.0896	1.3492	0.0892	1.3581	0.0981	1.3539	0.0939	0.133	0.148833333	0.281833	205	205	130	95325	0.1368369	10%	0.1231532	1.079956	1.208522	2.288477
YO3SEP14	472	15	1.2661	1.3531	0.087	1.3529	0.0868	1.3624	0.0963	1.3596	0.0935	0.088107	0.210826667	0.298933	310	275	115	152525	0.2169169	40%	0.1301501	0.676962	1.619873	2.296835
YO4SEP14	474	15	1.2647	1.3526	0.0879	1.3515	0.0868	1.3612	0.0965	1.3575	0.0928	0.11692	0.1896	0.30652	225	215	70	79175	0.1142269	10%	0.1028042	1.137308	1.844282	2.98159
YO5SEP14	390	15	1.2621	1.35	0.0879	1.3488	0.0867	1.3657	0.1036	1.3597	0.0976	0.156	0.2834	0.4394	305	110	40	50150	0.0735919	50%	0.036796	4.239597	7.701935	11.94153
DRA1SEP1	415	15	1.269	1.3576	0.0886	1.353	0.084	1.3718	0.1028	1.3654	0.0964	0.177067	0.343066667	0.520133	90	55	20	7850	0.0143719	30%	0.0100603	17.60048	34.10094	51.70142
DRA2SEP1	440	15	1.267	1.3547	0.0877	1.3495	0.0825	1.3647	0.0977	1.3593	0.0923	0.1584	0.287466667	0.445867	70	40	25	5550	0.0111519	10%	0.0100367	15.78206	28.64152	44.42359
DRA3SEP1	493	15	1.2568	1.3446	0.0878	1.3393	0.0825	1.3513	0.0945	1.3476	0.0908	0.121607	0.272793333	0.3944	50	45	20	4150	0.0091919	20%	0.0073535	16.5372	37.09697	53.63418
DRA4SEP1	555	15	1.2565	1.3434	0.0869	1.3392	0.0827	1.353	0.0965	1.3478	0.0913	0.1924	0.3182	0.5106	75	40	20	5300	0.0108019	40%	0.0064811	29.68614	49.0963	78.78244
DRA5SEP1	530	15	1.2561	1.3461	0.09	1.3412	0.0851	1.366	0.1099	1.3598	0.1037	0.219067	0.6572	0.876267	70	65	20	7250	0.0135319	50%	0.006766	32.37781	97.13344	129.5113

			Pre-heat bare weight(g)			ost heat bare weight(g		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	Total matter
Sample	Tot vol	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filter	Filter	AFDM (g)	Inorganic(	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1OCT14	253	15	1.267	1.3555	0.0885	1.3517	0.0847	1.3615	0.0945	1.3587	0.0917	0.047227	0.118067	0.165293	130	120	35	24350	0.0374719	10%	0.0337247	1.400357977	3.500895	4.901252919
ED2OCT14	375	15	1.2672	1.3554	0.0882	1.3506	0.0834	1.4409	0.1737	1.4254	0.1582	0.3875	1.87	2.2575	225	130	45	45225	0.0666969	40%	0.0400181	9.68310871	46.72881	56.4119172
ED3OCT14	350	15	1.2544	1.3433	0.0889	1.339	0.0846	1.3619	0.1075	1.3546	0.1002	0.170333	0.364	0.534333	170	110	50	32700	0.0491619	0%	0.0491619	3.464742684	7.404108	10.86885034
ED4OCT14	352	15	1.2571	1.3462	0.0891	1.342	0.0849	1.3908	0.1337	1.3757	0.1186	0.354347	0.790827	1.145173	170	105	75	38475	0.0572469	50%	0.0286235	12.37959319	27.62863	40.0082217
ED5OCT14	359	15	1.2686	1.3567	0.0881	1.3524	0.0838	1.3662	0.0976	1.3614	0.0928	0.11488	0.2154	0.33028	170	110	20	24300	0.0374019	30%	0.0261813	4.387859593	8.227237	12.61509633
TAN1OCT1	350	15	1.2662	1.3564	0.0902	1.3516	0.0854	1.3718	0.1056	1.3675	0.1013	0.100333	0.371	0.471333	120	105	25	18225	0.0288969	10%	0.0260072	3.857904532	14.26527	18.12317943
TAN2OCT1	348	15	1.2726	1.3604	0.0878	1.3566	0.084	1.374	0.1014	1.3708	0.0982	0.07424	0.32944	0.40368	160	125	55	35675	0.0533269	30%	0.0373288	1.988811329	8.82535	10.8141616
TAN3OCT1	333	15	1.2585	1.3485	0.09	1.3438	0.0853	1.3659	0.1074	1.3592	0.1007	0.14874	0.34188	0.49062	210	135	25	36975	0.0551469	0%	0.0551469	2.697159768	6.199442	8.896601622
TAN4OCT1	345	15	1.2662	1.3537	0.0875	1.3494	0.0832	1.3784	0.1122	1.3719	0.1057	0.1495	0.5175	0.667	140	85	35	19775	0.0310669	0%	0.0310669	4.812195617	16.6576	21.46979583
TAN5OCT1	320	15	1.2608	1.3489	0.0881	1.3445	0.0837	1.3683	0.1075	1.3639	0.1031	0.093867	0.413867	0.507733	150	110	90	39900	0.0592419	40%	0.0355451	2.640773582	11.64341	14.28418437
PET1OCT14	373	15	1.2758	1.3643	0.0885	1.3608	0.085	1.4496	0.1738	1.438	0.1622	0.288453	1.919707	2.20816	175	75	55	26875	0.0410069	40%	0.0246041	11.72377223	78.02373	89.74749778
PET2OCT14	353	15	1.279	1.3659	0.0869	1.3618	0.0828	1.401	0.122	1.3953	0.1163	0.13414	0.788367	0.922507	180	90	55	31050	0.0468519	50%	0.023426	5.726128503	33.65356	39.37969076
PET3OCT14	491	15	1.2534	1.3415	0.0881	1.3375	0.0841	1.4289	0.1755	1.4118	0.1584	0.55974	2.432087	2.991827	175	75	60	28125	0.0427569	50%	0.0213785	26.18244073	113.7635	139.9459113
PET4OCT14	505	15	1.2603	1.3483	0.088	1.3447	0.0844	1.3772	0.1169	1.3686	0.1083	0.289533	0.804633	1.094167	170	100	55	31850	0.0479719	10%	0.0431747	6.706086349	18.63668	25.34276818
PET5OCT14	482	15	1.2525	1.3414	0.0889	1.3381	0.0856	1.361	0.1085	1.3552	0.1027	0.186373	0.54948	0.735853	160	55	30	15250	0.0247319	50%	0.012366	15.07149336	44.43492	59.50641344
YO1OCT14	450	15	1.2582	1.3472	0.089	1.3434	0.0852	1.3527	0.0945	1.3616	0.1034	-0.267	0.546	0.279	170	120	35	30550	0.0461519	0%	0.0461519	dry<ash	11.8305	6.045254908
YO2OCT14	464	15	1.2572	1.3468	0.0896	1.3484	0.0912	1.366	0.1088	1.357	0.0998	0.2784	0.266027	0.544427	160	75	50	23750	0.0366319	10%	0.0329687	8.444370435	8.069065	16.51343552
YO3OCT14	513	15	1.2586	1.3473	0.0887	1.3441	0.0855	1.4158	0.1572	1.3492	0.0906	2.27772	0.17442	2.45214	165	130	90	48000	0.0705819	30%	0.0494073	46.10085184	3.530245	49.63109725
YO4OCT14	350	15	1.2684	1.3549	0.0865	1.3538	0.0854	1.3653	0.0969	1.3587	0.0903	0.154	0.114333	0.268333	140	100	70	30800	0.0465019	40%	0.0279011	5.519487734	4.097801	9.617289234
YO5OCT14	345	15	1.2666	1.3553	0.0887	1.3518	0.0852	1.3751	0.1085	1.3785	0.1119	-0.0782	0.6141	0.5359	180	85	75	35175	0.0526269	10%	0.0473642	dry<ash	12.96549	11.3144503
DRA1OCT1	370	15	1.265	1.3541	0.0891	1.3536	0.0886	1.354	0.089	1.4082	0.1432	-1.33693	1.3468	0.009867	180	180	35	45000	0.0663819	30%	0.0464673	dry<ash	28.9838	0.212335563
DRA2OCT1	378	15	1.2622	1.3523	0.0901	1.352	0.0898	1.4335	0.1713	1.422	0.1598	0.2898	1.764	2.0538	135	135	50	31725	0.0477969	40%	0.0286781	10.10525787	61.51027	71.61552318
DRA3OCT1	405	15	1.2527	1.3421	0.0894	1.3415	0.0888	1.4144	0.1617	1.4045	0.1518	0.2673	1.701	1.9683	180	145	45	40725	0.0603969	50%	0.0301985	8.851447674	56.32739	65.17884196
DRA4OCT1	475	15	1.2525	1.3412	0.0887	1.3403	0.0878	1.4149	0.1624	1.4042	0.1517	0.338833	2.0235	2.362333	215	125	50	43875	0.0648069	10%	0.0583262	5.809280825	34.69281	40.50208874
DRA5OCT1	472	15	1.2516	1.3415	0.0899	1.3405	0.0889	1.4363	0.1847	1.4225	0.1709	0.43424	2.580267	3.014507	160	100	60	31600	0.0476219	0%	0.0476219	9.118493802	54.18235	63.30084828

			Pre-heat bare weight(g)			ost heat bare weight(g		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	Total matter
Sample	Tot vol	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filter	Filter	AFDM (g)	Inorganic(	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1NOV14	474	15	1.2638	1.3523	0.0885	1.3523	0.0885	1.4018	0.138	1.3921	0.1283	0.30652	1.25768	1.5642	180	110	45	32850	0.0493719	20%	0.0394975	7.760487	31.842	39.60248643
ED2NOV14	201	15	1.2638	1.3515	0.0877	1.3516	0.0878	1.4779	0.2141	1.4426	0.1788	0.47302	1.2194	1.69242	190	85	25	23025	0.0356169	50%	0.0178085	26.56155	68.47311	95.03466051
ED3NOV14	265	15	1.2507	1.3386	0.0879	1.3383	0.0876	1.4323	0.1816	1.4139	0.1632	0.325067	1.3356	1.660667	225	160	80	66800	0.0969019	50%	0.048451	6.709191	27.56602	34.27521373
ED4NOV14	248	15	1.253	1.6424	0.3894	1.3421	0.0891	1.4112	0.1582	1.396	0.143	0.251307	0.891147	1.142453	170	110	45	31300	0.0472019	40%	0.0283211	8.873466	31.46578	40.33924246
ED5NOV14	171	15	1.2643	1.3542	0.0899	1.3539	0.0896	1.3602	0.0959	1.3572	0.0929	0.0342	0.03762	0.07182	275	150	70	71000	0.1027819	0%	0.1027819	0.332743	0.366018	0.698761163
TAN1NOV	300	15	1.2624	1.3523	0.0899	1.352	0.0896	1.3696	0.1072	1.3636	0.1012	0.12	0.232	0.352	165	110	55	33275	0.0499669	0%	0.0499669	2.40159	4.643074	7.044663567
TAN2NOV	283	15	1.2699	1.3576	0.0877	1.3569	0.087	1.378	0.1081	1.3722	0.1023	0.109427	0.28866	0.398087	190	140	110	62900	0.0914419	0%	0.0914419	1.19668	3.156759	4.353438267
TAN3NOV	405	15	1.2545	1.344	0.0895	1.3438	0.0893	1.4127	0.1582	1.3921	0.1376	0.5562	1.3041	1.8603	140	85	65	26525	0.0405169	40%	0.0243101	22.87934	53.64428	76.52362348
TAN4NOV	291	15	1.2621	1.3517	0.0896	1.3555	0.0934	1.4462	0.1841	1.4172	0.1551	0.5626	1.19698	1.75958	185	90	55	31775	0.0478669	30%	0.0335068	16.79061	35.72346	52.51406952
TAN5NOV	341	15	1.2574	1.3447	0.0873	1.345	0.0876	1.6091	0.3517	1.5815	0.3241	0.62744	5.376433	6.003873	135	100	60	27600	0.0420219	10%	0.0378197	16.59029	142.1596	158.7498512
PET1NOV1	466	15	1.2731	1.361	0.0879	1.3603	0.0872	1.4128	0.1397	1.3948	0.1217	0.5592	1.0718	1.631	230	160	65	62150	0.0903919	40%	0.0542351	10.31066	19.7621	30.07275357
PET2NOV1	253	15	1.2749	1.364	0.0891	1.3704	0.0955	1.5581	0.2832	1.5268	0.2519	0.527927	2.637947	3.165873	190	150	110	65900	0.0956419	50%	0.047821	11.03965	55.16299	66.2026441
PET3NOV1	464	15	1.2507	1.3392	0.0885	1.338	0.0873	1.3955	0.1448	1.3855	0.1348	0.309333	1.469333	1.778667	240	150	55	57450	0.0838119	60%	0.0335248	9.227011	43.8283	53.05531394
PET4NOV1	503	15	1.2566	1.3462	0.0896	1.3458	0.0892	1.5663	0.3097	1.5359	0.2793	1.019413	6.374687	7.3941	250	100	85	54750	0.0800319	0%	0.0800319	12.73759	79.65182	92.38940972
PET5NOV1	450	15	1.2487	1.3378	0.0891	1.337	0.0883	1.3645	0.1158	1.3583	0.1096	0.186	0.639	0.825	125	115	25	20375	0.0319069	0%	0.0319069	5.82946	20.02702	25.85647619
YO1NOV14	550	15	1.2633	1.3514	0.0881	1.3418	0.0785	1.3841	0.1208	1.3799	0.1166	0.154	1.397	1.551	138	115	110	43700	0.0645619	70%	0.0193686	7.951026	72.12716	80.07818853
YO2NOV14	402	15	1.2539	1.3438	0.0899	1.3434	0.0895	1.3701	0.1162	1.363	0.1091	0.19028	0.52528	0.71556	200	150	50	47500	0.0698819	10%	0.0628937	3.025422	8.351869	11.37729035
YO3NOV14	385	15	1.2563	1.3428	0.0865	1.3416	0.0853	1.3749	0.1186	1.3655	0.1092	0.241267	0.613433	0.8547	180	100	80	40400	0.0599419	30%	0.0419593	5.750012	14.61971	20.36972468
YO4NOV14	389	15	1.2657	1.3525	0.0868	1.3519	0.0862	1.3787	0.113	1.3714	0.1057	0.189313	0.5057	0.695013	160	120	50	33200	0.0498619	40%	0.0299171	6.327922	16.90335	23.2312759
YO5NOV14	526	15	1.2744	1.3618	0.0874	1.3507	0.0763	1.373	0.0986	1.3683	0.0939	0.164813	0.617173	0.781987	230	120	70	52100	0.0763219	20%	0.0610575	2.699313	10.10806	12.80737683
DRA1NOV	451	15	1.2654	1.3559	0.0905	1.3554	0.09	1.4358	0.1704	1.422	0.1566	0.41492	2.00244	2.41736	150	145	85	46825	0.0689369	50%	0.0344685	12.03768	58.09487	70.1325415
DRA2NOV	471	15	1.2626	1.3494	0.0868	1.349	0.0864	1.3938	0.1312	1.3822	0.1196	0.36424	1.04248	1.40672	145	105	70	32725	0.0491969	30%	0.0344378	10.57674	30.27136	40.84810222
DRA3NOV	455	15	1.254	1.3425	0.0885	1.3408	0.0868	1.3832	0.1292	1.3741	0.1201	0.276033	1.0101	1.286133	140	100	50	26000	0.0397819	0%	0.0397819	6.938666	25.39094	32.32961053
DRA4NOV	425	15	1.2544	1.3406	0.0862	1.3415	0.0871	1.4143	0.1599	1.3952	0.1408	0.541167	1.5215	2.062667	175	125	35	32375	0.0487069	10%	0.0438362	12.3452	34.70875	47.05394619
DRA5NOV	450	15	1.2519	1.3412	0.0893	1.3403	0.0884	1.4226	0.1707	1.4027	0.1508	0.597	1.872	2.469	260	80	75	46300	0.0682019	20%	0.0545615	10.94178	34.30989	45.25167187

			Pre-heat bare weight(g)			ost heat bare weight(g		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol Tot	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filter	Filter	AFDM (g)	Inorganic(	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1DEC14	461	15	1.2635	1.3508	0.0873	1.3504	0.0869	1.3878	0.1243	1.3808	0.1173	0.215133	0.934293	1.149427	280	120	105	75600	0.1092219	0%	0.1092219	1.96969	8.554084	10.52377
ED2DEC14	421	15	1.264	1.3525	0.0885	1.3519	0.0879	1.4003	0.1363	1.3916	0.1276	0.24418	1.114247	1.358427	140	100	20	18800	0.0297019	30%	0.0207913	11.74432	53.59189	65.33621
ED3DEC14	501	15	1.2509	1.3395	0.0886	1.3396	0.0887	1.3854	0.1345	1.3784	0.1275	0.2338	1.29592	1.52972	150	70	55	22600	0.0350219	10%	0.0315197	7.417581	41.11459	48.53217
ED4DEC14	415	15	1.2536	1.3431	0.0895	1.3448	0.0912	1.3601	0.1065	1.356	0.1024	0.113433	0.309867	0.4233	205	90	65	37625	0.0560569	50%	0.0280285	4.047078	11.05543	15.10251
ED5DEC14	550	15	1.265	1.3533	0.0883	1.3524	0.0874	1.3833	0.1183	1.3747	0.1097	0.315333	0.817667	1.133	180	125	70	43850	0.0647719	20%	0.0518175	6.085458	15.77973	21.86519
TAN1DEC14	339	15	1.2634	1.4419	0.1785	1.4401	0.1767	1.4735	0.2101	1.4657	0.2023	0.17628	0.57856	0.75484	110	85	40	17150	0.0273919	30%	0.0191743	9.193542	30.17367	39.36722
TAN2DEC14	468	15	1.27	1.358	0.088	1.3571	0.0871	1.4134	0.1434	1.3984	0.1284	0.468	1.28856	1.75656	230	140	85	63650	0.0924919	50%	0.046246	10.11981	27.8632	37.983
TAN3DEC14	477	15	1.255	1.3447	0.0897	1.3437	0.0887	1.3985	0.1435	1.3826	0.1276	0.50562	1.23702	1.74264	270	170	35	61300	0.0892019	60%	0.0356808	14.17066	34.6691	48.83977
TAN4DEC14	161	15	1.2627	1.3516	0.0889	1.3506	0.0879	1.3884	0.1257	1.3816	0.1189	0.072987	0.332733	0.40572	145	120	20	22700	0.0351619	10%	0.0316457	2.306368	10.51433	12.8207
TAN5DEC14	365	15	1.258	1.3471	0.0891	1.3478	0.0898	1.4003	0.1423	1.3881	0.1301	0.296867	0.980633	1.2775	100	90	65	21350	0.0332719	0%	0.0332719	8.922444	29.47332	38.39576
PET1DEC14	496	15	1.2728	1.3634	0.0906	1.3622	0.0894	1.3858	0.113	1.38	0.1072	0.191787	0.588587	0.780373	205	100	45	34225	0.0512969	0%	0.0512969	3.738757	11.47412	15.21288
PET2DEC14	303	15	1.2762	1.3638	0.0876	1.3626	0.0864	1.3865	0.1103	1.3731	0.0969	0.27068	0.2121	0.48278	110	100	40	19400	0.0305419	10%	0.0274877	9.84731	7.716176	17.56349
PET3DEC14	384	15	1.2511	1.3392	0.0881	1.3525	0.1014	1.4483	0.1972	1.431	0.1799	0.44288	2.0096	2.45248	175	155	80	53525	0.0783169	30%	0.0548218	8.078534	36.65693	44.73546
PET4DEC14	403	15	1.2581	1.3464	0.0883	1.3463	0.0882	1.4126	0.1545	1.4004	0.1423	0.327773	1.453487	1.78126	110	65	35	13275	0.0219669	40%	0.0131801	24.86873	110.2785	135.1473
PET5DEC14	340	15	1.2497	1.3373	0.0876	1.3359	0.0862	1.371	0.1213	1.3645	0.1148	0.147333	0.648267	0.7956	120	85	50	20450	0.0320119	20%	0.0256095	5.753069	25.3135	31.06657
YO1DEC14	350	15	1.2568	1.3449	0.0881	1.3415	0.0847	1.3712	0.1144	1.3628	0.106	0.196	0.497	0.693	190	120	100	53800	0.0787019	10%	0.0708317	2.767122	7.016631	9.783754
YO2DEC14	435	15	1.2542	1.3431	0.0889	1.3411	0.0869	1.3607	0.1065	1.3528	0.0986	0.2291	0.3393	0.5684	110	90	65	22900	0.0354419	5%	0.0336698	6.804316	10.07728	16.88159
YO3DEC14	462	15	1.2549	1.3453	0.0904	1.3463	0.0914	1.3609	0.106	1.354	0.0991	0.21252	0.23716	0.44968	130	90	60	24900	0.0382419	30%	0.0267693	7.938936	8.859392	16.79833
YO4DEC14	414	15	1.266	1.3547	0.0887	1.3551	0.0891	1.4	0.134	1.3866	0.1206	0.36984	0.8694	1.23924	160	100	75	35500	0.0530819	0%	0.0530819	6.967347	16.37846	23.34581
YO5DEC14	472	15	1.2655	1.3545	0.089	1.3529	0.0874	1.3981	0.1326	1.3865	0.121	0.365013	1.05728	1.422293	250	180	75	77250	0.1115319	40%	0.0669191	5.454543	15.79937	21.25391
DRA1DEC14	278	15	1.2657	1.3555	0.0898	1.3545	0.0888	1.4053	0.1396	1.3936	0.1279	0.21684	0.724653	0.941493	120	100	25	17500	0.0278819	20%	0.0223055	9.72136	32.48762	42.20898
DRA2DEC14	466	15	1.2629	1.3526	0.0897	1.3495	0.0866	1.4369	0.174	1.4204	0.1575	0.5126	2.202627	2.715227	155	145	45	35975	0.0537469	50%	0.0268735	19.07459	81.96293	101.0375
DRA3DEC14	508	15	1.2533	1.341	0.0877	1.3429	0.0896	1.5123	0.259	1.5004	0.2471	0.403013	5.334	5.737013	145	125	30	26225	0.0400969	40%	0.0240581	16.75164	221.7129	238.4645
DRA4DEC14	489	15	1.2533	1.3409	0.0876	1.3399	0.0866	1.3799	0.1266	1.3732	0.1199	0.21842	1.08558	1.304	210	170	165	98400	0.1411419	60%	0.0564568	3.868802	19.22852	23.09732
DRA5DEC14	375	15	1.2534	1.3413	0.0879	1.3401	0.0867	1.5275	0.2741	1.4968	0.2434	0.7675	3.9175	4.685	270	180	20	57600	0.0840219	70%	0.0252066	30.44841	155.4158	185.8642

