Headwater Stream Biofilm Structure and Function at High Resolution Spatial-Temporal Scales

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Declaration

I hereby declare that the information on which this research project is based has been collected by me personally, has not been plagiarised from any unacknowledged sources and has not been previously submitted for another higher degree.

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Abstract

Spatial and temporal patterns in benthic diatom assemblage composition and chlorophyll-*a* production were investigated over a 25 month period within three headwater sub-catchments of the River Eden catchment, NW England. This was undertaken as structural and functional attributes of headwater stream have at large been neglected, despite the long-standing recognition of the importance of interconnectivity of upstream and downstream reaches. Results demonstrated that, catchment specific quasi-seasonal patterns in Shannon-Wiener diversity and *in situ* fluorometry chlorophyll-*a* in response to variable discharge-nutrient regimes demonstrating the ability of benthic diatom communities to recover from event-driven disturbances to their underlying equilibrium with water quality. This highlights the stability and resilience of benthic biofilms, in particular their ability to discriminate nutrient pressures between catchments, even in dynamic streams. Investigation of how diatom community attributes (diversity and chlorophyll-*a*) varied in relation to one another was explored through Shannon-Wiener diversity and *in situ* fluorometry (ISF) chlorophyll-*a* revealing a unimodal productivity-diversity relationship. Examination of the critical antecedent period of temporal dynamics in discharge and nutrient conditions (TP and NO_3) demonstrated that at-a-point in time community composition is a product of factors, such as phosphorus availability, related to discharge over the preceding 15 - 21 days. Secondly, investigation of patterns in benthic communities at fine spatial scales defined by physical flow biotope units, demonstrated that riffles were more productive than pools but pools displayed greater diversity in benthic diatom composition. Finally, reach-scale temporal dynamics in stream hydraulics and nutrient concentrations and their relation to benthic composition and chlorophyll-*a* production in riffle biotope units was explored over a 28 day period. This demonstrated a threshold relationship between change in benthic diatom composition and chlorophyll-*a* with highest diversity and chlorophyll-*a* observed during sub-scouring rainfall-discharge events preceded by quasi-stable periods in discharge-nutrient condition. Therefore, these investigations of fine-spatial high-temporal patterns in benthic diatom biofilm community structure and ecological functional processes, such as chlorophyll-*a* production, revealed the time-scale of response and physicochemical sensitivities of benthic ecosystems in headwaters which are important considerations for environmental monitoring and policy decision making.

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Do mo thuistí, do mo chlann uilig, agus do Mark, le grá mór ó mo chraoí, go raibh míle maith agaibh as creidúint ionam agus as bheith ann dom i gcónaí.

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Abbreviations

Alkalinity (Alk)

Ammonia (NH_4)

Analysis of variance (ANOVA)

Before-After Control-Impact (BACI)

Biological quality elements (BQE)

Canonical correspondence analysis (CCA)

Centre for Ecology and Hydrology (CEH)

Conductivity (EC)

Demonstration Test Catchments (DTC)

Detrended correspondence analysis (DCA)

Digital Elevation Model (DEM)

Dissolved organic carbon (DOC)

Dissolved oxygen (DO)

Ecological Quality Ratio (EQR)

Ecological status (ES)

Eden Rivers Trust (ERT)

Environment Agency (EA)

European Union (EU)

Extracellular polymorphic substances (EPS)

Fliess Wasser Stammtisch (FST)

Hours (Hrs)

In situ fluorometry (ISF)

In situ fluorometry probe (ISFP)

Intermediate Disturbance Hypothesis (IDH)

Limit of detection (LOD)

Mean Trophic Rank (MTR)

Member States (MS)

Multivariate productivity – diversity (MPD)

National Water Quality Instrumentation Service (NWQIS)

Nitrate (NO_3)

Nitrite (NO_2)

Nitrogen (N)

Non-metric multidimensional scaling (MDS)

Particular phosphorus (PP)

Pearson correlation coefficient (r)

Per annum (pa)

Phosphorus (P)

Principal component analysis (PCA)

Rainfall Event (RE)

Rapid assessment techniques (RAT)

Redundancy analysis (RDA)

River Habitat Survey (RHS)

River Invertebrate Predication and Classification System (RIVPACS)

Schreiner index (SHE)

Soluble reactive phosphorus (SRP)

Suspended solids (SS)

Total phosphorus (TP)

Total reactive phosphorus (TRP)

Total suspended solids (TSS)

Transient storage zone (TSZ)

Trophic diatom index (TDI)

UK Technical Advisory Group (UK TAG)

Water Framework Directive (WFD)

Weighted mean sensitivity (WMS)

Chapter 1: Introduction

1.1 Overview

Headwater streams are small first- and second order streams (Strahler, 1957; Lowe and Likens, 2005) accounting for up to 80% of cumulative river network length internationally (Benda *et al.*, 2005) from which 90% of river flow can be derived (Kirby, 1978). Consequently, headwater streams are important intermediaries in the hydrological connectivity of a river network linking terrestrial and aquatic ecosystems (Likens and Bormann, 1974; Lowe and Likens, 2005). Headwater streams receive pulse inputs of allochthonous material including nutrients and carbon (e.g. N, P, C) that are processed and transported downstream, therefore providing coupling of upstream-downstream biogeochemical processes and function (Vannote *et al.*, 1980; Fisher *et al.*, 2004; Wipfli *et al.*, 2007). Headwater streams are also hotspots of biodiversity in the landscape, serving as a habitat not only for species specialised according to headwater environments, but also for species that migrate between headwaters and larger downstream systems during specific life stages (Meyer *et al.*, 2007). However, relative food web complexity can be significantly simpler than that observed further downstream, which is in part attributed to a typically dynamic hydrological regime and smaller stream size (Poff *et al.*, 1997; Woodward and Hildrew, 2002).

Diatom-dominated benthic biofilms develop ubiquitously on stream bed cobble substrates within headwater streams (Lock *et al.*, 1984; John, 2003).

Photoautotrophic organisms, including diatoms, cyanobacteria and green algae, within the biofilm often dominate primary production and represent the base of the aquatic food web which is comprised primarily of macrophytes, macroinvertebrates and fish (Geesey *et al.*, 1978; Lock *et al.*, 1984; Lamberti, 1996; Giller and Malmqvist, 1998b; Hodoki, 2005; Brown *et al.*, 2008). Moreover, this benthic diatom biofilm represents a critical interface within the environment (Rusanov *et al.*, 2012). A high benthic surface area-to-water volume ratio results in increased interaction between the biota and the water column (Peterson *et al.*, 2001; Alexander *et al.*, 2007). This interaction is important in terms of optimizing nutrient uptake and export downstream but also as an indicator of water quality. Diatoms have long been employed in assessments of ecosystem health. This can be attributed to their sessile nature and relatively short life cycles, compared to that of macro-invertebrates and fish, which results in rapid response times to perturbation in environment condition (Kolkwitz and Marsson, 1908; Fisher *et al.*, 1982a; Power and Stewart, 1987; Stevenson, 1990; Rott, 1991; Stevenson and Pan, 1999; Stoermer and Smol, 1999; McGrady-Steed and Morin, 2000; Winter and Duthie, 2000; Brown *et al.*, 2008). Metric development and application has generally focused on structural assessment of diatom composition in larger downstream reaches (e.g. in UK Trophic Diatom Index (TDI) - (Kelly and Whitton, 1995a), in France Generic Diatom Index - (Rumeau and Coste, 1988), in Germany the Schreiner (SHE) index - (Schiefele and Schreiner, 1991). Structural and functional attributes of diatom communities in headwater reaches have largely been ignored, with limited application of functional attributes of benthic diatom assemblages across river networks as a whole (Sandin and Solimini, 2009). This is despite the long-standing recognition of the importance of functionally based classification as part of assessments of ecosystem health (Cummins, 1974) and interconnectivity of upstream and downstream reaches (Vannote *et al.*, 1980).

Dynamics in diatom community stability is highly dependent on the scale of observation (Wu and Loucks, 1995). In particular, given stream-landscape interconnectivity, perturbation in terms of precipitation can influence the magnitude, timing, and route of water into streams (Fisher *et al.*, 2004), thereby impacting in-stream chemistry (e.g. dissolved nutrient loads, sediment) and hydrology (e.g. shear stress, velocity). Diatoms are particularly sensitive to variation in climate and landscape variables which are key determinants of morphological, hydrological and chemical environments in streams (Stevenson, 1997). Hierarchical theory provides a useful framework to explore patterns in benthic diatom composition and how chlorophyll-*a* responds to dynamic allogeneic and autogenic shifts in benthic biofilm state. Moreover, it illustrates the impact of, often interacting, multiple environmental parameters on benthic functioning and stability which vary in importance along both spatial and temporal gradients (Ormerod *et al.*, 2010).

1.2 High frequency *in situ* monitoring: Eden Demonstration Test Catchment platform

Recent advances in monitoring technology have led to the opportunity for near-continuous measurements of environmental variables such as water chemistry and discharge (Jordan *et al.*, 2005; Jordan *et al.*, 2007; Cassidy and Jordan, 2011; Bowes *et al.*, 2012; Owen *et al.*, 2012; Wade *et al.*, 2012) to better determine these salient drivers of ecological communities at high spatial-temporal resolution. Study sites within this research form part of the Defra (Department for the Environment and Rural Affairs)-funded Demonstration Test Catchments (DTC) programme in England, a catchment-scale research platform testing measures for addressing the effects and impact of diffuse pollution from agriculture on stream ecosystems and sustainable food production (Jordan *et al.*, 2007; ADAS, 2010; Bowes *et al.*, 2012; Wade *et al.*, 2012; EdenDTC, 2013; NDTCN, 2013; UEA, 2013). The EdenDTC is one of three main national catchment consortia selected to incorporate differences in key landscape and farming types (EdenDTC, 2013). Sister consortia include the Hampshire Avon and Tamar in southern England and the Wensum in Norfolk, eastern England (ADAS, 2010; UEA, 2013).

These research platforms have adopted a Before-After Control-Impact (BACI) experimental approach which enabling comparison of a manipulated stream with a non-manipulated stream, before and after implementation of a mitigation measure. Within the catchment of the river Eden, each 10 km^2 sub-catchment; Morland, Dacre and Pow, contains two nested 2 km^2 catchments providing a control and mitigation zone (Figure 1.1). This allows for an additional spatial reference to account for confounding environmental factors such as differences in geology, land use, rainfall, discharge and riparian zone shading. Water quality and quantity as well as ecological investigation are undertaken at the confluence of each of the two 2 km^2 nested catchment confluences and at the outflow of the 10 km^2 sub-catchments. This provides a baseline against which to compare post-mitigation conditions in the stream and detect impact (Owen *et al.*, 2012). Note, this research has been undertaken before mitigation measures were employed.

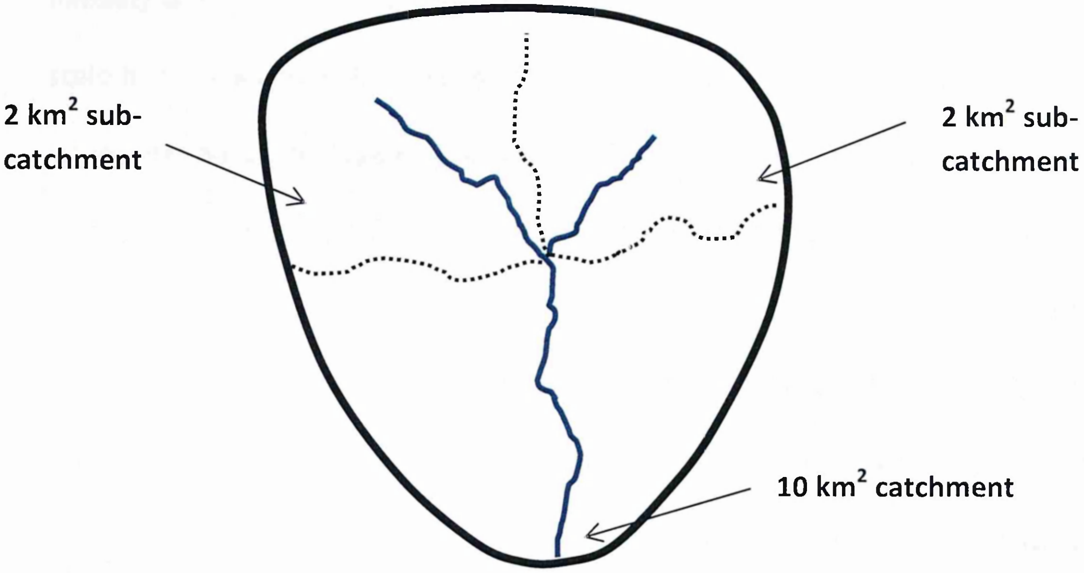


Figure 1.1: Schematic of experimental design illustrating a 10 km^2 sub-catchment of the River Eden containing two 2 km^2 nested catchments providing a control and mitigation zone facilitating a BACI experimental approach.

1.3 Research aims and objectives

Appreciation of scalar influences on benthic diatom communities and the impact of, often interacting, multiple pressures on these communities is important for advancing ecological understanding of the effects of different factors on headwater diatom assemblage structure and function (Stevenson, 1997; Biggs *et al.*, 1998b; Soininen, 2004). Within this research spatial-temporal pattern in headwater benthic diatom attributes (richness, diversity and chlorophyll-*a*) and their salient environmental drivers were investigated. This allowed for investigation into the hypothesis that biofilm structure and function varies according to specific physical and/or chemical variables, which themselves are influenced by biotope (physical habitat) properties. Investigation into benthic diatom communities at the biotope scale had the additional objective of allowing for more accurate representation and interpretation of biological monitoring efforts. In particular, this small scale of investigation facilitated focus on key research questions relating to spatial and temporal variability in biologically important benthic diatom biofilm attributes (richness, evenness, diversity and chlorophyll-*a*). This allowed for evaluation of the value of high frequency routine monitoring and spatial characterisation of physical and chemical habitats characteristics for cost-effective ecological assessment. Such a research approach will facilitate ecological theory and biological monitoring to proceed to the next stage in its development as an informative, representative and reliable means of ecological status determination and water quality evaluation for headwater streams (Ormerod *et al.*, 2010). This research addresses the following research objectives derived from knowledge gaps outlined above and discussed in the following chapter;

1) To investigate spatial and temporal patterns in benthic diatom biofilm assemblage within and among three headwater 10 km^2 catchments in the Eden Catchment, Cumbria Northwest England, over 25 month period;
2) To explore these patterns ['structure'] and their statistical relationships with measured physical and chemical parameters;

3) To quantify the importance of physical flow biotope properties in determining composition ['structure'] and productivity ['function'] of benthic diatom assemblages; and

4) To investigate how reach-scale temporal dynamics in stream discharge, velocity, shear stress and nutrient condition (TP and NO_3) determines benthic composition ['structure'] and chlorophyll-*a* production ['function'].

1.4 Thesis structure

More specific hypotheses and objectives are presented in each chapter, the contents are of which summarised below.

Chapter 2 provides an overview of current knowledge and understanding, identifying knowledge gaps from the scientific literature.

Chapter 3 provides site descriptions of all nine sites studied within this research. It also presents the methods employed and approaches adopted to answer the research aims.

Chapter 4 addresses the first and second research aims through investigation of patterns in benthic diatom composition and chlorophyll-*a* production among three headwater 10 km^2 sub-catchments in the River Eden. To achieve this aim, monthly diatom sampling was conducted over a 25 month period incorporating analysis of diatom assemblage composition, productivity in terms of chlorophyll-*a* and water chemistry. Therefore, this chapter assessed the implication of sub-catchment scale spatial and temporal variability in headwater benthic diatom communities and the physicochemical parameters influencing this variability.

Chapter 5 sought to address research aim three by better understanding within-reach spatial variability in biotope flow habitat and its relation to diatom diversity and chlorophyll-*a* production. In addition, this chapter also addressed research aim two by exploring if differences in biofilm attributes were determined by key physical variables. This was achieved through capturing spatial differences in diatom composition and chlorophyll-*a* production in riffle and pool biotope units.

Chapter 6 aimed to identify critical thresholds in the relationship between change in benthic diatom composition and chlorophyll-*a* production. It sought to determine if observed changes in communities were a result of discharge, velocity and nutrient pulses (TP and NO_3) over a 28 day period in riffle biotopes. Hence, this chapter primarily sought to address research aim four.

In **Chapter 7** a discussion of the results found in this thesis are presented in the context of the wider literature both theoretical and applied. In addition, key conceptual models are provided to summarise key research findings.

Chapter 8 reviews and summarises the key findings including the limitations of this thesis, presents recommendations for critiquing current biomonitoring protocols and highlights further research areas requiring investigation.

Chapter 2: Literature review

2.1 Headwater streams

2.1.1 Overview

Headwater streams, defined within this study as small first and second order permanent flowing streams in the upper reaches of a catchment, represent unique habitats due to their strong connectivity with the adjacent landscape and characteristic hydromorphology (Strahler, 1957; Lowe and Likens, 2005; Richardson and Danehy, 2007; Wipfli *et al.*, 2007). The spatial extent of headwater drainage accounts for the majority of the total catchment area within a catchment (Selby, 1985; Gomi *et al.*, 2002). For example headwater streams can represent between 50 to 85% of total length of river network (Shreve, 1969; Leopold, 1964; Peterson *et al.*, 2001; Benda *et al.*, 2005). This together with high benthic surface area-to-water volume ratio, results in increased interaction with the water column leading to headwaters being important intermediaries linking terrestrial and aquatic ecosystems (Figure 2.1; Liken and Bormann, 1974, Peterson 2001, Lowe and Likens, 2005, Alexander *et al.*, 2007). Headwater streams are central to the functioning of river systems (Meyer *et al.*, 2007) and have been shown to be critical sites in river networks for processes such as nutrient uptake and retention (Peterson *et al.*, 2001). Thus biogeochemical processes which occur in the upper reaches of river networks provide important ecosystem services in terms of improved water quality, nutrient cycling, autochthonous production, allochthonous material processing and subsequent transportation downstream, thereby influencing downstream ecosystem function (Newbold *et al.*, 1981; Minshall *et al.*, 1985; Meyer *et al.*, 1988; Meyer and Wallace, 2001; Reddy and DeLaune, 2008; Noble *et al.*, 2010). Despite this the ecological function of headwaters is poorly understood (Berkowitz *et al.*, 2014). The main reasons cited in the literature for their poor representation is their small size, number, accessibility and perceived lack of resources such as fish (Benda *et al.*, 2005). This lack of knowledge on headwater stream functioning has led to an underestimation of the importance of dynamic headwater flow processes in shaping both upstream and downstream environments upon which stream biota depend for habitat.

2.1.2 Flow Generation

Within headwater catchments hydrological flow paths are of primary importance for stream flow generation and vary as a function of climate, geology, vegetation, soil characteristics and anthropogenic pressure (Freeze, 1974; Sidle *et al.*, 2000; Gomi *et al.*, 2002; Heathwaite, 2010). These flow paths provide conduits for inputs from terrestrial systems that begin with precipitation and flow downhill through or over soils to streams channels (McDowell and Likens, 1988). More specifically, flow paths are pervaded by field drainage that finely dissect the land, thereby creating high connectivity between streams and the landscapes they drain (Heathwaite *et al.*, 2005a; Heathwaite *et al.*, 2006). Due to small catchment areas and short resident times of precipitation, the relative and absolute size and connectivity among flow paths within a headwater system varies as a function of precipitation (Fisher *et al.*, 2004). Agricultural-based land use within these catchments may further accentuate connectivity in stream response to rainfall events, leading to sub-surface or saturated overland flow (Figure 2.1). This is dependent on soil characteristics, an important intermediary which contributes to temporally rapid change in, and generation of, peak flow conditions (Ward and Robinson, 1990; Sidle *et al.*, 2000; Burt, 2001; Gomi *et al.*, 2002). When rainfall intensity exceeds infiltration capacity infiltration-excess overland flow results (Figure 2.1). This can contribute to large in-stream flow peaks and increased sediment load through erosional processes (Burt, 2001). Sediment-associated transfer of pollutants to water quality include nutrients, in particular phosphorus (P), which may bind to fine particles or be transported as fine particles in sediment (Haygarth *et al.*, 1997; Citeau *et al.*, 2001; Citeau *et al.*, 2003; Heathwaite *et al.*, 2005b; Oliver *et al.*, 2005a; Oliver *et al.*, 2005b). While P and suspended solids (SS) are generated as a result of surface runoff, nitrate (NO_3) dominated runoff from agricultural land is attributed to subsurface and groundwater composition which is reflective of soil processes, for example nitrification (Edwards and Withers, 2008). Both subsurface and groundwater flows move at lower velocities through longer flow paths (Burt, 2001). Differences in patterns of transport and delivery of nutrients (nitrogen (N), P and their fractions) to streams throughout the year can contribute to differences in the bioavailability of N and P and shifts in the N:P ratio (Conley, 1999). Peak flow generation and associated chemical (especially N and P) and sediment transport processes can therefore shift in intensity from the natural baseline hydrological response. This fluctuation in flow rate is a function of seasonal changes in farming practice, land use and climactic variables (Boardman *et al.*, 1994; Newson, 1997; Burges *et al.*, 1998; Dunbar *et al.*, 2010).

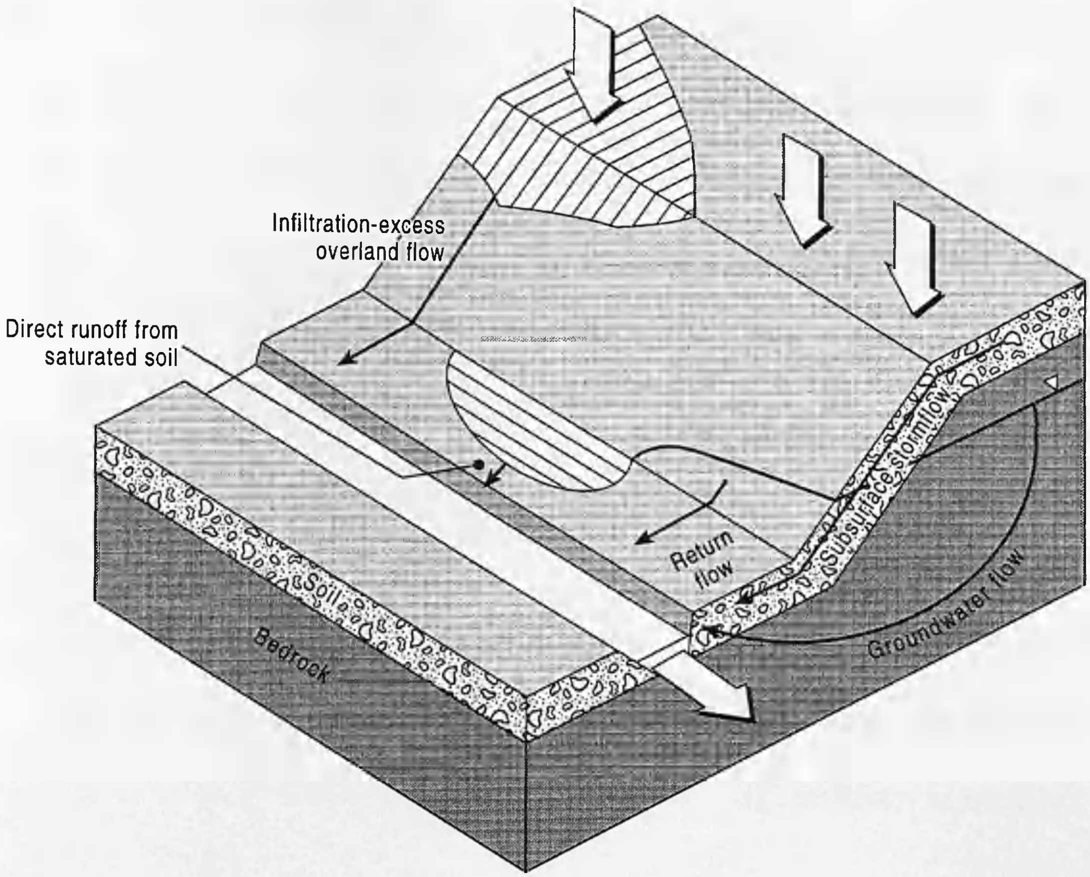


Figure 2.1: Hydrological pathways: infiltration-excess overland flow; saturation-excess overland flow; direct run-off from saturated soil; return flow; subsurface flow; and groundwater flow. (Reproduced from Burt, 2001).

Variability associated with rainfall and its interactive effects with terrain, soil texture, evapotranspiration and riparian zone characteristics results in catchment specific local flow regimes (Minshall, 1988) which vary over timescales of hours to days to seasons and longer (Poff *et al.*, 1997). Catchment specific differences in hydrological pattern can be further emphasised by critical components of the flow regime: duration, magnitude, frequency and rate of change in flow (Poff and Ward, 1989; Richter, 1996; Poff *et al.*, 1997). These components of flow create and maintain in-stream flow dynamics and habitat which is critical for phytobenthos and other biota, including macro-invertebrate and fish, by influencing temperature, SS, nutrient availability and physical habitat structure (Poff and Ward, 1989; Wallace *et al.*, 1997). Changes in riparian vegetation can affect water quality by altering terrestrial run-off patterns (Naiman and Decamps, 1997; Dosskey *et al.*, 2010) and increasing water temperatures through loss of shade from, for example, tree removal (Brown and Krygier, 1970; Johnson and Jones, 2000). The riparian zone thereby acts as a buffer shielding in-stream biota and benthos from the adverse effects of catchment processes (Pan *et al.*, 2004). Thus in order to understand the biodiversity and productivity of headwater phytobenthos and biota is it necessary to appreciate the central organising role played by a dynamically varying physical and chemical environment (Resh *et al.*, 1988; Bunn and Arthington, 2002; Dudgeon *et al.*, 2006; Monk *et al.*, 2008).

2.1.3 Water Chemistry

An important function of headwater streams is their role in naturally improving water quality (Clinton and Vose, 2006). Nutrients, pesticides, herbicides, and sediment can all become pollutants, which are generally attributed to agriculture activities, within headwater catchments and have profound effects on the riverine food web at the landscape scale (Osborne and Wiley, 1988; Johnson *et al.*, 1997a; Woodward and Hildrew, 2002; Scanlon *et al.*, 2007). Headwater streams are particularly sensitive to pollutants due to their relative shallow depth and the volume of water in physical contact with the stream channel (Gomi *et al.*, 2002; Ensign and

Doyle, 2006). Concentrations and retention of nutrients in the water column are therefore flow dependent. P adsorbs to soil particles which are then transported from the terrestrial system to the stream through the surface water (Pionke *et al.*, 1996). When flows are high relatively more of the discharge reaching the stream arrives as surface flow thereby decreasing the time for vegetative uptake and microbial transformation of nutrients (Gilliam, 1994; Martin *et al.*, 1999). Furthermore, high discharge results in a smaller degree of P removal as there is less time for finer sediment particles to settle out. This prevents storage in headwater sediments resulting in increased P transport downstream (Brooks *et al.*, 2005). Therefore, headwater streams tend to flush rather than store fine sediments when compared to lower gradient streams resulting in headwater streams been key source areas of sediment and associated nutrients (Gomi *et al.*, 2002; Benda *et al.*, 2005). Headwater streams have the most rapid uptake and transformation of inorganic N which, unlike P, primarily moves through ground water as dissolved NO_3, ammonia (NH_4) and organic N (Peterjohn and Correll, 1984; Peterson *et al.*, 2001; Starry *et al.*, 2005; Alexander *et al.*, 2007). Catchment nutrient concentration is therefore mediated by nutrient transport and in-stream processing. This in turn is regulated by the prevalent flow regime with the cycling of these nutrients to a large extent controlled by phytobenthos production and microbial decomposition (Pionke *et al.*, 1996; Alexander *et al.*, 2000; Peterson *et al.*, 2001; Bernhardt *et al.*, 2003; Bernhardt *et al.*, 2005).

For algae, it is not only the concentration but the relative ratio of N:P (Si is also important for diatoms) which is important for growth. Tilman's (1982) resource ratio theory predicts that changes in the ratio of these two essential nutrients will result in changes in plant community structure due to exploitative competition among taxa with different optimal nutrient ratios. Therefore, N:P and their respective concentrations effect algal community composition and production (Stelzer and Lamberti, 2001). Differential concentrations of N and P can accentuate P limiting conditions, which is considered as 'limiting' in UK streams (Reynolds and Davies, 2001; Mainstone and Parr, 2002). In marine systems at least, carbon, N and P occur in algae tissue at a consistent ratio of atomic weights of 106:16:1 (Redfield, 1958). Therefore, when N:P falls below 16:1, algae will have less N available compared to P and thus experience nitrogen limitation whereas ratios above 16:1 indicate phosphorus limitation. At N:P ratios between 10:1 and 20:1 there is potential for joint limitation by N and P (Allan, 1995). Therefore, variation in nutrient concentration and in N:P ratio, which is driven by terrestrial flow patterns and the in-stream discharge regime in headwater catchments, can consequently influence stream metabolism.