			Pre-heat bare weight(g)			ost heat bare weight(		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol Tot	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filter	Filter	AFDM (g)	Inorganic(	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1JAN15	325	15	1.2633	1.3514	0.0881	1.3519	0.0886	1.4883	0.225	1.4745	0.2112	0.299	2.656333	2.955333	175	120	55	37225	0.0554969	30%	0.0388478	7.696698	68.37791	76.07461
ED2JAN15	445	15	1.2636	1.352	0.0884	1.352	0.0884	1.6949	0.4313	1.6589	0.3953	1.068	9.1047	10.1727	130	110	70	31100	0.0469219	40%	0.0281531	37.93538	323.3991	361.3345
ED3JAN15	420	15	1.2512	1.3403	0.0891	1.3398	0.0886	1.6795	0.4283	1.6442	0.393	0.9884	8.5232	9.5116	140	100	70	30800	0.0465019	10%	0.0418517	23.61672	203.6524	227.2691
ED4JAN15	400	15	1.2544	1.3441	0.0897	1.3428	0.0884	1.417	0.1626	1.4017	0.1473	0.408	1.570667	1.978667	155	110	40	27650	0.0420919	20%	0.0336735	12.11635	46.64397	58.76032
ED5JAN15	300	15	1.2647	1.354	0.0893	1.3534	0.0887	1.4102	0.1455	1.3949	0.1302	0.306	0.83	1.136	125	85	80	27425	0.0417769	30%	0.0292438	10.46375	28.38206	38.8458
TAN1JAN1	275	15	1.2617	1.3508	0.0891	1.3506	0.0889	1.4722	0.2105	1.4523	0.1906	0.364833	1.8645	2.229333	210	105	35	33075	0.0496869	20%	0.0397495	9.178308	46.90623	56.08453
TAN2JAN1	312	15	1.2709	1.3574	0.0865	1.3574	0.0865	1.4372	0.1663	1.4162	0.1453	0.4368	1.22304	1.65984	135	75	40	18525	0.0293169	40%	0.0175901	24.83209	69.52986	94.36196
TAN3JAN1	350	15	1.2577	1.3462	0.0885	1.343	0.0853	1.4536	0.1959	1.433	0.1753	0.480667	2.1	2.580667	150	105	20	20850	0.0325719	10%	0.0293147	16.39677	71.63639	88.03316
TAN4JAN1	300	15	1.2621	1.3515	0.0894	1.3506	0.0885	1.4451	0.183	1.4306	0.1685	0.29	1.6	1.89	205	150	25	39625	0.0588569	20%	0.0470855	6.159006	33.98072	40.13973
TAN5JAN1	453	15	1.2581	1.345	0.0869	1.3445	0.0864	1.5338	0.2757	1.5077	0.2496	0.78822	4.92864	5.71686	145	100	80	34100	0.0511219	30%	0.0357853	22.02634	137.7279	159.7543
PET1JAN15	350	15	1.2735	1.36	0.0865	1.3594	0.0859	1.578	0.3045	1.5516	0.2781	0.616	4.484667	5.100667	120	125	55	28475	0.0432469	40%	0.0259481	23.73966	172.8319	196.5716
PET2JAN15	445	15	1.2752	1.3627	0.0875	1.3631	0.0879	1.7801	0.5049	1.7376	0.4624	1.260833	11.11017	12.371	180	135	110	58950	0.0859119	50%	0.042956	29.35177	258.6409	287.9927
PET3JAN15	375	15	1.2509	1.3387	0.0878	1.3388	0.0879	1.5395	0.2886	1.5112	0.2603	0.7075	4.31	5.0175	155	100	65	32075	0.0482869	0%	0.0482869	14.65201	89.25816	103.9102
PET4JAN15	345	15	1.258	1.3455	0.0875	1.3448	0.0868	1.4593	0.2013	1.4362	0.1782	0.5313	2.1022	2.6335	125	120	95	38275	0.0569669	10%	0.0512702	10.36274	41.00237	51.36511
PET5JAN15	350	15	1.249	1.338	0.089	1.3456	0.0966	1.4398	0.1908	1.4188	0.1698	0.49	1.708	2.198	220	175	60	62200	0.0904619	30%	0.0633233	7.738064	26.97268	34.71075
YO1JAN15	370	15	1.2547	1.3433	0.0886	1.3426	0.0879	1.462	0.2073	1.4311	0.1764	0.7622	2.183	2.9452	160	135	65	40775	0.0604669	10%	0.0544202	14.00583	40.11377	54.1196
YO2JAN15	435	15	1.2538	1.3426	0.0888	1.3423	0.0885	1.42	0.1662	1.4021	0.1483	0.5191	1.7342	2.2533	185	110	65	39525	0.0587169	0%	0.0587169	8.840726	29.53494	38.37566
YO3JAN15	355	15	1.2557	1.3437	0.088	1.3425	0.0868	1.4012	0.1455	1.3841	0.1284	0.4047	0.984533	1.389233	125	95	40	20675	0.0323269	40%	0.0193961	20.86498	50.75924	71.62422
YO4JAN15	361	15	1.2666	1.354	0.0874	1.3519	0.0853	1.4126	0.146	1.3973	0.1307	0.36822	1.092627	1.460847	195	130	45	39975	0.0593469	0%	0.0593469	6.204536	18.41085	24.61538
YO5JAN15	448	15	1.2636	1.3544	0.0908	1.354	0.0904	1.3974	0.1338	1.384	0.1204	0.400213	0.896	1.296213	175	100	60	34000	0.0509819	0%	0.0509819	7.850106	17.57486	25.42497
DRA1JAN1	403	15	1.256	1.3522	0.0962	1.352	0.096	1.5074	0.2514	1.4848	0.2288	0.607187	3.567893	4.17508	185	70	60	28250	0.0429319	0%	0.0429319	14.14302	83.10588	97.2489
DRA2JAN1	430	15	1.2636	1.3496	0.086	1.349	0.0854	1.6701	0.4065	1.6481	0.3845	0.630667	8.5742	9.204867	185	95	60	34375	0.0515069	10%	0.0463562	13.60479	184.9634	198.5681
DRA3JAN1	535	15	1.2531	1.3417	0.0886	1.3411	0.088	1.6015	0.3484	1.5794	0.3263	0.788233	8.499367	9.2876	140	120	55	31100	0.0469219	0%	0.0469219	16.79884	181.1386	197.9374
DRA4JAN1	325	15	1.2534	1.3415	0.0881	1.341	0.0876	1.4266	0.1732	1.4125	0.1591	0.3055	1.549167	1.854667	135	110	70	32000	0.0481819	0%	0.0481819	6.340555	32.15246	38.49302
DRA5JAN1	355	15	1.253	1.3411	0.0881	1.3403	0.0873	1.4589	0.2059	1.4274	0.1744	0.7455	2.061367	2.806867	230	145	75	61475	0.0894469	20%	0.0715575	10.41819	28.80713	39.22532

			Pre-heat bare weight(g)			ost heat bare weight(g		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol Tot	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filte	Filter	AFDM (g)	Inorganic(g)	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1FEB15	324	15	1.275	1.3633	0.0883	1.3591	0.0841	1.4456	0.1706	1.4373	0.1623	0.17928	1.68912	1.8684	240	175	35	56525	0.0825169	30%	0.0577618	3.10378	29.24284	32.34662
ED2FEB15	355	15	1.2547	1.3439	0.0892	1.3394	0.0847	1.5841	0.3294	1.5662	0.3115	0.423633333	5.3676	5.791233333	190	110	95	49400	0.0725419	0%	0.0725419	5.839843	73.9931	79.83294
ED3FEB15	250	15	1.2664	1.3535	0.0871	1.3503	0.0839	1.721	0.4546	1.7077	0.4413	0.221666667	5.956666667	6.178333333	175	105	90	43575	0.0643869	20%	0.0515095	4.303412	115.6421	119.9455
ED4FEB15	250	15	1.2623	1.3514	0.0891	1.347	0.0847	1.3969	0.1346	1.3905	0.1282	0.106666667	0.725	0.831666667	135	90	35	20025	0.0314169	0%	0.0314169	3.3952	23.07675	26.47195
ED5FEB15	329	15	1.2677	1.3578	0.0901	1.3529	0.0852	1.4954	0.2277	1.4877	0.22	0.168886667	2.956613333	3.1255	170	155	90	55600	0.0812219	10%	0.0730997	2.31036	40.44631	42.75667
TAN1FEB1	150	15	1.269	1.3584	0.0894	1.3541	0.0851	1.5675	0.2985	1.5607	0.2917	0.068	2.066	2.134	155	130	25	27275	0.0415669	40%	0.0249401	2.726528	82.83835	85.56488
TAN2FEB1	200	15	1.274	1.3612	0.0872	1.3578	0.0838	1.4276	0.1536	1.4231	0.1491	0.06	0.870666667	0.930666667	150	75	40	20250	0.0317319	40%	0.0190391	3.151403	45.73036	48.88176
TAN3FEB1	370	15	1.2599	1.3484	0.0885	1.3443	0.0844	1.4675	0.2076	1.4628	0.2029	0.115933333	2.923	3.038933333	155	110	50	30300	0.0458019	60%	0.0183208	6.327976	159.5458	165.8738
TAN4FEB1	305	15	1.2558	1.3433	0.0875	1.3386	0.0828	1.3712	0.1154	1.3675	0.1117	0.075233333	0.587633333	0.662866667	140	100	10	16400	0.0263419	80%	0.0052684	14.28016	111.5397	125.8198
TAN5FEB1	353	15	1.2589	1.3478	0.0889	1.3442	0.0853	1.4332	0.1743	1.4248	0.1659	0.19768	1.896786667	2.094466667	115	85	25	14775	0.0240669	30%	0.0168468	11.73396	112.5901	124.3241
PET1FEB1	229	15	1.2683	1.357	0.0887	1.3533	0.085	1.4847	0.2164	1.4763	0.208	0.12824	1.8778	2.00604	155	140	70	42350	0.0626719	0%	0.0626719	2.046212	29.96239	32.0086
PET2FEB1	348	15	1.2812	1.3684	0.0872	1.364	0.0828	1.6595	0.3783	1.6439	0.3627	0.36192	6.49368	6.8556	120	85	50	20450	0.0320119	10%	0.0288107	12.562	225.3912	237.9532
PET3FEB1	250	15	1.2783	1.3671	0.0888	1.3634	0.0851	1.4555	0.1772	1.4484	0.1701	0.118333333	1.416666667	1.535	240	175	45	60675	0.0883269	0%	0.0883269	1.33972	16.0389	17.37862
PET4FEB1	250	15	1.2844	1.371	0.0866	1.3671	0.0827	1.4606	0.1762	1.4551	0.1707	0.091666667	1.466666667	1.558333333	125	80	60	22300	0.0346019	0%	0.0346019	2.64918	42.38688	45.03606
PET5FEB1	300	15	1.28	1.3685	0.0885	1.3648	0.0848	1.8421	0.5621	1.8242	0.5442	0.358	9.188	9.546	145	110	55	29975	0.0453469	10%	0.0408122	8.771885	225.1287	233.9006
YO1FEB15	278	15	1.2691	1.3574	0.0883	1.3536	0.0845	1.3898	0.1207	1.3972	0.1281	-0.13714667	0.808053333	0.670906667	190	80	60	31400	0.0473419	0%	0.0473419	dry<ash	17.06846	14.17152
YO2FEB15	350	15	1.2584	1.3474	0.089	1.3435	0.0851	1.3889	0.1305	1.3855	0.1271	0.079333333	0.98	1.059333333	205	155	70	56975	0.0831469	40%	0.0498881	1.590224	19.64395	21.23417
YO3FEB15	276	15	1.2663	1.3535	0.0872	1.3494	0.0831	1.4032	0.1369	1.3977	0.1314	0.1012	0.88872	0.98992	130	85	60	23950	0.0369119	0%	0.0369119	2.741663	24.07679	26.81845
YO4FEB15	450	15	1.272	1.3606	0.0886	1.357	0.085	1.4582	0.1862	1.453	0.181	0.156	2.88	3.036	110	95	80	26850	0.0409719	0%	0.0409719	3.807488	70.29208	74.09957
YO5FEB15	300	15	1.2623	1.3503	0.088	1.3442	0.0819	1.4271	0.1648	1.4184	0.1561	0.174	1.484	1.658	135	125	50	29875	0.0452069	0%	0.0452069	3.84897	32.82685	36.67582
DRA1FEB1	248	15	1.2526	1.3407	0.0881	1.335	0.0824	1.4895	0.2369	1.4921	0.2395	-0.04298667	2.597386667	2.5544	120	60	45	15300	0.0248019	50%	0.012401	dry<ash	209.4506	205.9842
DRA2FEB1	361	15	1.2677	1.3561	0.0884	1.3533	0.0856	1.5984	0.3307	1.5902	0.3225	0.197346667	5.701393333	5.89874	160	130	65	39650	0.0588919	40%	0.0353351	5.584997	161.3519	166.9369
DRA3FEB1	273	15	1.2619	1.3504	0.0885	1.3462	0.0843	1.4441	0.1822	1.4407	0.1788	0.06188	1.7199	1.78178	225	85	55	36175	0.0540269	30%	0.0378188	1.636222	45.47735	47.11357
DRA4FEB1	250	15	1.2525	1.3423	0.0898	1.3406	0.0881	2.003	0.7505	1.988	0.7355	0.25	10.79	11.04	205	160	165	93025	0.1336169	50%	0.0668085	3.742042	161.5065	165.2486
DRA5FEB1	380	15	1.2662	1.3554	0.0892	1.3519	0.0857	1.5902	0.324	1.5805	0.3143	0.245733333	5.7912	6.036933333	165	125	90	46725	0.0687969	60%	0.0275188	8.929666	210.4455	219.3752

			Pre-heat bare weight(g)			ost heat bare weight(		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol TOT	Filtered	Foil	Foil+Filter	Filter	Foil+Filte	Filter	Foil+Filte	Filter	Foil+ Filte	Filter	AFDM (g)	Inorganic(g)	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1MAR1	287	15	1.2741	1.362	0.0879	1.3405	0.0664	1.4301	0.156	1.4147	0.1406	0.294653333	1.419693333	1.714346667	140	100	85	34400	0.0515419	50%	0.025771	0.663159	55.0889	66.52245
ED2MEDR	300	15	1.2537	1.3411	0.0874	1.339	0.0853	1.5375	0.2838	1.5123	0.2586	0.504	3.466	3.97	195	75	70	33525	0.0503169	30%	0.0352218	dry<ash	98.40488	112.7142
ED3MAR1	356	15	1.2633	1.3519	0.0886	1.3521	0.0888	1.5217	0.2584	1.4809	0.2176	0.96832	3.056853333	4.025173333	175	160	50	44750	0.0660319	50%	0.033016	1.683097	92.58717	121.916
ED4MAR1	471	15	1.2598	1.3484	0.0886	1.348	0.0882	1.8165	0.5567	1.7701	0.5103	1.45696	13.25394	14.7109	170	100	40	27800	0.0423019	30%	0.0296113	0.722801	447.5969	496.7997
ED5MAR1	352	15	1.2647	1.3534	0.0887	1.3536	0.0889	1.44385	0.17915	1.4306	0.1659	0.310933333	1.806933333	2.117866667	185	145	30	36725	0.0547969	10%	0.0493172	dry<ash	36.639	42.94376
TAN1MAR	450	15	1.268	1.3549	0.0869	1.3552	0.0872	1.4762	0.2082	1.4472	0.1792	0.87	2.76	3.63	175	80	35	22925	0.0354769	30%	0.0248338	0.257952	111.1387	146.1716
TAN2MAR	376	15	1.2726	1.3596	0.087	1.3598	0.0872	1.4621	0.1895	1.4403	0.1677	0.546453333	2.017866667	2.56432	135	110	40	24650	0.0378919	20%	0.0303135	0.615289	66.56656	84.59328
TAN3MAR	300	15	1.2579	1.3442	0.0863	1.3431	0.0852	1.4095	0.1516	1.3912	0.1333	0.366	0.962	1.328	160	100	40	26400	0.0403419	20%	0.0322735	1.253647	29.80772	41.14829
TAN4MAR	322	15	1.255	1.3414	0.0864	1.3401	0.0851	1.362	0.107	1.358	0.103	0.085866667	0.384253333	0.47012	185	95	30	25975	0.0397469	10%	0.0357722	2.557886	10.74167	13.14205
TAN5MAR	400	15	1.2576	1.3439	0.0863	1.3433	0.0857	1.4003	0.1427	1.388	0.1304	0.328	1.192	1.52	150	85	30	19800	0.0311019	40%	0.0186611	dry<ash	63.87605	81.45269
PET1MAR1	193	15	1.2661	1.3538	0.0877	1.3535	0.0874	1.4376	0.1715	1.4176	0.1515	0.257333333	0.824753333	1.082086667	160	85	20	18500	0.0292819	30%	0.0204973	3.825461	40.23711	52.79159
PET2MAR1	474	15	1.2792	1.3675	0.0883	1.3665	0.0873	1.4424	0.1632	1.4279	0.1487	0.4582	1.94024	2.39844	130	75	40	17950	0.0285119	0%	0.0285119	4.331397	68.05018	84.12067
PET3MAR1	414	15	1.2764	1.3637	0.0873	1.3626	0.0862	1.7066	0.4302	1.6791	0.4027	0.759	8.7354	9.4944	210	95	40	32150	0.0483919	40%	0.0290351	1.576425	300.8561	326.9969
PET4MAR1	450	15	1.2819	1.3712	0.0893	1.3701	0.0882	1.4947	0.2128	1.4799	0.198	0.444	3.294	3.738	185	110	75	42475	0.0628469	50%	0.0314235	2.334544	104.8262	118.9557
PET5MAR1	408	15	1.2804	1.3683	0.0879	1.3667	0.0863	1.6064	0.326	1.5883	0.3079	0.49232	6.02752	6.51984	190	140	40	39800	0.0591019	50%	0.029551	data missi	203.9704	220.6305
YO1MAR1	303	15	1.2671	1.355	0.0879	1.3545	0.0874	1.4249	0.1578	1.4101	0.143	0.29896	1.12312	1.42208	125	105	35	21175	0.0330269	0%	0.0330269	0.603042	34.00622	43.05823
YO2MAR1	400	15	1.2566	1.344	0.0874	1.3432	0.0866	1.3876	0.131	1.3839	0.1273	0.098666667	1.085333333	1.184	170	120	100	49400	0.0725419	40%	0.0435251	1.423351	24.93578	27.20267
YO3MAR1	350	15	1.2644	1.3539	0.0895	1.3536	0.0892	1.3912	0.1268	1.3793	0.1149	0.277666667	0.599666667	0.877333333	170	140	75	47050	0.0692519	20%	0.0554015	1.457292	10.82401	15.83591
YO4MAR1	403	15	1.27	1.3587	0.0887	1.3582	0.0882	1.4298	0.1598	1.4091	0.1391	0.55614	1.367513333	1.923653333	180	95	65	34975	0.0523469	60%	0.0209388	9.394983	65.31014	91.87045
YO5MAR1	400	15	1.258	1.349	0.091	1.3453	0.0873	1.3889	0.1309	1.3709	0.1129	0.48	0.682666667	1.162666667	135	105	95	36975	0.0551469	0%	0.0551469	1.093375	12.37906	21.08308
DRA1MAR	412	15	1.2571	1.3446	0.0875	1.3402	0.0831	1.4804	0.2233	1.4557	0.1986	0.678426667	3.1724	3.850826667	140	110	50	27900	0.0424419	0%	0.0424419	0.384073	74.74689	90.73172
DRA2MAR	509	15	1.2652	1.3527	0.0875	1.3538	0.0886	1.6631	0.3979	1.6424	0.3772	0.70242	9.79316	10.49558	160	135	30	30450	0.0460119	0%	0.0460119	0.586289	212.8397	228.1058
DRA3MAR	271	15	1.2607	1.348	0.0873	1.3473	0.0866	1.4711	0.2104	1.4522	0.1915	0.34146	1.895193333	2.236653333	180	105	20	24600	0.0378219	30%	0.0264753	dry<ash	71.58337	84.48066
DRA4MAR	400	15	1.2524	1.3392	0.0868	1.339	0.0866	1.5572	0.3048	1.5483	0.2959	0.237333333	5.581333333	5.818666667	160	100	45	27700	0.0421619	40%	0.0252971	1.13654	220.631	230.0128
DRA5MAR	279	15	1.2643	1.3534	0.0891	1.3527	0.0884	1.608	0.3437	1.5848	0.3205	0.43152	4.31706	4.74858	190	110	40	32900	0.0494419	40%	0.0296651	133.2568	145.5264	160.0727

			Pre-heat bare weight(g)			ost heat bare weight(		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol Tot	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filte	Filter	AFDM (g)	Inorganic(g)	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1APR15	200	15	1.2734	1.3613	0.0879	1.3604	0.087	1.6846	0.4112	1.6507	0.3773	0.452	3.870666667	4.322666667	230	150	75	63000	0.0915819	0%	0.0915819	4.935473	42.26454	47.20001
ED2APR15	425	15	1.252	1.3397	0.0877	1.3391	0.0871	1.7014	0.4494	1.6582	0.4062	1.224	9.041166667	10.26516667	140	120	95	41500	0.0614819	30%	0.0430373	28.44043	210.0773	238.5177
ED3APR15	362	15	1.2649	1.3528	0.0879	1.3526	0.0877	1.4282	0.1633	1.4083	0.1434	0.480253333	1.344226667	1.82448	185	105	85	44075	0.0650869	10%	0.0585782	8.198498	22.94755	31.14605
ED4APR15	305	15	1.2606	1.349	0.0884	1.3486	0.088	1.646	0.3854	1.5887	0.3281	1.1651	4.882033333	6.047133333	180	110	95	47350	0.0696719	30%	0.0487703	23.88952	100.1025	123.9921
ED5APR15	279	15	1.2649	1.353	0.0881	1.3569	0.092	1.3923	0.1274	1.3735	0.1086	0.34968	0.30876	0.65844	110	110	55	24200	0.0372619	10%	0.0335357	10.42709	9.206902	19.634
TAN1APR1	262	15	1.2688	1.3579	0.0891	1.3571	0.0883	1.4209	0.1521	1.4037	0.1349	0.300426667	0.813946667	1.114373333	160	125	85	44225	0.0652969	40%	0.0391781	7.668222	20.77553	28.44375
TAN2APR1	308	15	1.272	1.3608	0.0888	1.3601	0.0881	1.3795	0.1075	1.3739	0.1019	0.114986667	0.28336	0.398346667	200	130	60	45800	0.0675019	10%	0.0607517	1.892731	4.664231	6.556962
TAN3APR1	347	15	1.2578	1.3445	0.0867	1.3434	0.0856	1.4266	0.1688	1.4155	0.1577	0.25678	1.667913333	1.924693333	175	55	35	17675	0.0281269	10%	0.0253142	10.14371	65.88842	76.03213
TAN4APR1	420	15	1.2532	1.3413	0.0881	1.3414	0.0882	1.3938	0.1406	1.3774	0.1242	0.4592	1.008	1.4672	195	175	55	54475	0.0796469	20%	0.0637175	7.206809	15.81982	23.02663
TAN5APR1	369	15	1.2624	1.3505	0.0881	1.3435	0.0811	1.3736	0.1112	1.3673	0.1049	0.15498	0.58548	0.74046	165	60	85	29025	0.0440169	0%	0.0440169	3.52092	13.30125	16.82218
PET1APR15	425	15	1.266	1.355	0.089	1.3538	0.0878	1.696	0.43	1.6555	0.3995	1.1475	8.8315	9.695666667	185	120	45	35925	0.0536769	10%	0.0483092	23.75323	182.8119	200.7002
PET2APR15	503	15	1.279	1.3574	0.0784	1.3673	0.0883	1.5941	0.3151	1.5469	0.2798	1.582773333	6.421633333	7.60536	110	95	35	17625	0.0280569	10%	0.0252512	62.68109	254.3099	301.1879
PET3APR15	376	15	1.302	1.3906	0.0886	1.3664	0.0644	1.5752	0.2732	1.4889	0.1969	2.163253333	3.321333333	5.23392	170	130	55	38600	0.0574219	30%	0.0401953	53.81852	82.62983	130.2121
PET4APR15	450	15	1.2835	1.3709	0.0874	1.3691	0.0856	1.5309	0.2474	1.4998	0.2263	0.933	4.221	4.854	195	100	90	46050	0.0678519	0%	0.0678519	13.75054	62.20902	71.53816
PET5APR15	500	15	1.2804	1.368	0.0876	1.3664	0.086	1.5478	0.2674	1.5173	0.2468	1.016666667	5.36	6.046666667	155	85	65	28775	0.0436669	30%	0.0305668	33.26045	175.3535	197.8179
YO1APR15	357	15	1.2782	1.3653	0.0871	1.3548	0.0766	1.3972	0.119	1.3825	0.1043	0.34986	0.65926	1.00912	170	80	45	24850	0.0381719	20%	0.0305375	11.45673	21.58852	33.04525
YO2APR15	368	15	1.2558	1.3449	0.0891	1.3438	0.088	1.3852	0.1294	1.3722	0.1164	0.318933333	0.696746667	1.01568	195	90	35	27525	0.0419169	30%	0.0293418	10.86958	23.74585	34.61543
YO3APR15	371	15	1.2644	1.353	0.0886	1.3521	0.0877	1.3923	0.1279	1.3806	0.1162	0.28938	0.7049	0.99428	210	160	75	61350	0.0892719	10%	0.0803447	3.601731	8.773446	12.37518
YO4APR15	400	15	1.271	1.3598	0.0888	1.3586	0.0876	1.4429	0.1719	1.4215	0.1505	0.570666667	1.677333333	2.248	150	125	80	40750	0.0604319	15%	0.0513671	11.10957	32.65384	43.76341
YO5APR15	413	15	1.2581	1.3456	0.0875	1.3455	0.0874	1.4895	0.2314	1.4704	0.2123	0.525886667	3.438913333	3.9648	245	125	50	49125	0.0721569	40%	0.0432941	12.14683	79.43138	91.57821
DRA1APR1	411	15	1.2508	1.3384	0.0876	1.3376	0.0868	1.5292	0.2784	1.4929	0.2421	0.99462	4.25522	5.24984	160	140	90	49400	0.0725419	20%	0.0580335	17.13872	73.32349	90.4622
DRA2APR1	424	15	1.2675	1.355	0.0875	1.354	0.0865	1.5804	0.3129	1.5586	0.2911	0.616213333	5.78336	6.399573333	145	120	105	45225	0.0666969	40%	0.0400181	15.39835	144.5185	159.9168
DRA3APR1	368	15	1.2598	1.3474	0.0876	1.3468	0.087	1.5314	0.2716	1.4963	0.2365	0.86112	3.667733333	4.528853333	175	110	65	37775	0.0562669	40%	0.0337601	25.507	108.6409	134.1479
DRA4APR1	422	15	1.2516	1.3393	0.0877	1.3389	0.0873	2.0365	0.7849	2.0444	0.7928	-0.22225333	19.84806667	19.62581333	210	145	70	55300	0.0808019	50%	0.040401	dry<ash	491.2772	485.776
DRA5APR1	367	15	1.268	1.356	0.088	1.3548	0.0868	1.4672	0.1992	1.4523	0.1843	0.364553333	2.3855	2.750053333	220	110	90	53900	0.0788419	30%	0.0551893	6.605504	43.22393	49.82944

			Pre-heat bare weight(g)			ost heat bare weight(		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol Tot	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filte	Filter	AFDM (g)	Inorganic(g)	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1MAY15	250	15	1.2736	1.3652	0.0916	1.3669	0.0933	1.3937	0.1201	1.3868	0.1132	0.115	0.331666667	0.446666667	200	120	70	46400	0.0683419	20%	0.0546735	2.103395	6.066313	8.169708
ED2MAY15	450	15	1.2531	1.3444	0.0913	1.3434	0.0903	1.3621	0.109	1.3566	0.1035	0.165	0.396	0.561	185	95	85	41375	0.0613069	10%	0.0551762	2.990419	7.177006	10.16743
ED3MAY15	388	15	1.266	1.355	0.089	1.3561	0.0901	1.3728	0.1068	1.3732	0.1072	-0.01034667	0.44232	0.431973333	205	100	90	47950	0.0705119	30%	0.0493583	dry<ash	8.961405	8.751782
ED4MAY15	50											#DIV/0!	#DIV/0!	#DIV/0!	240	95	30	32850	0.0493719	30%	0.0345603	data missi	#DIV/0!	#DIV/0!
ED5MAY15	240	15	1.2654	1.3575	0.0921	1.3571	0.0917	1.3643	0.0989	1.3618	0.0964	0.04	0.0752	0.1152	125	110	85	33725	0.0505969	30%	0.0354178	1.129375	2.123224	3.252599
TAN1MAY	350	15	1.2895	1.3775	0.088	1.3578	0.0683	1.3641	0.0746	1.3627	0.0732	0.032666667	0.114333333	0.147	145	95	50	25775	0.0394669	10%	0.0355202	0.919664	3.218825	4.138489
TAN2MAY	321	15	1.273	1.362	0.089	1.3612	0.0882	1.4255	0.1525	1.4193	0.1463	0.13268	1.24334	1.37602	190	95	35	28025	0.0426169	30%	0.0298318	4.447598	41.6783	46.1259
TAN3MAY	450	15	1.2577	1.35	0.0923	1.3499	0.0922	1.3605	0.1028	1.3581	0.1004	0.072	0.246	0.318	190	125	25	31625	0.0476569	0%	0.0476569	1.510799	5.161897	6.672696
TAN4MAY	373	15	1.2548	1.3464	0.0916	1.3451	0.0903	1.3578	0.103	1.354	0.0992	0.094493333	0.221313333	0.315806667	230	80	45	32350	0.0486719	0%	0.0486719	1.941435	4.547045	6.48848
TAN5MAY	238	15	1.2578	1.3501	0.0923	1.3487	0.0909	1.3808	0.123	1.3731	0.1153	0.122173333	0.387146667	0.50932	220	135	25	38575	0.0573869	20%	0.0459095	2.661176	8.432819	11.094
PET1MAY1	458	15	1.2669	1.3593	0.0924	1.359	0.0921	1.4341	0.1672	1.421	0.1541	0.399986667	1.893066667	2.293053333	140	90	40	21800	0.0339019	10%	0.0305117	13.10928	62.04394	75.15322
PET2MAY1	386	15	1.2805	1.3731	0.0926	1.3725	0.092	1.4466	0.1661	1.4315	0.151	0.388573333	1.518266667	1.90684	185	125	50	38625	0.0574569	0%	0.0574569	6.762866	26.42444	33.18731
PET3MAY1	350	15	1.2778	1.3699	0.0921	1.3694	0.0916	1.5621	0.2843	1.5359	0.2581	0.611333333	3.885	4.496333333	210	105	65	42525	0.0629169	20%	0.0503335	12.14565	77.18514	89.33079
PET4MAY1	364	15	1.2832	1.3734	0.0902	1.3731	0.0899	1.4266	0.1434	1.4142	0.131	0.300906667	0.99736	1.298266667	150	75	70	27000	0.0411819	10%	0.0370637	8.118633	26.90934	35.02797
PET5MAY1	465	15	1.2809	1.3711	0.0902	1.3706	0.0897	1.5504	0.2695	1.5281	0.2472	0.6913	4.8825	5.5738	155	110	80	38250	0.0569319	0%	0.0569319	12.14258	85.76036	97.90293
YO1MAY15	374	15	1.2678	1.3556	0.0878	1.3555	0.0877	1.3881	0.1203	1.3762	0.1084	0.296706667	0.51612	0.812826667	150	105	75	34875	0.0522069	10%	0.0469862	6.314761	10.9845	17.29926
YO2MAY15	396	15	1.2564	1.3479	0.0915	1.3475	0.0911	1.381	0.1246	1.3726	0.1162	0.22176	0.66264	0.8844	170	120	70	40700	0.0603619	40%	0.0362171	6.123068	18.29631	24.41938
YO3MAY15	350	15	1.2653	1.357	0.0917	1.3565	0.0912	1.4537	0.1884	1.4327	0.1674	0.49	1.778	2.268	180	95	55	32225	0.0484969	0%	0.0484969	10.10374	36.66214	46.76588
YO4MAY15	424	15	1.271	1.3615	0.0905	1.3611	0.0901	1.4061	0.1351	1.3907	0.1197	0.435306667	0.836693333	1.272	205	105	80	46325	0.0682369	0%	0.0682369	6.379344	12.2616	18.64094
YO5MAY15	517	15	1.2591	1.3498	0.0907	1.3491	0.09	1.3884	0.1293	1.3782	0.1191	0.35156	1.00298	1.35454	130	130	105	44200	0.0652619	0%	0.0652619	5.38691	15.36854	20.75545
DRA1MAY	408	15	1.2504	1.3423	0.0919	1.3425	0.0921	1.509	0.2586	1.4937	0.2433	0.41616	4.11264	4.5288	165	85	75	32775	0.0492669	10%	0.0443402	9.385612	92.75193	102.1375
DRA2MAY	450	15	1.2686	1.3602	0.0916	1.3605	0.0919	1.7263	0.4577	1.7055	0.4369	0.624	10.35	10.974	175	100	45	29875	0.0452069	30%	0.0316448	19.71886	327.0676	346.7865
DRA3MAY	388	15	1.2607	1.3521	0.0914	1.3516	0.0909	1.434	0.1733	1.4264	0.1657	0.196586667	1.934826667	2.131413333	155	85	65	28775	0.0436669	20%	0.0349335	5.627451	55.38596	61.01341
DRA4MAY	298	15	1.2524	1.3455	0.0931	1.3451	0.0927	1.7258	0.4734	1.706	0.4536	0.39336	7.16988	7.56324	175	130	70	44100	0.0651219	30%	0.0455853	8.629092	157.2848	165.9139
DRA5MAY	448	15	1.2683	1.3612	0.0929	1.361	0.0927	1.4439	0.1756	1.4406	0.1723	0.09856	2.377386667	2.475946667	170	145	25	32525	0.0489169	10%	0.0440252	2.238717	54.00058	56.23929

			Pre-heat bare weight(g)			ost heat bare weight(		Dry weight(g)		Ash weight(g)				Total					Total		Realised	AFDM	Inorganic	otal matte
Sample	Vol Tot	Filtered	Foil	Foil+Filter	Filter	Foil+Filter	Filter	Foil+Filter	Filter	Foil+ Filte	Filter	AFDM (g)	Inorganic(g)	Matter (g)	X (m)	y (m)	Z (m)	xy*xz*yz	stone area	embedded	stone area	g.m2	g.m2	g.m2
ED1JUN15	370	15	1.274	1.3656	0.0916	1.3637	0.0897	1.3769	0.1029	1.3754	0.1014	0.037	0.2886	0.3256	210	95	90	47400	0.0697419	20%	0.0557935	0.663159	5.172644	5.835803
ED2JUN15	426	15	1.253	1.3429	0.0899	1.3406	0.0876	1.3463	0.0933	1.3465	0.0833	-0.00568	-0.12212	0.16188	170	70	60	26300	0.0402019	0%	0.0402019	dry<ash	-3.03767	4.026675
ED3JUN15	450	15	1.2739	1.3626	0.0887	1.3531	0.0792	1.3662	0.0923	1.3637	0.0898	0.075	0.318	0.393	155	110	60	32950	0.0495119	10%	0.0445607	1.683097	7.136332	8.819429
ED4JUN15	250	15	1.2614	1.34	0.0786	1.3492	0.0878	1.363	0.1016	1.3612	0.0998	0.03	0.2	0.23	165	110	45	30525	0.0461169	10%	0.0415052	0.722801	4.818672	5.541473
ED5JUN15	378	15	1.2644	1.3566	0.0922	1.3555	0.0911	1.3637	0.0993	1.3648	0.0972	-0.02772	0.15372	0.20664	125	100	45	22625	0.0350569	0%	0.0350569	dry<ash	4.384871	5.894417
TAN1JUN1	300	15	1.27	1.3627	0.0927	1.3805	0.1105	1.3804	0.1104	1.3799	0.1099	0.01	-0.012	-0.002	165	85	45	25275	0.0387669	0%	0.0387669	0.257952	-0.30954	-0.05159
TAN2JUN1	350	15	1.2711	1.3642	0.0931	1.3663	0.0952	1.3856	0.1145	1.385	0.1139	0.014	0.436333333	0.450333333	120	70	50	17900	0.0284419	20%	0.0227535	0.615289	19.17652	19.79181
TAN3JUN1	400	15	1.2573	1.3464	0.0891	1.3445	0.0872	1.3613	0.104	1.3598	0.1025	0.04	0.408	0.448	150	95	25	20375	0.0319069	0%	0.0319069	1.253647	12.7872	14.04085
TAN4JUN1	400	15	1.254	1.3395	0.0855	1.3436	0.0896	1.4131	0.1591	1.4094	0.1554	0.098666667	1.754666667	1.853333333	185	80	65	32025	0.0482169	20%	0.0385735	2.557886	45.48889	48.04678
TAN5JUN1	358	15	1.2567	1.345	0.0883	1.3473	0.0906	1.3867	0.13	1.4502	0.1279	-1.51553333	0.890226667	0.940346667	155	85	80	32375	0.0487069	30%	0.0340948	dry<ash	26.11031	27.58033
PET1JUN1	450	15	1.2711	1.3641	0.093	1.36	0.0889	1.4991	0.228	1.4923	0.2212	0.204	3.969	4.173	170	145	35	35675	0.0533269	0%	0.0533269	3.825461	74.42773	78.25319
PET2JUN1	455	15	1.2816	1.3685	0.0869	1.3679	0.0863	1.5132	0.2316	1.5076	0.226	0.169866667	4.237566667	4.407433333	230	70	55	32600	0.0490219	20%	0.0392175	4.331397	108.0529	112.3843
PET3JUN1	350	15	1.2776	1.3695	0.0919	1.3686	0.091	1.4685	0.1909	1.4652	0.1876	0.077	2.254	2.331	170	120	55	36350	0.0542719	10%	0.0488447	1.576425	46.14625	47.72267
PET4JUN1	360	15	1.2832	1.3745	0.0913	1.373	0.0898	1.5245	0.2413	1.5195	0.2363	0.12	3.516	3.636	175	90	70	34300	0.0514019	0%	0.0514019	2.334544	68.40214	70.73668
PET5JUN1	50	15	1.2873	1.3812	0.0939	1.3734	0.0861		-1.2873		-1.2873	0	-4.578	-4.578	160	95	80	35600	0.0532219	0%	0.0532219	data missi	-86.0172	-86.0172
YO1JUN15	407	15	1.2652	1.3559	0.0907	1.3562	0.091	1.3743	0.1091	1.3732	0.108	0.029846667	0.461266667	0.491113333	195	90	85	41775	0.0618669	20%	0.0494935	0.603042	9.319739	9.92278
YO2JUN15	400	15	1.2559	1.3438	0.0879	1.3431	0.0872	1.3555	0.0996	1.3539	0.098	0.042666667	0.288	0.330666667	135	85	45	21375	0.0333069	10%	0.0299762	1.423351	9.607619	11.03097
YO3JUN15	450	15	1.2666	1.3549	0.0883	1.3526	0.086	1.3844	0.1178	1.3819	0.1153	0.075	0.879	0.954	200	140	65	50100	0.0735219	30%	0.0514653	1.457292	17.07946	18.53675
YO4JUN15	450	15	1.2709	1.3593	0.0884	1.3585	0.0876	1.3912	0.1203	1.3881	0.1172	0.093	0.888	0.981	14	85	35	4655	0.0098989	0%	0.0098989	9.394983	89.70694	99.10192
YO5JUN15	478	15	1.2582	1.3491	0.0909	1.3483	0.0901	1.3701	0.1119	1.3684	0.1102	0.054173333	0.64052	0.694693333	145	95	80	32975	0.0495469	0%	0.0495469	1.093375	12.92755	14.02092
DRA1JUN1	200	15	1.2501	1.3414	0.0913	1.3414	0.0913	1.4936	0.2435	1.4922	0.2421	0.018666667	2.010666667	2.029333333	130	110	75	32300	0.0486019	0%	0.0486019	0.384073	41.37012	41.7542
DRA2JUN1	324	15	1.2686	1.3597	0.0911	1.3589	0.0903	1.4027	0.1341	1.4017	0.1331	0.0216	0.92448	0.94608	130	110	40	23900	0.0368419	0%	0.0368419	0.586289	25.09317	25.67946
DRA3JUN1	469	15	1.2619	1.3536	0.0917	1.352	0.0901	1.2915	0.0296	1.4886	0.0267	-6.16266	-1.98230667	-1.89163333	140	95	80	32100	0.0483219	0%	0.0483219	dry<ash	-41.0229	-39.1465
DRA4JUN1	400	15	1.2541	1.346	0.0919	1.344	0.0899	1.4826	0.2285	1.48	0.2259	0.069333333	3.626666667	3.696	220	100	75	46000	0.0677819	10%	0.0610037	1.136543	59.44994	60.58648
DRA5JUN1	350	15	1.2679	1.3599	0.092	1.3588	0.0909	1.634	0.3661	1.4727	0.3448	3.763666667	5.924333333	6.421333333	100	100	50	20000	0.0313819	10%	0.0282437	133.2568	209.7576	227.3545

Appendix 2

Chlorophyll a calculations for each month at each sampling site

Sample	Tot vol	Filtered volume (ml)	Abs (665)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone area (m^{-2})	embedded	Realised stone area	chl a (mg/m2)
A1JUN14	126	15	0.185	0.131	0.04	0.52000704	115	85	25	14775	0.0240669	0%	0.0240669	21.60673124
A2JUN14	191	15	0.706	1.239	0.04	0	140	110	55	29150	0.0441919	50%	0.022096	phaeopigment
B1JUN14	133	15	0.102	0.188	0.04	0	120	85	30	16350	0.0262719	30%	0.0183903	phaeopigment
B5JUN14	326	15	0.017	0.082	0.04	0	165	95	20	20875	0.0326069	20%	0.0260855	phaeopigment
C2JUN14	177	15	0.89	0.597	0.04	3.96356336	156	95	550	152870	0.2173999	0%	0.2173999	18.23167058
C3JUN14	309	15	0.516	0.347	0.04	3.99107696	195	120	60	42300	0.0626019	0%	0.0626019	63.753288
D4JUN14	284	15	0.54	0.366	0.04	3.77670016	125	60	45	15825	0.0255369	5%	0.0242601	155.6756636
D5JUN14	313	15	0.345	0.281	0.04	1.530978987	155	80	40	21800	0.0339019	30%	0.0237313	64.51298712

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	xy+xz+yz	stone area (m2)	embedded	Realised stone area	chl a (mg/m2)
A1JUL14	359	15	0.977	0.954	0.01	0.157764	185	120	32	31960	0.0481259	20%	0.03850072	4.097683
A2JUL14	295	15	0.47	0.461	0.01	0.050728	185	110	45	33625	0.0504569	70%	0.01513707	3.351256
A3JUL14	330	15	0.024	0.12	0.04	0	135	115	75	34275	0.0513669	40%	0.03082014	phaeopigr
B1JUL14	378	15	0.027	0.017	0.04	0.288893	175	120	45	34275	0.0513669	20%	0.04109352	7.03013
B2JUL14	384	15	0.454	0.439	0.01	0.110054	150	95	20	19150	0.0301919	10%	0.02717271	4.050181
B3JUL14	361	15	0.778	0.764	0.02	0.19313	130	85	67	25455	0.0390189	0%	0.0390189	4.949657
C2JUL14	325	15	0.029	0.134	0.04	0	215	150	30	43200	0.0638619	40%	0.03831714	phaeopigr
C3JUL14	335	15	0.088	0.539	0.02	0	150	90	10	15900	0.0256419	0%	0.0256419	phaeopigr
C5JUL14	311	15	0.702	0.695	0.02	0.08319	210	160	50	52100	0.0763219	80%	0.01526438	5.449971
D2JUL14	198	15	0.84	0.013	0.01	3.12864	240	115	40	41800	0.0619019	10%	0.05571171	56.15768
D4JUL14	320	15	0.649	0.65	0.02	0	160	90	50	26900	0.0410419	20%	0.03283352	phaeopigr
D5JUL14	311	15	0.777	0.779	0.02	0	210	120	110	61500	0.0894819	50%	0.04474095	phaeopigr

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A1AUG14	325	15	0.16	0.1	0.04	1.49032	170	130	70	43100	0.063722	40%	0.03823314	38.9798
A4AUG14	395	15	0.29	0.186	0.04	3.139607	175	115	50	34625	0.051857	50%	0.02592845	121.0874
A5AUG14	421	15	0.032	0.023	0.04	0.289581	130	80	45	19850	0.031172	0%	0.0311719	9.289798
B3AUG14	474	15	0.352	0.266	0.04	3.115457	250	160	50	60500	0.088082	10%	0.07927371	39.3
B4AUG14	377	15	0.062	0.04	0.04	0.633883	180	100	45	30600	0.046222	0%	0.0462219	13.71391
B5AUG14	583	15	0.036	0.018	0.04	0.802021	110	75	20	11950	0.020112	20%	0.01608952	49.84744
C1AUG14	509	15	0.24	0.163	0.04	2.99539	165	101	55	31295	0.047195	10%	0.04247541	70.52058
C4AUG14	428	15	0.244	0.171	0.04	2.387875	130	95	50	23600	0.036422	40%	0.02185314	109.2692
C5AUG14	386	15	0.67	0.507	0.04	4.808613	175	85	65	31775	0.047867	0%	0.0478669	100.458
D1AUG14	500	15	1.583	1.075	0.04	19.41237	205	75	55	30775	0.046467	0%	0.0464669	417.7678
D3AUG14	531	15	0.359	0.29	0.04	2.800197	150	135	60	37350	0.055672	0%	0.0556719	50.29821
D4AUG14	429	15	0.663	0.462	0.04	6.590195	135	93	90	33075	0.049687	0%	0.0496869	132.6345

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xz+xz+yz)	stone surface area(m2)	embedded	Realised stone area	chl a (mg/m2)
A3SEP14	414	15	0.184	0.12	0.02	1.0125	145	90	47	24095	0.037115	0%	0.037115	27.28016
A4SEP14	442	15	0.191	0.127	0.02	1.080979	190	110	40	32900	0.049442	40%	0.029665	36.43936
A5SEP14	421	15	0.466	0.323	0.02	2.300557	135	110	75	33225	0.049897	40%	0.029938	76.8437
B2SEP14	381	15	0.442	0.305	0.02	1.994621	110	85	35	16175	0.026027	20%	0.020822	95.79615
B3SEP14	378	15	0.153	0.105	0.02	0.693343	130	55	40	14550	0.023752	40%	0.014251	48.65174
B4SEP14	470	15	0.73	0.474	0.02	4.597828	160	75	30	19050	0.030052	30%	0.021036	218.5661
C1SEP14	431	15	1.469	0.982	0.02	8.020864	125	110	50	25500	0.039082	10%	0.035174	228.0358
C2SEP14	515	15	0.499	0.331	0.02	3.306218	100	75	35	13625	0.022457	30%	0.01572	210.3215
C3SEP14	425	15	0.496	0.345	0.02	2.452341	155	150	50	38500	0.057282	20%	0.045826	53.51474
D1SEP14	565	15	0.726	0.5	0.02	4.879461	155	150	40	35450	0.053012	0%	0.053012	92.04463
D2SEP14	475	15	0.295	0.197	0.02	1.778831	205	205	130	95325	0.136837	10%	0.123153	14.44405
D3SEP14	472	15	0.499	0.352	0.02	2.651394	310	275	115	152525	0.216917	40%	0.13015	20.37181
E1SEP14	415	15	0.213	0.143	0.02	1.110097	90	55	20	7850	0.014372	30%	0.01006	110.344
E2SEP14	440	15	1.022	0.693	0.02	5.531762	70	40	25	5550	0.011152	10%	0.010037	551.1529
E5SEP14	530	15	0.355	0.238	0.02	2.369609	70	65	20	7250	0.013532	50%	0.006766	350.2256

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area(m2)	embedded	Realised stone area	chl a (mg/m2)
A2OCT14	253	15	0.117	0.076	0.04	0.792774	130	120	35	24350	0.0374719	40%	0.02248314	35.26081
A4OCT14	352	15	0.052	0.035	0.04	0.457337	170	105	75	38475	0.0572469	50%	0.02862345	15.97771
A5OCT14	359	15	0.234	0.094	0.04	3.841204	170	110	20	24300	0.0374019	30%	0.02618133	146.7154
B1OCT14	350	15	0.013	0.153	0.04	0	120	105	25	18225	0.0288969	10%	0.02600721	phaeopigr
B2OCT14	348	15	0.108	0.069	0.04	1.037263	160	125	55	35675	0.0533269	30%	0.03732883	27.78717
B5OCT14	320	15	0.636	0.311	0.04	7.948373	150	110	90	39900	0.0592419	40%	0.03554514	223.6135
C1OCT14	373	15	1.807	1.216	0.04	16.84772	175	75	55	26875	0.0410069	40%	0.02460414	684.7516
C2OCT14	353	15	0.418	0.288	0.04	3.50722	180	90	55	31050	0.0468519	50%	0.02342595	149.7152
C5OCT14	482	15	0.373	0.272	0.04	3.720603	160	55	30	15250	0.0247319	50%	0.01236595	300.8748
D1OCT14	450	15	0.354	0.148	0.04	7.084752	170	120	35	30550	0.0461519	0%	0.0461519	153.5094
D3OCT14	513	15	0.818	0.222	0.04	23.3673	165	130	90	48000	0.0705819	30%	0.04940733	472.9521
D4OCT14	350	15	0.608	0.165	0.04	11.84995	140	100	70	30800	0.0465019	40%	0.02790114	424.7122
E1OCT14	370	15	0.313	0.15	0.04	4.609292	180	180	35	45000	0.0663819	30%	0.04646733	99.19426
E2OCT14	378	15	0.829	0.166	0.04	19.15359	135	135	50	31725	0.0477969	40%	0.02867814	667.8813
E4OCT14	472	15	0.459	0.235	0.04	8.080439	215	125	50	43875	0.0648069	10%	0.05832621	138.5387

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area(m2)	embedded	Realised stone area	chl a (mg/m2)
A1NOV14	474	15	0.201	0.133	0.04	2.463384	180	110	45	32850	0.049372	20%	0.03949752	62.36808
A2NOV14	201	15	1.0772	0.7176	0.04	5.524089	190	85	25	23025	0.035617	50%	0.01780845	310.1948
A3NOV14	265	15	0.7346	0.4741	0.04	5.275924	225	160	80	66800	0.096902	50%	0.04845095	108.8921
B3NOV14	405	15	1.004	0.641	0.04	11.23587	140	85	65	26525	0.040517	40%	0.02431014	462.1885
B4NOV14	291	15	2.418	1.8801	0.04	11.96298	185	90	55	31775	0.047867	30%	0.03350683	357.0312
B5NOV14	341	15	1.5291	1.0367	0.04	12.83268	135	100	60	27600	0.042022	10%	0.03781971	339.3119
C3NOV14	464	15	0.7025	0.4736	0.04	8.117246	240	150	55	57450	0.083812	60%	0.03352476	242.1269
C4NOV14	503	15	0.961	0.68	0.04	10.80237	250	100	85	54750	0.080032	0%	0.0800319	134.9759
C5NOV14	450	15	0.6317	0.4219	0.04	7.215442	125	115	25	20375	0.031907	0%	0.0319069	226.1405
D1NOV14	550	15	0.891	0.5655	0.04	13.68228	138	115	110	43700	0.064562	70%	0.01936857	706.4168
D2NOV14	402	15	0.546	0.351	0.04	5.991086	200	150	50	47500	0.069882	10%	0.06289371	95.25732
D5NOV14	526	15	0.1716	0.1093	0.04	2.504487	230	120	70	52100	0.076322	20%	0.06105752	41.01848
E1NOV14	451	15	1.0996	0.8035	0.04	10.2061	150	145	85	46825	0.068937	50%	0.03446845	296.0998
E3NOV14	455	15	1.184	0.786	0.04	13.84011	140	100	50	26000	0.039782	0%	0.0397819	347.8995
E4NOV14	425	15	0.539	0.4591	0.04	2.595259	175	125	35	32375	0.048707	10%	0.04383621	59.20353