2.1.4 Lateral, vertical and longitudinal energy flux

Headwaters offer the greatest opportunity for energy flux and exchange between the terrestrial and water environment, providing upstream-downstream connectivity mediated by interconnected local stream structure (e.g., the River Continuum Concept - Vannote *et al.*, 1980; the Link Discontinuity Concept - Rice *et al.*, 2001; the Network Dynamics Hypothesis - Benda *et al.*, 2004). Three major axes of energy transfer are present; upstream/downstream, lateral transfer from land mediated by riparian zone and surficial/groundwater transfer (Figure 2.2). Headwater streams receive large volumes of organic matter and nutrients from the surrounding catchment making lateral transfer important between terrestrial and stream environments. Association between headwaters streams and the terrestrial landscape is mediated through riparian vegetation which plays a key role in the ecology and productivity of headwater streams. For example, through litter fall, shading, influencing incident light reaching the stream bed and overland flow (Wipfli *et al.*, 2007). Openness with land and ecological hydro-chemical coupling means that headwater stream ecosystems are an important unit of *in situ* biogeochemical processing (Fisher *et al.*, 2004). Moreover, the concentration and forms of energy and material delivered to streams integrate a suit of abiotic and biotic transformations that occur en route in the soil matrix. Hence processing in soils is linked to in-stream processing (Neill *et al.*, 2001). Upon reaching the stream, solutes are subject to additional biological, chemical and physical processes which influence catchment nutrient balance and in-stream properties (Alexander *et al.*, 2007). These include organic matter decomposition, nitrogen transformations (e.g. nitrogen fixation, ammonification, nitrification and denitrification) and nutrient mineralization, all of which are directly or indirectly mediated by microbes within the phytobenthos on streambeds. In turn, these processes influence dissolved and particulate nutrient transport with the spiralling length that a particle travels before being removed from water column similar to larger streams (Ensign and Doyle, 2006). The river continuum concept of Vannote *et al.* (1980) further supports this demonstrating how food web dynamics are influenced not only by allochthonous inputs from the surrounding terrestrial landscape but also from within the system through, for example, primary productivity (Polis *et al.*, 1997). Comprehension of the structure and function of phytobenthos communities in headwater is therefore critical for understanding of biogeochemical linkages in small catchments and wider catchment functioning.

Vertical energy flux is also evident in headwater streams which facilitate the greatest exchange with groundwater (Gomi *et al.*, 2002). In particular, it is the hyporheic zone, the area of saturated sediments beneath the water, which provides the potential for exchange in water, nutrients, organic matter between surface and ground water. This vertical exchange is important for the aforementioned ecological processes within streams. For example up-dwelling water provides nutrients to stream biota while down-dwelling water increases dissolved oxygen and organic matter (Boulton *et al.*, 1998). More specifically, the hyporheic zone influences stream chemistry as a result of storage and retention via ground water, surface water and biogeochemical transformations (Hill and Lymburner, 1998). The hyporheic zone is also the primary site for N chemical reactions; ammonification and nitrification and denitrification, mediated through oxygen availability. These reactions are facilitated by stream bed irregularities, small pore space, organic matter deposits, variations in discharge and anoxic and hypoxic pockets (Boulton *et al.*, 1998).

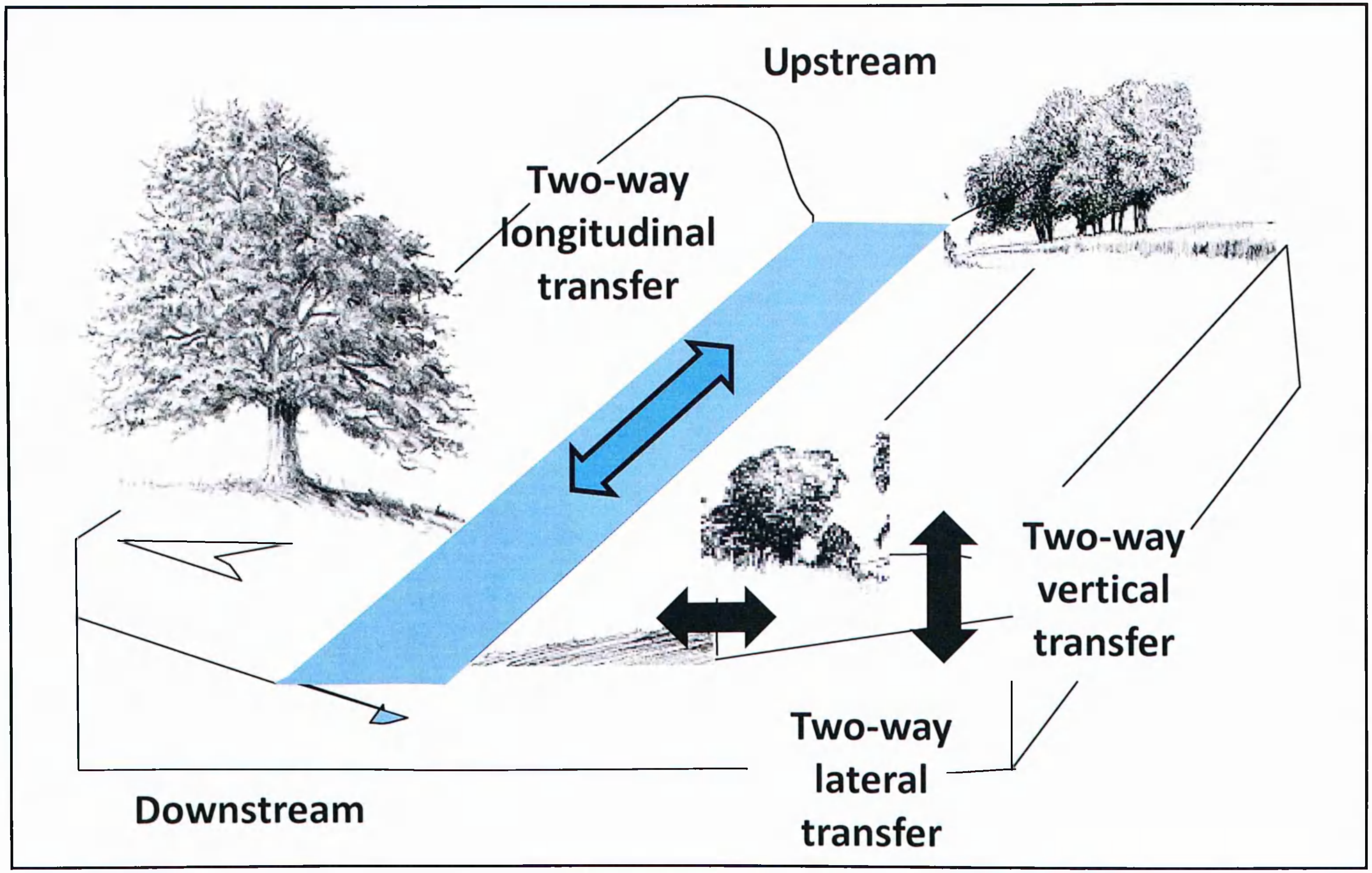


Figure 2.2: A natural connectivity model demonstrating the central role of headwater stream and their position within catchments, linking upland and downstream systems. Headwater streams are sources and conduits for nutrient processing and export mediated by hydraulic conditions. Major axis of transfer in headwater systems; two way longitudinal transfer (upstream/downstream), two way lateral transfer (channel/catchment) and two way vertical transfer (surficial/groundwater). (Adapted from Wipfli *et al.*, 2007, Piégay and Schumm, 2003).

2.1.5 Biotic assemblages of headwater streams

Food web structure in headwater streams is highly dependent on environmental stability in terms of flow (Woodward and Hildrew, 2002). Together with high hydrological coupling between terrestrial and aquatic systems, as presented in section 2.1.2, this results in a short food chain length (Marks *et al.*, 2000). Headwater food chains are primarily comprised of organic matter, phytobenthos, macrophytes, macroinvertebrates and fish. Phytobenthos, or benthic biofilms, form a dominant component of stream biota (Kupe *et al.*, 2008). These biofilms develop ubiquitously on the benthic substrate (Lock *et al.*, 1984; John, 2003) and comprise photoautotrophic algae and heterotrophic fungi, bacteria and protozoa contained within an extracellular polymeric matrix (EPS; see Figure 2.5; Geesey *et al.*, 1978; Lock *et al.*, 1984; Flemming and Wingender, 2010). The benthic biofilm is an important intermediary of two headwater sub- food chains; the microbial loop and algae-macroinvertebrate grazing. The microbial loop incorporates the nutritional relationship between bacteria, protozoa, algae and top consumers (Figure 2.3). Algae uptake inorganic matter and give out dissolved organic carbon (DOC). DOC derived from algae source is of a higher nutritional quality than DOC from other sources and thus preferentially used by bacteria and macroinvertebrate, making biofilms an important source of carbon cycling. This has importance in terms of understanding trophic interaction and energy transfer within the aquatic food web (Meyer, 1994). Therefore, benthic biofilms represent an important component of the base of the aquatic food web (Hodoki, 2005), the composition of which is shaped to varying degrees by many different biological, physical and chemical factors such as stream flow variability, light, temperature, substrata properties, nutrient availability, pH, predation and grazing (Patrick, 1970; Pringle, 1990; Biggs *et al.*, 1998b; Suren *et al.*, 2003; Biggs *et al.*, 2005; Telford *et al.*, 2006; Brown *et al.*, 2008; Law, 2011). This research will focus on the photoautotrophic algae component comprising cyanobacteria, green algae and wholly diatoms which are abundant and temporally persistent component of benthic biofilms (Brown *et al.*, 2008).

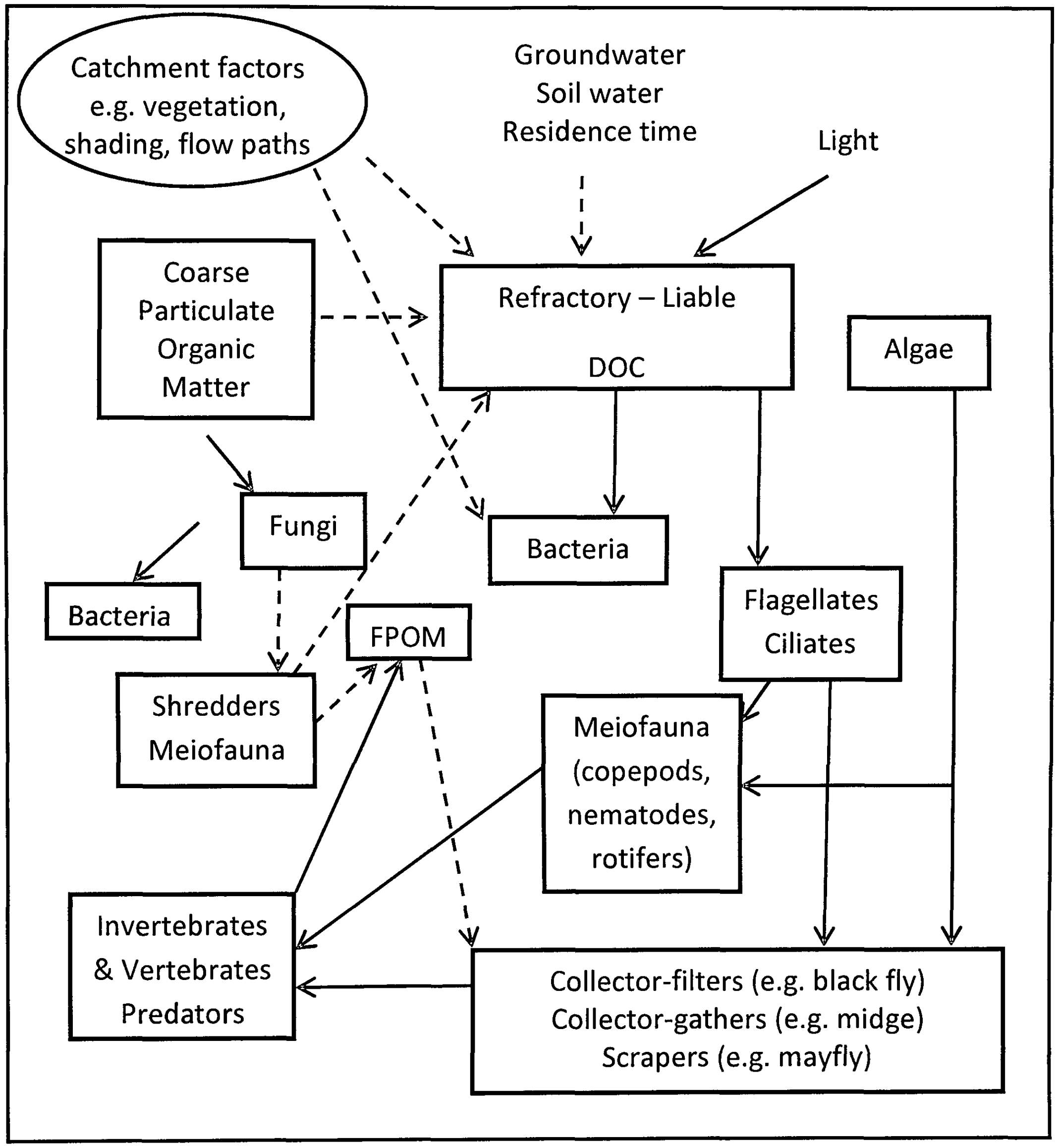


Figure 2.3: Lotic food web emphasizing the role of microbial production and demonstrating the sources and pathways of organic carbon. Dotted lines are flows within the microbial food web but not in planktonic system. (Reproduced from Meyer, 1994).

Macroinvertebrates are themselves a diverse group of biota and are a food source for higher trophic levels such as fish (Wallace and Webster, 1996; Wallace and Eggert, 2009). Macroinvertebrates are also involved in allochthonous and autochthonous carbon breakdown, primary production, nutrient recycling, decomposition and translocation of materials (Wallace and Webster, 1996). For example grazing, through the removal of sediment and senescent cells, may increase light intensities within the biofilm resulting in increased biomass (Pringle *et al.*, 1993). Within headwater streams dominant grazers can significantly reduce biofilm biomass and influence taxonomic composition (Jordan and Lake, 1996). Grazing can also increase local nutrient supply through increasing turnover rates and leaching of dissolved organic material (Lock *et al.*, 1984; Steinman *et al.*, 1995). The impact of grazing is dependent on the architecture of the benthic biofilm with adnate attachment providing not only resistance to scour but also grazing (Steinman *et al.*, 1987; Peterson and Stevenson, 1992). Therefore, macroinvertebrate diversity plays a vital part in the structure and functioning of river ecosystems influencing both algae composition and total biofilm biomass, thus contributing to the concepts of top-down and bottom-up control in aquatic communities (Wallace and Webster, 1996).

2.1.6 Physical-biological coupling and benthic biofilms in headwater catchments

Benthic biofilms are a major energy supporting the stream food web (McIntire, 1973; Minshall, 1978; Paerl and Pinckney, 1996; Hall *et al.*, 2000). The biofilm itself plays an important role in determining stream community composition through providing attachment sites for algal propagules and invertebrate larvae (Lock *et al.*, 1984;

Chapman, 1995), thus influencing recruitment processes (Huang and Boney, 1985; Thompson *et al.*, 1998). Micro-organisms within the biofilm matrix are a major source of primary production (Geesey *et al.*, 1978; Bustamante *et al.*, 1995; Lamberti, 1996) with positive correlation observed between benthic chlorophyll-*a*, gross primary production and community respiration (Dodds *et al.*, 2006).

Many of the flow forces and processes affecting benthic organisms and their biomass (e.g. drag, drift, diffusivity, mass transfer and shear stress) vary as a function of velocity (Biggs *et al.*, 1998a). A velocity gradient is created by friction between the moving water and the stationary bed where the biofilm is found (Nowell and Jumars, 1984). Within this interphase, known as the transient storage zone (TSZ), biofilms form a sink for inorganic nutrients by buffering release into the water column (Wetzel, 1996) and transforming inorganic nutrient into organic forms that are readily available for secondary production (Lock *et al.*, 1984). Furthermore, biofilms have the potential to recycle N and carbon within the polysaccharide matrix serving as a primary carbon reserve during low nutrient conditions (Freeman and Lock, 1995). Algae association, between for example diatoms and cyanobacteria, can convert atmospheric N to NH_4 to amino acids providing a source of N within the biofilm matrix enhancing biofilm productivity (Peterson and Grimm, 1992).

Flow characteristics related to the velocity field can strongly affect diatom habitat characteristics; dispersal, resource acquisition, competition and predation (Hart and Finelli, 1999). For example, the rate of nutrient uptake is species specific and related to nutrient concentration in the water column and size of the TSZ (Bothwell, 1989;

Mulholland *et al.*, 1994; Vogel, 1994; Stevenson, 1996). High water velocities may reduce biofilm thickness and affect internal nutrient cycling (Battin *et al.*, 2003a). This can alter the composition of the phytobenthic assemblage of benthic algae from diatoms, a preferred food source for many invertebrates, to filamentous green and blue green algae that detritivores tend to avoid (Hart and Robinson, 1990; Sullivan and Currin, 2000). Alternatively increases in current velocity and runoff during rainfall events may positively affect remaining algae by increasing nutrient and dissolved gas supplies to the cell (Stevenson, 1990). Thus, flow characteristics can influence phytobenthic habitat and food source for higher trophic communities such as macroinvertebrates and fish, thus illustrating an important control of the benthic diatom biofilm on trophic dynamics.

Phytobenthic accumulation on the stream bed can be an important determinant of near-bed hydraulic characteristics. Through changing the roughness profile of the stream bed, phytobenthic growth, stimulated by velocity, can influence both the dispersion coefficient and the TSZ volume (Reiter, 1989; Nikora *et al.*, 1997; Nikora *et al.*, 1998; Dodds and Biggs, 2002; Labiod *et al.*, 2007). Moreover, growth of the benthic biofilm can change the fine scale hydrologic profile decreasing drag forces and bed roughness (Biggs and Hickey, 1994; Graba *et al.*, 2010). Thus the level of nutrient cycling and retention within the biofilm depends on the development stage of a phytobenthic mat. This shows that an understanding of physical-biological coupling is important for the ecological organisation of streams (Hart and Finelli, 1999).

2.1.7 Headwater streams: summary

Small catchments in England and their phytobenthos communities are spatially and temporally sensitive indicators of change in ecosystems arising primarily from agricultural pressures. Hydrology presents a key control of major in-stream biological, chemical and physical processes coupling hydrology to terrestrial processes, such as plant decomposition, soil cation exchange, chemistry, biological uptake and mineralization. Nutrient cycles (N, P, C) of headwater catchments are to a large extent determined by phytobenthos especially by the primary production of algae and by microbial decomposition. However, there is incomplete information on how complex and interactive hydrological, chemical and biological processes impact upon benthic diatom assemblages. This research aims to assess changes in stream water quality over a 25 month period following changes in climate (rainfall), land use and nutrient input in order to determine the influence of hydrochemistry on stream benthic diatom biofilms.

2.2 Diatoms: biological and ecological characteristics

2.2.1 Biological characteristics

Diatoms, eukaryotic microalgae algae 2 – 200 µm in length, are among the most species rich algae group (Mann, 1999) with over 10,000 species across 260 genera found globally (Round *et al.*, 1990). Diatoms have certain distinctive features which make them unique amongst algae. Most notably they have a highly differentiated and heavily impregnated siliceous outer cell wall (frustule) consisting of transverse and short lateral ribs separated by tiny "pores" or "areolae" (Round *et al.*, 1990). These pores also facilitate secretion of mainly polysaccharides, proteins, nucleic acids and lipids which together form a hydrated matrix of EPS. The EPS forms the immediate environment of diatoms providing mechanical stability and support for a three dimensional architecture of the biofilm. It also mediates their adhesion to surfaces and nutrient exchange (Flemming and Wingender, 2010). Additional distinctive features of the diatom cell are the possession of unique photosynthetic pigments (chlorophyll a/c and green light absorbing xanthophylls) and specific storage products (oil and chrysolaminarin) (Round *et al.*, 1990). As a result of their abundance and photosynthetic ability diatoms are significant primary producers especially in streams with low turbidity and high incident light (Cummins, 1974; Giller and Malmqvist, 1998a; John, 2003). Hence, the ecological functional role of diatoms can be attributed to their role in oxygen production, organic carbon fixation, deposition and nutrient cycling (N, P, Si; Dodds *et al.*, 2000; Gibson *et al.*, 2000; Dodds *et al.*, 2002; Battin *et al.*, 2003b; Lyon and Ziegler, 2009).

Within the phytobenthos diatoms can live on, or in, sediments (epipelic and endopelic respectively), or attached to substratum, including cobbles, and macrophytes, classified according to the substrata they attach to; sand grains, rock, plants, and animals which are referred to as epipsammic, epilithic, epiphytic and epizoic communities respectively (Round *et al.*, 1990). In this study, the focus is on epilithic communities on cobble substrate on the stream bed. Modes of attachment to cobbles are predominately adnate (tightly appressed to the substratum) or pedunculate (attached to the substrata by stalks or pads) and colonial. Differential attachment therefore allows for a highly organised benthic diatom biofilm varying along a succession gradient from a single layer of cells to a three dimensional structure with 'upper' and 'lower' tiers (Bryers, 1987; McCormick and Stevenson, 1991; Kelly *et al.*, 2009c). Figure 2.4 illustrates the 'upper' and 'lower' tiers within an established biofilm. Lower tiers are represented through diatom attachment via mucilage pads or held by a pad at one end of a long cell with upper tier species held on simple or branched stalks. Such morphological traits, differentiated through species attachment, provide a way to explore the sensitivity of species within three dimensional structure of the biofilm to environmental condition to environment perturbation and associated resource supply (e.g. Biggs *et al.*, 1998; Passy 2007; Lange *et al.*, 2011). The origins of morphological classification are based on terrestrial plants which were placed into groups based on their resource supply requirements and ability to withstand disturbance (CSR classification of Grimes, 1979). This sensitivity of the benthic diatom biofilm to environmental factors is associated with their sessile nature, relatively short life cycles and high growth rate which facilitates complete turnover in species within days to changes in water quality (Fisher, 1982; Schinder, 1987; Round, 1991; Rott, 1991; Pan, 1996; McGrady-Steed and Morin, 2000; Taylor *et al.*, 2007). Heterogeneity in stream biofilm community structure and function is also observed due to substrate type, current velocity, light intensity, nutrients and grazing (Mosisch *et al.*, 2001; Jarvie *et al.*, 2002; Battin *et al.*, 2003a; Lange *et al.*, 2011) which may confound changes in assemblage composition to pollutants (Tien *et al.*, 2009). Moreover, the range of water column and interface properties which govern benthic diatom composition serves to further accentuate spatial and temporal differences in biofilm architecture (Davey and O'Toole, 2000). Therefore, the three dimensional structural composition of diatoms within the biofilm is related to its function and to the survival of its constituent organisms all of which are inter-dependent and influenced by environmental condition (Wimpenny *et al.*, 2000; Hall-Stoodley *et al.*, 2004).

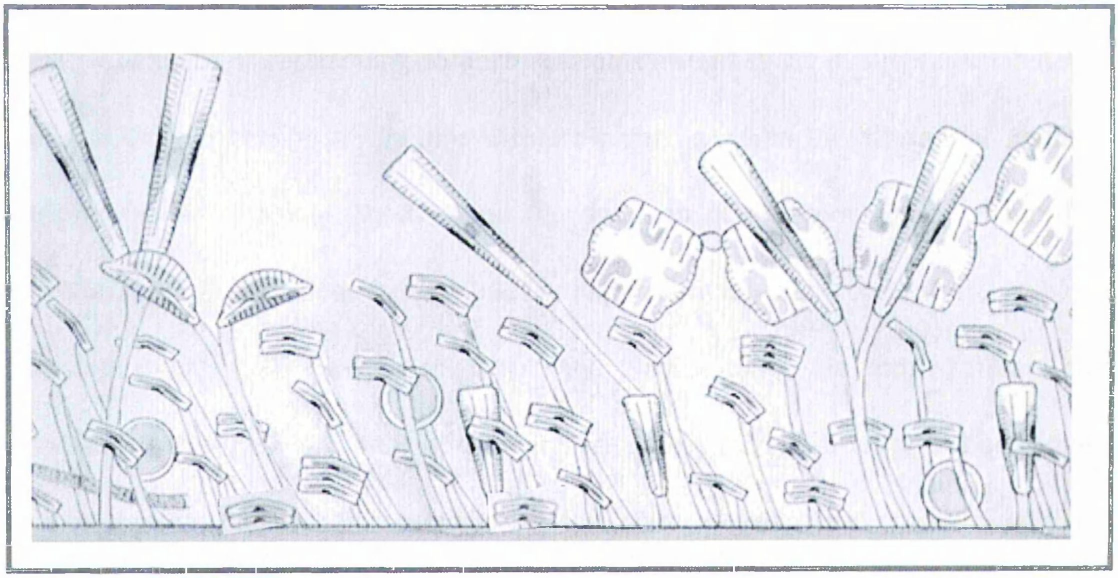


Figure 2.4: Three dimensional structural composition of diatoms within the biofilm. The understory of the biofilm is composed low profile species, typically *Achnanthidium minutissimum*. High profile species stalked species such as *Gomphonema olivacuem* form the upper canopy layer. Filamentous and motile species can also grow entangled within the biofilm extra cellular matrix. (Adapted from Kelly *et al.*, 2009c).

2.2.2 Diatom biofilm development: succession and perturbation

Biofilms, as a function of their position in the food web of stream systems, demonstrate sensitivity in key attributes of benthic biofilm successional state of composition, density and productivity to changes in biological, chemical and physical stream condition (Biggs *et al.*, 1998b; Soininen, 2007). Therefore, temporal factors have an important impact on the structure of benthic diatom communities (Passy, 2006) with diatoms responding to perturbation through shifts in successional state (Kelly, 2003). Succession of diatoms within biofilms is driven by differential species performance in dispersal, survival and reproduction at different stages of biofilm development (Burns and Ryder, 2001). For example, early colonisers, which characteristically have rapid immigration and growth rates, are more tolerant to disturbance than high profile species which are competitively superior but sensitive to disturbance (Passy, 2007b). As the benthic diatom biofilm matures competitive interactions between species increase as different species perform differently at different resource levels (McCormick and Stevenson, 1991; Paul *et al.*, 1991). Nutrients, light and space in terms of available substrate form the basic resources for biofilms (Burns and Ryder, 2001). Key mediatory factors include ecophysiology, life history and disturbance (Pickett and McDonnell, 1989). In addition to these factors, substratum type, water temperature, sediment type, hydrodynamics current velocity, discharge and turbidity can be considered drivers of observed successions (King *et al.*, 2006). Of these environmental factors it has been suggested that frequency of high flow events, nutrient and light availability and effects of grazing impose the greatest constraints on diatom assemblage structure and function

(Werner and Kohler, 2005). Furthermore, processes that control resources and structural composition also affect biomass accumulation with disturbances leading to losses (Biggs, 1996b). Therefore, successional development and truncation can be a source of uncertainty and variation. Instances where this can become particularly acute is when a newly colonized surface is compared with a surface with a mature biofilm, thus making natural environmental variation an important consideration in assessment (King *et al.*, 2006).

2.2.3 Discharge and nutrient pulses: A key determinant of headwater benthic diatom biofilm attributes.

Benthic diatom biofilm composition and productivity are determined by four key factors water quality, the energy budget (temperature regime, organic matter, nutrients), the physical structure of the channel and the flow regime (Maddock, 1999). While many factors influence the phytobenthic assemblage in headwater streams nutrient concentration and flow disturbances have long been considered the key driving variables of benthic diatom biofilm colonization and development (Lohman *et al.*, 1992). Within the dynamic headwater environment diatom assemblages have characteristics of immature or pioneer communities due to dynamic flow regime which confers advantage to species with adnate attachment (Biggs *et al.*, 1998b). Therefore, colonisation, rapid growth, and loss are the distinctive characters of the communities and accrual is the dominant process (Biggs, 1996b). Under such circumstances competition is greater for space rather than nutrients.

Fluctuations in discharge results in nutrient pulses which cause short-term changes in nutrient concentrations (Johnson *et al.*, 1969a; Biggs and Close, 1989; Poff and Ward, 1989; Hinton *et al.*, 1997). Nutrient pulses within the biofilm can also be generated by invertebrate grazing in the benthos through mechanical disruption of biofilms and excretion by grazers (Davies and Bothwell, 2012). Development of the biofilm in response to nutrient delivery is dependent upon the differential uptake of a cell within the biofilm and individual species differences in physiological attributes such as nutrient storage, cellular ratio and efficiency of use (Bothwell, 1989; Paul *et al.*, 1991; Borchardt, 1996; Passy, 2007b). However, successional processes rarely reach climax in headwater streams due to frequent periodic sloughing. This can be attributed to high shear stress with turbulence and sediment instability resulting in loss from the biofilm matrix which arises as the drag of the developing mat exceeds its tensile strength (Biggs, 1996a).

The role of physical and biological disturbance in regulating key resources to the biofilm (light, substrate and nutrients) and their interaction with other components of the aquatic systems (macroinvertebrate grazers) in determining both the structure and the function of the biofilm is summarised in Figure 2.5. However, despite this understanding, our comprehension of how these factors change and interact along a temporal gradient over fine-spatial scales to determine benthic diatom biofilm composition and chlorophyll-*a* is in its infancy. Therefore, a key aim of this research is to investigate high temporal and fine spatial patterns in benthic diatom communities so as to determine the role of two key environmental variables;

discharge and nutrient (TP and NO_3) availability in regulating observed patterns in benthic diatom biofilm attributes.

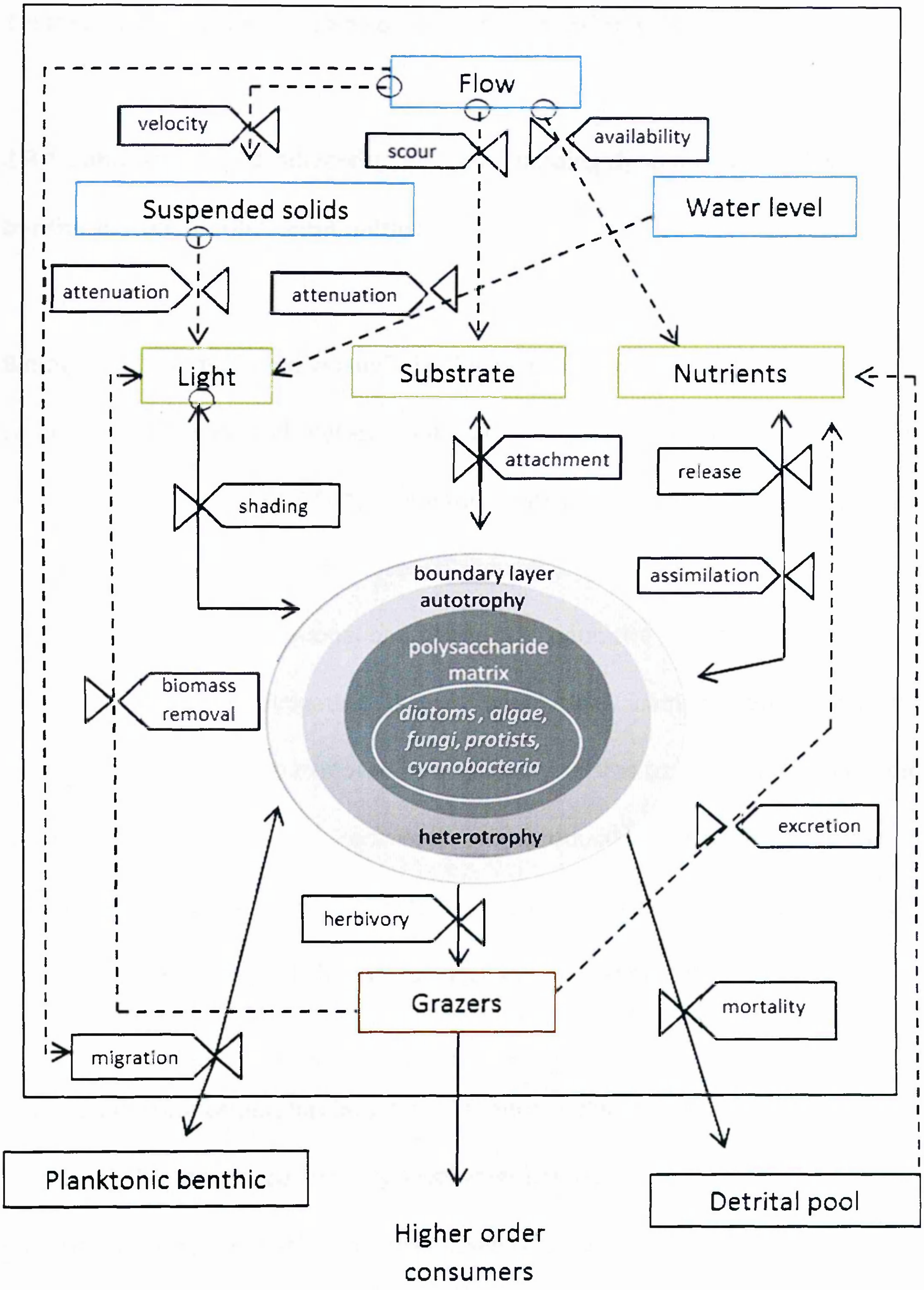


Figure 2.5: Resources (green text box) and key agents of physical (blue text box) and biological (red text box) disturbance important for determining biofilm structure and function. Matter and energy flow are connected by solid arrows and indicate processes, modulators of community function are shown by arrows originating from small circles. The benthic biofilm is composed of autotrophs (cyanobacteria and algae) and heterotrophs (bacteria, fungi and protists) in a polysaccharide matrix forming the boundary layer of the biofilm which is at interphase with the environment. (Adapted from Burns and Ryder, 2001).

2.3 Diversity in benthic diatom community composition

2.3.1 Components of biodiversity for understanding dynamics in headwater stream benthic diatom biofilm communities.

Biological diversity, "biodiversity", is the diversity or variety of species within a community (Wilson and Peter, 1988) and is important for the functioning of ecosystems (Giller *et al.*, 2004). Community attributes; composition, structure and function both determine and constitute biodiversity (Noss, 1990). Therefore, biodiversity is multidimensional and dependent upon the diversity of habitats within an area. Studies investigating diatom assemblage composition in relation to ecosystem function have explored how diversity relates to both community stability (resistance, resilience and recovery) and productivity. However, despite this, patterns in biodiversity and the relationship between its primary components are poorly understood, especially in headwater streams (Kominoski *et al.*, 2010).

Community composition has important taxonomic dimensions of richness, evenness, and rarity. Moreover, community level interactions between species depend, in a complex fashion, on species identity, assembly sequence and productivity (Fukami and Morin, 2003). Therefore, an important aspect of biodiversity is diversity in species and interspecific interactions among species present within the community (Mougi and Kondoh, 2012). Finlay *et al.* (2002) distinguished two types of diatom species, cosmopolitan species with broad ecological tolerance and diatoms with more exacting requirements (rare species) which consequently occur at few locations. The relative proportion of these species can provide information on resource use strategies and competition among species (Mihuc, 1997). For example a community dominated by rare species is suggestive of a high level of interspecific competition. In addition, rare species are important in contributing to taxonomic richness and the maintenance of stability in ecosystem function (Loreau *et al.*, 2001). Rare species are especially important in headwater streams in which the dynamic physical environment constitutes a key driver of natural selection (Molinos and Donohue, 2010). Understanding these multiple interactions within a community can therefore provide a greater understanding of community dynamics and maintenance of biodiversity.

While both rare and common species constitute traditional measures of structural diversity (Harper and Hawksworth, 1994), the contribution of rare species to biodiversity is not well understood (Ellingsen *et al.*, 2007). Moreover, empirical ecological studies tend to focus on common species due to their richness, sampling practicality and function significance (Ellingsen *et al.*, 2007; Gillett *et al.*, 2011). This is based on the assumption that rare species fulfil the same ecological roles as common species, thus leading to functional redundancy (Mouillot *et al.*, 2013). However, determination of the effects that species loss may have on overall community function is context dependant especially in heterogeneous environments (Wellnitz and Poff, 2001). Considerable debate exits in the literature on the role of rarity and the importance of its definition in terms of abundance and distribution (Gaston, 1994; Nijboer and Schmidt-Kloiber, 2004). In particular, debate centres on their ability to adequately represent ecological and environmental conditions (Lavoie *et* *al.*, 2009; Gillett *et al.*, 2011; Rimet and Bouchez, 2012a). However, rare species with small distribution ranges often disappear first after disturbance and recolonize slowly, therefore acting as early warning indicators of change from good ecological status (Cao *et al.*, 2001; Nijboer and Schmidt-Kloiber, 2004). Moreover, recent studies by Mykra *et al.* (2012), have demonstrated that rarity can influence ecological status classification under the Water Framework Directive (WFD; European Council, 2000). Concluding from this, there is a need to assess the role of common and those more specialists' species in assessments of diversity for lotic habitats at fine scales given the natural dynamic flow regime.

The functional diversity of a community quantifies the value and range of species traits that influences their performances (Villeger *et al.*, 2008). Thus, morphological traits present a useful method of assessing diversity through grouping of taxa with similar morphological characteristics referred to as guilds (Passy, 2007b). For example, growth morphologies can be grouped into four ecological guilds, i.e. low profile, high profile, motile and planktonic species based on their potential to tolerate nutrient limitation and physical disturbance. Species can also be grouped based on low or high trait values (physiological guilds). Examples include low and high nutrient taxa, native and non-native taxa, taxa sensitive and tolerant to pollution, or low pH and high pH taxa (e.g. van Dam *et al.*, 1994; Stevenson *et al.*, 2008). Consequently, functional diversity is viewed as an important driver of ecosystem function (Diaz and Cabido, 2001) including resilience to disturbance (Dukes, 2001; Bellwood *et al.*, 2004) and productivity (Tilman *et al.*, 1997; Petchey *et al.*, 2004; Hooper *et al.*, 2005). Resilience is conferred through the process of succession and the rate of recovery after an event disturbance, which is dependent on the initial assemblage composition (Stevenson, 1990). The resistance and resilience of benthic diatom communities and their morphological organisation in relation to stream velocity is complexly related to habitat factors, such as substrate size, bottom roughness (Fisher *et al.*, 1982b; Power and Stewart, 1987) and channel morphology (Resh *et al.*, 1988). The structural diversity of habitat in turn is dependent upon the physical characteristics of a stream system from microhabitat to reach to landscape scale (Biggs *et al.*, 2005). Thus, diversity of habitat influences aspects of community stability (resistance, resilience and recovery) with high diversity facilitating a biologically diverse ecological community. Hence, a space-time habitat matrix is an important concept when studying relationships between biodiversity and ecosystem functioning in headwater streams.

2.3.2 The relationship between benthic diatom assemblage diversity and its productivity

Productivity, the amount of energy available for ecosystem development in a given location, plays a fundamental role in determining variation in biodiversity (Fukami and Morin, 2003; Chase, 2010). Traditionally, uni-modal relationships in which diversity peaked at intermediate productivity levels were understood to be the prevalent pattern (Rosenzweig, 1992; Rosenzweig, 1995). However, no clear linear tendency has been observed between the dimensions of biodiversity (e.g. richness, evenness) and productivity with several different studies reporting monotonic, U-shaped, positive, negative or non-significant relationships (Abrams, 1995; Waide *et* *al.*, 1999; Mittelbach *et al.*, 2001; Mittelbach *et al.*, 2003; Whittaker and Heegaard, 2003; Bonn *et al.*, 2004; Gillman and Wright, 2006; Simova *et al.*, 2013). Possible reasons for the observed variation in the relationship between productivity and diversity include disturbance (Kondoh, 2001), higher trophic consumers (Leibold *et al.*, 1997; Worm *et al.*, 2002) and niche specialisation, all of which cause variation between taxonomic groups and habitat type (Waide *et al.*, 1999; Mittelbach *et al.*, 2001). Moreover, productivity-diversity relationships depend on the history of community assembly as well as structural and functional diversity at the habitat level (Fukami and Morin, 2003). Therefore, measures of diversity within headwater streams should include not only aspects of their organisation (species composition, biodiversity, food web structure) but also their robustness (disturbance mediated nutrient cycling and production rates) and resilience (Rapport *et al.*, 1998). This research aims to apply this conceptual approach to the investigation of patterns in biodiversity and its relation to productivity and in doing, provide a more unified conceptual framework of headwater stream ecology relating benthic diatom biofilm process and pattern to flow condition (Hart and Finelli, 1999).

2.4 Determinants of benthic diatom scalar heterogeneity

2.4.1 Hierarchical organisation of headwater streams

Headwater streams can be classified at the catchment level by broad biogeoclimatic variables, at the system level by boundaries such as drainage divides and at reach through differences in substrate type (e.g. riffle-pool; Frissell *et al.*, 1986).

Consequently, within the landscape headwater streams can be viewed as hierarchical systems within spatially nested scales (Stevenson, 1997). Spatial factors contribute substantially to small and large scale heterogeneity of benthic algal assemblages (Stevenson, 1997; Passy, 2001).Therefore, the view of pattern in diatom assemblage composition and function as 'stable', 'quasi-stable' or 'dynamic' is highly dependent on the sale of observation (Wu and Loucks, 1995).

Predicting those factors which regulate scalar dependant patterns in the structure and function of benthic diatom assemblages presents a key challenge in benthic algal ecology. Such predictions require that the spatial and temporal scale of observation be defined. Hierarchical theory provides a framework for assessing how higher levels of ecological and environmental organisation incorporates and constrains benthic algal heterogeneity at lower levels (Allen and Starr, 1982; Stevenson, 1997; Habersack, 2000; Parsons and Thoms, 2007; Gothe *et al.*, 2014). A model of the hierarchical framework of environmental factors affecting benthic algal spatial heterogeneity is presented in Figure 2.6. This shows that higher level factors, such as climate and geology, referred to as ultimate determinants constrain the expression of intermediate and proximate determinants which directly affect diatom function and structure. Important intermediaries include land use, while proximate factors such as pH, temperature, light, shear stress due to current velocity, abrasion due to bed load, and substratum collision result in fine scale differences in diatom assemblage composition. Hence, heterogeneity in benthic diatom communities arises from these complex interactions among multiple causes that operate at many spatial and temporal scales (O'Neill *et al.*, 1989). In effect this emphasises the importance of quantification of interactions among the multiple factors which influence environmental and consequently diatom assemblage heterogeneity that vary through time or space or both. Hierarchical theory, therefore, advocates that no single level of organization is fundamental and environmental stress will be expressed in different ways according to the level of biological organisation (Noss, 1990; Whittaker *et al.*, 2001; Wu and David, 2002). Thus, the hierarchical concept provides a useful framework to assess biodiversity while providing recognition of the effects of environmental factors and their interaction, suggesting, therefore that for a holistic understanding of biodiversity, diatom communities should be monitored at multiple levels of organization within the hierarchical framework.

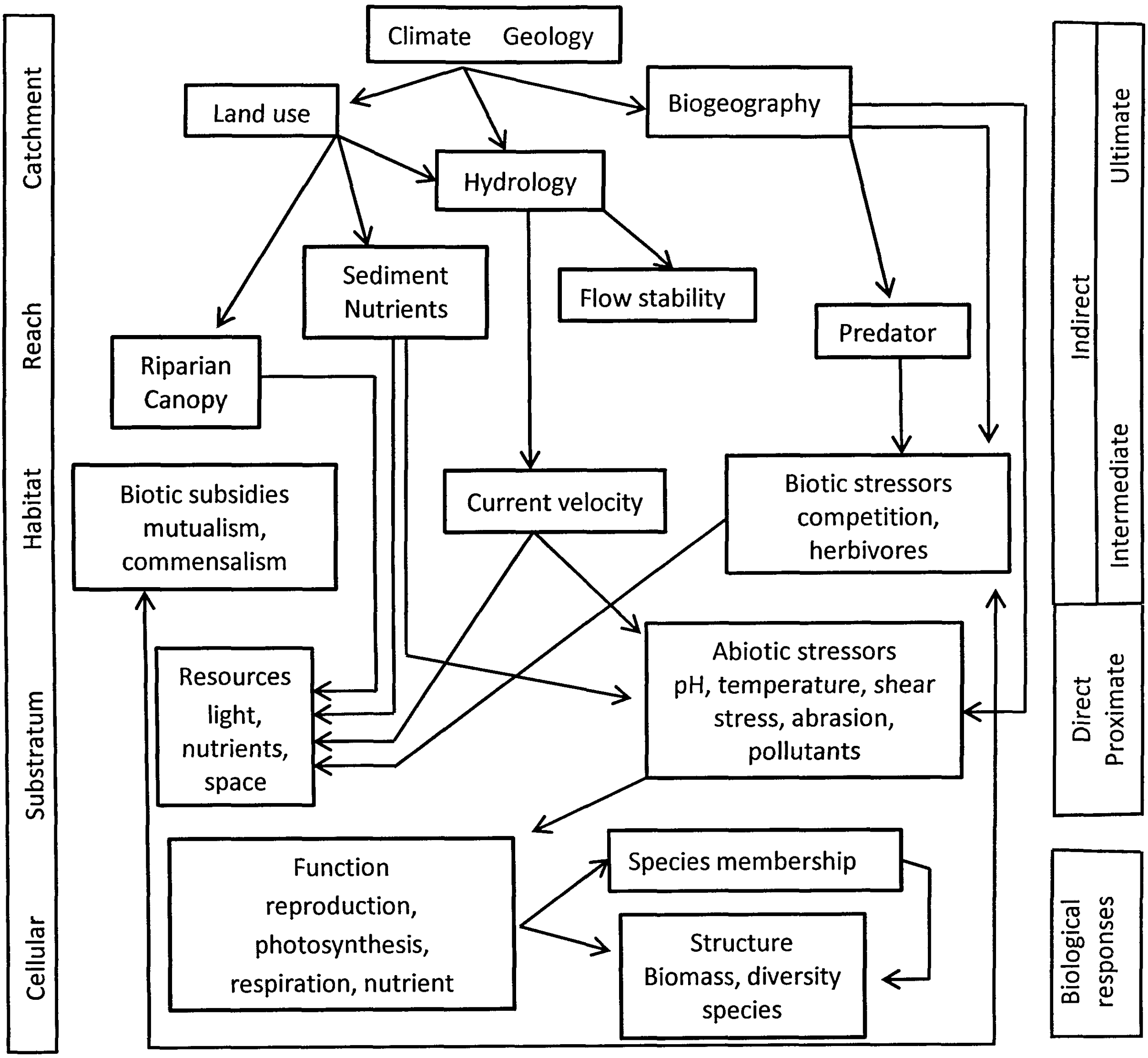


Figure 2.6: Hierarchical interrelationships among proximate, intermediate and ultimate determinants of benthic diatom biofilm composition and chlorophyll-*a*. Relative spatial scale included. (Reproduced from Stevenson, 1997).

2.4.2 Physical flow biotope for understanding pattern in diatom composition and function.

Hierarchical theory proposes that emergent properties at one scale arise from ecological processes within finer spatial and temporal scales (Wu and David, 2002). Finer level hierarchical constraints arise from reach-scale stream channel dimensions (width, depth, slope) confined by differences in stream habitat (e.g. riffle, pool, glides) and micro-habitats due to the impact of substrate and hydrology on the physical and chemical factors effecting algae growth (Figure 2.7). Thus lower levels in a hierarchy contain the details in species composition (e.g. species identities and abundances) providing the mechanistic basis for higher-order pattern (Noss, 1990). While the relationship between ultimate factors and diversity is well established within the literature few studies have examined if diatom communities are structured spatially at fine scales confined by habitat properties, which forms a central aim of this research.

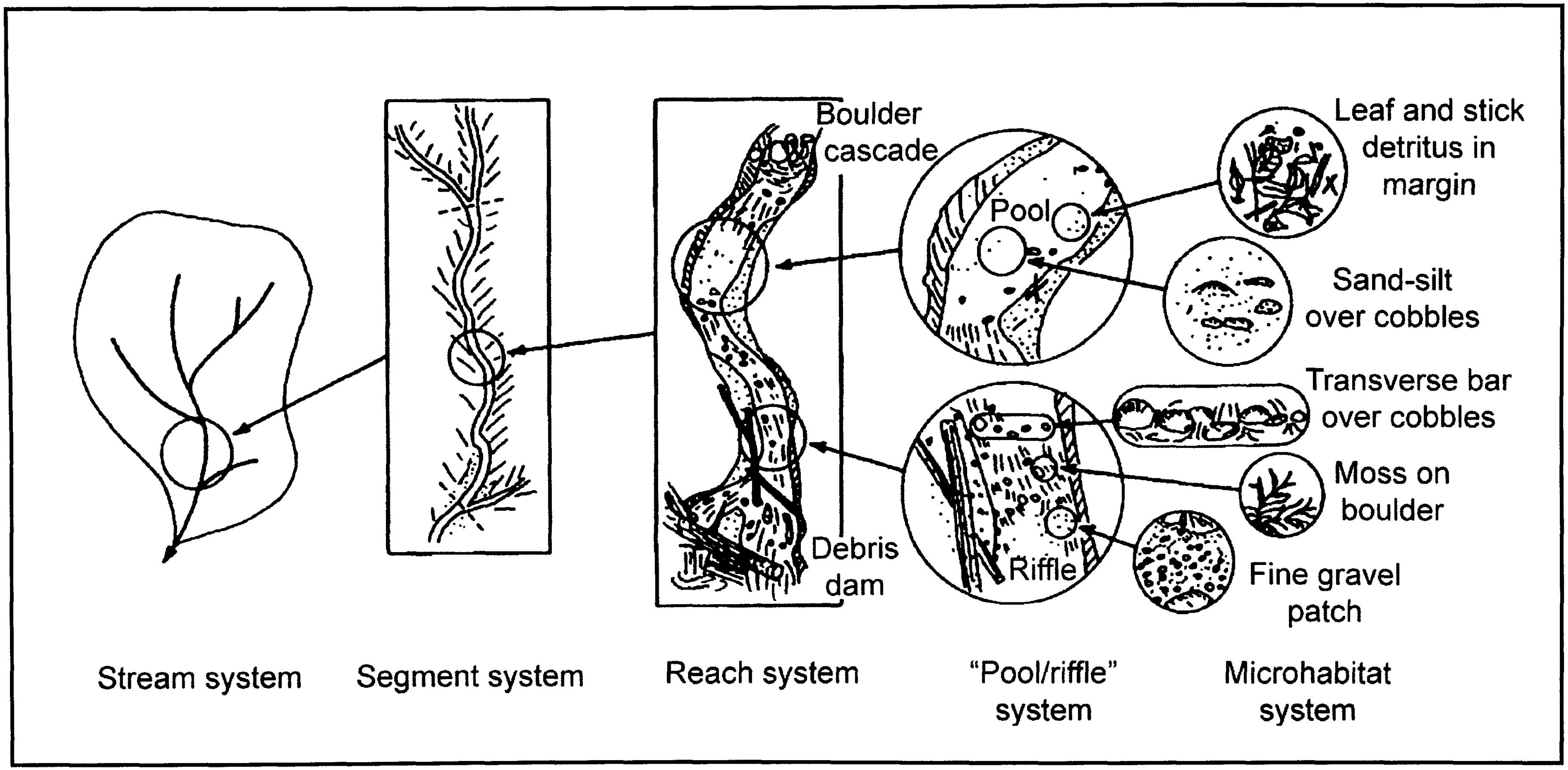


Figure 2.7: The hierarchical organization of a stream illustrating how habitat specific characteristics are nested within and confined by larger hierarchical units (Reproduced from Frissell *et al.*, 1986).

The importance of 'mesoscale' or 'habitat-scale' (Harper and Everard, 1998) sub-reach hydraulic 'patches' (Newson *et al.*, 1998) and microscale flow hydrodynamics for understanding the pattern in diatom diversity has long been recognised (Crowder and Diplas, 2000). In the 1920s, habitat was identified as a filter for biological traits with patterns in these traits related to spatial habitat variability and disturbance (Statzner *et al.*, 2001a; Statzner *et al.*, 2001b). Subsequently, Southwood (1977) put forward the concept of the "habitat template concept", linking gradients in habitat spatial-temporal characteristics and variation to gradients in ecological response (Townsend and Hildrew, 1994). Arising from the habitat template concept, differences within stream conditions were viewed as a mosaic of patches (Pringle *et al.*, 1988), each of which have similar substrata and hydraulic conditions, giving rise to the term biotope (Palmer and O'Keefe, 1992). As flow defined physical biotope units refer specifically to fluvial features such as riffle, runs, pools and glides, they provide a basic unit of in-stream habitat underpinned by the concept of ecohydraulics (Padmore, 1998). Consequently, flow-defined biotope units are an important element in defining habitat biodiversity (Padmore, 1998; Dodkins *et al.*, 2005). Physical flow biotope can also be used as a surrogate for diatom composition and diversity. This approach to investigation is important because interactions between the timing of disturbance, the successional state of the benthic algal community and habitat may influence temporal and spatial heterogeneity in stream ecosystems (Peterson and Stevenson, 1992). Therefore, through providing a means of constraining flow variability within headwater streams, physical biotopes provide a means of understanding system dynamism and unpicking potentially confounding relationships between biofilms and in-stream and broader catchment characteristics.

2.4.3 Determinants of benthic diatom scalar heterogeneity: summary

Spatial and temporal heterogeneity in diatom assemblage composition is dependent on the scale at which it is observed with local factors, confined by catchment scale characteristics, influencing the species which are present (Tison *et al.*, 2005a). Headwater streams are well positioned to assess these questions of spatial variance due to their hierarchical framework and changes in major abiotic variables over high and fine scales (Finn and Poff, 2005). Appreciation of the relation of fine scale spatial-temporal distribution of diatom composition and productivity to environmental factors is critical for understanding community dynamics and the appropriate design of diatom-based biological assessment strategies.

2.5 Application of diatoms in headwater biomonitoring

Stream biomonitoring, the assessment of the extent of anthropogenic influence on aquatic ecology, is an important branch of applied ecology (Passy, 2007a). Most assessments of riverine ecosystems are based on structural attributes of communities, providing a reliable and relatively inexpensive way to record conditions (Bellinger and Sigee, 2010). Phytobenthic diatom communities have been chosen as a key ecological group in assessment as they occupy a pivotal position at the interface between biological communities and their physical-chemical environment (Rusanov *et al.*, 2012). Furthermore, diatoms, which have been noted to dominate stream biofilms, especially in spring, are ubiquitous with variability spanning most ecological conditions of aquatic environment (Moore, 1977; Taylor *et al.*, 2007; Feio *et al.*, 2009). This is due to species rich communities that have different auto-ecologies along a continuum in environmental condition (Lowe, 1974; Dixit *et al.*, 1992; Van Dam *et al.*, 1994). Broad distribution, short generation times and temporal sensitivity in response to a wide variety of both natural and anthropogenic pressures in their environment (for example stream size, nutrients, light, organic matter, pH, salinity, toxicants) exhibited through changes in composition and abundance (Vilbaste and Truu, 2003; Sabater *et al.*, 2007; Feio *et al.*, 2009; Elias *et al.*, 2012) favours the widespread use of benthic diatoms in water quality assessment (King *et al.*, 2006).

Despite the dominance of benthic diatom biofilms in headwater systems and their use in biomonitoring, ecological knowledge is limited on their spatial-temporal patterns at fine-spatial and high-temporal scales in headwater catchments. In part this is due to the fact that assemblage composition can respond within days to various pressures (Schindler, 1987; Steinman and McIntire, 1990; Biggs *et al.*, 2005; Taylor *et al.*, 2009). While, this means they facilitate an integrated temporal measure of water quality, given the dynamic environment within headwater this also imparts considerable variability into assessment and emphasising the need for determination of appropriate sampling resolution (Rusanov *et al.*, 2012). This research aims to determine the appropriate resolution for sampling through investigation of variability amongst benthic communities at the biotope scale and determination of the critical period of antecedent temporal dynamics in discharge and nutrient condition that benthic diatom communities respond to.

Numerous studies provide illustration of how the diversity of diatoms within the biofilm reflects water quality (Soininen, 2002; Kupe *et al.*, 2008; Tien *et al.*, 2009). Typically biological assessment methods have been developed to address ecological response along single pressure gradients, such as P through the Trophic Diatom Index (TDI; Kelly and Whitton, 1995a). However, the ecology of stream biota including, diatoms, macrophytes and macroinvertebrates, rarely reflect pressures from a single gradient but from numerous interacting pressures (Ormerod *et al.*, 2010). Interacting environmental pressures have an influence on the structure and functioning of diatom communities over a multitude of scales ranging from micro- to macro- habitats (Stevenson, 1997). Consideration of multiple interacting physical-chemical factors is therefore important as this can obscure the effects of individual environmental stressors and the response of the biofilm to them (Kelly *et al.*, 1998b; Mosisch *et al.*, 2001; Jarvie *et al.*, 2002; Tien *et al.*, 2009). In order to define and establish true relationships between both physico-chemical and biological status near-continuous monitoring is required together with investigations of individual species response to specific and interacting environmental stressors (see Round, 1991). This is important for advancing ecological understanding of these communities, having the following on objective of allowing for more accurate representation and interruption of diatom structure, function and processes.

A key driver of biomonitoring tool development has been requirements which have arisen as a direct result of environmental policy frameworks such as European Directive 2000/60/EC, commonly known as the Water Framework Directive (WFD; European Council, 2000), represents a new paradigm in biological evaluation proceeding to type-specific ecological assessment and classification. It creates statutory obligation for European Union (EU) Member States (MS) to monitor the ecological status of water bodies to achieve at least 'good ecological status' by 2015 with ecological status quantified as 'an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface water' Article 2 (Sandin and Solimini, 2009). An integral part of this approach is the determination of baseline or "natural" conditions via which to compare various anthropogenic disturbances (Tornes *et al.*, 2012). Furthermore, it advocates an integrated approach to monitoring and ecosystem management with the inclusion of structural and functional aspects of four biological quality elements (BQE): 'phytoplankton'; 'macrophytes and phytobenthos'; 'benthic invertebrate fauna'; and 'fish fauna'. These are employed in conjunction with supporting physico-chemical variables and other factors including hydromorphological attributes and quantification of uncertainty in measurements. The WFD requires member states to assess the ecological status of its rivers based on appropriately informative aspects of these BQEs. Therefore, classification of ecological status (ES) can be based on either a single BQE or a combination of BQEs (WFD, Annex V, Section 1.2). The choice of BQEs and the metrics to be used within each BQE should depend upon their ability (statistical power and precision), cost-effectiveness at quantifying the ecological status, and ability to detect and quantify changes in ES. Inter-calibration of ecological status among MS is facilitated by conversion of BQE metrics to an Ecological Quality Ratio (EQR) score from which ecological status is determined on a 5 point classification scale (0 – 1; see WFD UK TAG, 2014 for further details). Hence, in effect, the WFD has provoked consideration to the limitations imposed by scale in experimental design and how to accurately report ecological data so that comparisons can be facilitated across the EU in a meaningful manner.