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xz+yz+xz)	stone surface area(m2)	embedded	Realised stone area	chl a (mg/m2)
A3DEC14	501	15	0.3113	0.1935	0.04	4.510534	150	70	55	22600	0.035022	10%	0.03151971	143.102
A4DEC14	415	15	0.5598	0.3726	0.04	5.937435	205	90	65	37625	0.056057	50%	0.02802845	211.836
A5DEC14	550	15	1.081	0.707	0.04	15.72097	180	125	70	43850	0.064772	20%	0.05181752	303.3909
B1DEC14	339	15	1.166	0.791	0.04	9.71574	110	85	40	17150	0.027392	30%	0.01917433	506.7056
B2DEC14	468	15	1.2777	0.8512	0.04	15.25492	230	140	85	63650	0.092492	50%	0.04624595	329.8649
B4DEC14	161	15	0.9358	0.6294	0.04	3.770158	100	90	65	21350	0.033272	10%	0.02994471	125.904
C2DEC14	303	15	0.7473	0.594	0.04	3.550011	110	100	40	19400	0.030542	10%	0.02748771	129.149
C3DEC14	384	15	1.01	0.662	0.04	10.21305	175	155	80	53525	0.078317	30%	0.05482183	186.2953
C4DEC14	403	15	0.1637	0.1091	0.04	1.681677	110	65	35	13275	0.021967	40%	0.01318014	127.5917
D1DEC14	350	15	2.122	1.432	0.04	18.45704	190	120	100	53800	0.078702	10%	0.07083171	260.5759
D3DEC14	462	15	0.6551	0.4325	0.04	7.85981	130	90	60	24900	0.038242	30%	0.02676933	293.6125
D4DEC14	414	15	0.7424	0.4991	0.04	7.698168	160	100	75	35500	0.053082	0%	0.0530819	145.0243
E1DEC14	278	15	1.3887	0.9158	0.04	10.04752	120	100	25	17500	0.027882	20%	0.02230552	450.4501
E2DEC14	466	15	2.507	1.797	0.04	25.28653	155	145	45	35975	0.053747	50%	0.02687345	940.9483
E5DEC14	375	15	0.3275	0.221	0.04	3.05229	270	180	20	57600	0.084022	70%	0.02520657	121.091

Sample	Tot Vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy + xz +yz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A2JAN15	445	15	0.109	0.094	0.02	0.255074	130	110	70	31100	0.046922	40%	0.02815314	9.060233
A3JAN15	420	15	0.286	0.21	0.02	1.21977	140	100	70	30800	0.046502	10%	0.04185171	29.14504
A4JAN15	400	15	0.085	0.061	0.02	0.366848	155	110	40	27650	0.042092	20%	0.03367352	10.89426
B1JAN15	275	15	0.228	0.182	0.02	0.483399	210	105	35	33075	0.049687	20%	0.03974952	12.16112
B2JAN15	312	15	0.194	0.137	0.02	0.679586	135	75	40	18525	0.029317	40%	0.01759014	38.63448
B4JAN15	300	15	0.042	0.036	0.02	0.068784	205	150	25	39625	0.058857	20%	0.04708552	1.460831
C1JAN15	350	15	1.454	0.88	0.02	7.677059	120	125	55	28475	0.043247	40%	0.02594814	295.8616
C2JAN15	445	15	0.665	0.458	0.02	3.520021	180	135	110	58950	0.085912	50%	0.04295595	81.9449
C3JAN15	375	15	0.717	0.544	0.02	2.47909	155	100	65	32075	0.048287	0%	0.0482869	51.34084
D1JAN15	370	15	0.347	0.247	0.02	1.413893	160	135	65	40775	0.060467	10%	0.05442021	25.98103
D2JAN15	435	15	0.109	0.099	0.02	0.166228	185	110	65	39525	0.058717	0%	0.0587169	2.831008
D3JAN15	355	15	0.368	0.275	0.02	1.261613	125	95	40	20675	0.032327	40%	0.01939614	65.04455
E1JAN15	403	15	1.263	0.905	0.02	5.51319	185	70	60	28250	0.042932	0%	0.0429319	128.4171
E2JAN15	430	15	0.653	0.547	0.02	1.741764	185	95	60	34375	0.051507	10%	0.04635621	37.57347
E4JAN15	325	15	0.308	0.232	0.02	0.943869	135	110	70	32000	0.048182	0%	0.0481819	19.58971

Sample	Tot Vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+yz+xz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A3FEB15	250	15	0.131	0.105	0.04	0.496773	175	105	90	43575	0.064387	20%	0.05150952	9.644301
A4FEB15	250	15	0.166	0.113	0.04	1.012653	135	90	35	20025	0.031417	0%	0.0314169	32.23276
A5FEB15	329	15	0.056	0.068	0.04	0	170	155	90	55600	0.081222	10%	0.07309971	phaeopigr
B1FEB15	150	15	0.095	0.066	0.04	0.332456	155	130	25	27275	0.041567	40%	0.02494014	13.33016
B4FEB15	305	15	0.358	0.259	0.04	2.307703	140	100	10	16400	0.026342	80%	0.00526838	438.029
B5FEB15	353	15	0.713	0.539	0.04	4.694279	115	85	25	14775	0.024067	30%	0.01684683	278.6446
C1FEB15	229	15	1.087	0.856	0.04	4.042894	155	140	70	42350	0.062672	0%	0.0626719	64.50888
C2FEB15	348	15	1.134	0.798	0.04	8.936417	120	85	50	20450	0.032012	10%	0.02881071	310.1769
C4FEB15	250	15	0.195	0.142	0.04	1.012653	125	80	60	22300	0.034602	0%	0.0346019	29.26583
D1FEB15	278	15	0.774	0.558	0.04	4.589268	190	80	60	31400	0.047342	0%	0.0473419	96.93883
D3FEB15	276	15	1.211	0.862	0.04	7.361722	110	95	80	26850	0.040972	0%	0.0409719	179.6773
D4FEB15	450	15	0.507	0.358	0.04	5.124408	135	125	50	29875	0.045207	0%	0.0452069	113.3546
E2FEB15	361	15	0.08	0.056	0.04	0.662161	160	130	65	39650	0.058892	40%	0.03533514	18.73944
E3FEB15	273	15	0.739	0.625	0.04	2.378551	225	85	55	36175	0.054027	30%	0.03781883	62.89329
E5FEB15	380	15	0.933	0.637	0.04	8.596471	165	125	90	46725	0.068797	60%	0.02751876	312.3859

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A1MAR15	287	15	0.708	0.767	0.04	0	140	100	85	34400	0.051542	50%	0.025771	phaeopigr
A2MAR15	300	15	0.536	0.483	0.04	1.215184	195	75	70	33525	0.050317	30%	0.035222	34.50088
A5MAR15	352	15	1.28	0.847	0.04	11.64865	185	145	30	36725	0.054797	10%	0.049317	236.1984
B1MAR15	450	15	1.91	1.428	0.04	16.57694	175	80	35	22925	0.035477	30%	0.024834	667.5146
B3MAR15	300	15	0.623	0.423	0.04	4.5856	160	100	40	26400	0.040342	20%	0.032274	142.0855
B5MAR15	400	15	0.499	0.395	0.04	3.179349	150	85	30	19800	0.031102	40%	0.018661	170.3727
C3MAR15	414	15	0.238	0.176	0.04	1.96172	210	95	40	32150	0.048392	40%	0.029035	67.56364
C4MAR15	450	15	0.655	0.443	0.04	7.291104	185	110	75	42475	0.062847	50%	0.031423	232.0275
C5MAR15	408	15	0.62	0.557	0.04	1.964471	190	140	40	39800	0.059102	50%	0.029551	66.47742
D2MAR15	400	15	0.203	0.134	0.04	2.109376	170	120	100	49400	0.072542	40%	0.043525	48.46339
D3MAR15	350	15	0.15	0.101	0.04	1.310717	170	140	75	47050	0.069252	20%	0.055402	23.65851
D4MAR15	403	15	0.197	0.127	0.04	2.155996	180	95	65	34975	0.052347	60%	0.020939	102.9668
E2MAR15	509	15	1.353	0.981	0.04	14.47124	160	135	30	30450	0.046012	0%	0.046012	314.5107
E3MAR15	271	15	0.009	0.006	0.04	0.062135	180	105	20	24600	0.037822	30%	0.026475	2.346897
E5MAR15	279	15	0.346	0.24	0.04	2.260242	190	110	40	32900	0.049442	40%	0.029665	76.19186

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A2APR15	425	15	0.058	0.04	0.04	0.584664	140	120	95	41500	0.061482	30%	0.0430373	13.58504
A4APR15	305	15	0.47	0.311	0.04	3.706311	180	110	95	47350	0.069672	30%	0.0487703	75.9952
A5APR15	279	15	1.321	0.881	0.04	9.382138	110	110	55	24200	0.037262	10%	0.0335357	279.7656
B1APR15	262	15	0.202	0.132	0.04	1.401665	160	125	85	44225	0.065297	40%	0.0391781	35.77671
B2APR15	308	15	0.649	0.443	0.04	4.849119	200	130	60	45800	0.067502	10%	0.0607517	79.81864
B3APR15	347	15	0.016	0.008	0.04	0.21216	175	55	35	17675	0.028127	10%	0.0253142	8.38108
C1APR15	425	15	0.736	0.48	0.04	8.315221	185	120	45	35925	0.053677	10%	0.0483092	172.125
C2APR15	503	15	0.527	0.408	0.04	4.574671	110	95	35	17625	0.028057	10%	0.0252512	181.1664
C3APR15	376	15	0.416	0.356	0.04	1.724186	170	130	55	38600	0.057422	30%	0.0401953	42.89517
D1APR15	357	15	0.101	0.065	0.04	0.982236	170	80	45	24850	0.038172	20%	0.0305375	32.16488
D3APR15	371	15	0.602	0.452	0.04	4.253144	210	160	75	61350	0.089272	10%	0.0803447	52.9362
D5APR15	413	15	0.132	0.093	0.04	1.231004	245	125	50	49125	0.072157	40%	0.0432941	28.43351
E1APR15	411	15	0.347	0.288	0.04	1.85327	160	140	90	49400	0.072542	20%	0.0580335	31.93448
E3APR15	368	15	0.255	0.21	0.04	1.265626	175	110	65	37775	0.056267	40%	0.0337601	37.48875
E5APR15	367	15	0.051	0.038	0.04	0.364632	220	110	90	53900	0.078842	30%	0.0551893	6.606923

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A1MAY15	250	15	0.175	0.0112	0.02	1.564836	200	120	70	46400	0.068342	20%	0.05467352	28.62146
A2MAY15	450	15	0.057	0.037	0.02	0.34392	185	95	85	41375	0.061307	10%	0.05517621	6.233121
A3MAY15	388	15	0.193	0.123	0.02	1.037874	205	100	90	47950	0.070512	30%	0.04935833	21.02733
B1MAY15	350	15	0.007	0.004	0.02	0.040124	145	95	50	25775	0.039467	10%	0.03552021	1.12961
B3MAY15	450	15	0.089	0.06	0.02	0.498684	190	125	25	31625	0.047657	0%	0.0476569	10.46405
B5MAY15	238	15	0.046	0.03	0.02	0.145516	220	135	25	38575	0.057387	20%	0.04590952	3.169634
C2MAY15	386	15	0.27	0.176	0.02	1.386533	185	125	50	38625	0.057457	0%	0.0574569	24.1317
C3MAY15	350	15	0.66	0.421	0.02	3.196545	210	105	65	42525	0.062917	20%	0.05033352	63.50729
C4MAY15	364	15	0.391	0.269	0.02	1.696978	150	75	70	27000	0.041182	10%	0.03706371	45.78542
D1MAY15	374	15	0.809	0.573	0.02	3.372862	150	105	75	34875	0.052207	10%	0.04698621	71.78408
D3MAY15	350	15	0.879	0.577	0.02	4.039149	180	95	55	32225	0.048497	0%	0.0484969	83.28675
D5MAY15	517	15	0.424	0.282	0.02	2.805394	130	130	105	44200	0.065262	0%	0.0652619	42.9867
E1MAY15	408	15	0.286	0.19	0.02	1.49674	165	85	75	32775	0.049267	10%	0.04434021	33.75581
E2MAY15	450	15	0.41	0.268	0.02	2.441832	175	100	45	29875	0.045207	30%	0.03164483	77.16369
E3MAY15	388	15	0.266	0.18	0.02	1.275103	155	85	65	28775	0.043667	20%	0.03493352	36.50083

Sample	Tot vol	Filtered volume (L)	Abs (665b)	Abs (665a)	extrant	Chl a (mg/sample)	x(mm)	y(mm)	z(mm)	(xy+xz+yz)	stone surface area	embedded	Realised stone area	chl a (mg/m2)
A1JUN15	370	15	0.159	0.115	0.02	0.622113	210	95	90	47400	0.069742	20%	0.0557935	11.15027
A2JUN15	426	15	0.049	0.036	0.02	0.211625	170	70	60	26300	0.040202	0%	0.0402019	5.264066
A3JUN15	450	15	0.044	0.029	0.02	0.25794	155	110	60	32950	0.049512	10%	0.0445607	5.788507
B2JUN15	350	15	0.471	0.463	0.02	0.106997	120	70	50	17900	0.028442	20%	0.0227535	4.702452
B3JUN15	400	15	0.289	0.261	0.02	0.427989	150	95	25	20375	0.031907	0%	0.0319069	13.41369
B4JUN15	400	15	0.191	0.13	0.02	0.932405	185	80	65	32025	0.048217	20%	0.0385735	24.17216
C1JUN15	450	15	0.995	0.159	0.02	14.37586	170	145	35	35675	0.053327	0%	0.0533269	269.5798
C4JUN15	350	15	0.707	0.527	0.02	2.40744	175	90	70	34300	0.051402	0%	0.0514019	46.83562
C5JUN15	360	15	0.778	0.558	0.02	3.026496	160	95	80	35600	0.053222	0%	0.0532219	56.86561
D2JUN15	400	15	0.304	0.192	0.02	1.711957	135	85	45	21375	0.033307	10%	0.0299762	57.11053
D3JUN15	450	15	0.3	0.193	0.02	1.839972	200	140	65	50100	0.073522	30%	0.0514653	35.75168
D4JUN15	450	15	0.19	0.12	0.02	1.20372	14	85	35	4655	0.009899	0%	0.0098989	121.6014
E2JUN15	324	15	0.146	0.1	0.02	0.569532	130	110	40	23900	0.036842	0%	0.0368419	15.4588
E3JUN15	469	15	0.135	0.086	0.02	0.878181	140	95	80	32100	0.048322	0%	0.0483219	18.17355
E4JUN15	400	15	0.168	0.113	0.02	0.840693	220	100	75	46000	0.067782	10%	0.0610037	13.78102

Appendix 3

Benthotorch calculations from March 2015 at each sampling site

Site C: Petrustroom						Site D: York Road						Site E: Reference					
Stone	Side	Green	Diatoms	Cyanobateria		Stone	Side	Green	Diatoms	Cyanobateria		Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.63	0.76		1	a	0	2.04	2.5		1	a	0	0.08	0.19	
	b	0	0.57	0.69			b	0	1.28	1.45			b	0	0.43	1.07	
	c	0.22	0.42	1.37			c	1.94	0.88	0.65			c	0.21	0.24	0.57	
	d	0	0.61	1.51			d	0	0.14	1.13			d	0.11	0.24	0.5	
	e	0.13	0.77	1.56			e	0	0.88	1.24			e	0	0.13	0.26	
		0.07	0.6	1.178	1.848	1	#DIV/0!	0.388	1.044	1.394	2.826	1	#DIV/0!	0.064	0.224	0.518	0.806
2	a	0	1.26	2.05		2	a	0	0.94	1.91		2	a	0.02	0.11	0.43	
	b	0	1.77	2.95			b	0	0.74	1.49			b	0	1.09	1.07	
	c	0	0	0.01			c	0	1.18	1.91			c	0.35	0.27	0.74	
	d	0.01	0	0.03			d	0	0.47	2.47			d	0.06	0.12	0.48	
	e	0.01	0	0.02			e	0	4.75	3.55			e	0	0.33	0.42	
		0.004	0.606	1.012	1.622	2	#DIV/0!	0	1.616	2.266	3.882	2	#DIV/0!	0.086	0.384	0.628	1.098
3	a	0.09	0.08	0.37		3	a	0	1.49	1.32		3	a	0.02	0.08	0.15	
	b	0	0.2	0.74			b	0	0.83	1.15			b	0	0.03	0.05	
	c	0.26	0.13	0.49			c	0	0.52	0.97			c	0	0.57	0.65	
	d	0	0	0.01			d	0.5	0.44	0.87			d	0.04	0	0	
	e	0.01	0	0.02			e	0.48	0.6	0.8			e	0	0.01	0.14	
		0.072	0.082	0.326	0.48	3	#DIV/0!	0.196	0.776	1.022	1.994	3	#DIV/0!	0.012	0.138	0.198	0.348
4	a	0.17	0.62	0.81		4	a	0.2	0.54	1.03		4	a	0	0.38	0.4	
	b	0	1.49	1.49			b	0	0.88	1.84			b	0.16	0.16	0.31	
	c	0	0.75	1.3			c	0	0.47	1.19			c	0.05	0.42	0.91	
	d	0.02	0.16	0.13			d	0.37	0.53	1.12			d	0.04	0	0.1	
	e	0	0.13	0.19			e	0	0.09	0.3			e	0.1	0.03	0.42	
		0.038	0.63	0.784	1.452	4	#DIV/0!	0.114	0.502	1.096	1.712	4	#DIV/0!	0.07	0.198	0.428	0.696
5	a	0	0.54	0.49		5	a	0.36	0.84	0.98		5	a	0	0.51	0.66	
	b	0	0.53	0.8			b	0	1.14	0.98			b	0.07	0.04	0.26	
	c	0	0.48	0.49			c	0.03	0	0.12			c	0	0.16	0.6	
	d	0	0	0			d	0.56	1.01	1.5			d	0	0.17	0.56	
	e	0	0.17	0.15			e	0.32	1.12	1.34			e	0	1.01	1.26	
		0	0.344	0.386	0.73	5	#DIV/0!	0.254	0.822	0.984	2.06	5	#DIV/0!	0.014	0.378	0.668	1.06

Site A: Edendale					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0.07	0.34	0.41	
	b	0	0.42	0.79	
	c	0	0.73	0.62	
	d	0	0.29	0.51	
	e				
		0.0175	0.445	0.5825	1.045
2	a	0.03	0.64	0.45	
	b	0.16	0.21	1.22	
	c	0.09	0.93	1.29	
	d	0	0.27	0.77	
	e	0	0	0.78	
		0.056	0.41	0.902	1.368
3	a	0.37	0.33	0.12	
	b	0	0.41	0.44	
	c	0	0.9	1.58	
	d	0	0.03	0.39	
	e	0	0.6	2.49	
		0.074	0.454	1.004	1.532
4	a	0	0.15	0.79	
	b	0.09	0.23	1.01	
	c	0.07	0.64	0.61	
	d	0.41	0.8	0.87	
	e	0.02	0.4	0.4	
		0.118	0.444	0.736	1.298
5	a	0	0.31	1.24	
	b	0	0.59	1.48	
	c	0	0.48	1.75	
	d	0	0.93	0.85	
	e	0	0	0.01	
		0	0.462	1.066	1.528

Site A: Tannery					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0.02	0	0.05	
	b	0	2.5	2.17	
	c	0	3.41	2.91	
	d	0	2.44	3.25	
	e	0.74	2.45	2.21	
1	#DIV/0!	0.152	2.16	2.118	4.43
2	a	0	0.03	0.04	
	b	0	0.37	0.73	
	c	0	0.57	0.89	
	d	0.7	0.33	0.46	
	e	0	0.3	0.45	
2	#DIV/0!	0.14	0.32	0.514	0.974
3	a	0	0	0.14	
	b	0.01	0	0.04	
	c	0	0.52	1.02	
	d	0.07	0.17	0.46	
	e	0	0	0.1	
3	#DIV/0!	0.016	0.138	0.352	0.506
4	a	0.1	0.08	1	
	b	0	0.92	1.8	
	c	0	1.56	1.75	
	d	0.01	0	0.03	
	e	0	0.21	0.48	
4	#DIV/0!	0.022	0.554	1.012	1.588
5	a	0	0.25	0.3	
	b	0.06	0.41	0.51	
	c	0	1.61	2.01	
	d	0.13	0.76	0.66	
	e	0	0.32	0.47	
5	#DIV/0!	0.038	0.67	0.79	1.498

Site C: Petrustroom					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.53	1.16	
	b	0.06	1.12	2.31	
	c	0	1.69	3.19	
	d	0.05	0.4	1.53	
	e	0	0.21	0.97	
1	#DIV/0!	0.022	0.79	1.832	2.644
2	a	0.62	0.59	1.1	
	b	0.23	1.03	1.91	
	c	0	1.52	1.52	
	d	0.42	0.55	1.67	
	e	0.09	0.07	1.87	
2	#DIV/0!	0.272	0.752	1.614	2.638
3	a	0.11	0.34	0.58	
	b	0	0.31	0.35	
	c	0	0.87	0.95	
	d	0.06	0.85	0.3	
	e	0.01	0.05	0.55	
3	#DIV/0!	0.036	0.484	0.546	1.066
4	a	0.87	0.25	1.35	
	b	0	0.86	1.29	
	c	0.21	0.4	1.09	
	d	0	0.04	0.12	
	e	0	0.12	0.31	
4	#DIV/0!	0.216	0.334	0.832	1.382
5	a	0.81	0.53	1.3	
	b	0.03	0.96	0.84	
	c	0	1.09	1	
	d	0	0.36	0.81	
	e	0.02	0	0.05	
5	#DIV/0!	0.172	0.588	0.8	1.56

Site D: York Road					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.8	0.95	
	b	0	1.06	0.61	
	c	0	2.71	2.46	
	d	0	0.42	1.03	
	e	0	0.39	1.12	
1	#DIV/0!	0	1.076	1.234	2.31
2	a	1.37	0.29	0.77	
	b	0	0.48	0.62	
	c	0	1.28	0.91	
	d	0	0.71	0.94	
	e	0	0.93	1.22	
2	#DIV/0!	0.274	0.738	0.892	1.904
3	a	0	0.67	0.62	
	b	0	0.52	0.92	
	c	0	1.08	0.96	
	d	0	0.32	0.72	
	e	0.13	0.36	0.95	
3	#DIV/0!	0.026	0.59	0.834	1.45
4	a	0	3.97	2.91	
	b	0	3.52	3.59	
	c	0.03	0	0.13	
	d	0.03	0	0.08	
	e	0	2.69	2.28	
4	#DIV/0!	0.012	2.036	1.798	3.846
5	a	0	1.83	2.67	
	b	0	1.83	5.05	
	c	0	1.9	2.24	
	d	0	5.02	4.01	
	e	0	0.12	0.22	
5	#DIV/0!	0	2.14	2.838	4.978

Site E: Reference					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.23	1.08	
	b	0	0.14	0.61	
	c	0	0.24	1.02	
	d	0.03	0.04	0.61	
	e	0.01	0	0.24	
1	#DIV/0!	0.008	0.13	0.712	0.85
2	a	0.37	0.08	0.54	
	b	0	0.29	0.91	
	c	0.37	0.1	0.79	
	d	0.1	0.28	0.96	
	e	0	0.38	1.61	
2	#DIV/0!	0.168	0.226	0.962	1.356
3	a	0.5	0.04	0.87	
	b	0.33	0.08	0.78	
	c	0.07	0	0.18	
	d	0	0.02	0.26	
	e	0	0.12	0.87	
3	#DIV/0!	0.18	0.052	0.592	0.824
4	a	0.19	0.03	0.27	
	b	0.09	0.21	1.16	
	c	0	0	0.29	
	d	0.06	0	0.36	
	e	0.45	0.08	1.26	
4	#DIV/0!	0.158	0.064	0.668	0.89
5	a	0	0.31	1.14	
	b	0.35	0.08	0.81	
	c	0	0.37	1.73	
	d	0	0.49	1.75	
	e	0.2	0	0.33	
5	#DIV/0!	0.11	0.25	1.152	1.512

Site A: Edendale					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0.13	0.15	0.32	
	b	0.56	0.35	0.52	
	c	0	3.32	2.45	
	d	0	0.24	0.62	
	e	0	1.45	1.46	
		0.138	1.102	1.074	2.314
2	a	0	1.2	2.25	
	b	0.26	0.05	0.33	
	c	2	0.58	1.59	
	d	0	3.69	2.82	
	e	0	1.49	2.25	
		0.452	1.402	1.848	3.702
3	a	2.81	0.58	1.15	
	b	0	0.22	0.38	
	c	0.09	0.09	0.16	
	d	0	1.73	7.55	
	e	0	1.18	2.82	
		0.58	0.76	2.412	3.752
4	a	0	2.5	2.16	
	b	0	2.43	1.36	
	c	0	2.25	7.33	
	d	0	2.57	2.3	
	e	0.03	0.66	1.47	
		0.006	2.082	2.924	5.012
5	a	0.04	0.18	0.49	
	b	0.16	0.58	0.51	
	c	0.04	0	0.1	
	d	0	1.74	3.01	
	e	0	0.32	0.61	
		0.048	0.564	0.944	1.556

Site B: Tannery					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.04	0.13	
	b	0.04	0.1	0.27	
	c	0	0.5	0.21	
	d	0	0.13	0.3	
	e	0	0.07	0.11	
1	#DIV/0!	0.008	0.168	0.204	0.38
2	a	0.08	0.35	0.26	
	b	0	0.52	0.25	
	c	0	0.11	0.38	
	d	0	0.13	0.3	
	e	0	0.22	0.5	
2	#DIV/0!	0.016	0.266	0.338	0.62
3	a	0	0.09	0.16	
	b	0	0.19	0.24	
	c	0	0.01	0.02	
	d	0	0	0	
	e	0	0.02	0.12	
3	#DIV/0!	0	0.062	0.108	0.17
4	a	0	0.54	0.45	
	b	0	0.45	0.32	
	c	0	0.43	0.7	
	d	0	0.4	0.37	
	e	0.04	0	0.1	
4	#DIV/0!	0.008	0.364	0.388	0.76
5	a	0	0.01	0.24	
	b	0	0.01	0.2	
	c	0	0.2	0.2	
	d	0	0.14	0.26	
	e	0	0.16	0.26	
5	#DIV/0!	0	0.104	0.232	0.336