Current approaches in using phytobenthos for monitoring freshwaters in Europe within the WFD policy framework generally select benthic diatoms as representative organisms of environmental conditions for "macrophytes and phytobenthos" across freshwater habitats (Kelly *et al.*, 2008a) sampled from a specified river habitat (Kelly, 1998b; Prygiel *et al.*, 2002; Rott *et al.*, 2003; King *et al.*, 2006). As most biomonitoring efforts employed under the WFD rely on structure as a community descriptor which reflects abiotic factors including nutrient concentrations, habitat disturbances and the presence of micropollutants (Blanco *et al.*, 2012), the selection of diatoms as surrogates of phytobenthos has become routine practice in the assessment of the ecological state of rivers (Kelly *et al.*, 2009a; Blanco *et al.*, 2012) not only in Europe but also in North America and Australia (Atazadeh *et al.*, 2007). Few studies adopt a holistic approach to benthic flora within Europe in contrast to North America (Stevenson and Bahls, 1999) and New Zealand (Biggs and Kilroy, 2000) where the entire phototrophic assemblage is analysed routinely.

Diatoms have been used as indicators for the assessment of environmental conditions since the beginning of the twentieth century (Kolkwitz and Marsson, 1908). However, there is scepticism surrounding the ability of diatoms indices, such as the TDI, to accurately reflect system complexity as most have been built to respond to general pollution or trophic gradients (Beck, 1954; Knopp; Pantle and Buck, 1955). This, together with the requirement of the WFD, has encouraged debate into which aspects, structure, function, or both, of biological communities should be measured and assessed in order to accurately communicate the state of the environment in a clear, concise and cost effective manner (Li *et al.*, 2010). This has led to the commencement of significant research focus into the ability of functional measures, including those that use diatoms, to assess ecosystem state and the relationship between community structure and ecosystem functioning. Investigations by Sandin *et al.* (2009) support the use of structural and functional measures to assess ecosystem health. Justification for the combined use of structure and function is also given by Young *et al.* (2008) who states that stressors can cause changes in structure but not function, both structure and function or function but not structure. It has also been argued that structure will influence function and vice versa but that each describe a different aspect of the ecosystem (Gessner and Chauvet, 2002; Sandin and Solimini, 2009). This demonstrates that there is a need to assess the usefulness of diatom indices as biomonitoring metrics for lotic habitats at fine scales given high natural environmental variability and, in particular, the role of generalists species that tolerate a wide range of environmental conditions (Heino and Soininen, 2006).

In summary, further investigations and intensive monitoring campaigns are required into the scale specific relationship between diatom community structure and function and their dependency on multiple environmental stressors. In order to evaluate the relationship between diatom structure and productivity year-round sampling at appropriate catchment scale spatial-temporal resolution is required and forms a central aim of this research. It is hypothesised that such an investigation will help to better inform understanding of the spatial and temporal scalar patterns in benthic diatom communities from which monitoring and classification is guided.

Chapter 3: Methods

The sites investigated in this thesis and the generic methods used are introduced in this chapter. The three results chapters that follow contain details of experimental design specific to those parts of the thesis.

3.1 Study location and site characteristics

3.1.1 Site location: the River Eden catchment

The River Eden, situated in northwest England, rises on the limestone hills of Mallerstang Common and drains parts of the Lake District and Pennine Hills before flowing a distance of 145 km northwards to Solway Firth where it enters the Irish Sea (Figure 3.1). The Eden valley is a fault-bounded basin 50 km long and 5-15 km wide covering an area of 2288 km^2. It contains Permian and Triassic strata which dip gently to the north-east. To the west, Permo-Triassic strata wedge out against Carboniferous limestone and overlying Coal measures which crop in a 5 - 15 km wide belt around the margins of the Lake District. The eastern boundary of the catchment is influenced by the Pennine Fault and associated North Pennine escarpment (Chadwick, 1995; Allen *et al.*, 2010). Further information on the lithostratigraphy and rock structure within the Eden catchment can be obtained from BGS Regional Geology of Northern England (Arthurton, 1981; Chadwick, 1995; Allen *et al.*, 2010; Stone, 2010).

Large variations are observed in rainfall across the catchment, from approximately 650 mm per year in the lowland valley to more than 2000 mm in the uplands (Mayes *et al.*, 2006). Elevations of up to 882 m asl contribute to variation in rainfall. The rain shadow effect from the Lake District Mountains in the west of the catchment is also a key factor in rainfall variability. As runoff is primarily determined by differences in topography and geology (Mayes *et al.*, 2006; Jencso *et al.*, 2009; Nippgen *et al.*, 2011) the upland areas of the catchment which have steep slopes and minimal soil cover are typified by small streams and flashy runoff. In the lowlands there are greater groundwater contributions from the lowland limestone and sandstone aquifers (Mayes *et al.*, 2006). The water table is found close to the surface along the River Eden, apart from the north of the basin where water is found 50 m below ground level (Butcher, 2005). Across the catchment the baseflow index ranges from 0.26 at source to 0.5 as it nears its mouth at Solway Firth, resulting in groundwater contributing less than 10% to river flow (Butcher, 2003).

The Eden catchment is predominantly agricultural with 97% of the land area covered by agricultural activities consisting of improved grassland (64%), arable (11%) and moorland and fell (11%). The predominant agricultural sector is livestock (breeding, meat and milk) production with sheep and cattle grazing in the upland areas of the Pennines, Lake District and the Howgill fells. In the lower parts of the catchment where the climate and soil type is more favourable for supporting agriculture, dairy production, mixed grazing and pockets of arable cropping dominate (Mayes *et al.*, 2006). Key catchment pressures include erosion of river banks by livestock, flash flooding from hills, fine siltation, NO_3 and P (Owen *et al.*, 2012).

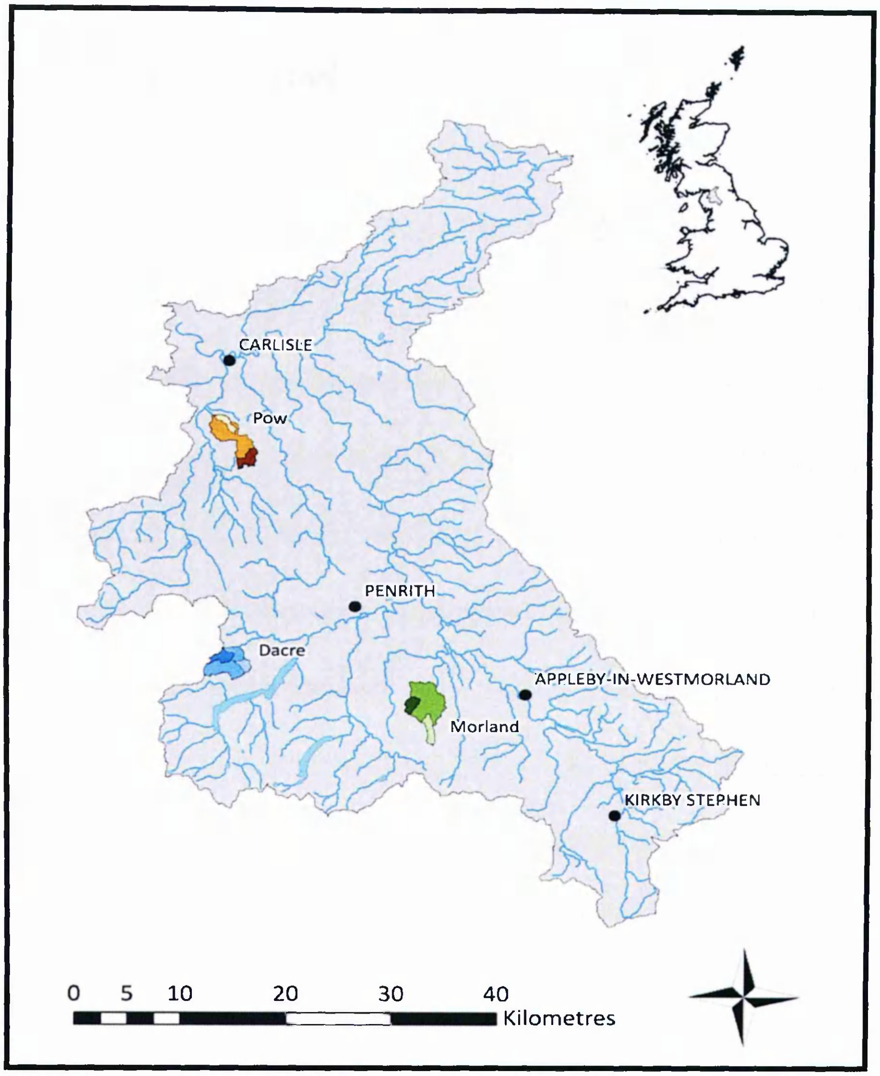


Figure 3.1: Location of the River Eden catchment, northwest England and the three focus 10 km^2 catchment; Morland, Dacre and Pow, with nested 2 km^2 sub-catchments. Consult Table 3.1 for catchment detail and colour code interpretation. (© Crown Copyright/database right 2014. An Ordnance Survey/EDINA supplied service).

Table 3.1: Catchment size and Becks studied including the colour code used to distinguish sites in chapter 3 and 4. Catchment size of 2 km^2 is approximate.

Catchment	Beck	Catchment size	Colour code
Morland	Newby	12.5 km^2	🟩
	Dedra Banks	2 km^2	🟩
	Sleagill	2 km^2	⬜
Pow	Pow	9.9 km^2	🟧
	Unnamed trib A	2 km^2	⬜
	Unnamed trib B	2 km^2	🟫
Dacre	Thackthwaite	10.6 km^2	🟦
	Mellfell	2 km^2	🟦
	Lowthwaite	2 km^2	⬜

3.1.2 Study locations: Pow, Newby and Dacre Becks

Three sub-catchments of the River Eden; Newby Beck (NY-59957,21249) which drains the headwaters of the Morland catchment, Pow Beck (NY-38685,50074) and Dacre Beck (NY-41190-25320), were selected for study due to their variable geographical, geological, local climate and land uses. The sub-catchments of Morland, Pow and Dacre drain areas of 12.5, 10.5 and 10.2 km^2 respectively. Nested within each sub-catchment are two smaller sub-catchments, each draining an area of approximately 2 km^2. Sampling locations occur at each of the sub-catchment outlets and also at the confluence of the 2 km^2 catchments (Figure 3.1 - 2, Table 3.1). Hereafter, Newby Beck (Morland), Pow Beck (Pow), and Thackthwaite Beck (Dacre) will be referred to as "catchments" while each of two smaller 2 km^2 catchments are referred to as "sub-catchments".

The upper section of the Morland catchment typically drains exposed, steeply dipping, fractured Carboniferous limestone, shale and sandstone units (Allen *et al.*, 2010). A significant proportion of its catchment consists of improved grassland, with rough grazing and arable being the dominant land uses (Owen *et al.*, 2012). Thackthwaite Beck lies in the upland west of the Eden catchment, on hard rock of volcanic andesite sheets of the Borrowdale Volcanic Group (Allen *et al.*, 2010), with similar land uses to the Morland catchment of improved grassland and rough grazing (Owen *et al.*, 2012). The Pow catchment is located in the north of the Eden catchment and consists of a significant till cover overlying the St Bees Sandstone (Allen *et al.*, 2010). Unlike Morland and Dacre catchments, agricultural practices are more intense in the Pow catchment which supports improved grassland, arable and rough grazing coinciding with intensive agricultural practices of dairy, sheep, pigs and poultry. Across all three catchments sediment-induced turbidity and P is of environmental concern with Pow having the additional pressure of elevated NO_3 concentrations (Owen *et al.*, 2012).

The hydrogeology of each catchment is dominated by superficial deposits, which are lithologically variable particularly in the Morland and Dacre catchments. Bedrock aquifers in the Morland catchment are localised within Carboniferous bedrock, while Dacre catchment is underlain by poorly permeable material. In the Pow catchment, major aquifers are present (e.g. St Bees Sandstone, Penrith Sandstone) but these do not support river flow (Allen *et al.*, 2010). Rivers draining these three catchments of the Eden instead gain their flow from a set of sources with different residence times. In each, baseflow originates from groundwater and typically dominates summer flows. Given the hydrogeological dominance of superficial deposits groundwater recharge may occur via relatively quick shallow groundwater flow. Delayed flow from near-surface or deeper groundwater flows occurs over a range of timescales due to subsurface flow mechanisms. For example in the Morland catchment, deeper and slower groundwater flow paths through Carboniferous bedrock aquifers may result in a component of older water in the surface flows from the catchment (Allen *et al.*, 2010), whereas rapid surface runoff to the stream and its tributaries dominates the flow regime. Consideration of the hydrogeology of these catchments is therefore critical to the understanding of effects of land-use and related changes in chemical and physical stream condition associated with dynamic stream flow.

3.2 Experimental Design

As this research formed part of the wider EdenDTC research framework (EdenDTC, 2013) a BACI experimental approach was undertaken to facilitate comparison of a manipulated stream with a non-manipulated stream, before and after implementation of a mitigation measure (Figure 1.1). Therefore, the three 10 km^2 catchments; Morland, Dacre and Pow, with two 2 km^2 nested sub-catchments provided a control and mitigation zone (Figure 3.2, Table 3.1). Note, this research was undertaken before mitigation measures were employed. Benthic diatom composition and chlorophyll-*a* samples from each of the nine sites were collected according to the sampling design of n = 3 sites per n = 3 catchments for 25 months (Figure 4.1, see section 3.3.1). This approach was adopted as it provided a spatial reference to account for confounding environmental factors such as differences in geology, land use, hydrology and riparian zone shading (Table 3.1).

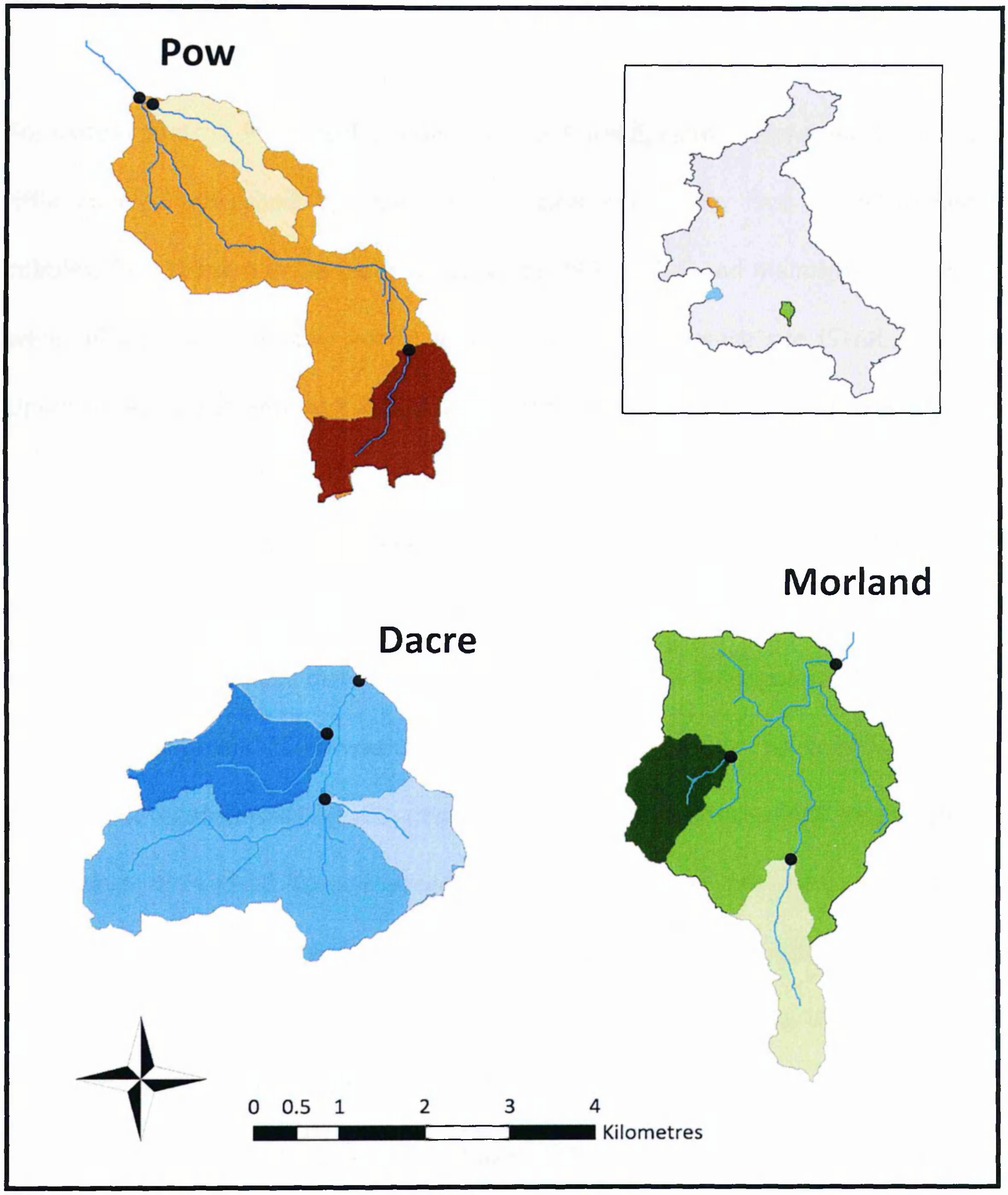


Figure 3.2: Sampling locations (black dots) of all nine sites within the River Eden catchment (Orange = Pow catchment with lighter shade representing Pow unnamed tributary A and darker shade representing Pow unnamed tributary B; Blue = Dacre catchment with lighter shade representing Lowthwaite Beck and darker shade representing Mellfell Beck; and Green = Morland catchment with lighter shade representing Sleagill Beck and darker shade representing Dedra Banks Beck) (consult Table 3.1). (© Crown Copyright/database right 2014. An Ordnance Survey/EDINA supplied service).

3.3 Biological sampling

For diatom analysis, in particular within chapter 4 and 6, cobbles were selected from riffle zones undisturbed by typical hydrological conditions. Five representative cobbles, 64 - 128 mm in diameter (Wentworth, 1992; Giller and Malmqvist, 1998b), were selected, both by colouration and feel, randomly at each site (STAR, 2002). Upper surface areas were scraped using a toothbrush (Kelly *et al.*, 1998b).

In the laboratory, a visual assessment of a sub-sample of the raw material at 400x magnification was undertaken to examine algal groups present and assess the proportion of live to dead diatoms cells. Samples were then homogenised by shaking the sample bottle and 50 ml was transferred to a 200 ml beaker. 50 ml of 30% (100 volume) hydrogen peroxide (H_2O_2) solution was added to the beaker and heated on a hotplate at 80 °C (± 10 °C) until all organic material had been oxidized (minimum of 120 minutes). Samples were then removed and beakers topped-up with distilled water. Following settling, for a minimum of 24 hours, the supernatant was decanted. The beaker containing the diatom suspension was then re-filled with distilled water. This settling period was repeated three times, with a minimum of 24 hours between each settling period (CEN, 2003).

Permanent slides were prepared by allowing a drop (approximately 100 µl) of diatom suspension to dry onto a clean coverslip at room temperature and fixed using Naphrax©, a diatom mountant with a refractive index of 1.73. Three hundred diatom valves were identified according to recommendations in Kelly *et al.* (1998b) for routine analysis and counted along transects at 1000x magnification, under oil immersion, with a Zeiss Axioskop microscope (CEN, 2004). Valves were identified using standard floras (primarily Krammer and Lange-Bertalot, 1986, 1988, 1991).

3.4 Assessment of chlorophyll-*a* concentration

Chlorophyll-*a* is the primary constituent of the molecule chlorophyll. When excited by UV light it emits fluorescence that reflects photosynthetic reactions within plant cells, inclusive of all eukaryotic algae and prokaryotic cyanobacteria (Krause and Weis, 1991; Beutler *et al.*, 2002b). Chlorophyll is composed of four molecules chlorophyll *a, b, c,* and *d*. The relative composition of these chlorophyll molecules and their peripheral antenna varies with algal taxonomic groups (van den Hoek, 1995), thus providing a robust characterisation of algal groups via their emission spectra. Chlorophyll-*a* is widely used as an indicator of algal benthic biomass and, given its relation to rate of production, benthic chlorophyll-*a* can be used as a surrogate variable for productivity (Dejong and Dejonge, 1995; Enriquez *et al.*, 1996; Morin *et al.*, 1999; Barranguet and Kromkamp, 2000; Lyon and Ziegler, 2009). Development of these principles has contributed to spectral group specific biomass determination (Beutler *et al.*, 2002a; Beutler *et al.*, 2002b; Aberle *et al.*, 2006). In this study, chlorophyll-*a* was used as a surrogate for stream productivity with estimates of chlorophyll-*a* concentration obtained using *in situ* fluorometry (ISF) techniques (see section 3.4.1) and *ex situ* using traditional techniques of extraction from disintegrated cells in an organic solvent, ethanol and spectrophotometric determination (Biggs and Kilroy, 2000).

3.4.1 *In situ* fluorometry

ISF is a technique used to characterise benthic biofilm community structure in headwater streams. It enables the composition and chlorophyll-*a* content of the phytobenthic component of biofilms to be resolved at high spatial and temporal resolution (Snell *et al.*, in prep). ISF chlorophyll-*a* measurements are taken using an *in situ* fluorometry probe (ISFP), in this case the Benthotorch©. The ISFP is placed on the cobble surface and a measurement is taken. It is based on the aforementioned principle that fluorescence is primarily emitted by the chlorophyll-*a* of the photosystem II antenna system, which contains evolutionally conserved chlorophyll-*a* core antenna and species dependant peripheral antennae (Aberle *et al.*, 2006). The technical principles and development of the Benthotorch© are discussed in detail in Aberle *et al.* (2006) and Beulter *et al.* (2002b) with subsequent testing presented in Carpentier *et al.* (2013). In brief, the ISFP exposes the benthic biofilm to pulses of excitation light from LEDs at wavelengths of 470 nm, 525 nm and 610 nm. The resulting fluorescence response from the phytobenthos is recorded at 690 nm, with an optical filter preventing excitation light from reaching the detector and causing an offset. Additional excitation light at 700 nm is used to correct for fluorescence due to reflection of light from the substratum. Individual 'spectral signature groups' of green algae (which contain chlorophyll a/b), cyanobacteria (containing phycobilisomes rich in phycocyanin) and diatoms (containing chlorophyll a/c and green light absorbing xanthophylls) facilitates determination of their respective densities. Following calibration, the fluorescence response recorded by the ISFP is deconvolved into chlorophyll-*a* concentration in μg/cm^2 providing an estimate of total phytobenthic chlorophyll-*a* based on an intact cell.

3.4.2 Laboratory chlorophyll-*a* analysis

Ex situ analysis of chlorophyll-*a* was adapted from (Biggs and Kilroy, 2000) and the following chlorophyll-*a* field procedure, extraction and spectrophotometric determination is based on this method.

3.4.2.1 Chlorophyll-a field procedure

Three representative cobbles were selected at random from riffle zones for benthic chlorophyll-*a* analysis. Upper surfaces were scraped using a toothbrush. The surface area of each scraped cobble was estimated by covering the scraped area with tin foil and comparing the weight of the tin foil to that of a known surface area. From a depth of between 10 - 30 cm, a 500 ml water sample was collected from the water column above riffle zone for planktonic chlorophyll-*a* analysis. Samples were transferred to the laboratory under subdued light and cool conditions and processed within 24 hours.

3.4.2.2 Extraction

15 ml of benthic and 300 ml of planktonic sample was filtered using 47 mm Whatman GF/C filter paper under suction and subdued light conditions. Fragments of

moss and invertebrates were removed from the filter paper using a forceps. Filter papers where then placed in vials containing 5 ml of 90% ethanol in a water bath at 78°C (boiling point of ethanol) for 5 minutes. Following cooling, the samples were refrigerated for 8 - 10 hours at 4°C. The samples were then centrifuged at a speed of 3000 rpm for 10 minutes. For calculations of chlorophyll-*a* total samples volumes were taken using a graduated cylinder.

3.4.2.3 Spectrophotometric determination

Spectrophotometric determination was undertaken at wavelengths 664 nm (spectrophotometer determined chlorophyll-*a* wavelength peak) and 750 nm (turbidity correction) using 3 ml of sample. Between each reading the cuvette was cleaned 3 times with ethanol, inverted and tapped dry. Between each sample a blank reading was taken. In instances when chlorophyll-*a* concentration was outside the range of 0 - 1, 90% ethanol was added in 5 ml increments. To correct for senescence material within the biofilm, concentrations of phaeophytin were estimated by adding 0.075 µl of 0.3 M hydrochloric acid. Absorbance readings were taken at 664 nm and 750 nm 1 minute after the addition of acid. Calculation of chlorophyll-*a* and phaeophytin concentration followed (Biggs and Kilroy, 2000).

3.4.3 Comparison of chlorophyll-*a* assessment methods: ISF v *ex situ* laboratory based chlorophyll-*a* measurement.

The relationship between the *in situ* ISF and *ex situ* lab methods, of Biggs and Kilroy (2000), for chlorophyll-*a* analysis was examined based on monthly samples collected from March 2011 to March 2013 (Figure 3.3). ISF chlorophyll-*a* concentrations were higher than *ex situ* laboratory determinations, which were all within error and less than 1.3 μg/cm^2. Median ISF chlorophyll-*a* concentrations were significantly different between the three individual catchments (Kruskal-Wallis ANOVA: H = 16.424, n = 22, $p < 0.001$) with a statistical significant difference observed between Newby Beck and Pow Outlet Beck (Turkey test: $p < 0.05$).

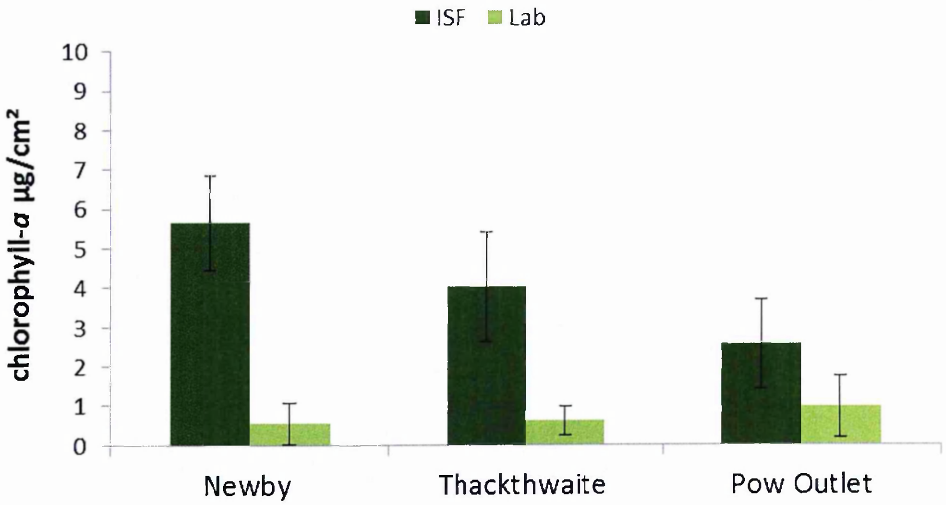


Figure 3.3: Difference in laboratory-determined chlorophyll-*a* methods minus senescence fraction (HCl correction) following Biggs and Kilroy (2000) (n = 25, ± standard deviation) and ISF methods (n = 22, ± standard deviation) for the outlet of the 10 km^2 catchments: Morland (Newby Beck), Dacre (Thackthwaite Beck) and Pow (Pow outlet Beck) from March 2011 to March 2013.

Investigation of ISF and *ex situ* laboratory determined chlorophyll-*a* concentrations within catchments revealed that lab determined chlorophyll-*a* concentrations were consistently lower than ISF determined concentrations at both the 10 km^2 and 2 km^2 catchment scale across Morland, Pow and Dacre (Figure 3.4). A maximum *ex situ* laboratory determined chlorophyll-*a* concentration of 1.27 µg/cm^2 was observed at Sleagill Beck, within the Morland catchment, where the highest ISF concentration of 6.64 µg/cm^2 was also observed. This demonstrates that patterns of *in situ* ISF chlorophyll-*a* concentration reflected laboratory determined chlorophyll-*a* but were consistently higher among all sites at both the 10 km^2 and 2 km^2 scale (see Figure 3.4).

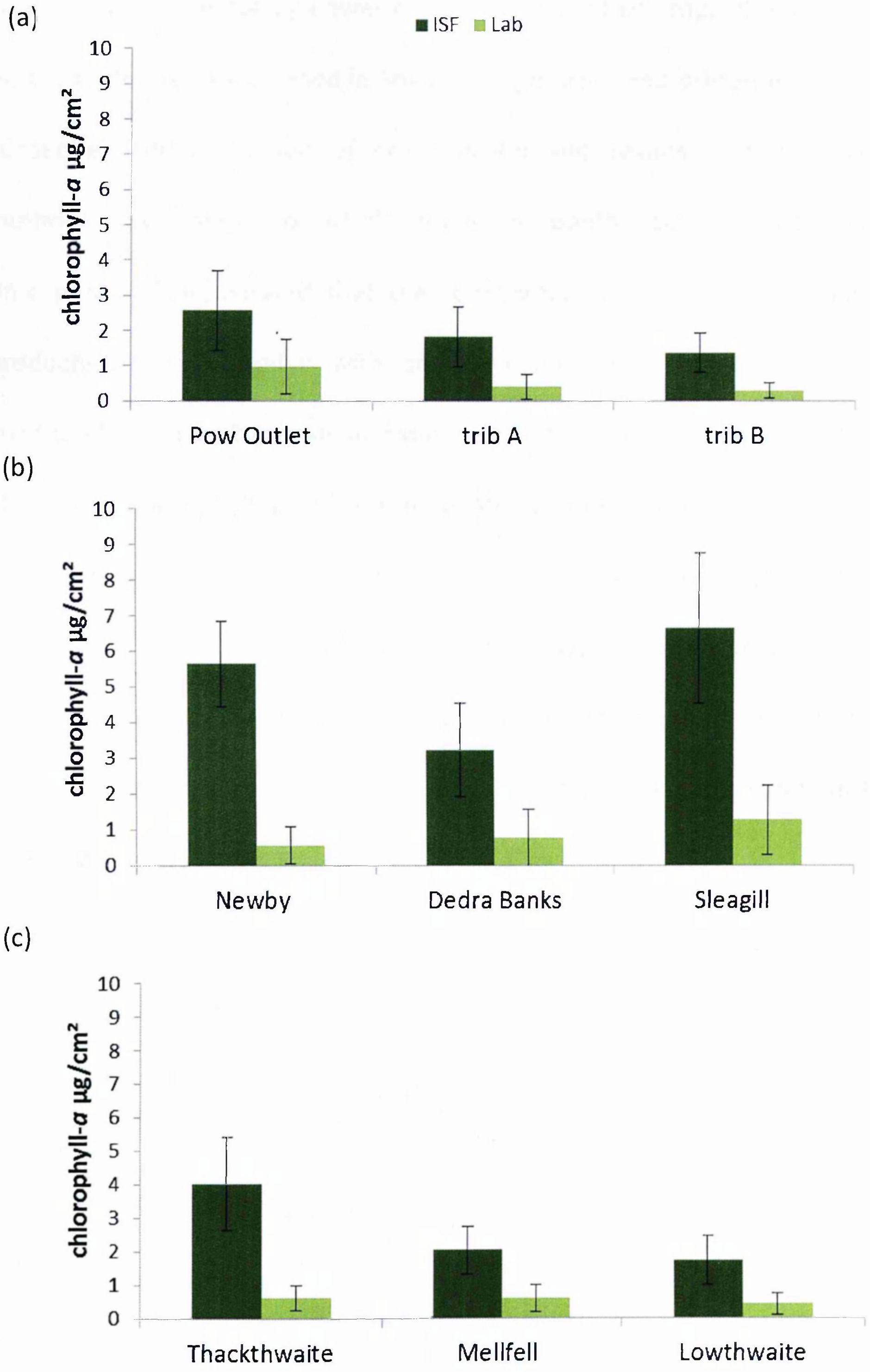


Figure 3.4: Difference in laboratory-determined chlorophyll-*a* methods minus senescence fraction (HCl correction) following Biggs and Kilroy (2000) (n = 25, ± standard deviation) and ISF methods (n = 22, ± standard deviation) for the three catchments (a) Pow, (b) Morland and (c) Dacre and their two 2km^2 sub-catchments.

Due to the discriminatory power of ISF determined chlorophyll-*a* and limitations of *ex situ* methods, as discussed in Snell *et al.* (in prep) and presented in section 7.6.2, subsequent determination of chlorophyll-*a* and results presented will use ISF methods only. Comparison of planktonic and benthic chlorophyll-*a* over the same time period demonstrated that the contribution of the water column to stream production was negligible with any observed chlorophyll-*a* trace likely to be attributed to material wash in from upstream or from bank erosion. Maximum planktonic chlorophyll-*a* concentration among the individual catchments reached 4.61 µg/L with chlorophyll-*a* concentrations demonstrating high variability but all within-error of each other (Figure 3.5). Moreover, monthly planktonic taxonomic analysis from February to October 2012 demonstrated that there were negligible algae present in the water column at both catchment and sub-catchment scale (Xing, pers. comm., 2013).

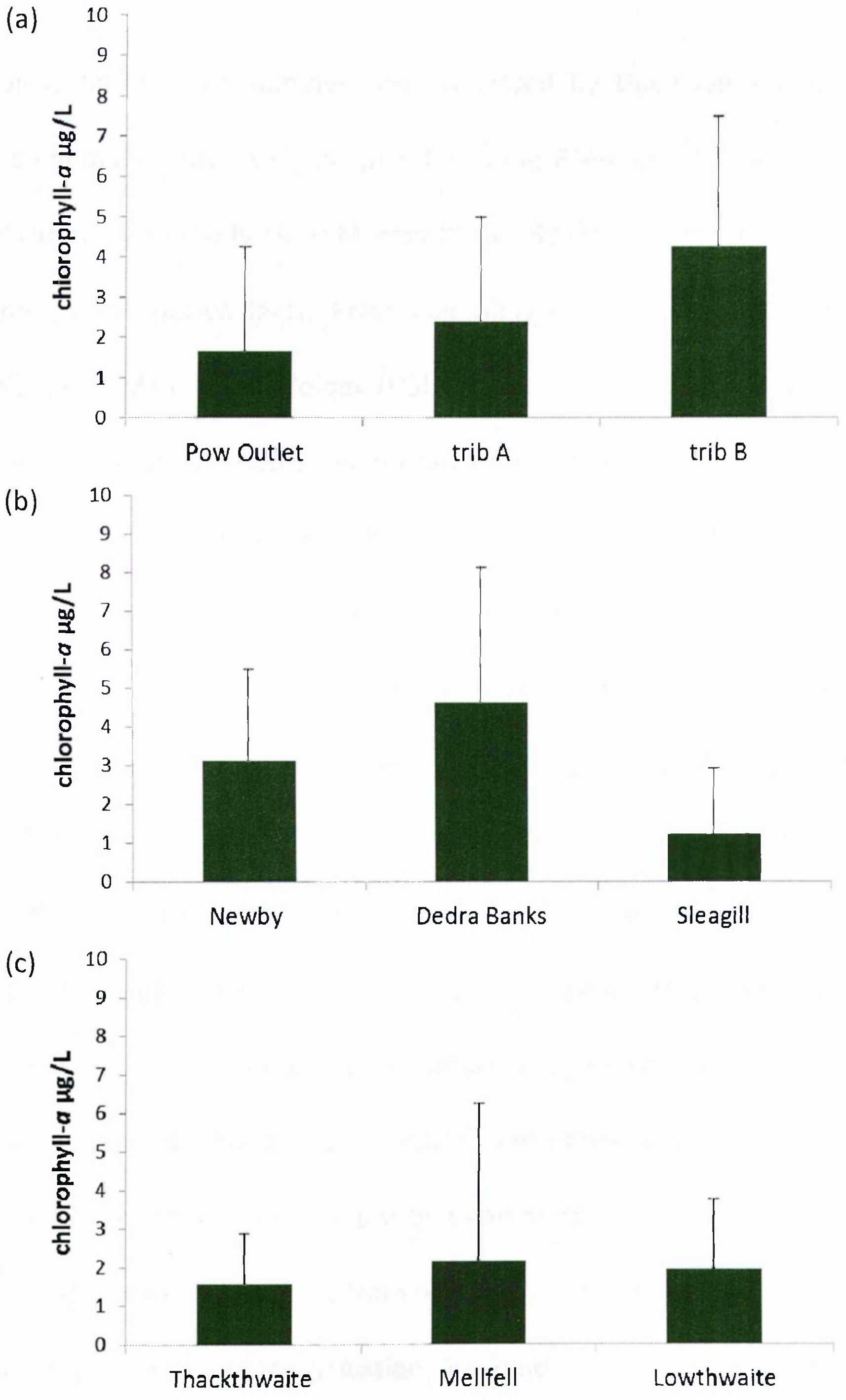


Figure 3.5: Difference in planktonic chlorophyll-*a* concentrations minus senescence fraction (HCl correction) among the three catchments a) Pow (n = 19, ± standard deviation), b) Morland (n = 22, ± standard deviation) and c) Dacre (n = 22, ± standard deviation) and their 2 km^2 sub-catchments from March 2011 to March 2013.

3.5 Assessment of biotic quality elements

Macroinvertebrate communities were assessed by the Environment Agency (EA) using semi-quantitative kick samples following River Invertebrate Predication and Classification System (RIVPACS; Murray-Bligh, 1997) with relative abundances of taxa determined to species level. River macrophytes were sampled using the Mean Trophic Rank (MTR) methodology (Holmes, 1999). Taxa were identified to species level with assessment based on presence/abundance within a 100 m section of reach. Fish surveys performed by the Eden River Trust (July – August 2012), consisted of timed 5 minute electrofishing runs, thus producing results in "number per 5 minute survey". Density results were then assigned to a class: absent, poor, fair, good or excellent, for each species fry (Chaffer, 2013). The hydromorphological diversity and condition of the river reaches was assessed using the River Habitat Survey (RHS; Environment Agency, 2003). Table 3.2 outlines the ecological groups monitored, frequency of assessment and source of work available within the research platform. This information was used to provide deeper understanding of benthic diatom diversity and productivity and metric interpretation. Comparisons between diatom community composition and metric score obtained through EA and corresponding monthly samples from within this research served as a quality control on diatom taxonomy and identification. An additional quality control was undertaken by taking part in the UK diatom ring-test, a quality assurance scheme for freshwater diatom analyses (Kelly, 2013).

Table 3.2: Biological analysis undertaken through the EdenDTC research platform (EA = Environment Agency, ERT = Eden Rivers Trust). NO = Newby Beck Outlet, DO = Dacre Beck Outlet, PO = Pow Beck Outlet, M = Morland Catchment, D = Dacre Catchment, P = Pow Catchment). The diatom and macroinvertebrate per annum (pa) samples were obtained seasonally (spring, summer and autumn). Both the macrophyte and fish assessment were undertaken within summer.

	Catchment	Frequency	Survey Period	Source
Diatoms	NO, DO, PO	3 pa	2011 - 13	EA
Macroinvertebrates	NO, DO, PO	3 pa	2011 - 13	EA
Macrophytes	NO, DO, PO	1 pa	2011 - 13	EA
Fish	M, D, P	1 pa	2012	EA/ERT

3.6 EdenDTC monitoring framework within the Morland, Dacre and Pow catchments

Monitoring technology that allows for near-continuous *in situ* measurements of environmental variables is provided through the Eden DTC project, a catchment-scale observatory and research platform in the Morland, Dacre and Pow catchments (EdenDTC, 2013). The near-continuous *in situ* physical and chemical measurements (present below and summarised in Table 3.3) are used as supporting data, together with monthly *ex situ* chemical samples, to explore key environmental drivers of headwater benthic diatom assemblage composition and productivity.

3.6.1 *In situ* physical and chemical analysis within the Morland, Dacre and Pow catchments

Discharge measurements were derived by applying stage-discharge relationships to 15 minute water level readings recorded by a pressure transducer, Schlumberger Baro-diver. The stage-discharge relationship was developed through the collection of manual current metering measurements using a Valeport Electromagnetic gauge model 801 and extrapolated beyond the gauged range using assumptions for the stage-velocity relationship and the hydrological water balance (Ewen *et al.*, 2010). Streamwise averaged velocity was calculated by comparing the local Sontek Argonaut-SW velocity measurements with average velocity measurements recorded during flow gauging. Reach averaged shear stress values were calculated following the method of Biron *et al.* (2004).

Automatic weather stations in each catchment provided a measure of rainfall at intervals of 15 minutes (Owen *et al.*, 2012). A centrally located weather station between the three catchments produced monthly average air temperature and total sunshine hours (Penrith Weather Station, 2010). Fixed monitoring stations, designed by National Water Quality Instrumentation Service (NWQIS) and built by AT Engineering (TEng, 2013), were located adjacent to biological sampling areas providing *in situ* water quality measurements (Table 3.3). A Hach Lange nutrient analyser combining a Sigmatax SC sampling and homogenisation unit with a Phosphax Sigma wet chemistry analyser measured total P (TP) and soluble reactive phosphorus (SRP) as PO_4-P. NO_3, which is turbidity compensated through a reference measurement, was measured using an optical Nitratax Plus SC probe as NO_3-N (Owen *et al.*, 2012; Wade *et al.*, 2012). A sample was taken from the watercourse using an intake pipe located mid-stream, via a peristaltic pump, which fills a flow cell located inside the monitoring station. The pump ran for five minutes every 30 minutes, allowing the flow cell to overflow with stream water. The Sigmatax draws a sample from the flow cell into a glass chamber, where it is homogenised by ultrasonication for 3 minutes. A 10 ml aliquot of the homogenised sample is delivered to a glass cuvette inside the Phosphax Sigma, from which TP analyses are conducted. Therefore, within the 30 minute sampling time, a single measurement of TP was made before the flow cell was re-filled. Due to asynchrony between pump timing and Sigmatax sampling frequency, the Hach Lange data was reported at hourly frequency, rather than at 30 minute intervals (Jordan *et al.*, 2005; Jordan *et al.*, 2007; Wade *et al.*, 2012).

Dissolved oxygen (DO; electrochemical sensor, 0 – 500% ± 2%), pH (0 – 14 units ± 0.2 units), temperature (-5 to + 50°C ± 0.15°C) and conductivity (0 – 100 mS cm^{-1} ± 0.5%) were analysed every 15 minutes using a YSI 6600 sonde. In Dacre, at Thackthwaite Beck, and each $2km^2$ sub-catchment, turbidity and water level were measured at 15 minutes intervals. Turbidity was measured by a McVan Analite 390 series probe which measured the degree of scattering at an angle of 90° to the incident light beam, operating in the range of 0 – 1000 NTUs, with 1% precision. Level was measured using a Schlumberger Baro-diver. The optical probes were equipped with self-cleaning sensors in order to minimise bio-fouling, which is further reduced by housing the sonde in a flow-through cell (Owen *et al.*, 2012).

For the continuous *in situ* time series (see Table 3.5), each determinant was averaged into daily time-steps. To identify major errors in the high-resolution determinant time series, each dataset was visually assessed to identify anomalies. Evident outliers for periods where the readings clearly demonstrated instrument drift were removed. Missing value replacement, based on averaging of neighbouring values, was undertaken when three days or less of missing data were observed, gaps greater than three days were left blank. Stream average flow velocity (cm/s) for the experiment period 16/04/2013 to 13/05/2013 was based on the linear relationship between average velocity (cm/s) and water level (m) ($r = 0.98$, $p < 0.05$, $n = 162$) from 16/04/2013 to 22/04/2013.

Table 3.3: EdenDTC monitoring infrastructure detailing monitoring station site, associated equipment and frequency of determinants measured

Site	Size (km^2)	Meteorological equipment, determinant measured and frequency	Flow parameters measured and equipment	Kiosk type and surface water monitoring equipment	Determinants monitored in kiosk & frequency	Determinants monitored in spot samples	Determinants monitored in -situ
Newby Beck (Morland outlet)	12.5	Air temperature, wind speed and wind direction measured by Automatic Weather Station; design from Environmental Measurements Limited, UK at frequency of 15 minutes. Rainfall measured by Casella 100000E tipping bucket rain gauges: 2x telemetered; 1x non-telemetered at frequency of 15 minutes.	Level and flow measured by Schlumberger Baro and Diver; Argonaut, at frequency of 15 minutes. Spot gauging using Valeport Electromagnetic gauge, model 801	*High-spec kiosk* Hach Lange Sigmatax, Phosphax Sigma and Nitratax probes YSI 6600 sonde	TP, TRP, NO_3-N, every 30 minutes. Turbidity, DO, pH, temperature, conductivity every 15 minutes.	TP, TRP, NO_3, NO_2, TON , SS, DOC, alkalinity, Si, turbidity. (CEH, EA Laboratories, measured monthly)	DO, pH, temperature, conductivity measured by HANNA HI 9828 Mulitparameter Meter with a HI 769828 Multiparameter Probe (measured monthly)
Sleagill Beck	2		Level measured by driver, Argonaut at frequency of 15 minutes. Spot gauging using Valeport Electromagnetic gauge, model 801	*Sub-station* Analite turbidity probe	Turbidity at frequency of 15 minutes		
Dedra Banks Beck	2						
Thackwaite Beck (Dacre outlet)	9.9	Air temperature, wind speed and wind direction measured by Automatic Weather Station; design from Environmental Measurements Limited, UK at frequency of 15 minutes. Rainfall measured by Casella 100000E tipping bucket rain gauges: 2x telemetered; 1x non-telemetered at frequency of 15 minutes.	Level measured by Schlumberger Baro and Diver; Argonaut, at frequency of 15 minutes. Spot gauging using Valeport Electromagnetic gauge, model 801	*Low-spec kiosk* Analite turbidity probe	Turbidity at frequency of 15 minutes		
Lowthwaite Beck	2		Level measured by driver, Argonaut at frequency of 15 minutes. Spot gauging using Valeport Electromagnetic gauge, model 801	*Sub-station* Analite turbidity probe	Turbidity at frequency of 15 minutes		
Mellfell Beck	2						
Pow Outlet Beck	10.6	Air temperature, wind speed and wind direction measured by Automatic Weather Station; design from Environmental Measurements Limited, UK at frequency of 15 minutes. Rainfall measured by Casella 100000E tipping bucket rain gauges: 2x telemetered; 1x non-telemetered at frequency of 15 minutes.	Level and flow measured by Schlumberger Baro and Diver; Argonaut, at frequency of 15 minutes. Spot gauging using Valeport Electromagnetic gauge, model 801	*High-spec kiosk* Hach Lange Sigmatax Phosphax Sigma and Nitratax probes YSI 6600 sonde	TP, TRP, NO_3-N, every 30 minutes. Turbidity, DO, pH, temperature, conductivity every 15 minutes.		
Pow unnamed trib A	2		Level measured by driver, Argonaut at frequency of 15 minutes. Spot gauging using Valeport Electromagnetic gauge, model 801	*Sub-station* Analite turbidity probe	Turbidity at frequency of 15 minutes		
Pow unnamed trib B	2						

3.6.2 *Ex situ* physical and chemical analysis within Morland, Dacre and Pow catchments

Coincident with monthly diatom sample collection, samples were collected at both the 10 km^2 outlets and the 2 km^2 sub-catchment confluences for water quality analysis (Table 3.3). From March 2011 to October 2011 analyses were undertaken at the Centre for Ecology and Hydrology (CEH) Lancaster laboratories with subsequent analysis to March 2013 carried out by the EA. Alkalinity, SS, NO_3, nitrite (NO_2), TP, SRP and DOC were measured through spot sample analysis. In addition, on each sampling occasion per site conductivity, temperature and %DO was measured using a HANNA HI 9828 multi-parameter meter with a HI 769828 multi-parameter probe. No filtration took place for the EA water analysis according to their standard protocols. For CEH analysis samples were filtered through Whatman GF/C filter papers with a pore size of 1.2 μm. Due to differences in pre-treatment, SRP was estimated in the CEH measurements, while TRP was estimated by the EA laboratories. Therefore, in the continuous dataset for TRP series the first 6 data points refer to SRP as determined by CEH. Given methodological differences, regression analysis was performed on CEH and EA water quality analysis results over a 6 month period from October 2011 and to March 2012 to ensure comparability between datasets. Method summary of water chemistry analysis, upper limit of detection and Pearson's correlation coefficient for CEH and EA water chemistry parameters over this 6 month period are presented in Appendix 1.1.

3.6.3 Comparison of *in situ* and *ex situ* chemical analysis across catchment: Morland and Pow

For both Newby Beck and Pow outlet Beck the relationship between water chemistry samples (*ex situ* water chemistry analysis; see section 3.6.2) collected on the day of sampling and its relation to both *in situ* water chemistry analysis on day of sampling (Kiosk water chemistry analysis; 3.6.1) and antecedent chemical condition over the preceding 21 days was explored. For Newby Beck a strong significant positive relationship was observed between *ex situ* and *in situ* water chemistry analysis taken on the same day for TP, TRP and NO_3 (see Table 3.4; $p < 0.05$). For Pow outlet Beck, only NO_3 was only significantly correlated between *ex situ* and *in situ* water chemistry analysis taken on the same day (see Table 3.4; $p < 0.05$). For both Newby Beck and Pow outlet Beck no significant relationship for TP, TRP and NO_3 was observed between *ex situ* water chemistry analysis and antecedent chemical condition over the previous 21 days (Table 3.4). Observed differences among Newby and Pow outlet Becks in trends in *in situ* and *ex situ* water chemistry analysis are likely a function of sample size with greater significance observed at Newby Beck where a fuller dataset was obtained over the 25 months from March 2011 to March 2013 (Table 3.4). Based on these findings, throughout this thesis analysis of pattern in community structure and chlorophyll-*a* are primarily investigated in terms of *in situ* water chemistry analysis.

Table 3.4: Relationship between *ex situ* laboratory- and *in situ* chemical analysis at Newby and Pow Becks. Day zero refers to *ex situ* laboratory analysis and its relation to 15 minute *in situ* chemical analysis averaged over that day. 'av 21 days' refers to 15 minute *in situ* chemical analysis averaged over 21 days. n = sample number. Red text refers to significant correlation based on Pearson's r coefficient at p = 0.05 two tailed.

	Newby Beck				**Pow outlet Beck**			
	day zero	***n***	***av 21 days***	***n***	***day zero***	***n***	***av 21 days***	***n***
NO_3 (mg/l)	0.750	19	0.392	18	0.906	10	0.743	9
TP (mg/l)	0.762	16	0.342	15	0.499	12	0.661	9
TRP (mg/l)	0.711	18	0.413	17	0.567	12	0.736	9

3.7 Data analysis

Non-metric multidimensional scaling (NMDS) were used to characterise assemblage similarities within and among catchments across seasons, using Primer v6. NMDS constructs a 'map' of the samples representing them in low dimensional space such that the relative distance between the points correspond to the same rank order of dissimilarities as measured by the underlying similarity matrix. The NMDS ordination technique makes few assumptions about the distribution of the species. It preserves the distance between communities in ordination space better than other ordination techniques and the 'dimensions' are linear and orthogonal (Clarke and Gorley, 2006). The ordination was run with 50 runs of real data and a Monte-Carlo test with 250 runs of randomized data following Clarke and Gorley (2006). All data were fourth root transformed to de-emphasis dominant species.

Multivariate statistical analysis was applied to identify the main gradients in the composition of the diatom community using CANOCO 5.01 software (terBraak and Smilauer, 2012). Detrended correspondence analysis (DCA) was employed to estimate the heterogeneity in community composition. It is an indirect analysis technique examining the variation within species data independent of environmental data. Similarities between assemblages (samples) are summarised in ordination diagrams whereby the distance between the samples are a measure of similarity. The closer two points are, the more similar their assemblage compositions are. Detrending is employed to overcome the resulting arch effect when values of second axis are compared to first axis and the compression of points towards the end of the axes (Kent and Coker, 1994). The linear ordination methods of Principal Component Analysis (PCA) and Redundancy Analysis (RDA) were employed to find key determinant environment variables. PCA relates species composition to measured environmental gradients by reducing the dimensionality of complex multivariate data. In order to distinguish structure in relationships between measured parameters, it finds a small number of linear combinations of variables which are visualized using an ordination diagram. The ordination axes are reflective of the greatest variability in the community composition for a set of samples. There are as many principle components as there are variables but normally only the first two axes are important in terms of explaining a significant proportion of the total variation (Kent and Coker, 1994). RDA is based on similar principles as PCA. It examines how much of the variation in one set of measured parameters can be explained by the variation in another set of measured parameters. RDA is employed when the expected relationship between dependent and independent variables is linear. In instances when the expected relationship is Gaussian, canonical correspondence analysis (CCA) was employed (Crawley, 2007).

Relationships between diatom assemblage composition and ISF chlorophyll-*a* were explored and subsequently investigated along primary environmental gradients of rainfall, discharge, TP and NO_3. Student t-tests, Mann-Whitney Rank Sum tests, Analysis of Variance (ANOVA) and Kruskal-Wallis ANOVA were used to examine differences between parameters. Linear regression together with Pearson's Product-moment, were used to explore relationships between parameters. Statistical analysis were conducted in R 2.11.1 (Team, 2010) and Sigmaplot 12 (SigmaPlot, 2010).

A Student's t-test was used to investigate if two sample means were drawn from the sample with the same average. It is based on the assumption that the samples are independent, the variances are constant and the errors are normally distributed. The test statistic is the number of standard errors by which the two sample means are separated (Crawley, 2007). In instances when samples were not drawn from normally distributed populations with the same variances a Mann-Whitney Rank Sum test was employed. This is a non-parametric procedure which does not assume normality or require equal variances. The Mann-Whitney Rank Sum test is based on the hypothesis that the two samples were not drawn from population with different medians. It ranks all the observations from smallest to largest without consideration for which group each observation comes from. The ranks for each group are summed and the rank sums compared. If the rank sums differ by a large amount then it can be concluded that samples were drawn from different populations (SigmaPlot, 2010).

Analysis of variance (ANOVA) is used to test the differences between two or more means. It is based on the assumption that the data is normally distributed, there is homogeneity in variance and that observations are obtained independently and randomly from populations defined by factor levels (Crawley, 2007). A Kruskal-Wallis ANOVA was used when samples were drawn from non-normal populations or populations which did not have equal variances. The Kruskal-Wallis ANOVA on ranks is similar in design and principal to the Mann-Whitney Rank Sum test but is employed when there are more than two experimental groups. The Kruskal-Wallis ANOVA is based on the null hypothesis that there is no difference in the distribution of values between the different groups (SigmaPlot, 2010).

To investigate whether two continuous variables, x and y, were correlated with each other within the dataset, Pearson product-moment correlation was employed. Correlation is defined as the variance of x, the variance of y and the covariance of x and y on the assumption that both variables are normally distributed and is therefore the expectation of the vector product x*y. To ensure that the covariance is 0 both x and y must be independent. The strength of a linear association between two x and y is denoted by Pearson correlation coefficient, *r*, which can take a range of values from +1 to -1. A value of 0 indicates that there is no association between x and y. A value greater than zero indicates a positive association and less than zero indicates a negative association (Crawley, 2007). Differences at $p < 0.05$ level were accepted as significant within this research.

3.8 Metrics employed to describe benthic diatom assemblage composition

Combinations of the indices provided below were employed to investigate key environmental drivers of diatom assemblage structure and relationship to functional aspects of the benthic assemblage as per the research hypothesis.

3.8.1 Common, pioneer and rare diatom species

The stability, resistance and resilience of assemblage composition were explored in terms of common (including pioneer species) and rare species. The presence of rarer species defined in terms of abundance rarity, or less than 5% percent occurrence, was investigated. This is particularly important in headwater streams where assemblage succession may be truncated and associated benthic primary productivity naturally low, causing metrics to potentially underestimate water quality and miss-classify components of diatom assemblage stability and ecological status.

To understanding community dynamics emphasis was placed on pioneer species that have a cosmopolitan distribution or broad ecological amplitude (such as *Achnanthidium minutissimum, Amphora pediculus* and *Cocconeis placentula* var. *euglypta* in the Eden) and exert clear dominance on assemblage composition. A key contributing factor to their dominance is that fast growing pioneer diatom species have optimal colonisation rates on the scoured cobble substrate and high resistance to discharge (Douglas, 1958; Rounick and Gregory, 1981; Peterson and Stevenson, 1990).

3.8.2 Species richness: Margalef richness index

Margalef richness index (d; Margalef, 1958) was used to provide an estimate of species richness. It is defined as the total number of species in each sample and given by the following equation:

$$d = \frac{(s-1)}{\ln(n)}$$

where *S* is the number of taxa, and *n* is the number of individuals. Margalef richness index was calculated in Primer v 6 (Clarke and Gorley, 2006).

3.8.3 Species evenness: Pielou's evenness index

Species evenness was estimated using Pielou's evenness index (J'; Pielou, 1969; Pielou, 1975) which estimates how close in numbers each species in a community are. J' is constrained between 0 and 1. The lower the variation in individuals among species the higher J' is (Magurran, 2004). J' was calculated using Primer v6 (Clarke and Gorley, 2006) and is represented by

$$J' = \frac{H'}{H'_{max}}$$

where H′ is the number of derived from Shannon-Wiener diversity index and H'_{max} is the maximum value of H′ equal to:

$$H'_{max} = - \sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S$$

3.8.4 Assemblage diversity: Shannon-Wiener diversity

Assemblage diversity was estimated using the Shannon-Wiener diversity index (H′) (Shannon and Weaver, 1949). H′ is based on species number and the distribution of individuals between species. Species rich communities with a uniform distribution of individuals to single species are indicative of high diversity. Conversely a value of 0 represents a community with just one species. The value of the Shannon Diversity Index range from 0 – 5, with typical values of 1.5 to 3.5 rarely exceeding 4 (Magurran, 2004). H′ was calculated as Shannon-Weaver index (H´) (log base = *e*)) in Primer v 6 (Clarke and Gorley, 2006) and is represented by the following formula:

$$H' = - \sum p_i \ln p_i$$

where p_i is the proportion of individuals found in the i*th* species.

3.8.5 Diatom cell size: biovolume

Cell sizes used were based on the literature following Rimet and Bouchez (2012b), and used to determine biovolume in µm^3 according to Hillerbrand *et al.*, (1999) (see Rimet and Bouchez, 2012 for specific details). For calculations within this thesis, each species counted within the datasets across the three result chapters were assigned a biovolume following a European dataset provided by Rimet and Bouchez (2012b). Species specific and total diatom community biovolumes estimates were then calculated and to investigate further the aims of this research.