Site C: Petrustroom					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.12	1.1	
	b	0	0.24	0.15	
	c	0	0.3	0.68	
	d	0	1.43	1.28	
	e	0.03	0.22	0.13	
1	#DIV/0!	0.006	0.462	0.668	1.136
2	a	0	0.46	0.86	
	b	0	0.22	0.35	
	c	0	0.14	0.13	
	d	0	0.15	1.17	
	e	0	0.71	1.04	
2	#DIV/0!	0	0.336	0.71	1.046
3	a	0.2	0.41	0.52	
	b	0.36	0.54	0.87	
	c	0	0.01	0.16	
	d	0	0	0.16	
	e	0.13	0.53	0.13	
3	#DIV/0!	0.138	0.298	0.368	0.804
4	a	0	0.36	0.65	
	b	0	0.22	0.42	
	c	0.1	0.75	1.09	
	d	0	0.72	0.8	
	e	0.03	0.09	0.4	
4	#DIV/0!	0.026	0.428	0.672	1.126
5	a	0	0.27	0.63	
	b	0.1	0.34	1.18	
	c	0	1.04	0.97	
	d	0	0	0.02	
	e	0.06	0.72	0.89	
5	#DIV/0!	0.032	0.474	0.738	1.244

Site D: York Road					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	1.03	1.42	
	b	0	5	2.99	
	c	0	0	0	
	d	0	1.83	1.15	
	e	0	2.11	0.38	
1	#DIV/0!	0	1.994	1.188	3.182
2	a	0.02	0	0.3	
	b	0	1.6	1.21	
	c	0	1.81	1.51	
	d	0.83	0.89	0.91	
	e	0	0.55	0.77	
2	#DIV/0!	0.17	0.97	0.94	2.08
3	a	0	0.53	1.32	
	b	0	1.36	2.48	
	c	0	2.05	2.82	
	d	0	0.87	1.51	
	e	0	0.4	0.65	
3	#DIV/0!	0	1.042	1.756	2.798
4	a	0	1.25	1.3	
	b	0	3.33	2.91	
	c	0	2.62	3.78	
	d	0	2.62	2.53	
	e	0	2.12	1.81	
4	#DIV/0!	0	2.388	2.466	4.854
5	a	0.49	0.21	0.26	
	b	0.19	1.46	0.48	
	c	0.42	0.93	1.28	
	d	0	1.9	1.31	
	e	0	0.7	0.82	
5	#DIV/0!	0.22	1.04	0.83	2.09

Site E: Reference					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0.16	0	0.48	
	b	0.1	0.24	0.49	
	c	0.36	0.36	0.54	
	d	0.13	0.13	0.22	
	e	0.08	0.08	0.99	
1	#DIV/0!	0.166	0.162	0.544	0.872
2	a	0.45	0	0.49	
	b	0.11	0.03	0.21	
	c	0	0.19	0.54	
	d	0.1	0.12	0.24	
	e	0.06	0.12	0.34	
2	#DIV/0!	0.144	0.092	0.364	0.6
3	a	0.06	0.07	0.53	
	b	0.04	0.33	0.87	
	c	0	0.28	0.47	
	d	0	0.09	0.43	
	e	0	0.23	0.34	
3	#DIV/0!	0.02	0.2	0.528	0.748
4	a	0.03	0.27	1.33	
	b	0.17	0.31	0.56	
	c	0.29	0.23	0.52	
	d	0	0.31	0.77	
	e	0.11	0.28	0.88	
4	#DIV/0!	0.12	0.28	0.812	1.212
5	a	0.35	0.19	0.8	
	b	0	[illegible]	0.01	
	c	0.17	0.19	0.6	
	d	0.49	0.41	0.69	
	e	0.09	0.1	0.47	
5	#DIV/0!	0.22	0.178	0.514	0.912

Site A: Edendale					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.32	0.56	
	b	0	0.18	0.24	
	c	0	0.3	0.28	
	d	0	0.5	0.73	
	e	0	0.19	0.32	
		0	0.298	0.426	0.724
2	a	0	0.23	0.33	
	b	0	0.33	0.55	
	c	0	0.32	0.41	
	d	0	0.21	0.38	
	e	0	0.3	0.35	
		0	0.278	0.404	0.682
3	a	0.48	0.97	0.46	
	b	0.13	0.42	1.04	
	c	0.01	0	0.07	
	d	0.11	0.03	0.53	
	e	0.03	0.81	1.03	
		0.152	0.446	0.626	1.224
4	a	2.1	1.74	1.17	
	b	0.11	0.39	0.82	
	c	0.02	0	0.31	
	d	0	1.04	1.09	
	e	0.04	0.39	0.94	
		0.454	0.712	0.866	2.032
5	a	0	5.04	2.54	
	b	0	0.16	0.33	
	c	0	0.01	0.26	
	d	0.07	0.54	0.51	
	e	0	0.73	0.49	
		0.014	1.296	0.826	2.136

Site B: Tannery					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0.04	0.12	0.13	
	b	0.51	1.25	0.86	
	c	0	0.33	0.35	
	d	0	0.75	0.59	
	e	0	0.62	0.82	
1	#DIV/0!	0.11	0.614	0.55	1.274
2	a	0.77	0.74	0.69	
	b	0	0.74	0.85	
	c	0	0.22	0.16	
	d	0	0.67	0.73	
	e	0.01	0	0.15	
2	#DIV/0!	0.156	0.474	0.516	1.146
3	a	0	0.05	0.03	
	b	0.01	0.04	0.14	
	c	0.02	0	0.13	
	d	0	0.06	0.11	
	e	0	0.6	0.97	
3	#DIV/0!	0.006	0.15	0.276	0.432
4	a	0	0	0.02	
	b	0	0.54	0.72	
	c	0.08	0.11	0.34	
	d	0	0.22	0.48	
	e	0	0.53	0.05	
4	#DIV/0!	0.016	0.28	0.322	0.618
5	a	0	0.49	0.51	
	b	0.42	0.42	0.65	
	c	0.08	0.03	0.21	
	d	0	0.65	0.53	
	e	0	1.01	1.16	
5	#DIV/0!	0.1	0.52	0.612	1.232

Site C: Petrustroom					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0.11	0.38	0.71	
	b	0.3	0.2	0.15	
	c	0.04	0.33	0.68	
	d	0.16	0.45	0.4	
	e	0	0.84	1.36	
1	#DIV/0!	0.122	0.44	0.66	1.222
2	a	0.01	0.44	0.78	
	b	0.17	0.62	0.94	
	c	0	0.77	0.83	
	d	2.53	1.64	1.3	
	e	0	2.6	2.25	
2	#DIV/0!	0.542	1.214	1.22	2.976
3	a	0	0.15	0.23	
	b	0	0.91	0.94	
	c	0.01	0	0.03	
	d	0	0.31	0.89	
	e	0	0.15	0.48	
3	#DIV/0!	0.002	0.304	0.514	0.82
4	a	0	0	0.03	
	b	0.01	0.45	0.85	
	c	0	0.01	0.01	
	d	0.55	0.93	0.39	
	e	0	3	2.94	
4	#DIV/0!	0.112	0.878	0.844	1.834
5	a	0	0.65	0.26	
	b	0.08	0.18	0.04	
	c	0.51	0.99	0.68	
	d	1.51	1.68	1.6	
	e	0	0.27	0.31	
5	#DIV/0!	0.42	0.754	0.578	1.752

Site D: York Road					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	1.08	1.04	
	b	0	1.26	0.9	
	c	0	3.49	2.62	
	d	0.11	0.39	0.81	
	e	0	0.8	1.18	
1	#DIV/0!	0.022	1.404	1.31	2.736
2	a	0.07	0.58	0.74	
	b	0	2.63	1.74	
	c	1.07	0.77	0.62	
	d	0	1.29	0.93	
	e	0.01	0.5	0.69	
2	#DIV/0!	0.23	1.154	0.944	2.328
3	a	0	0.01	0.15	
	b	0.24	0.32	0.21	
	c	0	1.46	0.84	
	d	0	0	0.15	
	e	0	0	0.31	
3	#DIV/0!	0.048	0.358	0.332	0.738
4	a	0	0.87	0.96	
	b	0	2.17	1.64	
	c	0	3.2	2.52	
	d	0	4.54	2.92	
	e	0	6.67	3.94	
4	#DIV/0!	0	3.49	2.396	5.886
5	a	0.28	0.94	1.18	
	b	0	1.45	1.54	
	c	0	5.67	3.53	
	d	0.06	1.3	1.55	
	e	0	9.68	4.35	
5	#DIV/0!	0.068	3.808	2.43	6.306

Site E: Reference					
Stone	Side	Green	Diatoms	Cyanobateria	
1	a	0	0.92	0.34	
	b	0.29	0.22	0.31	
	c	0.2	0.01	0.28	
	d	0.19	0	0.22	
	e	0.61	1	0.28	
1	#DIV/0!	0.258	0.43	0.286	0.974
2	a	0.19	0.26	0.46	
	b	0.08	1.38	0.47	
	c	0.24	0.07	0.44	
	d	0.15	0.11	0.35	
	e	0	2.82	0.71	
2	#DIV/0!	0.132	0.928	0.486	1.546
3	a	0.08	0.15	0.28	
	b	0.42	0.25	0.62	
	c	0	0	0	
	d	0.4	0.07	0.86	
	e	0	2.82	0.92	
3	#DIV/0!	0.18	0.658	0.536	1.374
4	a	0.25	0.18	0.51	
	b	0.01	0	0.04	
	c	0	0.03	0.09	
	d	0	0.2	0.35	
	e	0.01	0	0.04	
4	#DIV/0!	0.054	0.082	0.206	0.342
5	a	0.19	0.14	0.46	
	b	0.38	0.71	0.77	
	c	0.21	0.47	0.68	
	d	0	0.33	0.94	
	e	1.73	0.21	0.78	
5	#DIV/0!	0.502	0.372	0.726	1.6

Appendix 4

Algal species counts for each month at each sampling site

	UNIT	Jun-14														
		Site A			Site B			Site C			Site D			Site E		
Sample		3	1	4	5	1	4	1	2	3	1	5	4			
Grids Counted	189 squares	1	1	1	1	1	1		1	1	1	1	1			
Squares counted	1 square							64								
Area counted	mm^2	9	9	9	9	9	9	4	9	9	9	9	9			
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.9	0.9	0.9	0.4	0.9	0.9	0.9	0.9	0.9	0	0	0
Volume sampled	ml	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0004	0.0009	0.0009	0.0009	0.0009	0.0009	0	0	0
Tot ball jar volume	ml	196	76	297	276	83	269	107	127	157	98	263	234			
Stone area m2	m^2	0.0501419	0.0240669	0.0244169	0.0326069	0.0262719	0.0277419	0.0378919	0.2174	0.062602	0.0464319	0.0339019	0.0255369			
TOT CELL COUNT (n)	n	42	24	22	4	7	23	206	220	203	840	386	889	0	0	0
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	cells.ml $^{-1}$	4666.666667	2666.666667	2444.444444	444.4444444	777.7777778	2555.555556	51500	24444.44	22555.56	93333.33333	42888.88889	98777.77778	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	cells.m $^{-2}$	18241563.78	8420970.988	29733504.25	3761984.938	2457209.245	24780005.86	145426859	14279880	56567328	196991005.5	332718159.7	905121608.3	0	0	0
Sample	ml	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum									4							
Achnanthidium oblongella																
Achnanthidium sp.		7	4		2	1	1	66	73	110	352	192	35			
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.										3	88		32			
Closterium																
Cocconeis sp.			1			2	3	6	3	4			2			
Coelastrum sp.																
Cosmarium sp.																
Crucigenia sp.																
Cymbella sp.		1						2	4			4				
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.																
Discotella sp.												2				
Encyonema sp.																
Encyonopsis leei var. sinensis																
Encyonopsis leei.		1	6	3	1			6			32	26	14			
Encyonopsis sp.			2			1		10	1	1		4				
Euastrum																
Eunotia sp									5			6				
Fragilaria sp		1		3	1		1	30	50	10		4	2			
Frustulia sp.								10								
Gimenella																
Gleocapsa sp.																
Gomphonema sp.			6	4		1	3	12	3	6	108		176			
Gyrosigma sp.												64				
Hantzschia sp.																
Heteroleibleinia		30									120	40	560			
Lynbya sp.											40					
Melosira sp.																
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.		2	2	1		2	3	64	61	37	16	10				
Netrium																
Nitzschia																
Nitzschia Sigma			1													
Oedogonium sp.				5								10	16			
Oocystis sp.																
other										22						
Pediastrum sp.																
Phormidium							12						12			
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.																
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis																
Stigeoclonium sp			2	6					16	10	84	24	40			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Jul-14														
		Site A			Site B			Site C			Site D			Site E		
Sample		4	5	1	5	2	1	1	5	3	2	5	4			
Grids Counted	189 squares	1	1	1	1	1	1	1		no algae						
Squares counted	1 square								16		32	64	32			
Area counted	mm^2	9	9	9	9	9	9	9	1	0	2	4	2			
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.1	0	0.2	0.4	0.2	0	0	0
Volume sampled	ml	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0001	0	0.0002	0.0004	0.0002	0	0	0
Tot ball jar volume	ml	329	318	309	300	334	328	209	261	285	148	261	270			
Stone area m2	m^2	0.04695	0.044192	0.048126	0.051262	0.030192	0.051367	0.030122	0.076322	0.025642	0.061902	0.089482	0.041042			
TOT CELL COUNT (n)	**n**	0	29	64	8	64	26	198	566	0	408	876	372	0	0	0
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	**cells.ml $^{-1}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	**cells.m $^{-2}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sample	**ml**	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	**ml**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella				19												
Achnanthidium sp.					3	14	2	76	250		298	566	97			
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.																
Closterium																
Cocconeis sp.			6		1	4	4	4	2							
Coelastrum sp.																
Cosmarium sp.																
Crucigenia sp.																
Cymbella sp.				1		1		4					2			
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.																
Discotella sp.											2					
Encyonema sp.																
Encyonopsis leei var. sinensis																
Encyonopsis leei.			1	5		1		3	2		22	112	2			
Encyonopsis sp.			2					1								
Euastrum																
Eunotia sp																
Fragilaria sp			2		3	1		4								
Frustulia sp.																
Gimenella																
Gleocapsa sp.																
Gomphonema sp.			4	2		3	16	5	2		66	108	185			
Gyrosigma sp.																
Hantzschia sp.																
Heteroleibleinia																
Lynbya sp.																
Melosira sp.						38		9								
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.			14	5	1	2	4	88	12		8	58				
Netrium																
Nitzschia																
Nitzschia Sigma																
Oedogonium sp.								4					59			
Oocystis sp.																
other																
Pediastrum sp.																
Phormidium																
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.																
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis																
Stigeoclonium sp				32					298		12	32	27			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Aug-14														
		Site A			Site B			Site C			Site D			Site E		
Sample		1	3	4	4	3	5	3	4	1	2	4	1			
Grids Counted	189 squares	1	1	1	1	1	1	1		1	1	1	1			
Squares counted	1 square								64							
Area counted	mm^2	9	9	9	9	9	9	9	4	9	9	9	9			
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.4	0.9	0.9	0.9	0.9	0	0	0
Volume sampled	ml	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0004	0.0009	0.0009	0.0009	0.0009	0	0	0
Tot ball jar volume	ml	375	361	345	262	424	533	423	378	459	209	379	450			
Stone area m2	m^2	0.063722	0.052907	0.051857	0.046222	0.088082	0.020112	0.027168	0.036422	0.047195	0.073395	0.049687	0.046467			
TOT CELL COUNT (n)	n	44	6	10	19	29	17	87	459	294	148	118	222	0	0	0
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	cells.ml $^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	cells.m $^{-2}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sample	ml	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.		16	4	2	2			36	269	150	109	82	8			
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.																
Closterium																
Cocconeis sp.		8		2	8	5	2		2							
Coelastrum sp.																
Cosmarium sp.										2						
Crucigenia sp.																
Cymbella sp.									1	4						
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.																
Discotella sp.																
Encyonema sp.																
Encyonopsis leei var. sinensis																
Encyonopsis leei.		8		1		2		3	67	58		6				
Encyonopsis sp.			1			1										
Euastrum																
Eunotia sp																
Fragilaria sp				1	3		2	4	2		4					
Frustulia sp.																
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		4	1	3				2		36	34	20	68			
Gyrosigma sp.					1											
Hantzschia sp.																
Heteroleibleinia																
Lynbya sp.																
Melosira sp.																
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.		8		1	4			39	114	4	1	10				
Netrium																
Nitzschia								3								
Nitzschia Sigma																
Oedogonium sp.										20			86			
Oocystis sp.																
other																
Pediastrum sp.																
Phormidium																
Pinnularia																
Placoneis																
Planothidium sp.					1											
Pleurotaenium sp.																
Scenedesmus sp.							4		4							
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis																
Stigeoclonium sp						21	9			20			60			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Sep-14														
		Site A			Site B			Site C			Site D			Site E		
Sample		5	1	2	3	4	5	5	2	1	3	4	2	1	3	2
Grids Counted	189 squares	1		1	1	1	1	1	1	1	1	1	1			
Squares counted	1 square		64											64	64	32
Area counted	mm^2	9	4	9	9	9	9	9	9	9	9	9	9	4	4	2
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.4	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.4	0.4	0.2
Volume sampled	ml	0.0009	0.0004	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0004	0.0004	0.0002
Tot ball jar volume	ml	371	416	416	328	420	323	395	465	381	422	424	425	365	443	390
Stone area m2	m^2	0.049897	0.071282	0.020322	0.023752	0.030052	0.037052	0.071772	0.022457	0.039082	0.216917	0.114227	0.136837	0.014372	0.009192	0.011152
TOT CELL COUNT (n)	**n**	12	252	198	54	41	101	734	582	303	214	742	65	590	752	327
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	**cells.ml $^{-1}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	**cells.m $^{-2}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sample	**ml**	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	**ml**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.		3	130	2	6	6	20	81	222	172	148	460		86	382	133
Adalfia ap.																
Anabaena sp?					8											
Ankistrodesmus								8	8					21	12	
Aulacoseira sp.							7									
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.			22							23		154				
Closterium																
Cocconeis sp.		3	3	4	12	14	16	11	8							
Coelastrum sp.									8					8		
Cosmarium sp.																
Crucigenia sp.								126	32							
Cymbella sp.			4	1		1	11		2					44		
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.																25
Discotella sp.																
Encyonema sp.								4						118		
Encyonopsis leei var. sinensis																
Encyonopsis leei.			17	6	1		2	13	24	9	13	10	3			
Encyonopsis sp.			10						2			2			40	39
Euastrum																
Eunotia sp																
Fragilaria sp			6	4	7	7	16		8			2		75	90	20
Frustulia sp.								15	2							
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		2	22	6	6	1	11	13	12	8	48	76	4	161	144	98
Gyrosigma sp.		1					1	1								
Hantzschia sp.																
Heteroleibleinia				50				250								
Lynbya sp.				120									24			
Melosira sp.								6								
Micrasterias sp.																
Mougeotia sp.														3	4	
Navicula sp.		3	30	4	6	11	17	92	88	15			2	45	78	
Netrium																
Nitzschia				1		1										
Nitzschia Sigma																
Oedogonium sp.											5	18	3			
Oocystis sp.								4	36							
other																
Pediastrum sp.			8											12		
Phormidium																
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.								52	64	12				16		12
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.								1							2	
Stauroneis																
Stigeoclonium sp					8			57	66	64		20	29			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.														1		
Tribonella																

	UNIT	Oct-14								
		Site A			Site B			Site C		
Sample		3	4	5	2	4	1	2	5	3
Grids Counted	189 squares	1	1	1	1					
Squares counted	1 square					64	64	16	64	64
Area counted	mm^2	9	9	9	9	4	4	1	4	4
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.9	0.4	0.4	0.1	0.4	0.4
Volume sampled	ml	0.0009	0.0009	0.0009	0.0009	0.0004	0.0004	0.0001	0.0004	0.0004
Tot ball jar volume	ml	300	302	309	298	295	300	303	432	441
Stone area m2	m^2	0.049162	0.057247	0.037402	0.053327	0.031067	0.028897	0.046852	0.024732	0.042757
TOT CELL COUNT (n)	n	13	10	32	247	430	406	476	228	786
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	cells.ml $^{-1}$	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	cells.m $^{-2}$	0	0	0	0	0	0	0	0	0
Sample	ml	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts										
Achnanthes oblongella										
Achnanthes sp.										
Achnanthidium exiguum										
Achnanthidium oblongella										
Achnanthidium sp.		3		3	5	78	80	76	46	154
Adalfia ap.										
Anabaena sp?										
Ankistrodesmus										
Aulacoseira sp.									2	
Batrachospermum sp.										
Capartogram sp.										
Chamaesiphon sp.										
Closterium										
Cocconeis sp.		3	2	5	7	4	29	10	11	8
Coelastrum sp.										
Cosmarium sp.										
Crucigenia sp.										
Cymbella sp.				2	6	16	3	10	4	
Cymbopluera sp.										
Desmococcus sp.										
Dichothrix sp.										
Discotella sp.										
Encyonema sp.						4				
Encyonopsis leei var. sinensis										
Encyonopsis leei.				3	7	8	6	93	38	122
Encyonopsis sp.				5	5			60		8
Euastrum										
Eunotia sp										
Fragilaria sp			3		141	262	211	90	20	90
Frustulia sp.										
Gimenella										
Gleocapsa sp.										
Gomphonema sp.		3	5	4	11	24	24	58	60	170
Gyrosigma sp.									2	
Hantzschia sp.										
Heteroleibleinia					40					
Lynbya sp.										
Melosira sp.										
Micrasterias sp.										
Mougeotia sp.										
Navicula sp.		4		6	21	34	53	55	33	224
Netrium										
Nitzschia										2
Nitzschia Sigma										
Oedogonium sp.										
Oocystis sp.										
other									6	
Pediastrum sp.										
Phormidium										
Pinnularia										
Placoneis										
Planothidium sp.										
Pleurotaenium sp.										
Scenedesmus sp.				4				24		8
Selenastrum sp									5	
Sellophora sp.										
Staurastrum sp.										
Stauroneis										
Stigeoclonium sp					4					
Surirella sp.										
Tabellaria sp									1	
Tetraedron sp.										
Tribonella										

	UNIT	Oct-14					
		Site D			Site E		
Sample		4	5	1	1	2	4
Grids Counted	189 squares	1	1	1			
Squares counted	1 square				32	64	64
Area counted	mm^2	9	9	9	2	4	4
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.2	0.4	0.4
Volume sampled	ml	0.0009	0.0009	0.0009	0.0002	0.0004	0.0004
Tot ball jar volume	ml	300	295	400	320	328	425
Stone area m2	m^2	0.046502	0.052627	0.046152	0.066382	0.047797	0.064807
TOT CELL COUNT (n)	n	793	958	158	293	484	518
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	cells.ml $^{-1}$	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	cells.m $^{-2}$	0	0	0	0	0	0
Sample	ml	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1
Cell counts							
Achnanthes oblongella							
Achnanthes sp.							
Achnanthidium exiguum							
Achnanthidium oblongella							
Achnanthidium sp.		24	10	40	43	224	66
Adalfia ap.							
Anabaena sp?							
Ankistrodesmus							
Aulacoseira sp.							
Batrachospermum sp.							
Capartogram sp.							
Chamaesiphon sp.			224	56			
Closterium							
Cocconeis sp.							
Coelastrum sp.							
Cosmarium sp.					2		
Crucigenia sp.							
Cymbella sp.							
Cymbopluera sp.							
Desmococcus sp.							
Dichothrix sp.							
Discotella sp.							
Encyonema sp.					42	26	
Encyonopsis leei var. sinensis							
Encyonopsis leei.		2		8			8
Encyonopsis sp.							36
Euastrum							
Eunotia sp		1					
Fragilaria sp		1				10	
Frustulia sp.							
Gimenella							
Gleocapsa sp.					24		
Gomphonema sp.		53	152	30	134	182	342
Gyrosigma sp.							
Hantzschia sp.							
Heteroleibleinia		640	440				
Lynbya sp.		30			40		
Melosira sp.							
Micrasterias sp.							
Mougeotia sp.							
Navicula sp.						42	66
Netrium							
Nitzschia							
Nitzschia Sigma							
Oedogonium sp.		12	36				
Oocystis sp.							
other							
Pediastrum sp.					8		
Phormidium							
Pinnularia							
Placoneis							
Planothidium sp.							
Pleurotaenium sp.							
Scenedesmus sp.			8				
Selenastrum sp							
Sellophora sp.							
Staurastrum sp.							
Stauroneis							
Stigeoclonium sp		30	88	24			
Surirella sp.							
Tabellaria sp							
Tetraedron sp.							
Tribonella							