3.9 Using taxonomy and trait based analysis of diatom assemblage composition to infer environmental condition

3.9.1 Trait-based analysis: Ecological guilds

Functional groups were calculated based on assemblage composition following Rimet and Bouchez (2012b). Diatom growth morphologies were grouped into three ecological guilds: low profile, high profile and motile. The low profile guild comprised species of short statures including prostrate (adhering to the substrate with the entire valve surface), adnate (apically attached but parallel to the substrate), erect (apically attached but perpendicular to the substrate) and slow moving species such as *Achnanthes, Achnanthidium, Amphora, Cocconeis, Cymbella, Meridion* and *Reimeria.* These taxa are expected to be adapted to high current velocities and to low nutrient concentrations. The high profile species were species of tall stature including erect, filamentous, branched, chain-forming, tube forming, stalked, and colonial centrics from the following genera; *Diatoma, Ellerbeckia, Eunotia, Fragilaria, Gomphonema, Melosira (Melosira varians)* and *Synedra*. Included in this guild are tube-forming diatoms such as *Encyonema* and *Frustulia,* examples of larger size taxa (< 1500 μm^3) as well as *Cymbella, Eucocconeis* and *Achnanthes* and filamentous benthic taxa such as *Melosira varians.* This guild is adapted to high nutrients concentrations and low current velocities. The motile guild includes "fast moving species" from the genera *Navicula, Nitzschia, Sellaphora* and *Surrirella*. This guild is sensitive to relatively high current velocities and high nutrient concentrations (Passy, 2007b). The planktonic guild includes solitary centrics, for example *Cyclotella* spp and *Stephanodiscus* spp. and taxa adapted to lentic environments with morphological adaptations that enable them to be resistant to sedimentation. Based on these definitions each species in the dataset was assigned to an ecological guild and the proportions of all species in each ecological guild calculated.

3.9.2 Taxonomy-based analysis: estimation of ecological status under the WFD using TDI and EQR metrics

The Trophic Diatom Index (TDI) was developed by (Kelly and Whitton, 1995a) and revised by Kelly *et al.* (2001). It is commonly employed in the UK to estimate the ecological sensitivity of diatoms to inorganic nutrient pressures (SRP and NO_3; Kelly, 2007b; Kelly *et al.*, 2008a; Kelly and Yallop, 2012). It is based on the weighted average equation of Zelinka and Marvan (1961):

$$\frac{\sum_{j=1}^{n} a_j \ x \ s_j}{\sum_{j=1}^{n} a_j}$$

where a_j = abundance of valves of species j in sample, s_j = pollution sensitivity of species j. Values of diatom sensitivity range from 1 (indicating low nutrient conditions) to 5 (indicating very high nutrient conditions). This equation provides the weighted mean sensitivity (WMS) of taxa present in a given sample. TDI is the WMS expressed on a scale of 0 – 100, with 0 indicating low nutrient condition and 100 indicating high nutrient condition. TDI is calculated as:

$$(\text{WMS x } 25) - 25$$

Calculation and interpretation of TDI v3 and EQR followed the WFD DARES protocol under the classification tool DARLEQ (Diatom Assessment of River and Lake Ecological Status; DARES, 2012; WFD-UKTAG, 2008; WFD-UKTAG, 2014). EQR is calculated based on the observed TDI value for a particular river system and that expected under reference conditions (Pardo *et al.*, 2012). The EQR is bound between 0 and 1 from which ecological classes: High, Good, Moderate, Poor and Bad, are calculated based on the WFD classification (EC, 2000). The boundaries between status classes for diatoms are provided in Table 3.5 (WFD-UKTAG, 2013).

Table 3.5: Status class boundaries and their corresponding EQR value based on WFD UK TAG under the DARLEQ classification tool (WFD-UKTAG, 2013).

Status class boundary	EQR
High-Good	1
Good-Moderate	0.75
Moderate-Poor	0.5
Poor-Bad	0.25
Bad	<0.25

3.9.3 Taxonomy-based analysis: calculation of antecedent forcing period

Relationships between environmental variables (daily averaged 15 minute rainfall, discharge and TP data) and diatom community composition and chlorophyll-*a* were explored through TDI and ISF chlorophyll-*a* analysis. The continuously sampled 15 minute rainfall, discharge and TP data were averaged over periods from 0 to 21 days. Day 0 corresponded with the day of monthly benthic diatom sampling. Calculations of antecedent forcing periods of TDI and ISF chlorophyll-*a* to rainfall were based on daily averaged data over 18 months for Pow outlet Beck and 25 months for Newby Beck. Daily averages for discharge and TP for Newby Beck are based over 23 and 16 months, and for Pow outlet Beck 18 and 10 months. Pearson's r statistic was calculated between monthly TDI and chlorophyll-*a* against mean discharge and TP for Pow outlet and Newby Becks. The quasi-continuously sampled discharge and TP data were averaged over periods from zero to 21 days.

Chapter 4: Spatial-temporal patterns in benthic diatom assemblages

4.1 Introduction

Headwater streams are a central feature of the landscape, with characteristic dynamic discharge attributed to their small catchment areas and therefore, short residence times of precipitation (Benda *et al.*, 2005; Meyer *et al.*, 2007). This results in frequent disturbance and resetting of community structure by high discharge events and episodic nutrient fluxes (Lohman *et al.*, 1992). To understand the biodiversity and ecology of headwater systems it is important to recognise that the natural flow regime of headwaters is dynamic (Poff *et al.*, 1997) and that this dynamism plays a central role in determining and maintaining ecosystem integrity (Resh *et al.*, 1988; Poff, 1992; Bunn and Arthington, 2002; Arthington *et al.*, 2006; Monk *et al.*, 2008). Benthic biofilms, dominated in many headwaters by diatoms, must respond to physical and chemical parameters that can vary in magnitude within hours, whereas their ecological regeneration times are much longer (McGrady-Steed and Morin, 2000; Kupe *et al.*, 2008; Tien *et al.*, 2009). Diatom community development in fluctuating, dynamic environments characteristic of headwaters is poorly understood. This chapter investigates patterns in benthic diatom biofilm composition, its chlorophyll-*a* production and multi-pressure sensitivity to discharge and nutrient condition within three headwater 10 km^2 sub-catchments of the River Eden Catchment, Cumbria Northwest England, over a 25 month period (March 2011 to March 2013). The influence of temporal flow variability and nutrient (TP and NO_3) resource supply on benthic diatom biofilm diversity and chlorophyll-*a* will be evaluated. These data will be used to test the hypotheses that 1) there is a distinct and reproducible seasonal pattern in benthic diatom community attributes of richness, evenness, diversity and chlorophyll-*a* among three 10 km^2 headwater catchments: Morland, Pow and Dacre; 2) descriptors of diatom community attributes (diversity and chlorophyll-*a*) are linearly and inversely related; and 3) at any given point in time, the benthic diatom community will reflect the accumulated effect of a critical period of antecedent temporal dynamics in discharge and TP and NO_3 conditions. The hypothesis that benthic diatom community attributes (diversity and chlorophyll-*a*) were inversely related was based on preliminary investigation of the data in 2011 which suggested a negative relationship between Shannon-Wiener diversity and chlorophyll-*a*.

In order to address these hypotheses this chapter will first characterise the physical and chemical attributes of each site. Then the species composition, structural biodiversity and chlorophyll-*a* of the benthic diatom communities will be presented for each catchment: Morland, Pow and Dacre. Through investigation of the aims of this chapter diatom life-forms were found to provide keys insights in controls on, and temporal functional dynamics of, the benthic assemblage. Therefore, structural diversity in life-forms of these assemblages and its relationship to environmental conditions will be explored. Following this, benthic diatom assemblage diversity and chlorophyll-*a* will be examined in the context of physico-chemical variables. Subsequently, the relationship between diatom assemblage diversity and its productivity, in terms of chlorophyll-*a*, will be investigated. Particular focus will be placed on nutrient conditions with N:P ratio's explored and a model of benthic diatom assemblage composition (mediated through nutrient concentrations), and chlorophyll-*a* to antecedent condition in discharge and nutrient condition (TP and NO_3), proposed. The importance of spatial-temporal variability when using benthic diatom assemblages as an indication of trophic and ecological status will then be considered. To place the understanding of benthic diatom community composition and chlorophyll-*a* within the context of stream ecosystem function and implication for ecological monitoring, the chapter concludes with a discussion of the concordance or otherwise among ecological groups (diatoms, macrophytes, macroinvertebrates and fish) within the context of WFD classification.

4.2 Results

4.2.1 Catchment characterisation

4.2.1.1 Atmospheric climate variables (rainfall, temperature, sunshine hours)

Similar patterns were observed across all three catchments in rainfall and discharge conditions with maximum rainfall and discharge recorded in the Dacre catchment. This is supported by Table 4.1 which illustrated that highest rainfall and rainfall:runoff ratios were observed in the Dacre catchment which was climatically different than both Morland and Pow catchments (Figure 4.2 a, b). The mean temperature between March 2011 and March 2013 was 9°C with cyclic seasonal patterns evident leading to higher temperatures in spring and summer (March - August) compared to autumn and winter (September - February). Low autumn and winter temperatures corresponded with periods of higher rainfall and discharge conditions. Total monthly sunshine hours tracked this seasonal cycle in temperature apart from summer 2012 when rainfall reached 120 mm in June (Figure 4.1 c).

Table 4.1: Rainfall and discharge characteristics for Morland, Pow and Dacre catchment over the hydrological years 2011-12 and 2012-13 (Source EdenDTC).

	Year	rainfall (mm)	discharge (mm)	rainfall:runoff
Morland	2011-12	1205	707	0.59
	2012-13	1190	708	0.59
Pow	2011-12	1014	498	0.49
	2012-13	801	850	0.62
Dacre	2011-12	1969	1490	0.76
	2012-13	1554	1120	0.72

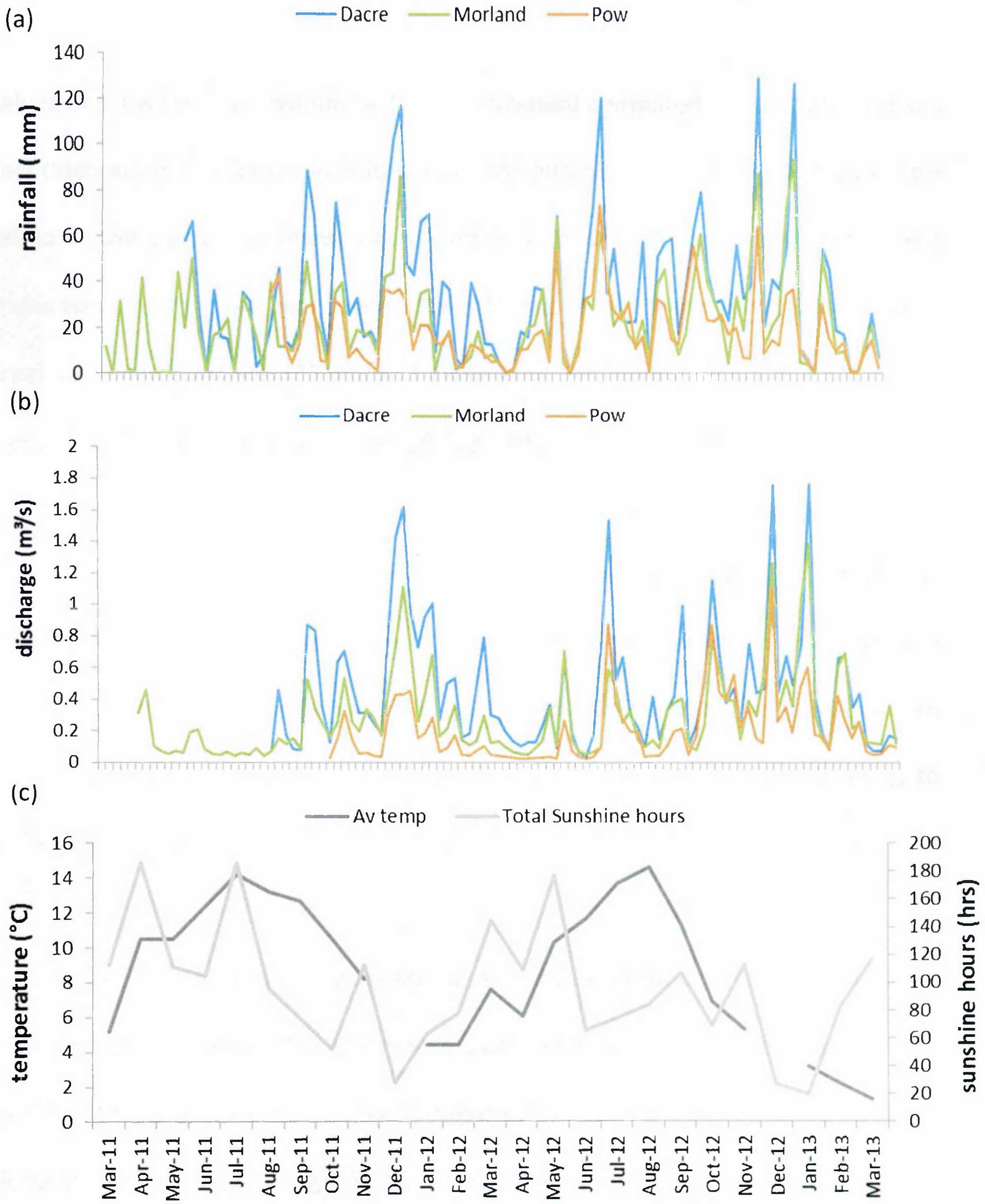


Figure 4.1: Weekly average (a) rainfall and (b) discharge for Morland, Pow and Dacre catchments from March 2011 to March 2013 (Source: EdenDTC). Mean air temperatures and total monthly sunshine hours recorded by Penrith weather station from March 2011 to March 2013 are given in (c) (Source: Penrith weather station: http://www.penrithweatherstation.com/weekrep.htm).

4.2.1.2 Water chemistry catchment characteristics: Morland, Pow and Dacre

Values of pH were lower within the Dacre catchment compared to both Morland and Pow catchments which demonstrated more comparable values (Table 4.2). Lower pH values in the Dacre catchment can be attributed to the fact that the catchment drains an area of upland peat soils to the outlet at Thackthwaite which lies between Great Mell Fell (altitude 537 m) and Little Mell Fell (altitude 505 m). Figure 4.2 presents a detailed overview of the geology and soil types of the Eden catchment and the three 10 km^2 catchments of Morland, Pow and Dacre. Pow catchment, and in particular, Morland catchment are underlain by limestone, mudstone and sandstone bedrock which contributes to higher pH values than the Dacre catchment (Figure 4.2b, Table 4.2). Differences in conductivity and alkalinity can be attributed to geology with waters flowing through the Morland catchment rich in $CaCO_3$ owing to the presence of calcite in soils and bedrock.

Mean nitrate concentrations across Morland, Dacre and Pow catchments were 2.01, 0.32 and 3.22 mg/l respectively. Similarly DOC and TSS were considerably higher in the Pow catchment (Mean: DOC 11.26 mg/l; TSS 36.12 mg/l) compared to both the Morland (Mean: DOC 2.53 mg/l; TSS 6.02 mg/l) and Dacre (Mean: DOC 4.86 mg/l; TSS 5.13 mg/l) catchments with intensive farming practice, including a relatively higher proportion of arable land, a key contributing factor (Neal *et al.*, 2006; Owen *et al.*, 2012). Overall, NO_3 concentrations comprised on average 96%, 91% and 86% of the inorganic dissolved nitrogen (NO_3 + NO_2 + NH_4) respectively for Morland, Pow and Dacre catchments.

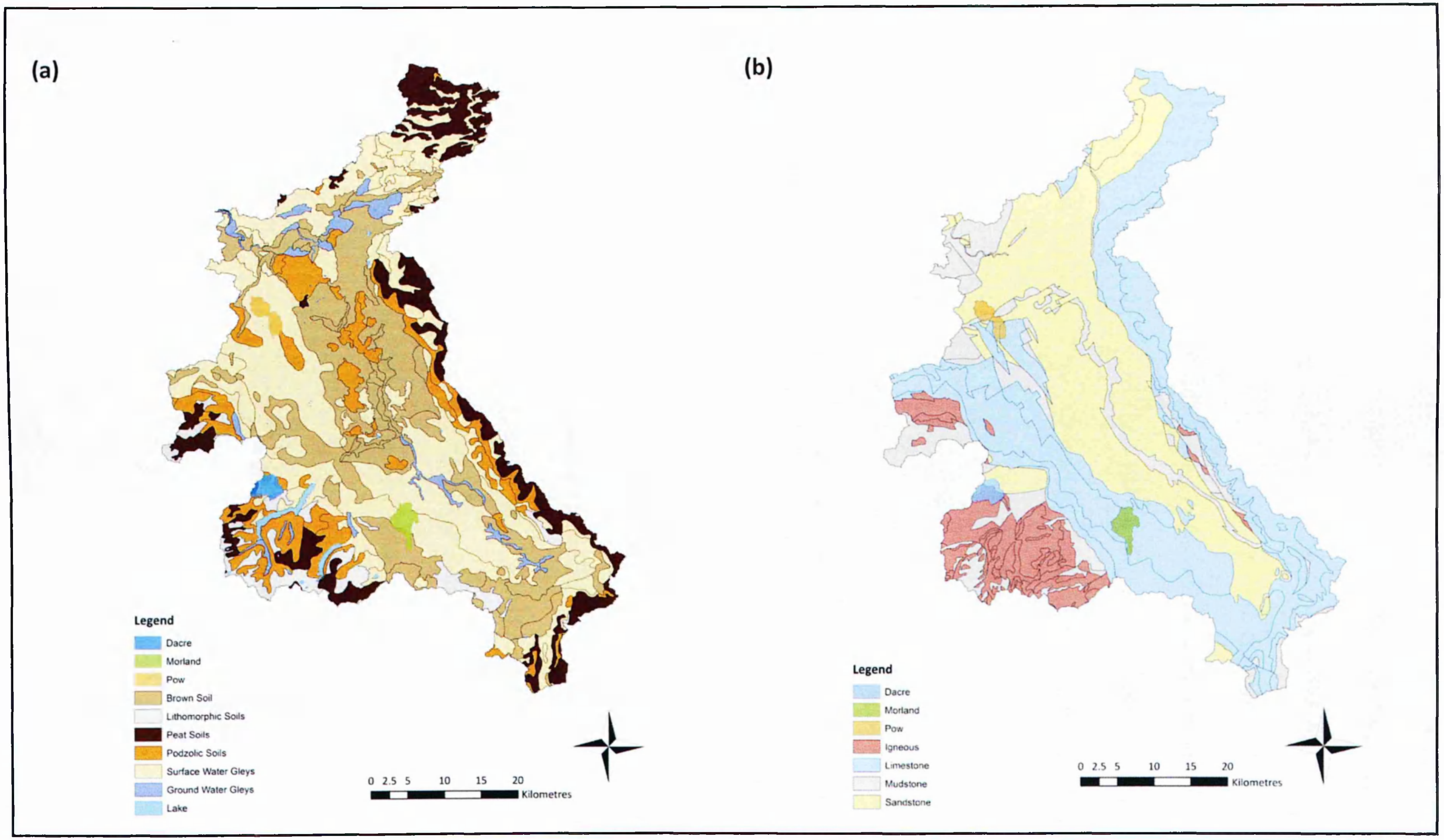


Figure 4.2: (a) Soil cover and (b) geology of the River Eden catchment and 10 km^2 sub-catchments of Morland (green), Pow (orange) and Dacre (blue) (© Crown Copyright/database right 2014. An Ordnance Survey/EDINA) supplied service).

TP and SRP concentrations were more variable across the catchments reflecting the importance of flow associated diffuse sources (Table 4.2). This is supported by Figure 4.1 demonstrating the variable hydrological regime and Table 4.1 which shows the high catchment connectivity through the rainfall:runoff ratios. Highest SRP concentrations of 0.18 mg/l were observed in Pow with mesotrophic concentrations in Morland of 0.03 mg/l and oligotrophic concentrations of 0.01 mg/l in Dacre (Environment Agency, 1998). Similar to NO_3, this is attributed with particulate transport of P from agricultural land due to farming practice and to the physical transport of sediment during hydrological events (Edwards and Withers, 2008). For Morland, Pow and Dacre catchments, SRP averages 59%, 64% and 45% of the TP. Components of TP unaccounted for are likely associated with particular phosphorus (PP). In terms of flux, this PP component will be higher since SRP concentrations decrease as flow increases while suspended sediment concentrations will increase (Neal *et al.*, 2008). Given the dynamic hydrological regime, throughout this research pattern in benthic biofilm attributes (composition, diversity and chlorophyll-*a*) will be explored in terms of TP rather than SRP. Rainfall:runoff ratios (Table 4.1) together with high correlation between TP and SRP (Morland: $r = 0.59$, $n = 75$, $p = 0.01$; Pow: $r = 0.95$, $n = 75$, $p = 0.01$; Dacre: $r = 0.52$, $n = 65$, $p = 0.01$) suggested that flow dependent TP supply to the benthic biofilm was likely to be as important as a measure of the bioavailable SRP fraction (Reynolds and Davies, 2001).

Table 4.2: Value range, mean and standard deviation (SD) for water chemistry parameters from March 2011 to March 2013 per catchment; Morland, Pow and Dacre. For Morland n = 75 for all parameters apart from temperature (n = 57), conductivity (EC) (n = 60), pH (n = 25). For Pow n = 75 for all parameters apart from temperature (n = 48), EC (n = 54), pH (n = 21). For Dacre n = 68 for all parameters apart from temperature (n = 50), EC (n = 52), pH (n = 22). Median value reported for pH. (Source: EdenDTC).

	Morland			Pow			Dacre		
	range	mean	SD	range	mean	SD	range	mean	SD
pH	7.81 - 8.42	8.2	-	7.3 - 8.27	8	8	6.79 - 7.81	7.4	7.4
Water Temp (°C)	0.49 - 14.81	7.09	3.26	1.98 - 17.96	8.56	4.51	1.98 - 17.96	7.11	3.98
EC (µg/cm)	415 - 1048	650.65	145.34	12.5 - 795	470.88	137.56	66 - 382	148.71	52.27
Alk (mg/l)	162 - 280	245.98	24.37	60 - 224	162.33	34.54	14 - 71	42.19	13.04
TSS (mg/l)	1.4 - 20	6.02	4.18	3 - 1400	36.2	161.3	3 - 31.4	5.13	5.67
DOC (mg/l)	1.19 - 7.3	2.53	1.26	5.1 - 26.3	11.26	4.14	1.56 - 16	2.53	1.26
TP (mg/l)	0.01 - 9.33	0.18	1.07	0.04 - 1.37	0.28	0.26	0.1 - 0.43	0.04	0.06
PO_4-P (mg/l)	0.01 - 0.2	0.03	0.03	0.02 - 0.95	0.18	0.18	0.01 - 0.02	0.01	0
NO_2-N (mg/l)	0.01 - 0.1	0.1	0.02	0.00 - 0.21	0.04	0.04	0.00 - 0.02	0.1	0
NO_3-N (mg/l)	1.06 - 3.74	2.01	0.67	0.25 -17.26	3.22	3.17	0.2 - 0.75	0.32	0.14
NH_4-N (mg/l)	0.03 - 0.77	0.07	0.11	0.03 - 3.23	0.2	0.45	0.02 - 0.15	0.04	0.02

4.2.2 Potential for nutrient limitation among catchments

For UK streams, P rather than N is generally considered to be the limiting nutrient (Withers and Lord, 2002; Mainstone and Parr, 2002). To explore whether P or N availability was potentially limiting plant growth across the Morland, Pow and Dacre catchments Figure 4.3 presents the ratio of SRP:NO_3 against SRP concentrations. Emphasis was placed on the main dissolved phases of NO_3 and SRP to provide a measure of the bioavailable form of the nutrient. Predication of nutrient limitation was based on the Redfield ratio (106C: 16N: 1P; Redfield, 1958). This is a compositional, optimal nutrient ratio, empirically determined for oceanic seston but has been applied to freshwater systems (Stelzer and Lamberti, 2001). P is considered 'limiting' at ratios of N:P greater than 16:1 and Figure 4.3 indicated that SRP concentrations were low and P was generally limiting within the Dacre catchment. For the Morland catchment, ratios were typically above 16:1 with only a relatively small proportion of samples spanning the mesotrophic threshold of 0.06 mg/l (Environment Agency, 1998). Higher phosphorus concentrations in the Pow catchment resulted in increasing phosphorus availability to the benthic algae contributing to both nitrogen and phosphorus limitation. Therefore while P 'limiting' conditions were observed across all three catchments, N 'limiting' conditions were mostly observed within the Pow catchment.

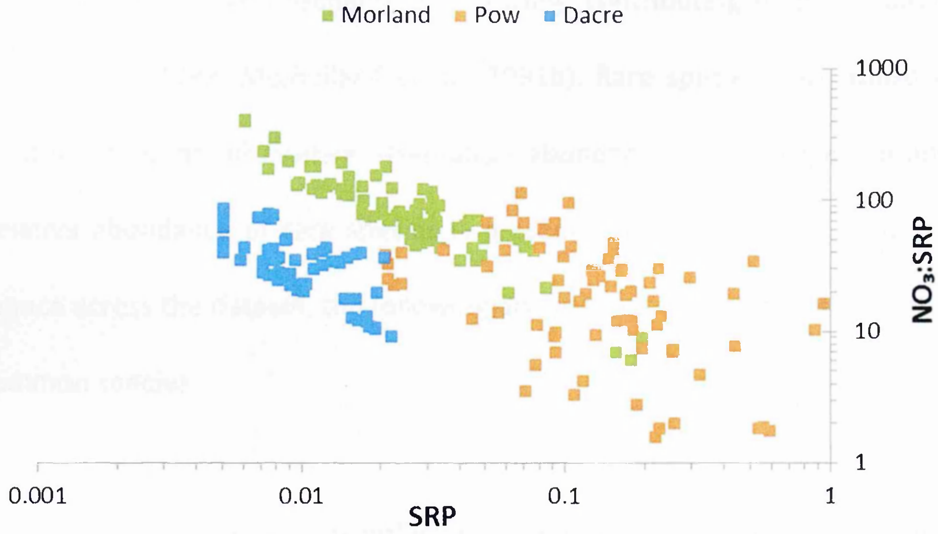


Figure 4.3: NO_3:SRP to SRP for Morland (n = 75) , Pow (n = 75) and Dacre (n = 67) catchments (modified from Mainstone and Parr, 2002). Nutrient limitation threshold is taken at 16:1 following the Redfield ratio. Trophic categories defined as per EA (1998): Oligotrophic 0.02 mg/l; Mesotrophic 0.06 mg/l; Meso-eutrophic 0.1 mg/l; Eutrophic 0.2 mg/l).

4.2.3 Diatom assemblage composition: Morland, Pow and Dacre catchments

Overall, 153 species were found belonging to 49 genera (see appendix 1.2 for further details). *Achnanthidium minutissimum* was the most widespread species across the three catchments with a mean relative abundance of 24%. All remaining species constituted 10% or less of diatom assemblage mean relative abundance. Based on species characteristics and relative abundances three groups of species were identified within the dataset; pioneer, common and rare. Pioneer species refer to those species with superior colonisation abilities and high growth rates characteristics (Korte and Blinn, 1983; Sabater *et al.*, 1998; Biggs *et al.*, 1998b).

Moreover, their prostrate growth forms and firm attachment to substrate offers resistance to disturbance (discharge and grazing) contributing to their dominance (Steinman *et al.*, 1987; Mulholland *et al.*, 1991b). Rare species were those which occupied less than 5% of relative assemblage abundance. Assemblage composition and relative abundance of rare species is presented in appendix 1.2. Given their dominance across the dataset, the following discussion is based primarily on pioneer and common species.

4.2.3.1 Pioneer species within assemblages of Morland, Pow and Dacre catchments

Across the three catchments key pioneer species dominated assemblage composition (Figure 4.4). The most widespread species was *A. minutissimum* which was present at all sampling sites. *A. minutissimum* had a mean relative abundance of 24%, more than twice as abundant as *Amphora pediculus,* the second most abundant species within the dataset. *A. minutissimum* and *A. pediculus,* together with *Cocconeis placentula,* dominated diatom assemblage composition across the three study catchments and reflected differences in flow dynamism and trophic condition (Figure 4.4). High mean abundance of *A. minutissimum* in the Dacre catchment, which demonstrated greatest dynamism in flow (section 4.2.1.1), could be associated with their superior dispersal and colonisation abilities and high growth rates characteristic (Korte and Blinn, 1983; Sabater *et al.*, 1998; Biggs *et al.*, 1998b). In addition, the prostrate growth form and rapid growth of *A. minutissimum* allows for firm attachment to substrate and formation of a monolayer of cells, offering resistance to scour (Steinman *et al.*, 1987; Mulholland *et al.*, 1991b). Thus, the high relative abundance of *A. minutissimum* at the upland catchment of Dacre was representative of the flashy hydrological regime (Peterson and Stevenson, 1992). In the Morland catchment, *A. pediculus* together with *A. minutissimum* dominated assemblage composition and provided support for the alternate trophic state evident within this catchment (see section 4.2.9.2). For example, *A. minutissimum* was dominant from March to August and is associated with good water quality while *A. pediculus,* which is associated with moderate water quality, dominated from September to February (Martin *et al.,* 2010). Thus, within the Morland catchment, dynamism in flow, together with trophic condition, controlled the relative abundance of key pioneer species. In the Pow catchment, *Cocconeis placentula* var. *euglypta* dominated the assemblage composition (Figure 4.4). *C. placentula* var. *euglypta* is tolerant of moderate nutrient enrichment and through its ability to fix N can be a key feature of assemblages in streams where N is limiting (Marcus, 1980). Therefore within the Pow catchment, and in contrast to Dacre catchment, trophic condition rather than flow dynamics is likely to influence assemblage composition.

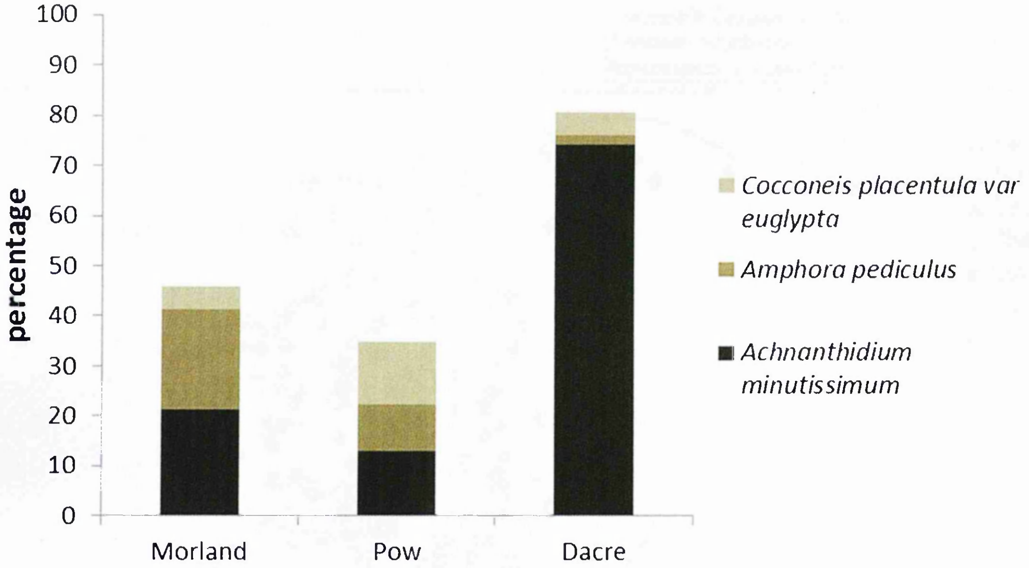


Figure 4.4: Relative percentage abundance of pioneer species *A. minutissimum, A. pediculus* and *C. placentula* var. *euglypta* to assemblage composition for Morland (n = 72), Pow (n = 75) and Dacre (n = 67) catchments from March 2011 to March 2013.

While the pioneer species, *A. minutissimum, A. pediculus* and *C. placentula* offered significant contributions to assemblage composition Figure 4.5 demonstrated that diatom assemblages were distinct and were more similar within than between catchments. Diatom assemblages in the Pow catchment demonstrated the greatest degree of clustering, while assemblages in the Dacre catchment demonstrated greater variability than those in the Morland catchment. This suggested that assemblages were more similar in the Pow catchment than Dacre and Morland catchments. Note, the stress value was high at 0.22, thus reducing the confidence of these representations (Clarke, 1993). Figure 4.5 therefore demonstrated the importance of diatom assemblage composition and in particular richness of rare species with the diatom assemblage.

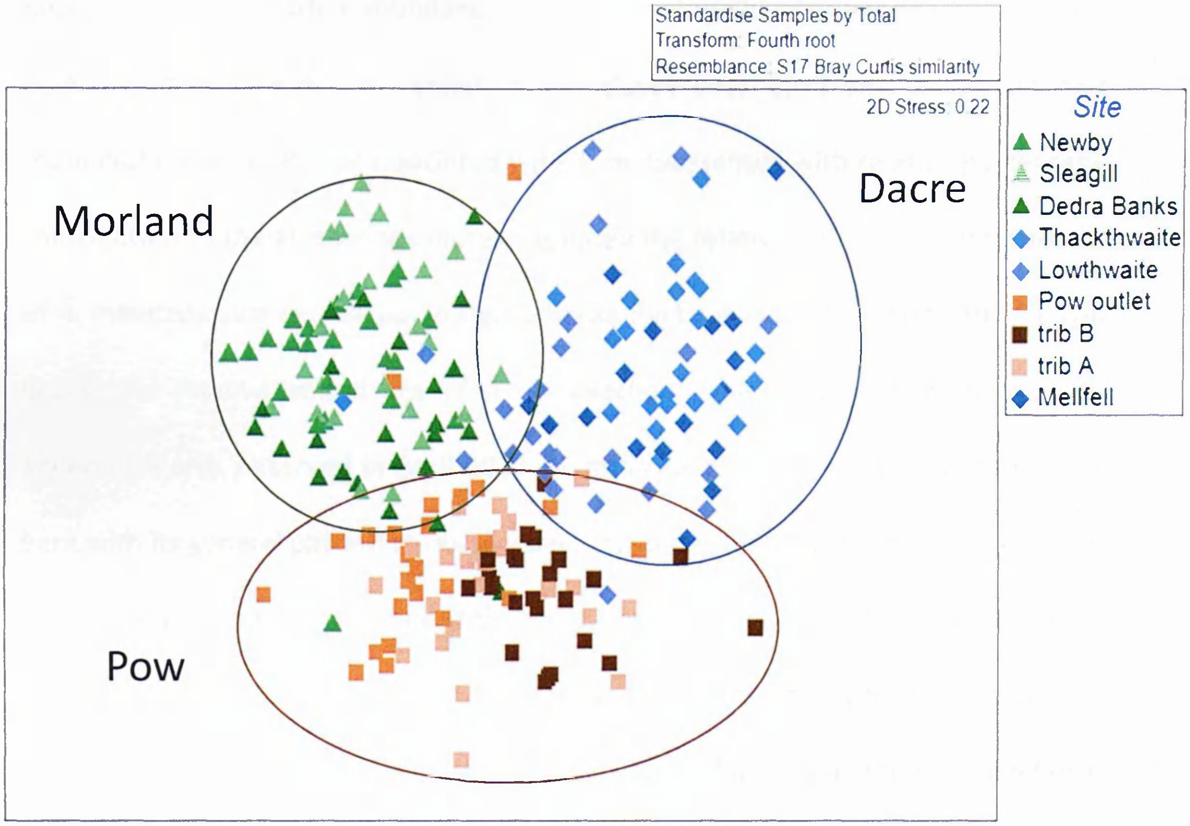


Figure 4.5: Diatom assemblages (n = 214) for all sites within Dacre, Morland and Pow demonstrating catchment similarity among assemblage composition. Data standardised, fourth root transform and based on Bray-Curtis distance.

4.2.3.2 Common species within assemblages of Morland, Pow and Dacre catchments

Figure 4.6 presents the assemblage composition within the Morland catchment with its alternate quasi-cyclic season pattern in relative abundance of *A. minutissimum* and *A. pediculus.* This pattern was reflected with the two 2 km^2 sub-catchments of Dedra Banks Beck and Sleagill Beck. *A. minutissimum* was dominant from March to August and *A. pediculus* was the dominant from September to February. At Newby

Beck, peaks in the relative abundance of *Achnanthidium biasolettiana* reflected those in *A. minutissimum* and increased in abundance over 2012 and 2013. *Navicula tripunctata* was negatively associated with *A. minutissimum* with relative percentage contribution to the assemblage increasing when the relative percentage contribution of *A. minutissimum* decreased. In Dedra Banks Beck, unlike both Newby and Sleagill Becks, the relative abundance of *A. biasolettiana* was low, with relatively high abundance only observed in April 2012. *N. tripuncata* also featured at Dedra Banks Beck with its general pattern in relative percentage abundance approximating that of *A. pediculus*. Within Sleagill Beck, different species were associated with the seasonal dominance of *A. minutissimum* and *A. pediculus. A. minutissimum, A. biasolettiana* and *Encyonema minutumn* demonstrated higher relative percentage abundance from March to August in both years. *C. placentula* var. *euglypta* and *N. tripunctata* were more closely associated with *A. pediculus,* showing higher relative percentage abundance from September to February in both years.

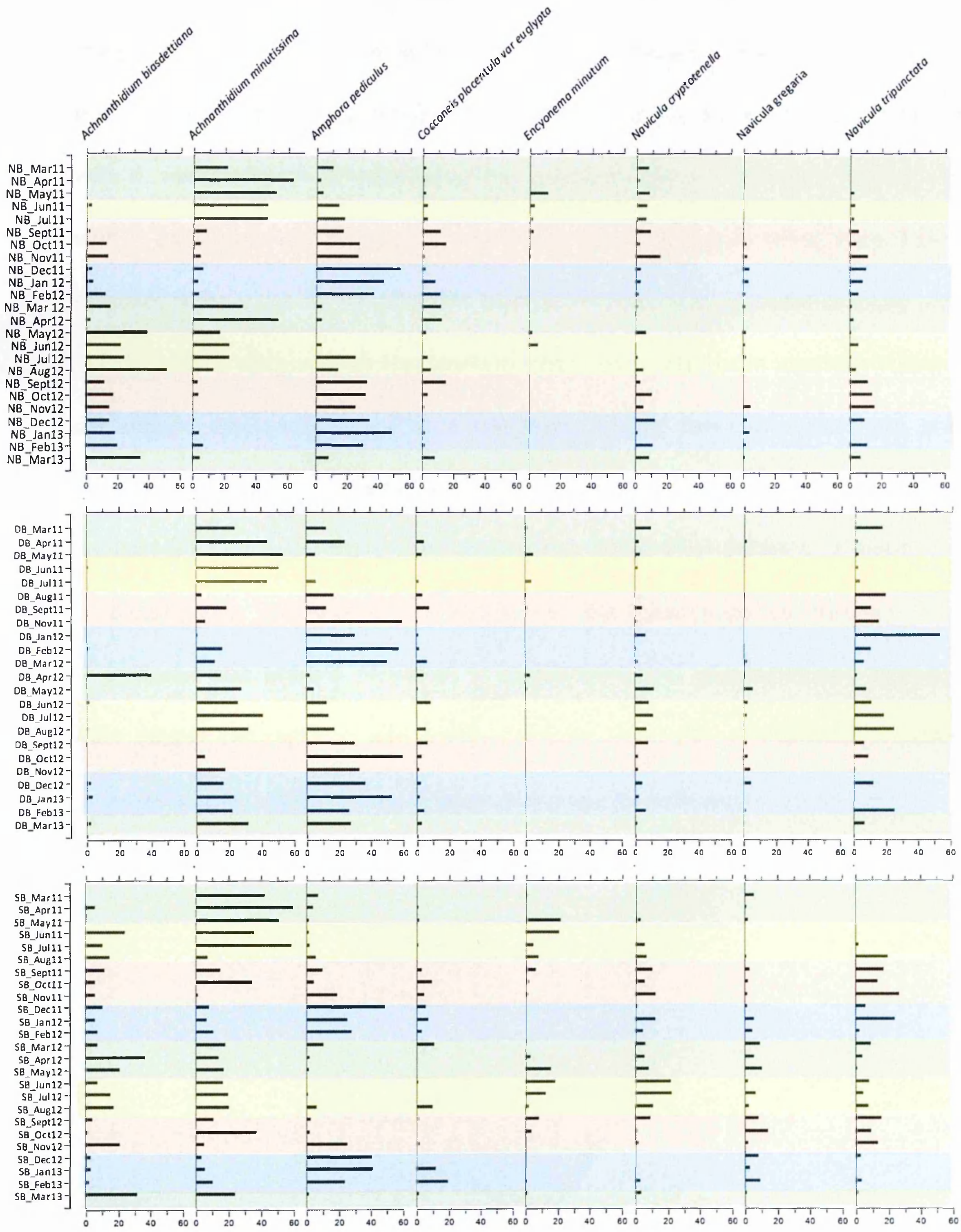


Figure 4.6: Relative percentage abundance of species contributing on average at least 5% to assemblage composition in the Morland catchment: Newby (NB), Dedra Banks (DB) and Sleagill (SB) Becks, from March 2011 to March 2013. (Green = spring, yellow = summer, brown = autumn, blue = winter).

A key feature of the diatom assemblage composition within the Pow catchment was the presence of *C. placentula* and *C. placentula* var. *euglypta* (Figure 4.7). Similar to the Morland catchment, *A. minutissimum* and *A. pediculus* all featured prominently with *A. minutissimum* demonstrating the greatest degree of alternate dominance with *C. placentula* var. *euglypta* at Pow Outlet and Tributary A. While there was a seasonal component to the alternate dominance observed, seasonal coupling was not as tight as observed for the Morland catchment. Patterns in alternate relative percentage abundance were also observed among *Navicula lanceolata* and *Planothidium lanceolatum*, although to a lesser extent. Pow Tributary B displayed a notably different assemblage composition than either Pow Outlet or Tributary A. *A. biasolettiana* remained a key feature of the assemblage composition with *A. pediculus* also present. However, *C. placentula* and *C. placentula* var. *euglypta* were absent and replaced with a higher relative abundance of *Navicula gregaria, N. lanceolata, Navicula minima, P. lanceolatum* and *Planothidium frequentissimum.*

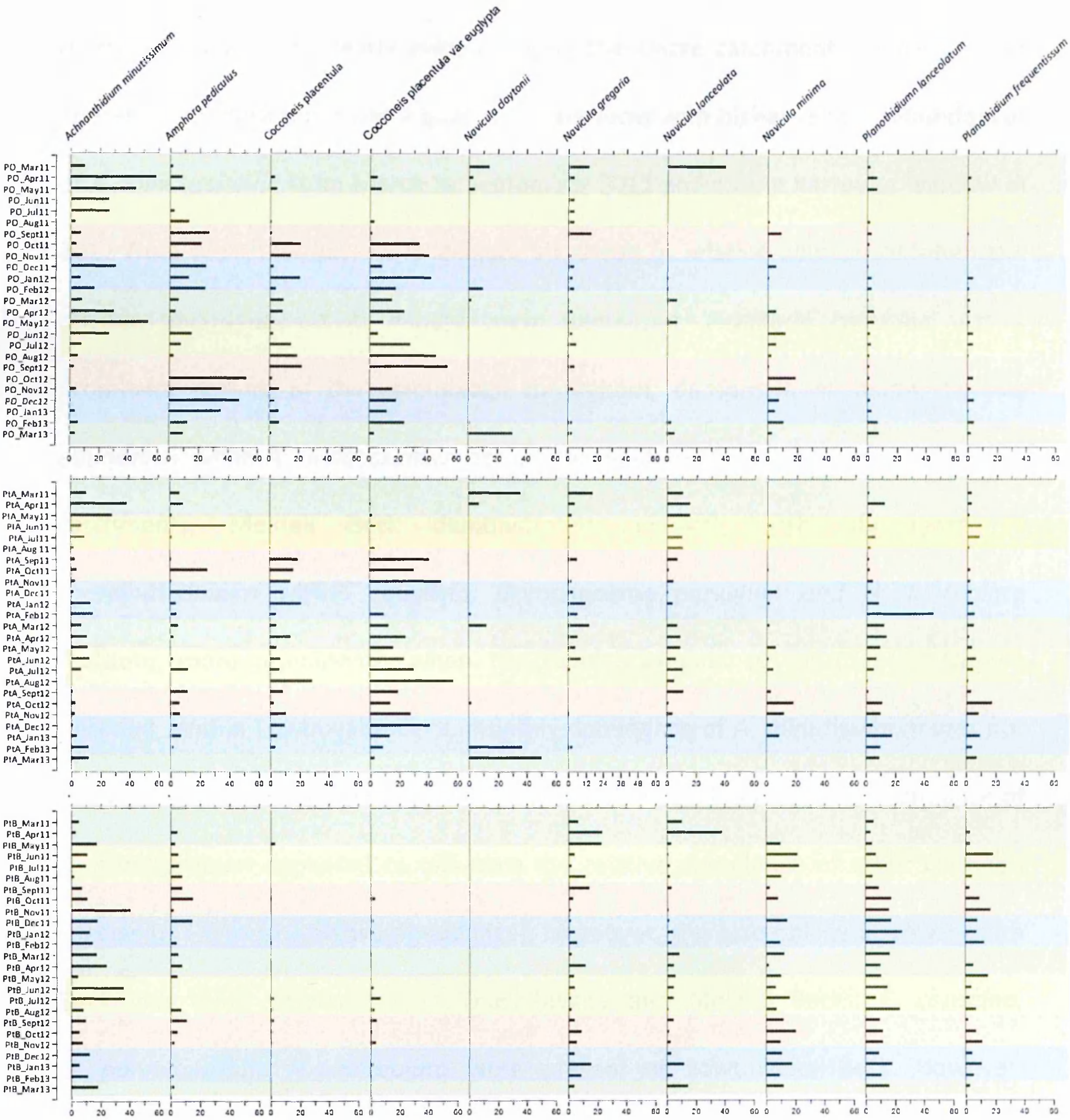


Figure 4.7: Relative percentage abundance of species contributing on average at least 5% to assemblage composition in the Pow catchment: Pow outlet (PO), Tributary A (PtA) and Tributary B (PtC) from March 2011 to March 2013. (Green = spring, yellow = summer, brown = autumn, blue = winter).

In comparison to both the Morland and Pow catchments, the dominance of *A. minutissimum* was clearly evident within the Dacre catchment (Figure 4.8). At Thackthwaite Beck there was a quasi-cyclic patterns with higher relative abundances of *A. minutissimum* from March to September 2011 and over a narrower window in 2012 from March to July. *N. lanceolata* increased in relative abundance when the relative abundance of *A. minutissimum* decreased. *Fragilaria capucina* was a prominent feature of the assemblage throughout, demonstrating higher relative abundance when *A. minutissimum* declined in relative abundance. Among the sub-catchments, Mellfell Beck demonstrated relatively high abundance of *A. minutissimum* wit *F. capucina, Gomphonema parvulum and N. lanceolata* featuring more prominently when the relative abundance to *A. minutissimum* declined. Within Lowthwaite Beck, monthly dominance of *A. minutissimum* was not as evident. Similar to both Thackthwaite and Mellfell Beck the relative abundance of *A. minutissimum* appeared to influence the relative abundance of other common species (> 5% mean relative abundance). Moreover, the assemblage at Lowthwaite Beck was more diverse. As in Thackthwaite and Mellfell Becks, *F. capucina, G. parvulum and N. lanceolata* were present at Lowthwaite Beck. However, *Gomphonema pumilum* and *Navicula claytonii* were also key constituents of the Lowthwaite Beck assemblage composition. For the Dacre catchment, assemblages were less diverse than Morland and Pow catchments demonstrating higher relative abundance by a single pioneer species, *A. minutissimum.* However, across all three catchments alternate quasi-cyclic patterns in dominance of species within assemblages was found.

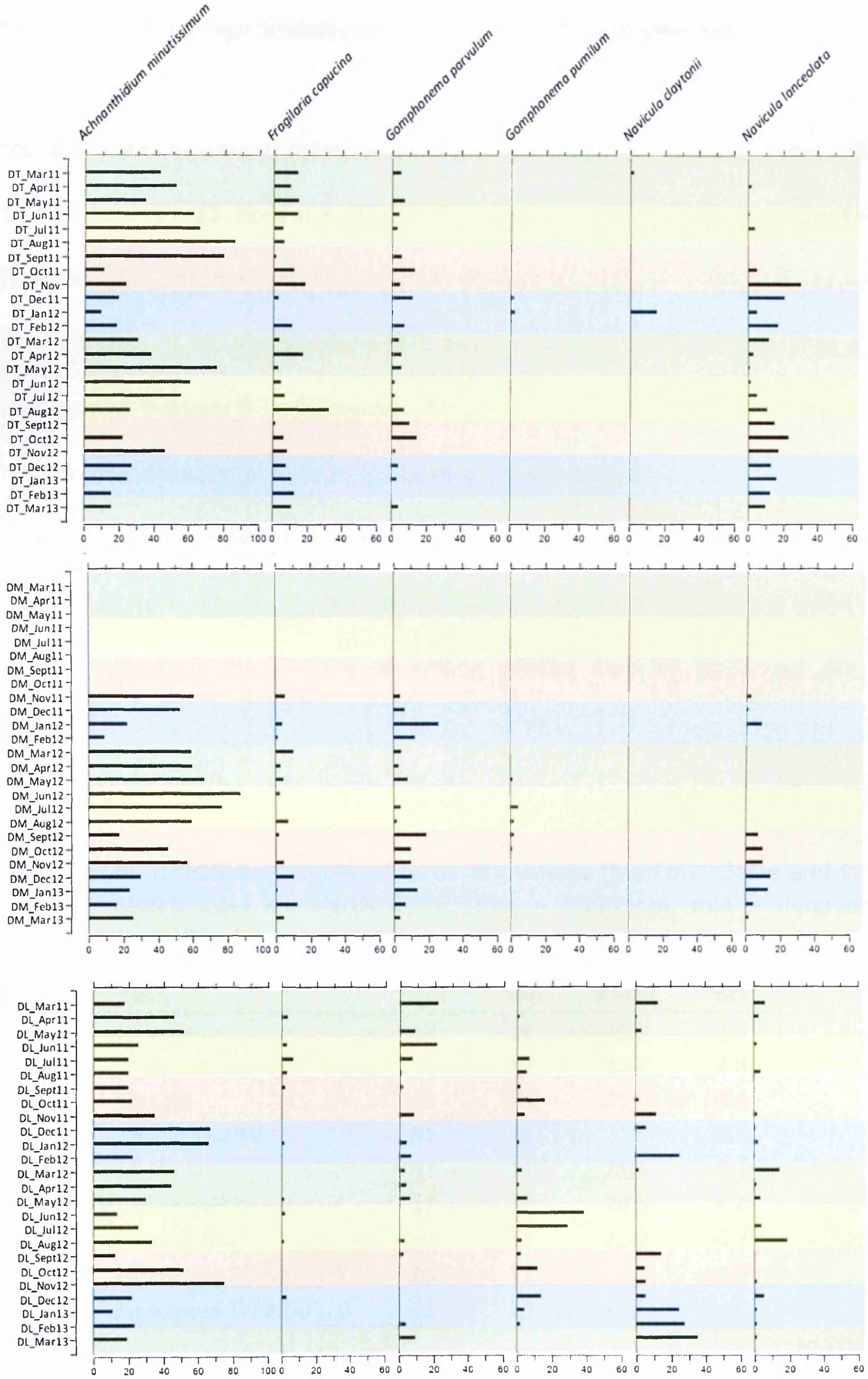


Figure 4.8: Relative percentage abundance of species contributing on average at least 5% to assemblage composition in the Dacre catchment: Thackthwaite (DT), Mellfell (DM) and Lowthwaite (DL) Becks from March 2011 to March 2013. (Green = spring, yellow = summer, brown = autumn, blue = winter).

4.2.4 Diatom assemblage diversity: Morland, Pow and Dacre catchments

Across the three catchments mean species richness (n) was similar from March 2011-2013 (Morland: n = 21; Pow: n = 21; Dacre: n = 23). However, spatial and temporal differences were observed within-catchment (Table 4.3). For example maximum total species richness of 41 was observed at Thackthwaite Beck in December 2011 and Pow unnamed Tribuary B in September 2011. The Dacre catchment demonstrated minimum total richness (n = 11) at Thackthwaite Beck in August 2011, Mellfell Beck in June 2012 and Lowthwaite Beck in November 2012. Subsequent discussion in this section will explore patterns in the total number of species present (species richness) and how individuals are distributed among species (species evenness) across catchments.

Table 4.3: Species richness within all three catchments (Morland, Dacre and Pow Beck) from March 2011 to March 2013. (max = maximum, min = minimum, SD = standard deviation).

Catchment	Beck	max	min	mean	SD
Morland	Newby	25	16	20.8	2.7
Morland	Dedra Banks	31	12	21.7	4.8
Morland	Sleagill	29	16	21.9	3.8
Dacre	Thackthwaite	41	11	21.6	6.3
Dacre	Mellfell	31	11	21.1	5.1
Dacre	Lowthwaite	32	11	20.9	5.4
Pow	Pow Outlet	30	12	21.2	4.7
Pow	Unnamed Tributary A	29	13	22.9	4.9
Pow	Unnamed Tributary B	41	13	23.8	6

Trends in the Margalef richness index at the Newby Beck outlet were similar to those observed further upstream in the 2 km^2 sub-catchments (Figure 4.9 a). The smaller sub-catchments were more variable and demonstrated the greatest range in values over the data series (Newby Beck: 2.6 - 4.2; Dedra Banks: 1.9 - 5.3; Sleagill Beck: 2.6 - 4.9). Values declined over the winter months with low Margalef richness index values observed in January of both years at Sleagill Beck. Trends in the Margalef richness index across the dataset demonstrated greater comparability in 2011 compared to 2012. From July to September Margalef richness index values were high with peak richness observed at Sleagill Beck in August 2011. Increasing variability was observed throughout 2012, with peaks in species richness in May 2012 at Sleagill Beck and then in September 2012 at both 2 km^2 sub-catchments. Comparisons of Pielou's evenness index and Margalef richness index in 2011 demonstrated that lower species richness corresponded with periods of lower Pielou evenness index values (Figure 4.9 b). An example of this is April 2011 when the total number of species present declined resulting in greater variability in the community between species. No clear pattern was observed through 2012 in terms of species richness and evenness. During this period, values of Pielou's evenness index indicated a more even distribution of species while Margalef richness index indicated more variable species richness.

Figure 4.9: Monthly (a) Margalef richness index and (b) Pielou's evenness index for Newby Beck (n = 24), Dedra Banks (n = 23) and Sleagill Beck (n = 25) from March 2011 to March 2013. Margalef richness index is (S-1)/ln (n), where S is the number of taxa, and n is the number of individuals. Pielou's evenness index is Shannon-Wiener diversity divided by the logarithm of number of taxa providing a measure of the evenness with which individuals are divided among the taxa present.

Similar to the diatom communities in the Morland catchment, trends in community structure at Pow outlet were broadly comparable with the two component upstream 2 km^2 sub-catchments (Pow Outlet: 1.9 to 5.1; Pow unnamed Tributary A: 0.44 – 0.8; Pow unnamed Tributary B: 2.1 to 7.01). Moreover, species richness was low in April and from October to December 2011. Higher values of the Margalef richness index were observed between May and September with a peak in species richness at unnamed Tributary B in in September 2011. Again, as for the Morland catchment, increasing variability was observed in species richness through 2012 with no clear pattern evident, and values did decline in December 2012 (Figure 4.10 a). Lower species richness over winter months within both Pow and Morland catchments suggested seasonal control in community development. Pielou's evenness index demonstrated no clear pattern in the distribution of individual taxa among species throughout the experimental series. However, the relatively high values suggested only a small degree of variation in communities present apart from August and September 2012 when values of Pielou's evenness index declined for unnamed Tributary A and Pow outlet respectively (Figure 4.10 b).