	UNIT	Nov-14														
		Site A			Site B			Site C			Site D			Site E		
Sample		2	5	1	4	3	1	5	2	1	1	3	2	4	2	1
Grids Counted	189 squares		1									1	1			
Squares counted	1 square	16		64	16	32	64	32	64	48	48			16	16	16
Area counted	mm^2	1	9	4	1	2	4	2	4	3	3	9	9	1	1	1
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.1	0.9	0.4	0.1	0.2	0.4	0.2	0.4	0.3	0.3	0.9	0.9	0.1	0.1	0.1
Volume sampled	ml	0.0001	0.0009	0.0004	0.0001	0.0002	0.0004	0.0002	0.0004	0.0003	0.0003	0.0009	0.0009	0.0001	0.0001	0.0001
Tot ball jar volume	ml	151	121	424	241	355	250	400	203	416	500	335	352	375	421	401
Stone area m2	m^2	0.035617	0.102782	0.049372	0.047867	0.040517	0.049967	0.031907	0.095642	0.090392	0.064562	0.059942	0.069882	0.048707	0.049197	0.068937
TOT CELL COUNT (n)	**n**	323	18	216	1146	380	211	310	263	183	352	146	331	313	300	480
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	**cells.ml $^{-1}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	**cells.m $^{-2}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sample	**ml**	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	**ml**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.								4								
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.		12	1	5	159	20	7	114	105	28	12		4	21	46	38
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.			2						7							
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.												10				
Closterium																
Cocconeis sp.			3	5	17	7	3			2						
Coelastrum sp.																
Cosmarium sp.																
Crucigenia sp.																
Cymbella sp.		5	1	5	17	20		1	13							4
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.													40			
Discotella sp.																
Encyonema sp.				4	15	12	6	20	16		4			89	45	58
Encyonopsis leei var. sinensis																
Encyonopsis leei.		11	2	39	27	20	11	19	10	5		14	5			2
Encyonopsis sp.										2			1			
Euastrum																
Eunotia sp																
Fragilaria sp			2		674	204	145	13	9	6			3	2	35	2
Frustulia sp.								1								
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		104	7	89	45	17	8	24	16	60	54	74	76	182	173	244
Gyrosigma sp.														2		
Hantzschia sp.																
Heteroleibleinia		130		60	115	30					170		110			80
Lynbya sp.		54				12		11			70	15				
Melosira sp.				4	2			20								
Micrasterias sp.																2
Mougeotia sp.																
Navicula sp.		7		1	25	36	10	76	86	12		4	7	17		34
Netrium																
Nitzschia									1							
Nitzschia Sigma																
Oedogonium sp.											18					
Oocystis sp.																
other																
Pediastrum sp.																
Phormidium							20			68			70			
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.				4		2										16
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.							1								1	
Stauroneis																
Stigeoclonium sp					50			7			24	29	15			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Dec-14														
		Site A			Site B			Site C			Site D			Site E		
Sample		3	1	4	4	1	3	1	5	3	5	2	3	2	1	3
Grids Counted	189 squares			1				1	1	1		1	1			
Squares counted	1 square	64	64		16	32	32				32			16	64	64
Area counted	mm^2	4	4	9	1	2	2	9	9	9	2	9	9	1	4	4
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.4	0.4	0.9	0.1	0.2	0.2	0.9	0.9	0.9	0.2	0.9	0.9	0.1	0.4	0.4
Volume sampled	ml	0.0004	0.0004	0.0009	0.0001	0.0002	0.0002	0.0009	0.0009	0.0009	0.0002	0.0009	0.0009	0.0001	0.0004	0.0004
Tot ball jar volume	ml	451	411	365	111	289	427	446	353	334	422	385	412	416	228	458
Stone area m2	m^2	0.034972	0.109172	0.056007	0.035112	0.027342	0.092442	0.051247	0.031962	0.078267	0.111482	0.035392	0.038192	0.053697	0.027832	0.040047
TOT CELL COUNT (n)	n	156	194	204	275	218	438	272	42	490	440	718	658	362	319	662
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	cells.ml $^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	cells.m $^{-2}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sample	ml	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.					43			44								
Achnanthidium exiguum									1							
Achnanthidium oblongella																
Achnanthidium sp.		7	15	3	5	8	66	16	5	98	24			36	11	
Adalfia ap.																
Anabaena sp?														12		
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.												25				
Closterium																
Cocconeis sp.		1	8		7	2		8	2	10						
Coelastrum sp.																
Cosmarium sp.																
Crucigenia sp.																
Cymbella sp.		10	32	3	12	13	60	2						1	2	8
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.				133									88			
Discotella sp.																
Encyonema sp.				6	4	10		1	2						4	32
Encyonopsis leei var. sinensis																
Encyonopsis leei.		94	108	7	28	56	40	5	3		17			18		54
Encyonopsis sp.														12		
Euastrum																
Eunotia sp								2								
Fragilaria sp		2			139	99	138	2	3					7	7	10
Frustulia sp.									1							
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		37	26	27	22	8	106	6	8	358	93	33	278	202	295	552
Gyrosigma sp.				1												
Hantzschia sp.																
Heteroleibleinia												593	232			
Lynbya sp.						10					218	50		43		
Melosira sp.				4												
Micrasterias sp.																
Mougeotia sp.																6
Navicula sp.		3	5		15	12	28	22	17	24	8	4		31		
Netrium																
Nitzschia																
Nitzschia Sigma				1												
Oedogonium sp.		2									52	13	60			
Oocystis sp.																
other																
Pediastrum sp.																
Phormidium				5												
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.								4								
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis																
Stigeoclonium sp				14				160			28					
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Jan-15														
		Site A			Site B			Site C			Site D			Site E		
Sample		2	1	5	4	1	3	2	5	3	4	1	5	5	2	3
Grids Counted	189 squares			1								1				
Squares counted	1 square	64	64		16	32	16	64	32	64	64		16	64	64	64
Area counted	mm^2	4	4	9	1	2	1	4	2	4	4	9	1	4	4	4
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.4	0.4	0.9	0.1	0.2	0.1	0.4	0.2	0.4	0.4	0.9	0.1	0.4	0.4	0.4
Volume sampled	ml	0.0004	0.0004	0.0009	0.0001	0.0002	0.0001	0.0004	0.0002	0.0004	0.0004	0.0009	0.0001	0.0004	0.0004	0.0004
Tot ball jar volume	ml	395	275	250	250	225	300	395	300	325	311	320	398	305	380	485
Stone area m2	m^2	0.046922	0.055497	0.041777	0.058857	0.0496869	0.0293169	0.0859119	0.0904619	0.0569669	0.0593469	0.0604669	0.0509819	0.0894469	0.0515069	0.0469219
TOT CELL COUNT (n)	n	311	482	320	438	474	1008	331	582	428	283	802	824	287	550	312
TOT CELL CONCENTRATION ($cells.ml^{-1}$)	$cells.ml^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY ($cells.m^{-2}$)	$cells.m^{-2}$	0	0	0	0	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Sample	ml	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella								3								
Achnanthes sp.											3					
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.			52	2					48	32	172	126		19	24	18
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.																
Closterium		1						2		2						
Cocconeis sp.			10	1	5				4	8		2				
Coelastrum sp.															2	
Cosmarium sp.		1						11		2				1	14	2
Crucigenia sp.																
Cymbella sp.		33	164	5		6	22		4	6	4	12		4		2
Cymbopluera sp.								1								
Desmococcus sp.																
Dichothrix sp.				67								196				
Discotella sp.																
Encyonema sp.					10			12		30	6			20		10
Encyonopsis leei var. sinensis																
Encyonopsis leei.		71	102	18		4	42	4	16	8	9	4			20	22
Encyonopsis sp.									20				2		36	
Euastrum															2	
Eunotia sp									2							
Fragilaria sp		5		33	404	422	286	63	130	118	7	6		36	70	60
Frustulia sp.														2		
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		48	94	46	9	26	2	16	32	32	68	130	58	154	310	150
Gyrosigma sp.																
Hantzschia sp.																
Heteroleibleinia				140			404					280	720			
Lynbya sp.		51						22								
Melosira sp.		42	38					48	22	20						
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.		28	22	3	10	10	12	149	12	98		10	2	38	70	28
Netrium																
Nitzschia				1												
Nitzschia Sigma																
Oedogonium sp.		30									14	36	42			
Oocystis sp.																
other																
Pediastrum sp.																12
Phormidium							240		192	24						
Pinnularia						6									2	
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.				4					8	32				12		8
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.														1		
Stauroneis																
Stigeoclonium sp									92	16						
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella		1														

	UNIT	Feb-15														
		Site A			Site B			Site C			Site D			Site E		
Sample		4	5	3	3	1	4	1	4	5	2	5	3	2	5	1
Grids Counted	189 squares	1	1	1	1	1	1				1	1	1			1
Squares counted	1 square							32	64	64				32	16	
Area counted	mm^2	9	9	9	9	9	9	2	4	4	9	9	9	2	1	9
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.9	0.9	0.9	0.2	0.4	0.4	0.9	0.9	0.9	0.2	0.1	0.9
Volume sampled	ml	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0002	0.0004	0.0004	0.0009	0.0009	0.0009	0.0002	0.0001	0.0009
Tot ball jar volume	ml	200	279	200	320	100	255	179	200	250	300	250	226	311	330	198
Stone area m2	m^2	0.0314169	0.0812219	0.0725419	0.0458019	0.0415669	0.0263419	0.0626719	0.0346019	0.0453469	0.0831469	0.0452069	0.0409719	0.0588919	0.0687969	0.0248019
TOT CELL COUNT (n)	n	30	219	325	115	370	112	72	450	592	170	372	34	210	288	258
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	cells.ml $^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	cells.m $^{-2}$	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Sample	ml	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.		2						4								
Achnanthidium exiguum																
Achnanthidium oblongella								2								
Achnanthidium sp.		1		17	10	128	13	19	72	162	21	18	4	37	8	14
Adalfia ap.															2	
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.									130							
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.																
Closterium																
Cocconeis sp.		1	11			12	5	2								
Coelastrum sp.																
Cosmarium sp.									2		2			2		2
Crucigenia sp.																
Cymbella sp.			4	27	2	6		1			1			1		2
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.																68
Discotella sp.																
Encyonema sp.											6			4		
Encyonopsis leei var. sinensis											2			1		
Encyonopsis leei.		4	12	101	4	44	1	8	24	14		8	2			6
Encyonopsis sp.				6		2				8						4
Euastrum																
Eunotia sp							1	3		8						
Fragilaria sp		1	4	3	22	34	1			6	27		2	33	148	18
Frustulia sp.			1						8	4						
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		13	89	134	27	128	69	8		22	70	50	18	102	64	60
Gyrosigma sp.																
Hantzschia sp.																
Heteroleibleinia					50							280				
Lynbya sp.											4					
Melosira sp.										16						
Micrasterias sp.																
Mougeotia sp.											4			2		
Navicula sp.		3	6	23				25	144	226	8			28	8	32
Netrium																
Nitzschia																2
Nitzschia Sigma										6						
Oedogonium sp.											4	4			8	
Oocystis sp.																
other																
Pediastrum sp.		5														
Phormidium			92	14			22			120					40	36
Pinnularia																
Placoneis																2
Planothidium sp.																
Pleurotaenium sp.											1				2	4
Scenedesmus sp.						16					4				8	8
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis																
Stigeoclonium sp									70		16	12	8			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Mar-15														
		Site A			Site B			Site C			Site D			Site E		
Sample		4	2	5	4	3	5	5	3	2	1	5	3	4	3	1
Grids Counted	189 squares		1		1			1	1	1	1		1	1	1	1
Squares counted	1 square	32		64		48	16					32				
Area counted	mm^2	2	9	4	9	3	1	9	9	9	9	2	9	9	9	9
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.2	0.9	0.4	0.9	0.3	0.1	0.9	0.9	0.9	0.9	0.2	0.9	0.9	0.9	0.9
Volume sampled	ml	0.0002	0.0009	0.0004	0.0009	0.0003	0.0001	0.0009	0.0009	0.0009	0.0009	0.0002	0.0009	0.0009	0.0009	0.0009
Tot ball jar volume	ml	421	250	302	272	250	350	358	364	424	253	350	300	350	221	362
Stone area m2	m^2	0.0423019	0.0503169	0.0547969	0.0397469	0.0403419	0.0311019	0.0591019	0.0483919	0.0285119	0.0330269	0.0551469	0.0692519	0.0421619	0.0378219	0.0424419
TOT CELL COUNT (n)	**n**	494	218	195	116	304	569	468	194	164	360	1028	822	124	433	428
TOT CELL CONCENTRATION ($cells.ml^{-1}$)	**$cells.ml^{-1}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY ($cells.m^{-2}$)	**$cells.m^{-2}$**	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Sample	**ml**	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	**ml**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.		26	20	2	8	5	62		10	26		132	398		44	80
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.						1										
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.											24					
Closterium																
Cocconeis sp.		6	3		16	1	14			2						
Coelastrum sp.																
Cosmarium sp.											4					
Crucigenia sp.																
Cymbella sp.		26	29	11		1	56		4					12		
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.				40						8		80	60		40	
Discotella sp.																
Encyonema sp.										1						
Encyonopsis leei var. sinensis																
Encyonopsis leei.		242	86	40		2	10	8	4	4	94	12	46		26	46
Encyonopsis sp.			1	4					2						1	
Euastrum																
Eunotia sp																
Fragilaria sp		8	18	9	77	93	312	22	22	19	66	2	48	30	18	12
Frustulia sp.								6	8		2					
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		30	31	31	7	4	105	32	38	15	64	68	204	38	240	176
Gyrosigma sp.																
Hantzschia sp.																
Heteroleibleinia						190		140				660				
Lynbya sp.																
Melosira sp.																
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.		22	30	6	8	5	10	232	104	23	42	2	4	14	16	14
Netrium						1										
Nitzschia									2							
Nitzschia Sigma																
Oedogonium sp.											26	18	58			
Oocystis sp.																
other																
Pediastrum sp.																
Phormidium		134		52						18				30	40	100
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.															8	
Selenastrum sp																
Sellophora sp.						1										
Staurastrum sp.																
Stauroneis																
Stigeoclonium sp								28		47	38	54	4			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella										1						

	UNIT	Apr-15														
		Site A			Site B			Site C			Site D			Site E		
Sample		1	5	2	3	2	5	5	2	4	3	4	1	3	1	2
Grids Counted	189 squares		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Squares counted	1 square	64														
Area counted	mm^2	4	9	9	9	9	9	9	9	9	9	9	9	9	9	9
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.4	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Volume sampled	ml	0.0004	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
Tot ball jar volume	ml	150	229	375	297	258	319	450	453	400	321	350	307	318	361	374
Stone area m2	m^2	0.0915819	0.0372619	0.0614819	0.0281269	0.0675019	0.0440169	0.0436669	0.0280569	0.0678519	0.0892719	0.0604319	0.0381719	0.0562669	0.0725419	0.0666969
TOT CELL COUNT (n)	**n**	272	56	106	122	48	72	146	390	808	740	714	330	300	358	297
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	**cells.ml $^{-1}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	**cells.m $^{-2}$**	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Sample	**ml**	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	**ml**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.		27		10	14	2	4	6	76	56	64	45		40	20	73
Adalfia ap.																
Anabaena sp?															26	
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.											54		42			
Capartogram sp.																
Chamaesiphon sp.													4			
Closterium				2												
Cocconeis sp.					18	16	33		4	8						
Coelastrum sp.																
Cosmarium sp.														2	6	11
Crucigenia sp.																
Cymbella sp.		17	6	12	4	2								2		2
Cymbopluera sp.																
Desmococcus sp.																3
Dichothrix sp.			20									30	20			
Discotella sp.																
Encyonema sp.			3		8									2		2
Encyonopsis leei var. sinensis																
Encyonopsis leei.		98	15	46				6	80	8	8	39		8	8	14
Encyonopsis sp.		14				1	2	6		12						
Euastrum																
Eunotia sp															2	
Fragilaria sp		22	1	14	32	18	22			24	4			14		
Frustulia sp.								2	4					2		
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		14	6	6	10	4	4		12	12	102	453	10	224	296	176
Gyrosigma sp.																
Hantzschia sp.																
Heteroleibleinia											320		160			
Lynbya sp.											24		50			
Melosira sp.				8					6	18						
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.		80	5	8	14	5	7	116	208	384				6		10
Netrium																
Nitzschia								4								
Nitzschia Sigma																
Oedogonium sp.								6				36	8			
Oocystis sp.																
other											14					
Pediastrum sp.																
Phormidium					18					124						
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.											8					6
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis					4											
Stigeoclonium sp										162	142	111	36			
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	May-15														
		Site A			Site B			Site C			Site D			Site E		
Sample		4	5	3	5	4	2	2	5	3	3	1	5	1	3	4
Grids Counted	189 squares		1	1	1	1		1	1	1				1		
Squares counted	1 square	48					64				48	32	32		32	32
Area counted	mm^2	3	9	9	9	9	4	9	9	9	3	2	2	9	2	2
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.3	0.9	0.9	0.9	0.9	0.4	0.9	0.9	0.9	0.3	0.2	0.2	0.9	0.2	0.2
Volume sampled	ml	0.0003	0.0009	0.0009	0.0009	0.0009	0.0004	0.0009	0.0009	0.0009	0.0003	0.0002	0.0002	0.0009	0.0002	0.0002
Tot ball jar volume	ml	400	190	338	188	323	271	336	415	300	300	324	467	358	338	248
Stone area m2	m^2	0.0493719	0.0505969	0.0705119	0.0573869	0.0486719	0.0426169	0.0574569	0.0569319	0.0411819	0.0484969	0.0522069	0.0652619	0.0492669	0.0436669	0.0651219
TOT CELL COUNT (n)	n	297	55	181	26	38	235	314	200	628	802	633	1171	186	558	336
TOT CELL CONCENTRATION ($cells.ml^{-1}$)	$cells.ml^{-1}$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY ($cells.m^{-2}$)	$cells.m^{-2}$	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Sample	ml	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	ml	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella							13									
Achnanthidium sp.			4	33		1	53	44	34	56	12	22	96	50	206	154
Adalfia ap.																
Anabaena sp?																
Ankistrodesmus															16	
Aulacoseira sp.																
Batrachospermum sp.													16			
Capartogram sp.																2
Chamaesiphon sp.											6		12			
Closterium																
Cocconeis sp.		2	2	3	6	12	56		4	28						
Coelastrum sp.																
Cosmarium sp.											2				4	14
Crucigenia sp.																
Cymbella sp.		2	1			2	4	4	4	12				2	6	
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.		254	20	90												
Discotella sp.									1							
Encyonema sp.				2	2	2	2	6						4		
Encyonopsis leei var. sinensis																
Encyonopsis leei.		13	4	14	1		5	16	46	28	40	8		5	44	
Encyonopsis sp.									2	8					14	20
Euastrum																
Eunotia sp																
Fragilaria sp				3	2	15		22		8	18			14	44	
Frustulia sp.					1		24		9					1		
Gimenella																
Gleocapsa sp.																
Gomphonema sp.		22	4	30	2		16	22	33	44	12	54	152	58	192	76
Gyrosigma sp.																
Hantzschia sp.													2			
Heteroleibleinia											240	450	680			
Lynbya sp.											20	60				
Melosira sp.								12								
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.		2		4	9	6	62	186	66	284	46		7	47	16	28
Netrium																
Nitzschia					1			2								
Nitzschia Sigma																
Oedogonium sp.		2	2	2								15	14			
Oocystis sp.																
other			18													
Pediastrum sp.															16	
Phormidium										160	216		192			
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.														4		16
Selenastrum sp														1		24
Sellophora sp.					1											2
Staurastrum sp.																
Stauroneis									1							
Stigeoclonium sp											190	24				
Surirella sp.					1											
Tabellaria sp																
Tetraedron sp.																
Tribonella																

	UNIT	Jun-15														
		Site A			Site B			Site C			Site D			Site E		
Sample		2	4	5	4	3	2	4	3	2	4	1	5	4	3	5
Grids Counted	189 squares	1	1	1	1	1	1		1			1	1	1		
Squares counted	1 square							32		64	32				64	16
Area counted	mm^2	9	9	9	9	9	9	2	9	4	2	9	9	9	4	1
chamber deapth	mm	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Volume sampled	mm^3	0.9	0.9	0.9	0.9	0.9	0.9	0.2	0.9	0.4	0.2	0.9	0.9	0.9	0.4	0.1
Volume sampled	ml	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0002	0.0009	0.0004	0.0002	0.0009	0.0009	0.0009	0.0004	0.0001
Tot ball jar volume	ml	376	200	328	350	350	300	310	300	405	400	357	428	350	419	300
Stone area m2	m^2	0.0402019	0.0461169	0.0350569	0.0482169	0.0319069	0.0284419	0.0514019	0.0533269	0.0542719	0.0098989	0.0618669	0.0495469	0.0677819	0.0483219	0.0313819
TOT CELL COUNT (n)	**n**	44	58	70	52	11	156	400	114	534	365	189	184	188	390	434
TOT CELL CONCENTRATION (cells.ml $^{-1}$)	**cells.ml $^{-1}$**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOT CELL DENSITY (cells.m $^{-2}$)	**cells.m $^{-2}$**	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Sample	**ml**	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Pellet Volume	**ml**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilution factor		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cell counts																
Achnanthes oblongella																
Achnanthes sp.																
Achnanthidium exiguum																
Achnanthidium oblongella																
Achnanthidium sp.			4	7	2		4	140	24	50	18	3	54	58	125	160
Adalfia ap.														1		
Anabaena sp?																
Ankistrodesmus																
Aulacoseira sp.																
Batrachospermum sp.																
Capartogram sp.																
Chamaesiphon sp.																
Closterium																
Cocconeis sp.		2	3	1	15	3	15	8						2		
Coelastrum sp.																
Cosmarium sp.							2	2								6
Crucigenia sp.																
Cymbella sp.				1	1		7						2	14	4	40
Cymbopluera sp.																
Desmococcus sp.																
Dichothrix sp.		22	28	30							30					
Discotella sp.																
Encyonema sp.		1		6				6		8					15	4
Encyonopsis leei var. sinensis																
Encyonopsis leei.			2	6		2	2	52	6	68	1	1	4		3	
Encyonopsis sp.					1				4							
Euastrum														1		
Eunotia sp																
Fragilaria sp							66	24		46			4	19	39	56
Frustulia sp.								8	26	2					1	
Gimenella												10				
Gleocapsa sp.																
Gomphonema sp.		7	16	10	4		5	22	4		52	15	40	85	175	164
Gyrosigma sp.					2											
Hantzschia sp.																
Heteroleibleinia											170	160	60			
Lynbya sp.																
Melosira sp.									6							
Micrasterias sp.																
Mougeotia sp.																
Navicula sp.			3	9	15	2	55	134	42	214					16	4
Netrium																
Nitzschia					12	3		4								
Nitzschia Sigma																
Oedogonium sp.													20			
Oocystis sp.																
other														8		
Pediastrum sp.																
Phormidium		12								110	80					
Pinnularia																
Placoneis																
Planothidium sp.																
Pleurotaenium sp.																
Scenedesmus sp.			2								4				12	
Selenastrum sp																
Sellophora sp.																
Staurastrum sp.																
Stauroneis						1										
Stigeoclonium sp									2	36	10					
Surirella sp.																
Tabellaria sp																
Tetraedron sp.																
Tribonella																

Appendix 5

Algal growth form divisions at each site during the sampling period

Site A													
	June	July	August	September	October	November	December	January	February	March	April	May	June
Cyanobacteria	4343229.5	0	0	139587806	0	302965778	33309388	81656160.2	13134131	264520399	4552361	247410078	26670814.8
Diatom	9265950.3	15338588	13570620	132498983	13459586	305997626	238913634	383140085	53808698	684557401	68813306	54795142.5	21822488.1
Green	5189499.9	7609670	0	3890655.7	1223942.6	2862627	5528557.9	23335193.9	1178889	0	451804.2	2433630.47	321244.811
Std Dev													
Cyanobacteria	7522694.1	0	0	214576588	0	418182243	57693552	72321285.1	19155255	354104429	7884921	380302451	11599626.2
Diatom	5009833.8	13284811	13240329	167921981	10705231	294000468	234550616	275358731	39067959	958622165	43420524	52623710.1	17183334.8
Green	8388088	13180335	0	6738813.4	2119930.7	4958215.4	5130985.5	38137625.7	2041896	0	782547.7	2572564.21	556412.334
Site **B**													
	June	July	August	September	October	November	December	January	February	March	April	May	June
Cyanobacteria	4309566.2	0	0	4091680.5	8278776.4	262671737	17616430	2196685188	20825896	130826207	7039524	0	0
Diatom	6023500.4	34105340	9341273	73267940	733523860	2.216E+09	993405115	2219493031	80629791	2241589944	66793589	137024633	78610689.8
Green	0	0	16504079	4091680.5	827877.64	87250775	0	0	1425636	688558.987	0	0	781319.892
std dev													
Cyanobacteria	7464387.7	0	0	7086998.4	14339261	285249083	30512552	3804770355	19562183	226597638	12192814	0	0
Diatom	5089018.4	39155753	4385197	21439383	526350810	2.438E+09	116024325	1361772331	26151474	3604744432	51387230	205081638	89371690.2
Green	0	0	19677171	7086998.4	1433926.1	142501100	0	0	2469274	1192619.15	0	0	1353285.75
Site **C**													
	June	July	August	September	October	November	December	January	February	March	April	May	June
Cyanobacteria	278656.79	0	0	59263263	0	57755578	0	125961337	55130560	45728609.6	27074217	43168911	68405565.3
Diatom	68494181	3.55E+08	5.34E+08	403981980	1.959E+09	715432170	129438335	441047870	4.33E+08	171470832	4.01E+08	248273164	664671296
Green	1275034.9	3.41E+08	18588210	242108889	65891629	14625886	52862457	84974724.1	34680177	32168207.8	37661218	0	24814294.7
std dev													
Cyanobacteria	482647.72	0	0	82022683	0	53051487	0	166687375	95488931	47173550.2	46893919	74770747.2	118481915
Diatom	68707818	4.92E+08	5.63E+08	385280644	988626537	965701506	92956250	87948812.9	2.91E+08	27011751.3	2.74E+08	114983296	567390647
Green	1408257.3	5.87E+08	23780859	219470585	77356483	25332777	91560461	76015072.6	60067828	40507810.5	59380823	0	36751858.1
Site **D**													
	June	July	August	September	October	November	December	January	February	March	April	May	June
Cyanobacteria	235863693	0	0	23932425	315918841	252737705	534523007	1966904795	57884018	799193765	1.22E+08	1913094505	1939122863
Diatom	207072760	6.77E+08	76204909	91707471	78025131	97187757	215566807	335651668	38918330	416828841	1.42E+08	481369241	515501672
Green	35340472	1.07E+08	52367025	9265489.4	45144119	44942125	79681727	122463663	9054078	105400716	84317276	208085609	102822501
std dev													
Cyanobacteria	328514643	0	0	34528671	229328894	322201576	304706392	3167475138	98872563	1341548998	95843779	1121633453	2296840903
Diatom	78231378	2.4E+08	27025008	118902884	21566252	72348152	963901572	157415889	20247123	238904623	1.79E+08	379451608	588933929
Green	19376097	1.53E+08	90702349	7456510.5	32293350	55185835	59497876	177902311	3822098	107508179	9201293	162797207	111701385
Site **E**													
	June	July	August	September	October	November	December	January	February	March	April	May	June
Cyanobacteria	0	0	0	145715080	51419639	155117699	142031787	0	94706702	58126843.1	4792122	0	0
Diatom	0	0	0	5.758E+09	735818392	2.397E+09	1.636E+09	654444393	5.9E+08	207179024	1.8E+08	901116400	307369915
Green	0	0	0	271335452	8034318.6	37753966	5718295.3	33994559	36440409	1731311.26	5678186	82062340.1	8862261.86
	0	0	0	252385922	89061427	268671737	246006271	0	95958125	33974722.3	8300199	0	0
	0	0	0	2.806E+09	180349747	168682551	887191149	383177959	4.91E+08	113013017	7277077	988700538	446403815
	0	0	0	100490809	13915848	58138479	9904378	22468220.2	43225236	2998719.07	5963870	69993727.1	14855788.2

Appendix 6

Invertebrate grazer family abundance at density at each site over the sampling period

Site A													
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	77	133	71	94	109	60	98	82	84	55	143	60	75
Chironom	1	24	24	34	7	1	10	0	205	140	123	17	24
Heptagen	0	1	4	4	4	8	10	2	4	6	4	3	7
Leptophle	1	21	11	24	0	1	4	24	3	2	14	33	29
Elmidae- L	1	1	15	6	2	11	0	4	10	6	1	0	1
stone surf	0.037087	0.048218	0.053166	0.045612	0.049596	0.066375	0.058905	0.046558	0.066417	0.052998	0.065017	0.060026	0.048126
	80	180	125	162	122	81	122	112	306	209	285	113	136
density	2157.096	3733.023	2351.131	3551.736	2459.881	1220.341	2071.135	2405.607	4607.261	3943.552	4383.476	1882.521	2825.921
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	2076.205	2758.289	1335.442	2060.884	2197.762	903.9562	1663.699	1761.248	1264.738	1037.777	2199.428	999.5685	1558.412
Chironom	26.9637	497.7363	451.4172	745.426	141.1407	15.06594	169.7652	0	3086.564	2641.614	1891.816	283.2111	498.692
Heptagen	0	20.73901	75.23619	87.69718	80.65183	120.5275	169.7652	42.95726	60.22564	113.212	61.52247	49.97843	145.4518
Leptophle	26.9637	435.5193	206.8995	526.1831	0	15.06594	67.90607	515.4872	45.16923	37.73734	215.3286	549.7627	602.5861
Elmidae- L	26.9637	20.73901	282.1357	131.5458	40.32591	165.7253	0	85.91453	150.5641	113.212	15.38062	0	20.77883
Site B													
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	504	353	449	110	80	61	8	32	19	29	199	151	287
Chironom	5	101	37	32	1	1	1	0	36	177	237	67	183
Heptagen	2	1	2	0	0	0	0	0	0	0	7	4	9
Leptophle	1	53	26	31	10	0	2	2	1	0	6	15	32
Elmidae- L	0	0	2	3	3	0	2	2	0	0	3	0	0
stone surf	0.041483	0.041456	0.047706	0.029065	0.045536	0.054363	0.055454	0.044311	0.033902	0.036912	0.056918	0.04716	0.039208
	512	508	516	176	94	62	13	36	56	206	452	237	511
density	12342.44	12253.87	10816.27	6055.414	2064.305	1140.484	234.429	812.4412	1651.825	5580.856	7941.263	5025.456	13033.09
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	12149.58	8514.99	9411.834	3784.634	1756.856	1122.089	144.264	722.1699	560.4406	785.6545	3496.264	3201.873	7319.953
Chironom	120.5316	2436.3	775.5854	1100.984	21.96069	18.3949	18.033	0	1061.887	4795.202	4163.892	1420.699	4667.427
Heptagen	48.21264	24.12179	41.92354	0	0	0	0	0	0	0	122.9842	84.81782	229.5456
Leptophle	24.10632	1278.455	545.006	1066.579	219.6069	0	36.06599	45.13562	29.49687	0	105.415	318.0668	816.162
Elmidae- L	0	0	41.92354	103.2173	65.88208	0	36.06599	45.13562	0	0	52.7075	0	0
Site C													
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	70	101	64	63	36	82	74	21	29	37	74	147	149
Chironom	400	179	36	37	3	0	1	225	255	136	153	128	157
Heptagen	0	1	0	3	0	0	0	0	1	0	0	0	1
Leptophle	0	20	6	7	1	0	2	2	0	0	0	0	2
Elmidae- L	0	0	0	3	1	0	1	0	1	1	0	0	0
stone surf	0.086275	0.053894	0.043898	0.043457	0.040664	0.076357	0.042777	0.064975	0.052592	0.045627	0.050135	0.050478	0.052249
	470	301	106	113	41	82	78	248	286	174	227	275	309
density	5447.728	5585.048	2414.694	2600.278	1008.265	1073.904	1823.414	3816.859	5438.1	3813.54	4527.784	5447.929	5914
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	811.3637	1874.053	1457.929	1449.712	885.3061	1073.904	1729.906	323.2017	551.4157	810.9251	1476.018	2912.166	2851.735
Chironom	4636.364	3321.341	820.0848	851.4183	73.77551	0	23.3771	3462.876	4848.655	2980.698	3051.766	2535.763	3004.848
Heptagen	0	18.55498	0	69.03392	0	0	0	0	19.01433	0	0	0	19.13916
Leptophle	0	371.0995	136.6808	161.0791	24.59184	0	46.75421	30.78112	0	0	0	0	38.27832
Elmidae- L	0	0	0	69.03392	24.59184	0	23.3771	0	19.01433	21.9169	0	0	0
Site D													
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	60	47	46	58	32	78	12	14	8	25	12	22	20
Chironom	259	82	59	34	2	0	0	14	11	9	5	58	64
Heptagen	0	0	1	3	0	0	3	2	0	0	2	1	0
Leptophle	0	7	8	8	0	0	0	0	0	0	0	0	0
Elmidae- L	0	0	1	2	0	0	1	1	0	0	0	0	0
stone surf	0.038585	0.055784	0.053043	0.118917	0.050499	0.064114	0.06335	0.052368	0.050716	0.056463	0.06039	0.058913	0.045628
	319	136	115	105	34	78	16	31	19	34	19	81	84
density	8267.483	2437.979	2168.065	882.9695	673.282	1216.585	252.5655	591.9657	374.636	602.1653	314.6221	1374.911	1840.963
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae	1555.012	842.537	867.2258	487.7356	633.6772	1216.585	189.4241	267.3393	157.7415	442.7686	198.7087	373.4326	438.3245
Chironom	6712.47	1469.958	1112.311	285.9139	39.60482	0	0	267.3393	216.8945	159.3967	82.7953	984.5042	1402.638
Heptagen	0	0	18.85274	25.2277	0	0	47.35603	38.19133	0	0	33.11812	16.97421	0
Leptophle	0	125.4842	150.8219	67.27387	0	0	0	0	0	0	0	0	0
Elmidae- L	0	0	18.85274	16.81847	0	0	15.78534	19.09567	0	0	0	0	0
Site E													
	June	July	August	Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae				14	27	24	105	71	105	19	60	47	84
Chironomidae				1	18	6	2	333	294	14	57	27	31
Heptageniidae				1	3	0	3	0	10	6	10	22	17
Leptophlebiidae				1	13	20	1	4	3	7	21	33	48
Elmidae- Larvae				2	0	0	0	0	5	0	0	2	0
stone surface area				0.01181	0.057401	0.054965	0.069328	0.055798	0.068027	0.043576	0.07103	0.050436	0.046586
				19	61	50	111	408	417	46	148	131	180
density				1608.82	1062.701	909.6714	1601.087	7312.103	6129.928	1055.629	2083.63	2597.356	3863.83
				Septembe	October	Novembe	Decembe	January	February	March	April	May	June
Baetidae				1185.446	470.3759	436.6423	1514.542	1272.449	1543.507	436.0208	844.7147	931.8759	1803.121
Chironomidae				84.67472	313.5839	109.1606	28.84841	5967.967	4321.82	321.2785	802.479	535.333	665.4374
Heptageniidae				84.67472	52.26399	0	43.27262	0	147.0007	137.6908	140.7858	436.1972	364.9173
Leptophlebiidae				84.67472	226.4773	363.8686	14.42421	71.68729	44.1002	160.6393	295.6501	654.2958	1030.355
Elmidae- Larvae				169.3494	0	0	0	0	73.50034	0	0	39.65429	0

Appendix 7

Total grazer invertebrate abundance and density at each site over the sampling period.

Adundance					
	Site A: Nu	Site B: Nu	Site C: Una	Site D: Alt	Site E: Ref
June	80	512	470	319	
July	180	508	301	136	
August	125	516	106	115	
Septembe	162	176	113	105	19
October	122	94	41	34	61
Novembe	81	62	82	78	50
Decembe	122	13	78	16	111
January	112	36	248	31	408
February	306	56	286	19	417
March	209	206	174	34	46
April	285	452	227	19	148
May	113	237	275	81	131
June	136	511	309	84	180
Density					
	Site A: Nu	Site B: Nu	Site C: Una	Site D: Alt	Site E: Ref
June	2157.096	12342.44	5447.728	8267.483	
July	3733.023	12253.87	5585.048	2437.979	
August	2351.131	10816.27	2414.694	2168.065	
Septembe	3551.736	6055.414	2600.278	882.9695	1608.82
October	2459.881	2064.305	1008.265	673.282	1062.701
Novembe	1220.341	1140.484	1073.904	1216.585	909.6714
Decembe	2071.135	234.429	1823.414	252.5655	1601.087
January	2405.607	812.4412	3816.859	591.9657	7312.103
February	4607.261	1651.825	5438.1	374.636	6129.928
March	3943.552	5580.856	3813.54	602.1653	1055.629
April	4383.476	7941.263	4527.784	314.6221	2083.63
May	1882.521	5025.456	5447.929	1374.911	2597.356
June	2825.921	13033.09	5914	1840.963	3863.83

Appendix 8

Actual and modelled water temperature at each site over the sampling period.

		Site C		Site D		Site E	
		y = 0.8911x - 1.8802		y = 0.724x + 2.7958		y = 1.1969x - 6.1708	
	Air Temp	Water Temp	Modelled WT	Water Temp	Modelled WT	Water Temp	Modelled WT
2014/06/01 01:00	18.40322		14.51890897		16.11973098		15.85601352
2014/06/02 01:00	16.83598		13.12234215		14.98504982		13.98018496
2014/06/03 01:00	15.24018		11.70032819		13.8296934		12.07017653
2014/06/04 01:00	20.29766		16.20704665		17.49130732		18.1234717
2014/06/05 01:00	14.09784		10.6823853		13.00263622		10.7029048
2014/06/06 01:00	9.94837		6.984792321		9.998419729		5.736403804
2014/06/07 01:00	9.639443		6.709507954		9.774756973		5.366649726
2014/06/08 01:00	14.59952		11.12943298		13.36585305		11.30336644
2014/06/09 01:00	17.17696		13.42618872		15.23191877		14.38830298
2014/06/10 01:00	10.701		7.655464479		10.54332675		6.637231438
2014/06/11 01:00	10.92112		7.851613374		10.7026936		6.900693016
2014/06/12 01:00	10.90094		7.833626075		10.68807929		6.876532991
2014/06/13 01:00	11.64532		8.496941273		11.22700893		7.76747897
2014/06/14 01:00	13.72834		10.35312151		12.73511632		10.2606471
2014/06/15 01:00	16.62428		12.93369327		14.83177658		13.72679719
2014/06/16 01:00	12.78919		9.516243422		12.05517048		9.136576424
2014/06/17 01:00	14.25279		10.82045913		13.1148183		10.88836161
2014/06/18 01:00	15.43125		11.87058591		13.96802422		12.29886183
2014/06/19 01:00	12.14392		8.941251233		11.58800143		8.364263384
2014/06/20 01:00	11.02708		7.946027201		10.77940284		7.027506965
2014/06/21 01:00	11.17315		8.076194188		10.88516078		7.202343534
2014/06/22 01:00	13.15624		9.843321974		12.32091492		9.575898968
2014/06/23 01:00	15.02922		11.51233349		13.67695166		11.81766743
2014/06/24 01:00	13.43463		10.09140006		12.52247315		9.909110343
2014/06/25 01:00	13.87342		10.48240412		12.84015572		10.4342958
2014/06/26 01:00	16.288		12.63404085		14.58831529		13.32431264
2014/06/27 01:00	12.75974		9.490003571		12.03385116		9.101331809
2014/06/28 01:00	14.39332		10.94568634		13.21656278		11.05656321
2014/06/29 01:00	15.57635		11.99988708		14.0730787		12.47253546
2014/06/30 01:00	16.2324		12.58449454		14.54805995		13.25776345
2014/07/01 01:00	18.4825		14.58955241		16.17712729		15.95089976
2014/07/02 01:00	16.22998		12.58233611		14.54630627		13.25486431
2014/07/03 01:00	16.05059		12.4224819		14.4164281		13.04015272
2014/07/04 01:00	15.70284		12.11260169		14.16465694		12.62393049
2014/07/05 01:00	16.67181		12.97605138		14.86619165		13.78369138
2014/07/06 01:00	9.684759		6.749888968		9.807565697		5.420888346
2014/07/07 01:00	8.854987		6.010478582		9.206810317		4.427733491
2014/07/08 01:00	8.177081		5.406396545		8.716006373		3.6163478
2014/07/09 01:00	8.434766		5.636020168		8.902570735		3.924771675
2014/07/10 01:00	9.143563		6.267629323		9.415739884		4.773131004
2014/07/11 01:00	11.02926		7.947970653		10.78098186		7.030117354
2014/07/12 01:00	13.07589		9.771725133		12.262744		9.479732143
2014/07/13 01:00	11.3546		8.237883206		11.01652971		7.419519593
2014/07/14 01:00	11.51874		8.384144981		11.13536432		7.615974221
2014/07/15 01:00	11.41803		8.294407721		11.06245469		7.495441703
2014/07/16 01:00	15.56039		11.98565937		14.06151898		12.45342521
2014/07/17 01:00	19.14256		15.17773388		16.65501235		16.74092827
2014/07/18 01:00	14.72707		11.24308844		13.45819572		11.4560252
2014/07/19 01:00	13.44986		10.10497199		12.53350006		9.927339778
2014/07/20 01:00	13.22946		9.90857292		12.37392995		9.66354217
2014/07/21 01:00	14.70993		11.22782055		13.44579089		11.43551781
2014/07/22 01:00	14.01058		10.60462565		12.93945814		10.59846026
2014/07/23 01:00	13.6758		10.30630245		12.69707682		10.19776108
2014/07/24 01:00	14.02383		10.61643265		12.94905108		10.61431908
2014/07/25 01:00	17.05251		13.31528758		15.14181392		14.23934373
2014/07/26 01:00	12.82328		9.546625588		12.07985535		9.177384879
2014/07/27 01:00	12.15509		8.951199845		11.59608447		8.377626074
2014/07/28 01:00	12.00224		8.81499198		11.48541844		8.19467557
2014/07/29 01:00	13.32732		9.995776189		12.44478077		9.780671103
2014/07/30 01:00	15.04688		11.52807521		13.68974148		11.83881127
2014/07/31 01:00	16.081		12.44958177		14.43844617		13.07655249

2014/08/01 01:00	14.72087	11.23756377	13.45370704	11.44860462
2014/08/02 01:00	17.04698	13.31036176	15.1378118	14.23272752
2014/08/03 01:00	19.12696	15.16383591	16.64372055	16.72226092
2014/08/04 01:00	19.07983	15.12183911	16.60959903	16.66585202
2014/08/05 01:00	16.87768	13.15950213	15.01524153	14.03009719
2014/08/06 01:00	16.65718	12.96301718	14.85560164	13.76618423
2014/08/07 01:00	17.72735	13.91663936	15.63039959	15.04706222
2014/08/08 01:00	18.16776	14.30908648	15.94925462	15.57418596
2014/08/09 01:00	17.76227	13.94775508	15.65568046	15.08885598
2014/08/10 01:00	16.87324	13.15554045	15.01202274	14.02477597
2014/08/11 01:00	17.6804	13.87480258	15.59640809	14.99086827
2014/08/12 01:00	18.28693	14.41527924	16.035534	15.71682103
2014/08/13 01:00	19.64807	15.62819295	17.02100087	17.34597199
2014/08/14 01:00	16.87843	13.16016675	15.01578151	14.03098987
2014/08/15 01:00	14.37742	10.93151636	13.20504997	11.03753051
2014/08/16 01:00	15.036	11.51837589	13.68186098	11.82578341
2014/08/17 01:00	16.2625	12.61131412	14.5698503	13.29378675
2014/08/18 01:00	18.76363	14.84007106	16.38066842	16.28738925
2014/08/19 01:00	22.56402	18.22659822	19.13215048	20.83607554
2014/08/20 01:00	20.36019	16.26276382	17.53657635	18.19830942
2014/08/21 01:00	17.44129	13.66173241	15.42329306	14.7046785
2014/08/22 01:00	13.81443	10.42983939	12.79744798	10.36369236
2014/08/23 01:00	12.55887	9.31100876	11.88842164	8.860911104
2014/08/24 01:00	13.09191	9.786004231	12.27434546	9.498911418
2014/08/25 01:00	13.04761	9.746523712	12.24226837	9.445882314
2014/08/26 01:00	15.50455	11.93590317	14.02109311	12.3865941
2014/08/27 01:00	19.64166	15.6224836	17.01636214	17.33830335
2014/08/28 01:00	16.52463	12.8448946	14.75962953	13.60752536
2014/08/29 01:00	12.30032	9.080619422	11.70123515	8.551458743
2014/08/30 01:00	13.57239	10.21415387	12.62220804	10.07398975
2014/08/31 01:00	14.21624	10.78789581	13.08836129	10.84462349
2014/09/01 01:00	14.68735	11.20769536	13.42943959	11.40848622
2014/09/02 01:00	13.90928	10.51435677	12.86611658	10.47721369
2014/09/03 01:00	16.02831	12.40262808	14.40029728	13.01348564
2014/09/04 01:00	17.43139	13.65291434	15.41612856	14.69283433
2014/09/05 01:00	17.1302	13.3845256	15.19806836	14.33234226
2014/09/06 01:00	20.18094	16.10303352	17.40679884	17.98376424
2014/09/07 01:00	18.96965	15.02365846	16.52982932	16.53397857
2014/09/08 01:00	18.81434	14.88525503	16.41737945	16.34807906
2014/09/09 01:00	20.26174	16.17504023	17.46530278	18.08048159
2014/09/10 01:00	17.85092	14.02675333	15.71986487	15.19496415
2014/09/11 01:00	17.9015	14.07183073	15.75648932	15.25551084
2014/09/12 01:00	18.49079	14.59694223	16.18313136	15.96082555
2014/09/13 01:00	19.92279	15.87300003	17.21990147	17.67478984
2014/09/14 01:00	22.18683	17.89048161	18.85906281	20.38461334
2014/09/15 01:00	26.52904	21.75982754	22.00282496	25.58180798
2014/09/16 01:00	21.68059	17.43937598	18.49254897	19.77870116
2014/09/17 01:00	21.85436	17.59422391	18.61835966	19.98668847
2014/09/18 01:00	24.41175	19.87311005	20.4699067	23.04762308
2014/09/19 01:00	14.45831	11.00360041	13.26361674	11.13435174
2014/09/20 01:00	13.52757	10.17421488	12.58975845	10.02034484
2014/09/21 01:00	16.1844	12.54171884	14.5133056	13.20030836
2014/09/22 01:00	18.69993	14.78330465	16.33454691	16.21114223
2014/09/23 01:00	22.16724	17.87302831	18.84488236	20.36117055
2014/09/24 01:00	22.42644	18.10400254	19.03254407	20.67140853
2014/09/25 01:00	20.26693	16.17966207	17.46905792	18.08668951
2014/09/26 01:00	21.20895	17.01909943	18.15108312	19.21419774
2014/09/27 01:00	17.30553	13.5407563	15.32500251	14.54218686
2014/09/28 01:00	12.79707	9.523265364	12.06087566	9.146008096
2014/09/29 01:00	18.24452	14.37748843	16.00482977	15.6660615
2014/09/30 01:00	18.2584	14.38985875	16.01488039	15.68267697

2014/10/01 01:00	15.63046	12.04809845	14.11224942	12.53729159
2014/10/02 01:00	12.24774	9.033757401	11.66316074	8.488515019
2014/10/03 01:00	13.68803	10.31720242	12.70593282	10.21240161
2014/10/04 01:00	15.58014	12.00326216	14.07582088	12.47706877
2014/10/05 01:00	16.39881	12.73277922	14.66853814	13.45693519
2014/10/06 01:00	20.55812	16.43914073	17.67987888	18.43521383
2014/10/07 01:00	25.05406	20.4454699	20.93493703	23.81640042
2014/10/08 01:00	18.30805	14.43410224	16.0508273	15.74210355
2014/10/09 01:00	13.64209	10.27626491	12.67267195	10.15741553
2014/10/10 01:00	12.35114	9.125901597	11.73802596	8.612280463
2014/10/11 01:00	19.95759	15.90401253	17.24509848	17.71644496
2014/10/12 01:00	21.36528	17.15839767	18.26426001	19.40129914
2014/10/13 01:00	17.92849	14.09588004	15.77602887	15.28781317
2014/10/14 01:00	16.5492	12.86679286	14.7774214	13.63693848
2014/10/15 01:00	16.71131	13.01124945	14.89478935	13.83096844
2014/10/16 01:00	14.9923	11.47944187	13.65022792	11.77348836
2014/10/17 01:00	14.51824	11.05700626	13.30700787	11.20608495
2014/10/18 01:00	15.21837	11.68088654	13.81389747	12.04406306
2014/10/19 01:00	15.49356	11.92611299	14.0131388	12.37344421
2014/10/20 01:00	15.01035	11.49552103	13.66329189	11.79508542
2014/10/21 01:00	15.14423	11.61482261	13.76022192	11.95532789
2014/10/22 01:00	16.10102	12.46742152	14.45294059	13.10051433
2014/10/23 01:00	18.67141	14.75789642	16.31390325	16.17701462
2014/10/24 01:00	19.81194	15.77422011	17.13964486	17.54211148
2014/10/25 01:00	18.00897	14.16759688	15.8342973	15.38414118
2014/10/26 01:00	14.40003	10.95166339	13.22141901	11.06459142
2014/10/27 01:00	18.72301	14.80387718	16.35126165	16.23877466
2014/10/28 01:00	21.41172	17.19978406	18.29788558	19.45688817
2014/10/29 01:00	23.99935	19.50561781	20.17132699	22.55401803
2014/10/30 01:00	23.29476	18.87776101	19.66120654	21.71069874
2014/10/31 01:00	15.94495	12.32834383	14.3399429	12.91370916
2014/11/01 01:00	14.71946	11.23630895	13.45268753	11.44691918
2014/11/02 01:00	19.75365	15.722279	17.09744381	17.47234568
2014/11/03 01:00	20.17629	16.09889165	17.40343366	17.978201
2014/11/04 01:00	22.75258	18.39462255	19.26866671	21.06176101
2014/11/05 01:00	18.09463	14.24392071	15.8963088	15.48665716
2014/11/06 01:00	15.49818	11.93022708	14.01648142	12.37897015
2014/11/07 01:00	14.86302	11.36423341	13.55662346	11.61874365
2014/11/08 01:00	18.79746	14.87021586	16.40516044	16.32787888
2014/11/09 01:00	24.59717	20.03834079	20.60415319	23.26955626
2014/11/10 01:00	18.42831	14.5412689	16.13789795	15.88604673
2014/11/11 01:00	16.78526	13.0771463	14.94832915	13.91947919
2014/11/12 01:00	21.79146	17.53816852	18.57281583	19.91139648
2014/11/13 01:00	21.5849	17.3541029	18.42326639	19.66416482
2014/11/14 01:00	20.47397	16.36415541	17.61895488	18.33449569
2014/11/15 01:00	17.91852	14.0869902	15.76880607	15.2758726
2014/11/16 01:00	13.87193	10.48108091	12.83908064	10.4325185
2014/11/17 01:00	15.19073	11.65625987	13.79388882	12.01098524
2014/11/18 01:00	16.17071	12.52952302	14.50339676	13.18392729
2014/11/19 01:00	17.03849	13.30280252	15.13167008	14.22257417
2014/11/20 01:00	18.57218	14.66946774	16.24205681	16.05823975
2014/11/21 01:00	16.37175	12.70867014	14.64895002	13.42455256
2014/11/22 01:00	21.1479	16.96469518	18.10688081	19.1411235
2014/11/23 01:00	21.17516	16.98898062	18.12661222	19.17374302
2014/11/24 01:00	20.42606	16.32145835	17.58426442	18.27714623
2014/11/25 01:00	18.06741	14.21967276	15.87660786	15.45408802
2014/11/26 01:00	18.0294	14.18579983	15.84908681	15.40859085
2014/11/27 01:00	22.17636	17.88115105	18.85148193	20.3720808
2014/11/28 01:00	18.48945	14.59575038	16.18216301	15.9592247
2014/11/29 01:00	17.10516	13.36221179	15.17993886	14.30237099
2014/11/30 01:00	16.42651	12.75746677	14.68859626	13.49009481

2014/12/01 01:00	19.38791			15.39636549			16.83264594			17.03458798
2014/12/02 01:00	20.78538			16.64165323			17.84441603			18.70722282
2014/12/03 01:00	19.3391			15.35287609			16.79731172			16.97617428
2014/12/04 01:00	21.37812			17.16984125			18.27355767			19.41666983
2014/12/05 01:00	17.96752			14.13065856			15.80428569			15.33452668
2014/12/06 01:00	17.17494			13.42439015			15.23045747			14.38588718
2014/12/07 01:00	19.83845			15.79784242			17.1588375			17.57384031
2014/12/08 01:00	18.50066			14.60573367			16.19027422			15.97263397
2014/12/09 01:00	20.19713			16.11746217			17.41852182			18.0031444
2014/12/10 01:00	19.89001			15.84378605			17.19616573			17.63555048
2014/12/11 01:00	21.76716			17.51651628			18.55522384			19.8823138
2014/12/12 01:00	23.561			19.11500636			19.8539634			22.0293599
2014/12/13 01:00	17.34581			13.57665166			15.35416674			14.59040049
2014/12/14 01:00	23.86726			19.38791687			20.07569745			22.39592549
2014/12/15 01:00	20.16925			16.09261793			17.3983364			17.96977433
2014/12/16 01:00	17.46228			13.68043994			15.43849253			14.72980592
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2014/12/18 01:00	23.5679			19.1211542			19.85895839			22.03761752
2014/12/19 01:00	22.03646			17.75648802			18.75019583			20.20463698
2014/12/20 01:00	20.25649			16.17035675			17.46149755			18.07419089
2014/12/21 01:00	22.82668			18.46065269			19.32231481			21.1504508
2014/12/22 01:00	20.59503		23.12608333	16.47203123			17.70660172		20.54291667	18.47939141
2014/12/23 01:00	24.77518		24.52858333	20.19695844		21.45025	20.7330267		22.66745833	23.48260696
2014/12/24 01:00	20.28708		24.949375	16.19761996		21.6685	17.48364833		23.30266667	18.11081004
2014/12/25 01:00	27.70464		25.10058333	22.80740359		21.53054167	22.85395846		23.568375	26.98888212
2014/12/26 01:00	20.94661		24.9865	16.78532417		21.40308333	17.96114564		21.29408333	18.90019751
2014/12/27 01:00	20.56249		23.60654167	16.44303187		19.59520833	17.68304035		20.10808333	18.44044029
2014/12/28 01:00	22.89919		21.06008333	18.52526524		18.831125	19.37481115		21.221	21.23723652
2014/12/29 01:00	21.49323		19.81320833	17.27241837		19.03625	18.35689943		22.32520833	19.55444848
2014/12/30 01:00	19.38743		21.87675	15.39594036		20.90554167	16.83230053		20.43716667	17.03401696
2014/12/31 01:00	18.12518		22.13158333	14.27115124		19.85308333	15.91843304		18.23258333	15.52323243
2015/01/01 01:00	21.67169		19.62191667	17.43144593		18.06116667	18.48610597		20.0285	19.76804975
2015/01/02 01:00	24.06706		18.95470833	19.56595494		18.99379167	20.22034963		22.17525	22.63506112
2015/01/03 01:00	23.38475		20.07195833	18.95794887		20.05504167	19.72635749		21.63904167	21.81840478
2015/01/04 01:00	22.69099		21.458625	18.33974008		21.22483333	19.22407586		22.97058333	20.98804443
2015/01/05 01:00	22.26351		22.26191667	17.95881302		20.620375	18.91458064		24.17104167	20.47639412
2015/01/06 01:00	20.89485		22.66370833	16.73920381		20.789125	17.92367381		23.18754167	18.83824995
2015/01/07 01:00	19.60237		22.67583333	15.58747154		21.173125	16.98791558		21.34141667	17.29127615
2015/01/08 01:00	23.78052		24.14383333	19.31062397		21.93779167	20.01289859		21.898625	22.29210788
2015/01/09 01:00	21.47403		25.07541667	17.25530479		22.3355	18.34299501		21.80270833	19.53146202
2015/01/10 01:00	22.02795		25.63741667	17.74890253		22.07125	18.74403278		22.60508333	20.19444837
2015/01/11 01:00	23.06238		24.63558333	18.67068348		21.18275	19.49296041		22.85204167	21.43255813
2015/01/12 01:00	26.39078		24.95245833	21.63662443		21.56816667	21.90272502		23.222375	25.41632508
2015/01/13 01:00	23.30592		25.29516667	18.88770643		21.46775	19.66928699		21.18879167	21.72405714
2015/01/14 01:00	22.04097		23.778	17.76050837		19.57433333	18.75346228		21.506125	20.21003699
2015/01/15 01:00	23.31438		21.2805	18.89524105		18.48920833	19.67540871		21.96766667	21.73417743
2015/01/16 01:00	24.6413		19.7895	20.07765797		18.53783333	20.63609758		20.11008333	23.32236599
2015/01/17 01:00	18.84458		19.857875	14.91220709		18.83741667	16.43927743		20.560875	16.3842803
2015/01/18 01:00	19.60306		21.28041667	15.58808677		20.65845833	16.98841544		20.62395833	17.29210251
2015/01/19 01:00	20.02558		22.00020833	15.96459285		20.83620833	17.29431871		20.61633333	17.79781471
2015/01/20 01:00	16.95364		24.13295833	13.22719157		20.43145833	15.07023777		21.591375	14.12101571
2015/01/21 01:00	20.46919		20.594125	16.35989855		19.03816667	17.61549628		22.56225	18.328778
2015/01/22 01:00	21.28321		20.44204167	17.08526769		18.73633333	18.20484344		24.012	19.30307305
2015/01/23 01:00	22.56275		19.87179167	18.22546875		20.69783333	19.13123281		24.770625	20.83455847
2015/01/24 01:00	24.67585		18.69829167	20.10844808		20.2725	20.66111389		24.37820833	23.36372237
2015/01/25 01:00	26.19292		19.63675	21.46030953		20.27208333	21.75947287		24.45758333	25.17950395
2015/01/26 01:00	26.39865		20.61183333	21.64363999		21.30383333	21.90842501		22.543	25.42574817
2015/01/27 01:00	22.55641		21.14320833	18.21982029		19.74779167	19.12664356		19.23766667	20.82697162
2015/01/28 01:00	19.2812		20.78066667	15.30127992		19.92741667	16.75539091		19.58329167	16.90687177
2015/01/29 01:00	18.28645		20.49283333	14.41485745		19.19570833	16.03519131		21.80145833	15.7162545
2015/01/30 01:00	21.08989		20.56220833	16.91300061		18.735125	18.06488006		21.55141667	19.07168884
2015/01/31 01:00	22.63991		20.40329167	18.29422677		19.996375	19.18709725		18.66545833	20.92691227

2015/02/01 01:00	17.51392		20.908625	13.72645226		20.56979167	15.47587657		18.33070833	14.79160835
2015/02/02 01:00	16.35977		21.067625	12.69798882		20.489125	14.64027167		20.52145833	13.41020572
2015/02/03 01:00	19.76606		19.71904167	15.73333718		20.19308333	17.10642835		22.22195833	17.48719871
2015/02/04 01:00	22.59781		20.59970833	18.25670626		20.109125	19.15661263		22.68	20.8765158
2015/02/05 01:00	22.80994		18.66183333	18.44574162		20.50341667	19.31019988		22.681375	21.13042267
2015/02/06 01:00	20.51873		21.02316667	16.40403622		21.1095	17.6513572		22.84591667	18.38806245
2015/02/07 01:00	21.55961		21.69191667	17.33156402		20.9515	18.40495402		23.91379167	19.63389122
2015/02/08 01:00	23.79433		21.433375	19.32292969		19.620875	20.02289673		24.24966667	22.30863657
2015/02/09 01:00	23.91825		20.56304167	19.43335332		20.11433333	20.1126136		24.49645833	22.45695442
2015/02/10 01:00	26.38095		20.28233333	21.62786492		20.5735	21.8956081		24.65004167	25.40455955
2015/02/11 01:00	24.27547		20.75175	19.75167095		20.34145833	20.37123998		24.989375	22.88450954
2015/02/12 01:00	24.80814		19.86520833	20.22633021		20.82804167	20.75689065		24.679125	23.52205828
2015/02/13 01:00	24.75969		20.10966667	20.18315939		20.56854167	20.72181526		23.16916667	23.46407246
2015/02/14 01:00	21.22262		18.86266667	17.03127483		19.61020833	18.16097537		19.162375	19.23055138
2015/02/15 01:00	17.15399		19.933	13.40571975		20.63133333	15.21528816		17.523875	14.36080963
2015/02/16 01:00	17.07702		19.66033333	13.33713512		20.44841667	15.15956459		18.60758333	14.26868873
2015/02/17 01:00	17.01409		20.20445833	13.28105968		21.44408333	15.11400448		21.58633333	14.19336981
2015/02/18 01:00	19.89289		18.84095833	15.84635688		21.290125	17.19825447		22.72375	17.63900353
2015/02/19 01:00	24.10829		18.11870833	19.60269611		20.6955	20.25020106		22.40804167	22.6844108
2015/02/20 01:00	21.52736		17.30116667	17.30282604		20.16829167	18.38160502		19.90095833	19.5952912
2015/02/21 01:00	23.05823		17.199	18.6669843		20.24879167	19.4899549		19.09616667	21.4275895
2015/02/22 01:00	16.55486		17.85608333	12.87183909		20.81741667	14.78152136		20.33804167	13.64371642
2015/02/23 01:00	19.68835		17.89779167	15.66408869		20.25979167	17.0501654		20.027	17.39418612
2015/02/24 01:00	20.63479		17.875875	16.50746285		20.22608333	17.73538917		20.88291667	18.52698215
2015/02/25 01:00	19.89877		17.9195	15.85159061		19.38429167	17.20250677		22.04608333	17.64603332
2015/02/26 01:00	23.58344		17.83020833	19.13500116		20.58720833	19.87020875		20.48870833	22.05621634
2015/02/27 01:00	21.0624		17.89275	16.8885039		19.917375	18.044977		19.067	19.03878556
2015/02/28 01:00	20.28107		17.27129167	16.19226445		19.88991667	17.47929709		18.62095833	18.10361667
2015/03/01 01:00	19.78186		17.88783333	15.74741656		20.65991667	17.11786755		18.72295833	17.50610973
2015/03/02 01:00	16.79396		17.87391667	13.08489404		20.78854167	14.95462402		19.543625	13.92988574
2015/03/03 01:00	18.07483		18.046375	14.22627656		20.359875	15.8819733		20.03566667	15.46295804
2015/03/04 01:00	20.10735		18.37229167	16.0374607		20.30233333	17.35352231		19.38066667	17.89568871
2015/03/05 01:00	22.0027		18.23054167	17.72640931		19.20466667	18.72575752		19.05895833	20.16423612
2015/03/06 01:00	20.51646		17.5965	16.40201305		19.09558333	17.64971342		19.29083333	18.38534499
2015/03/07 01:00	21.85958		16.83595833	17.59887434		18.752625	18.62213803		19.15020833	19.99293479
2015/03/08 01:00	22.23662		16.29220833	17.93485542		19.231375	18.8951156		20.05845833	20.44421497
2015/03/09 01:00	22.26657		15.97720833	17.96153978		19.75875	18.91679608		20.48904167	20.48005664
2015/03/10 01:00	25.6542		15.55466667	20.98026059		19.333625	21.36944321		18.52591667	24.53471597
2015/03/11 01:00	21.57819		15.55091667	17.34812437		19.680625	18.41840896		18.935	19.65613461
2015/03/12 01:00	23.01168		15.28825	18.62550656		19.90370833	19.45625511		20.54566667	21.3718778
2015/03/13 01:00	23.10854		15.2235	18.71182408		19.73220833	19.52638628		19.48920833	21.48781701
2015/03/14 01:00	22.35789		15.26325	18.04291949		20.01166667	18.98291538		19.75875	20.58936353
2015/03/15 01:00	26.46814		14.91754167	21.7055603		18.53270833	21.95873396		19.69225	25.50891776
2015/03/16 01:00	22.95787		14.58679167	18.5775535		18.31479167	19.41729426		18.48033333	21.30746862
2015/03/17 01:00	18.62412		14.94570833	14.7157537		18.28908333	16.27966318		19.313625	16.12040973
2015/03/18 01:00	22.05023		15.17379167	17.76876181		18.87366667	18.76016803		19.35220833	20.22112278
2015/03/19 01:00	23.80377		15.187625	19.33133573		18.44170833	20.02972646		19.82991667	22.31992733
2015/03/20 01:00	21.66942		15.17591667	17.42942425		18.4615	18.4844634		19.95108333	19.76533428
2015/03/21 01:00	25.14568		14.67954167	20.52711879		18.91220833	21.00127504		19.92225	23.92606888
2015/03/22 01:00	22.53556		15.19266667	18.20124049		19.23445833	19.11154785		18.68445833	20.80201575
2015/03/23 01:00	20.55562		15.335	16.4369115		18.632875	17.67806767		18.35933333	18.43221958
2015/03/24 01:00	22.68842		15.27425	18.33744958		19.118375	19.22221487		18.66754167	20.9849679
2015/03/25 01:00	24.316		14.91558333	19.78778389		19.074875	20.40058098		18.23554167	22.93301541
2015/03/26 01:00	22.62264		14.6275	18.27883413		19.29575	19.17459106		18.83895833	20.90623732
2015/03/27 01:00	19.2683		14.370125	15.28977953		19.23041667	16.74604709		17.61029167	16.89142478
2015/03/28 01:00	17.32288		14.2065	13.55621466		19.28691667	15.3375621		19.146125	14.56295008
2015/03/29 01:00	18.77683		14.1365	14.85183507		19.089625	16.39022643		19.34745833	16.30319032
2015/03/30 01:00	18.42329		13.86154167	14.53679261		18.27325	16.13426106		19.60033333	15.8800343
2015/03/31 01:00	18.64078		13.677375	14.73059609		18.04529167	16.29172231		19.968625	16.14034559

2015/04/01 01:00	20.78771		13.43295833	16.64372764		14.357875	17.84610144		20.15975	18.7100091
2015/04/02 01:00	21.6192		13.53029167	17.38466801		12.246375	18.4480999		19.42783333	19.70521898
2015/04/03 01:00	20.94639		13.65916667	16.78513147		18.14645833	17.96098908		19.557625	18.89993868
2015/04/04 01:00	20.64736		13.367625	16.51866398		19.21929167	17.74448985		18.567625	18.54202718
2015/04/05 01:00	18.06061		13.21720833	14.21361068		17.71441667	15.87168255		17.22333333	15.44594561
2015/04/06 01:00	16.58283		13.20483333	12.89676167		17.90070833	14.80177043		16.47045833	13.67719172
2015/04/07 01:00	15.15062		13.863875	11.62051451		17.77375	13.76484647		16.30783333	11.96297309
2015/04/08 01:00	16.66506		14.15558333	12.97003311		17.77770833	14.86130193		16.45158333	13.77560782
2015/04/09 01:00	17.87959		13.79329167	14.05230488		17.174	15.74062497		16.34045833	15.22928426
2015/04/10 01:00	16.91534		12.298	13.19305799		17.75008333	15.04250495		16.45441667	14.07516845
2015/04/11 01:00	18.55103		10.76425	14.65062655		16.64966667	16.22674874		16.54883333	16.03293279
2015/04/12 01:00	20.13428		10.4585	16.06145654		16.45875	17.37301842		16.72491667	17.92791923
2015/04/13 01:00	20.6717		10.271625	16.54035336		15.86291667	17.76211201		17.23841667	18.57115972
2015/04/14 01:00	20.21362		9.81725	16.13215493		16.16054167	17.43045937		15.78529167	18.02287928
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2015/04/16 01:00	13.73544		9.397583333	10.3594517		15.84870833	12.74025947		14.12616667	10.26914963
2015/04/17 01:00	14.36435		9.593041667	10.91987006		15.63354167	13.19558759		15.09241667	11.02188752
2015/04/18 01:00	15.20438		9.992791667	11.6684209		15.42254167	13.8037694		15.56983333	12.02731958
2015/04/19 01:00	15.66465		10.1	12.0785685		15.00566667	14.1370057		15.2155	12.57821809
2015/04/20 01:00	16.4491		9.865541667	12.77759104		15.1335	14.7049468		15.886125	13.51712515
2015/04/21 01:00	15.57802		9.701541667	12.00137704		16.11345833	14.07428926		16.50066667	12.47453673
2015/04/22 01:00	21.56095		9.516958333	17.33276069		14.92891667	18.40592629		16.0425	19.63549856
2015/04/23 01:00	18.54612		9.107375	14.64624679		15.03116667	16.22319028		16.77216667	16.02705003
2015/04/24 01:00	17.04797		8.877833333	13.31124681		14.38325	15.13853088		16.63475	14.23391629
2015/04/25 01:00	20.49909		8.936958333	16.38653576		15.79208333	17.63713845		16.4725	18.36455633
2015/04/26 01:00	18.81924		8.926541667	14.88962402		16.371125	16.42092916		16.61729167	16.35394736
2015/04/27 01:00	17.92393		8.930416667	14.09181736		15.20629167	15.77272804		16.40495833	15.28235631
2015/04/28 01:00	18.77997		8.840708333	14.85463275		14.29408333	16.39249949		16.201625	16.30694809
2015/04/29 01:00	19.82473		8.698958333	15.78561802		12.19295833	17.14890543		15.388875	17.55742083
2015/04/30 01:00	18.07519		8.525166667	14.22660144		11.02225	15.88223726		14.62479167	15.46339441
2015/05/01 01:00	15.85474		8.8185	12.24796026		12.55883333	14.27463294		13.65158333	12.80574025
2015/05/02 01:00	16.68773		9.1765	12.9902401		12.044	14.87771969		13.411125	13.80274927
2015/05/03 01:00	16.4442		9.561041667	12.77322721		11.88516667	14.70140128		13.315	13.51126378
2015/05/04 01:00	16.63121		9.528958333	12.93986689		11.73166667	14.83679251		13.6385	13.73508941
2015/05/05 01:00	19.75604		9.91425	15.72440316		12.62729167	17.09916964		13.95441667	17.47519879
2015/05/06 01:00	17.10506		10.19658333	13.36212194		14.00520833	15.17986585		13.74779167	14.3022503
2015/05/07 01:00	15.10137		10.20954167	11.57663426		13.28629167	13.72919469		13.83175	11.90403439
2015/05/08 01:00	16.69924		9.884833333	13.00048939		13.00525	14.88604701		13.11066667	13.81651582
2015/05/09 01:00	19.79808		9.651833333	15.76187169		12.76845833	17.12961203		12.197	17.52552544
2015/05/10 01:00	17.2657		9.731166667	13.50526193		12.41520833	15.29616409		12.69925	14.49451184
2015/05/11 01:00	16.63401		10.03791667	12.94236557		12.59983333	14.83882264		13.5655	13.73844557
2015/05/12 01:00	16.1365		10.761375	12.49903441		11.93245833	14.4786254		13.41116667	13.14297585
2015/05/13 01:00	16.89732		11.21425	13.1769974		11.89008333	15.02945606		13.58975	14.05359632
2015/05/14 01:00	17.32485		11.86054167	13.55797395		12.22891667	15.33899149		13.15691667	14.56531311
2015/05/15 01:00	17.11847		12.24004167	13.37407159		12.20358333	15.18957469		14.03029167	14.31830073
2015/05/16 01:00	16.68244		12.58554167	12.98552377		11.91995833	14.87388777		13.68020833	13.79641443
2015/05/17 01:00	16.31624		12.68520833	12.65920332		11.858625	14.60875927		13.22704167	13.35811015
2015/05/18 01:00	19.13222		12.84216667	15.1685179		11.58891667	16.64752457		12.79154167	16.72854963
2015/05/19 01:00	19.75237		12.083	15.72113802		11.41041667	17.09651679		12.36158333	17.47081315
2015/05/20 01:00	16.81461		11.568125	13.10329452		13.05308333	14.96957402		12.03770833	13.95460072
2015/05/21 01:00	19.71924		11.752875	15.69161328		12.26570833	17.07252855		12.01533333	17.43115636
2015/05/22 01:00	16.92855		12.24516667	13.20482979		11.992375	15.0520693		11.73166667	14.09098
2015/05/23 01:00	18.80209		11.73175	14.8743398		11.582625	16.40851105		11.23691667	16.33341803
2015/05/24 01:00	17.53531		11.04220833	13.74551582		12.48233333	15.49136531		11.14808333	14.81721399
2015/05/25 01:00	18.22836		10.67729167	14.36308736		12.35704167	15.9931292		11.18320833	15.6467184
2015/05/26 01:00	16.53165		11.12204167	12.85115291		11.445	14.76471427		11.677	13.61593134
2015/05/27 01:00	16.35893		11.65254167	12.69724245		11.455	14.63966526		11.6245	13.40920322
2015/05/28 01:00	18.98609		11.77075	15.0383048		11.63883333	16.54172916		11.1335	16.55365112
2015/05/29 01:00	15.76683		11.77070833	12.16961954		12.06391667	14.21098275		10.759625	12.70051524
2015/05/30 01:00	18.38567		11.38925	14.50327262		12.362375	16.10702677		11.024	15.83501122
2015/05/31 01:00	16.48578		10.8275	12.8102767		13.443125	14.73150321		12.385625	13.56102759

2015/06/01 01:00	18.06115		10.50025	14.21408965		12.66820833	15.8720717		11.9605	15.44658894
2015/06/02 01:00	18.68367		10.046	14.76882019		13.43608333	16.32277859		10.26895833	16.19168712
2015/06/03 01:00	20.00603		9.84325	15.94717185		13.47604167	17.28016451		8.447041667	17.77441531
2015/06/04 01:00	15.1886		9.790875	11.65436079		13.15675	13.79234586		7.0125	12.00843444
2015/06/05 01:00	11.72828		10.28808333	8.570867449		13.029125	11.2870724		7.434375	7.866774492
2015/06/06 01:00	10.11587		10.22066667	7.13404797		13.475	10.1196868		6.366666667	5.936879716
2015/06/07 01:00	12.54756		9.601041667	9.300926261		12.12425	11.88022982		6.630833333	8.84736858
2015/06/08 01:00	12.03495		9.588458333	8.844141717		12.39466667	11.50910199		6.485208333	8.233828663
2015/06/09 01:00	11.70805			8.552842984		13.05091667	11.2724279		6.781083333	7.842564546
2015/06/10 01:00	12.33386			9.110501384		13.08229167	11.72551361		7.851208333	8.591595338
2015/06/11 01:00	14.79058			11.29968472		12.16166667	13.50417902		5.674833333	11.53204371
2015/06/12 01:00	15.47463			11.90924179		12.194625	13.99943131		3.9675	12.3507833
2015/06/13 01:00	13.91517			10.51960918		10.3275	12.87038405		4.169416667	10.48426857
2015/06/14 01:00	14.35608			10.91250604		14.212875	13.18960448		3.469833333	11.01199639
2015/06/15 01:00	14.85491			11.35701004		12.63533333	13.55075463		4.057291667	11.60904143
2015/06/16 01:00	13.46091			10.11481872		13.24970833	12.54150032		2.93475	9.940565623
2015/06/17 01:00	14.00281			10.59770321		12.914625	12.93383381		2.861	10.58916224
2015/06/18 01:00	13.19931			9.881703619		13.484125	12.3520992		4.30075	9.627452094
2015/06/19 01:00	12.99441			9.69911708		13.41679167	12.20375148		3.835958333	9.382207085
2015/06/20 01:00	13.157			9.844000101		13.061625	12.32146589		3.603458333	9.576809809
2015/06/21 01:00	13.91624			10.52056521		13.24454167	12.87116081			10.48555269
2015/06/22 01:00	13.82839			10.44228011		12.84866667	12.80755581			10.38040238
2015/06/23 01:00	13.89478			10.50144202		12.09904167	12.85562362			10.45986697
2015/06/24 01:00	14.31401			10.87501765		12.902875	13.15914596			10.96164306
2015/06/25 01:00	13.01353			9.716158588		13.11720833	12.21759735			9.40509675
2015/06/26 01:00	13.3524			10.01812594		12.69554167	12.46293947			9.810690652
2015/06/27 01:00	12.97978			9.68608025		10.17145833	12.19315933			9.364696388
2015/06/28 01:00	13.13787			9.826960264		8.556541667	12.30762138			9.553922388
2015/06/29 01:00	12.07444			8.879331368		14.30833333	11.53769284			8.281094393