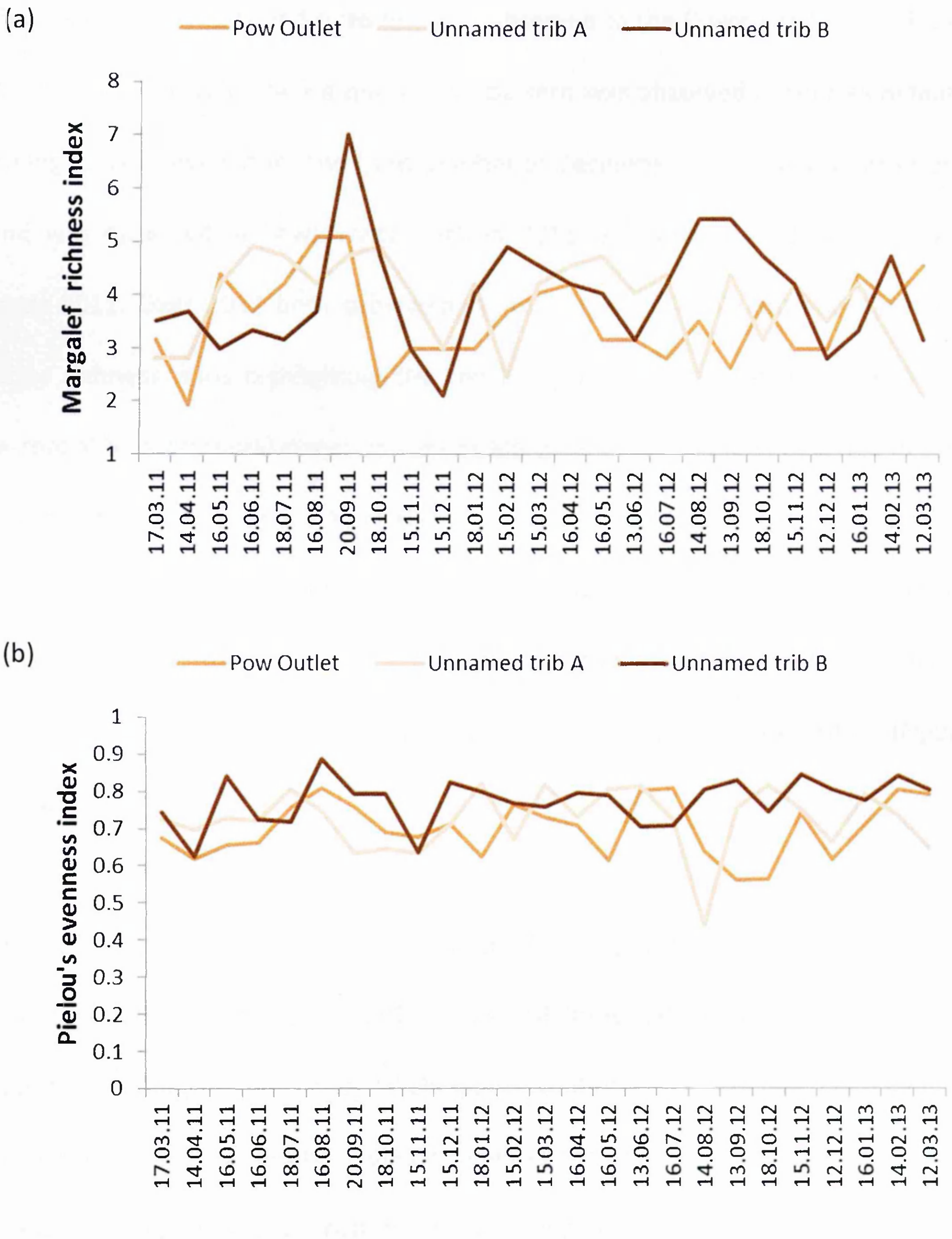
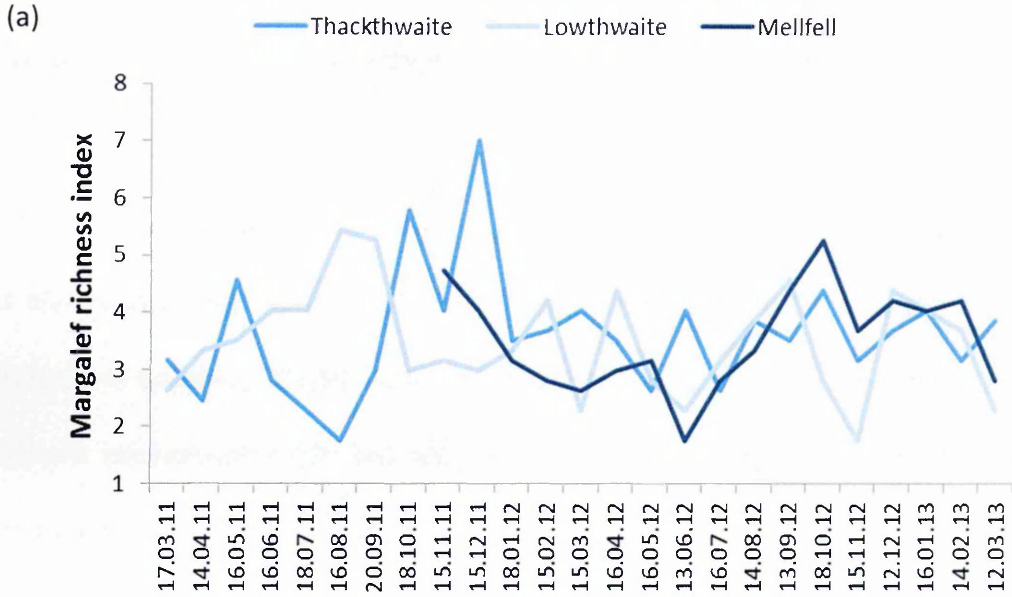


Figure 4.10: Monthly (a) Margalef richness index and (b) Pielou's evenness index for Pow outlet (n = 25), unnamed Tributary A (trib A) (n = 25) and unnamed Tributary B (trib B) (n = 25) from March 2011 to March 13. Margalef richness index is (S-1)/ln (n), where S is the number of taxa, and n is the number of individuals. Pielou's evenness index is Shannon-Wiener diversity divided by the logarithm of number of taxa providing a measure of the evenness with which individuals are divided among the taxa present.

Unlike both the Morland and Pow catchments, a greater degree of coupling in trends of both species richness and evenness was observed in the Dacre catchment (Figure 4.11). For Thackthwaite Beck a quasi-cyclic pattern was observed in species richness with higher richness in April and from October to December in both years. An inverse trend was observed at Lowthwaite Beck in 2011 with higher species richness in August 2011. Over 2012 both sub-catchments influenced minimum and maximum species richness, thus highlighting the sensitivity of diatom structural diversity at fine-spatial high-temporal scales. Values of Margalef richness index declined in June and November 2012 with peak richness observed at Mellfell Beck in October 2012 (Figure 4.11 a). Pielou's evenness values were higher between October to February across both years, thus suggesting that in autumn and winter when highest species richness was observed, there was less variation in community composition (Figure 4.11 b).

Catchments specific patterns were observed in species richness but greater heterogeneity was observed in 2012 across all three catchments. A contributing factor to this may be the variable climatic conditions, in particular high rainfall, observed in June 2012. Overall, species richness and evenness were related to one another with higher species richness contributing to a more even distribution of individuals amongst species. Overall structural composition was robust and reproducible among catchments; Morland, Dacre and Pow, with the smaller nested sub-catchments capturing maximum and minimum species richness and evenness.



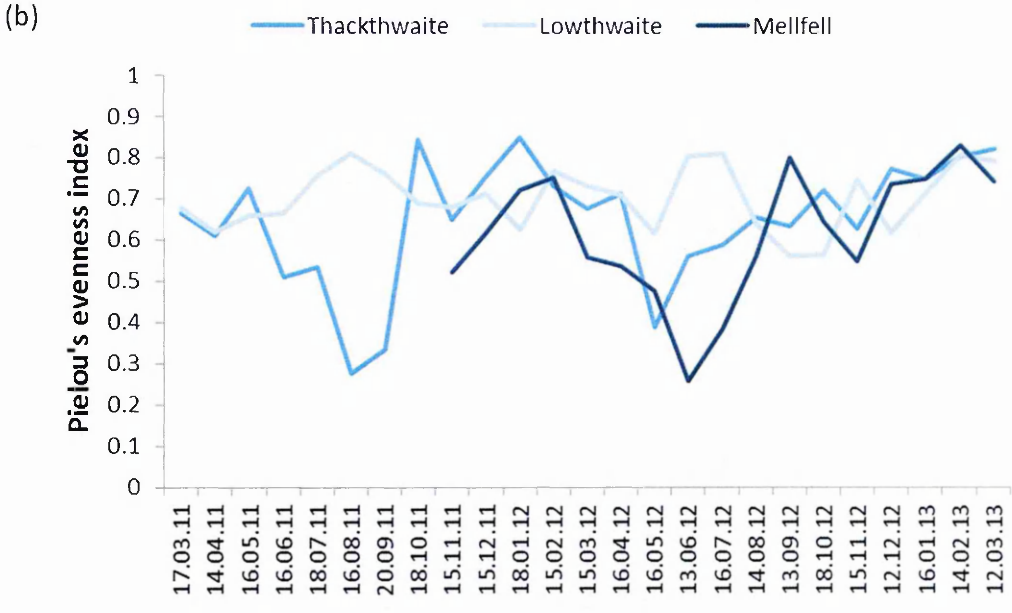


Figure 4.11: Monthly (a) Margalef richness index and (b) Pielou's evenness index for Thackthwaite Beck (n = 25), Mellfell Beck (n = 17) and Lowthwaite Beck (n = 25) catchment from March 2011 to March 2013. Margalef richness index is (S-1)/ln (n), where S is the number of taxa, and n is the number of individuals. Pielou's evenness index is Shannon-Wiener diversity divided by the logarithm of number of taxa providing a measure of the evenness with which individuals are divided among the taxa present.

4.2.5 Response of diatom assemblage functional diversity to variability in discharge-nutrient (TP, NO_3) condition

In this section, the functional diversity of the diatom assemblage is explored in terms of life-forms re-organised into larger ecological groups termed "Ecological Guilds" (Rimet and Bouchez, 2012b). Guild diversity is examined in relation to discharge and nutrient concentration (TP and NO_3) at the outflow of Morland, Pow and Dacre catchments.

4.2.5.1 Diatom assemblage functional diversity at Newby Beck

At Newby Beck in April 2012 low profile species dominated the species assemblage with percentage contribution ranging from 41 to 90% (Figure 4.12). Seasonal shifts in the relative proportion of the low profile and motile species were observed. From September 2011 to January 2012 and again from October 2012 to February 2013, the relative abundance of motile species increased corresponding with periods of higher rainfall and thus higher in-stream discharge, NO_3 and TP condition. This therefore suggested that motile species are superior competitors in the presence of elevated nutrient conditions and can physical avoid stress by moving within the biofilm to favourable micro-habitats. Hence within dynamic discharge and nutrient regime there is a trade-off between low profile species which can tolerate physical disturbance and those motile species which can utilise resources and move from stress.

Timing of high rainfall events which elicit an in-stream response in discharge was important. This is because discharge spikes and pulse delivery of nutrients to the stream, together with quiescent periods, influenced the relative contribution of each functional group to the structural diversity of the assemblage. For example in May and June 2012, an intense rainfall event and associated spike in discharge, NO_3 and TP preceding a quiescent period, contributed to an increase in relative proportion of high profile species and motile species. This suggested that quiescent periods facilitate development of the biofilm by allowing high profile and motile species to avail of higher nutrient concentration. Highest proportions of low profile species were observed in April 2012. This followed a period of quiescent conditions. However, increased rainfall is likely to have scoured the biofilm providing the opportunity for low profile species, which are resistant to physical abrasion, to colonise and increase in relative abundance. Thus, high diversity of conditions ranging from pulse to cluster events, promotes the coexistence of growth morphologies and differences in the relative abundances of the different functional groups.

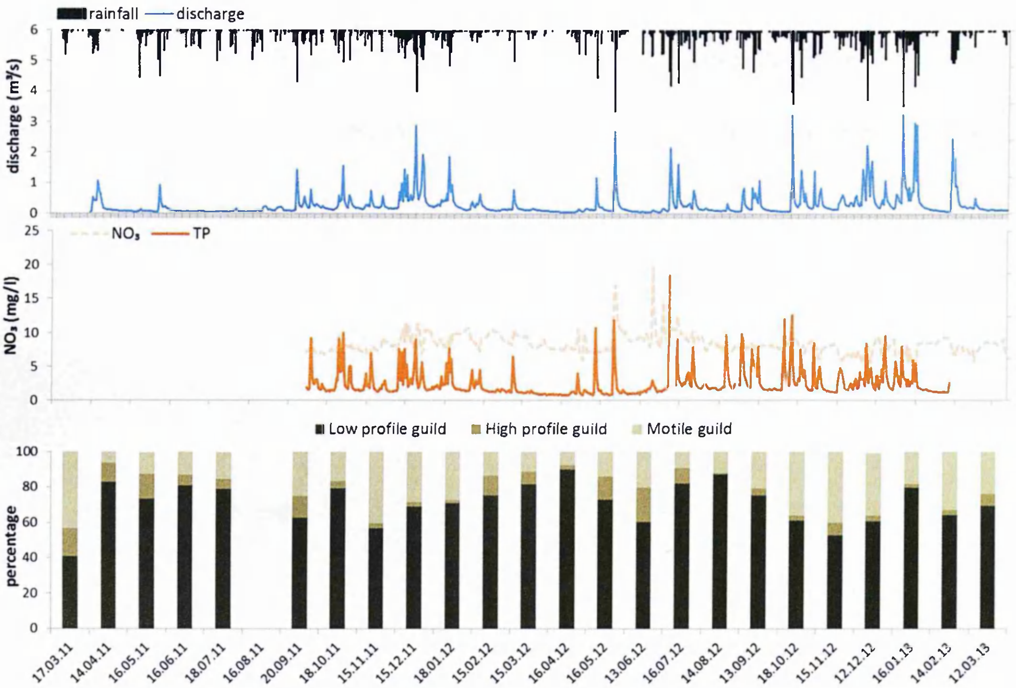


Figure 4.12: Rainfall, discharge, NO_3 and TP values were collected at 15 minute intervals from March 2011 to March 2013 at Newby Beck presented as daily averages (Source: EdenDTC). Relative abundance (percentage) of each ecological guild to assemblage structural diversity was calculated following Rimet and Bouchez (2012) and based on monthly diatom sampling from March 2011 to March 2013.

4.2.5.2 Diatom assemblage functional diversity at Pow Outlet Beck

At Pow outlet the low profile guild dominated occupying 21 to 93% of relative assemblage composition (Figure 4.13). Throughout 2012 intense rainfall events and associated spikes in discharge resulted in an initial high proportion of low profile species followed by an increased relative proportion of motile species. This was observed in April and September 2012. Quiescent periods between October 2011 and February 2012 resulted in a higher proportion of low profile species. In particular this is evident in October 2011, November 2011 and January 2012. These months corresponded with assemblage dominance by *C. placentula* var. *euglypta*. Therefore, a high proportion of low profile species during this relative quiescent period may be attributed to catchment specific species effects, seasonal demand and N:P (see section 4.2.2 and Figure 4.3).

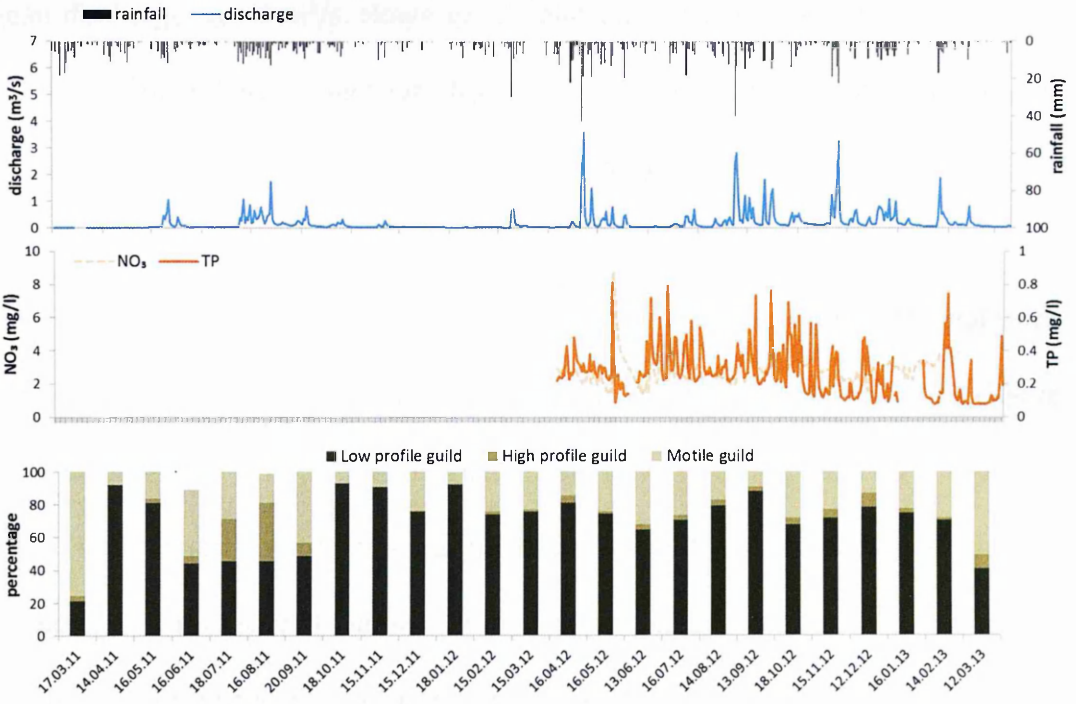


Figure 4.13: Rainfall, discharge, NO_3 and TP values were collected at 15 minute intervals from March 2011 to March 2013 at Pow Outlet and presented as daily averages (Source: EdenDTC). Relative abundance (percentage) of each ecological guild to assemblage structural diversity was calculated following Rimet and Bouchez (2012) and based on monthly diatom sampling from March 2011 to March 2013.

4.2.5.2 Diatom assemblage functional diversity at Thackthwaite Beck

Dacre, and the Morland and Pow catchments, differed in rainfall characteristics (Figure 4.14). Over the period of investigation higher amounts of rainfall fell in the Dacre catchment where high intense events were evident. This variable rainfall resulted in a more dynamic discharge regime with frequent spikes in discharge evident. For example on the 23/06/2012, average rainfall was 75.45 mm and daily mean discharge was 4 m^3/s. However, despite this dynamism assemblage diversity and successional development was higher than observed in the Morland and Pow catchments with high profile species featuring throughout.

In the Dacre catchment, as observed within the Morland catchment, seasonal shifts were observed in the relative abundance of low profile and motile species (Figure 4.14). Between the periods October 2011 to March 2012 and October 2012 to March 2012, the highest percentage contribution of motile species to assemblage composition was observed. Declines in the relative percentage contribution of motile species to assemblage composition corresponded with higher proportions of low profile species. An example of this was May 2012 when low profile species represented 77% of the assemblage composition and motile species only 8%. This again emphasised the role played by peak events and their timings in resetting succession processes. For example, following a peak event June 2012, the relative proportion of high profile species increased to a maximum of 53% in August 2012, indicative of elevated nutrient condition following this particular event. The importance of temporal variability was also illustrated at Thackthwaite Beck. From

March to September 2011 the relative proportion of low profile species was greatest reaching 84% in September 2011. From March to September 2011 high profile species also featured prominently with motile species only making a minor contribution. Hence, this demonstrated how patterns in the relative contribution of ecological groups can vary through time despite similar rainfall regimes.

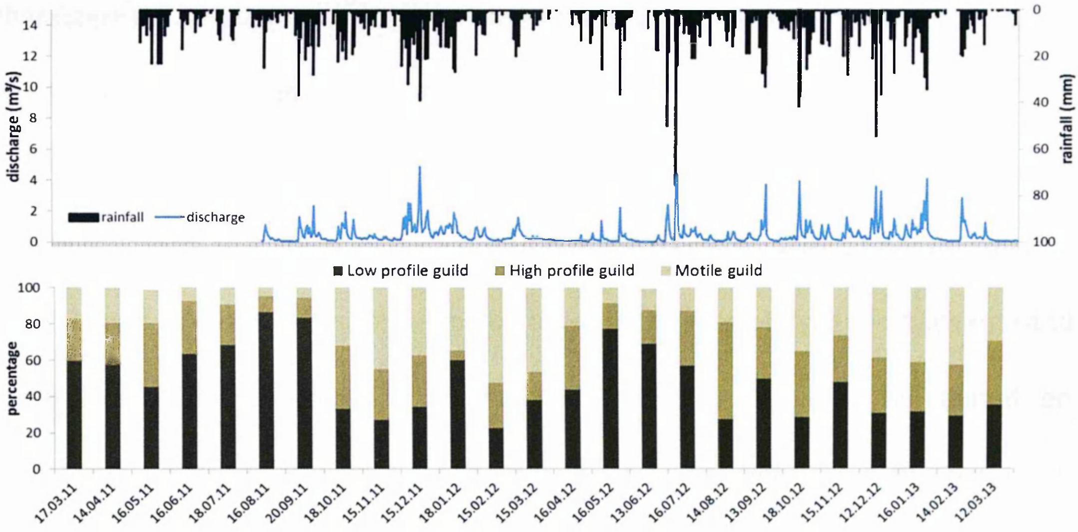


Figure 4.14: Rainfall and discharge values were collected at 15 minute intervals from March 2011 to March 2013 at Thackthwaite Beck and presented as daily averages (Source: EdenDTC). Relative abundance (percentage) of each ecological guild to assemblage structural diversity was calculated following Rimet and Bouchez (2012) and based on monthly diatom sampling from March 2011-13.

4.2.5.4 Summary of the response of diatom assemblage functional diversity to variability in discharge-nutrient (TP, NO_3) condition

The relative composition of ecological guilds demonstrated distinct catchment specific distribution patterns and predictable behaviour in response to peak events in discharge-nutrient conditions and associated quiescent periods. For example, within the Morland and Pow catchments, the high profile guild was not a dominant feature of assemblage composition. Instead, assemblage composition was dominated by the low profile guild. In the Dacre catchment, despite higher rainfall and more dynamic discharge-nutrient conditions, high profile species featured prominently. Therefore, this illustrated that functional diversity of assemblage structural composition can vary even at the 10 km^2 scale and that is important to consider catchment characteristics at appropriate spatial-temporal resolution when designing ecological monitoring assessments.

Ecological guilds also provided insight into the ecological stability of benthic diatom assemblages in terms of resilience and recovery in the face of dynamic discharge and nutrient condition. Through ecological guilds insights can also be gained on competitive interactions within the biofilm, in particular between high profile and mobile species. Relative abundances within the assemblage corresponded with a shift from competition to facilitation, with such changes in composition highlighting that, as well as taxonomic change, there are pronounced physiognomic shifts. In addition to interspecies competition other biotic factors such as grazing (physical disturbance) can also lead to changes in physiognomic and taxonomic biofilm structure (Feminella and Hawkins, 1995; Lange *et al.*, 2011; Berthon *et al.*, 2011). Low profile species are generally single celled, prostrate, and erect compared to high profile species which are filamentous chain-forming diatoms (Passy, 2007b). Different invertebrate demonstrate preferential grazing on guild diversity depending on their mouthpart morphology (Lange *et al.*, 2011). In general, morphological adaption of low profile species (e.g. adnate attachment) confers resistance to discharge which also offers resistance to grazing (Robinson and Rushforth, 1987), increasing their relative abundance. Peaks in the relative abundance of low profile species observed in spring and autumn months within all three catchments may be attributed to grazing pressure. Therefore, the influence of resources (TP, NO_3) and physical disturbance (discharge) on diatom composition can be assessed through both taxonomic and functional groups. However, consideration must also be given to secondary factors such as grazing which may offer an additional seasonal control on assemblage on diversity.

4.2.6 Diatom assemblage chlorophyll-*a*: Morland, Pow and Dacre catchments

ISF chlorophyll-*a* concentration differed significantly between the three catchments over the period March 2011 to March 2013 (Kruskal-wallis test: $H = 52.22$, $df = 2$, $p < 0.001$). Mean ISF chlorophyll-*a* concentration was higher in the Morland catchment than both Dacre and Pow catchments (Figure 4.15), with Dunn's Pairwise multiple comparison revealing differences in median chlorophyll-*a* concentrations among catchments ($p < 0.05$).

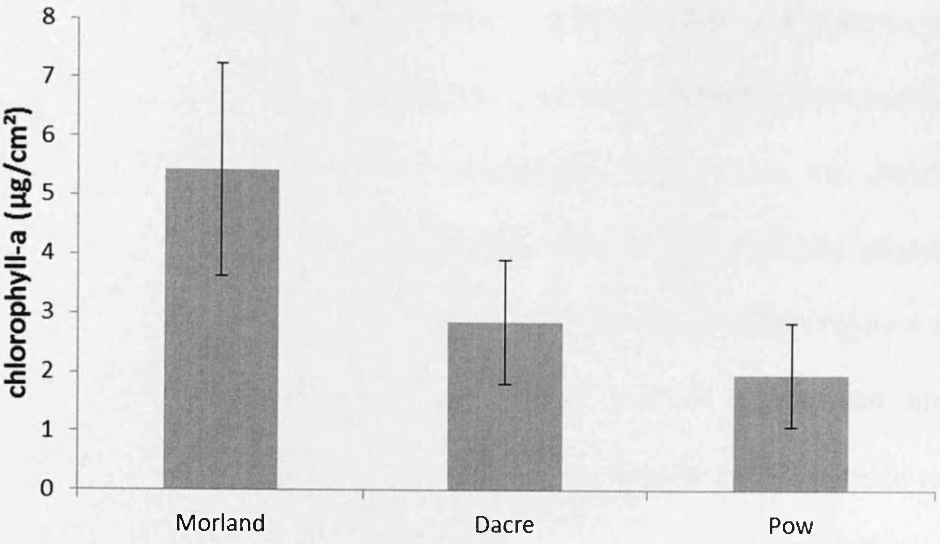


Figure 4.15: Monthly chlorophyll-*a* concentrations for Morland (n = 66), Pow (n = 66) and Dacre (n = 61) catchments from March 2011 to March 2013.

4.2.6.1 Influence of nutrient concentration and cell biovolume on chlorophyll-a

Key determinants of chlorophyll-*a* concentration in the dynamic headwater streams were assemblage composition, cell size and nutrient concentration (Figure 4.16). Chlorophyll-*a* and SRP were significantly correlated among catchments ($r = -0.255$, $n = 66$, $p < 0.05$). Pow catchment, the most SRP enriched catchment, had the lowest chlorophyll-*a* concentration, typically below 5 µg/cm^2 but highest cell biovolume. The Dacre catchment which demonstrated lowest SRP concentrations, displayed similar chlorophyll-*a* concentrations to the Pow catchment but comparable diatom biovolumes to the Morland catchment (Environment Agency, 1998). Morland catchment with mesotrophic SRP concentration had the highest chlorophyll-*a* concentrations, reaching a maximum of 21 µg/cm^2. This suggested that biovolume is a key intermediary in the inverse relationship between chlorophyll-*a* and SRP concentration. Potential for N:P limitation is greater in Pow and is attributed to the diatom assemblage been dominated by *C. placentula*, which has an individual species biovolume of 2533 µm^3. This is considerably higher than the biovolume of *A. minutissimum* (76 µm^3) and *A. pediculus* (72 µm^3) which feature prominently in the Morland and Dacre catchments (Rimet and Bouchez, 2012; see Figure 4.3). Hence the Pow catchment, despite having higher relative diatom cell biovolume, demonstrated lowest chlorophyll-*a* concentration. Mesotrophic condition within the Morland catchment, together with the presence of *A. minutissimum* and *A. pediculus,* had a significant influence on chlorophyll-*a* concentration. This may be attributed the dominance of *A. minutissimum,* which similar to *A. pediculus,* has high colonisation and rates of reproduction (Korte and Blinn, 1983; Biggs *et al.*, 1998b; Sabater *et al.*, 1998). Therefore, this illustrates the importance of diatom assemblage composition and trophic condition in determining chlorophyll-*a* concentration.

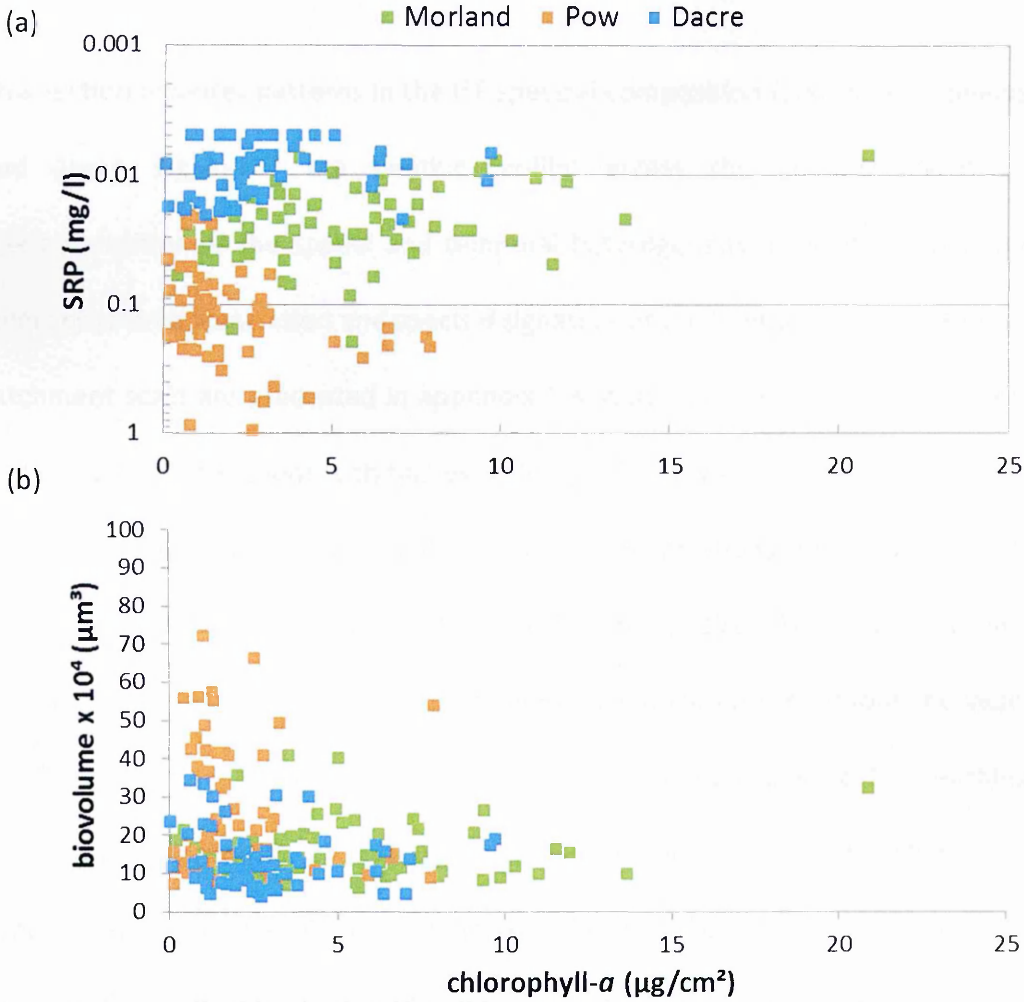


Figure 4.16: Monthly (a) chlorophyll-*a* concentrations and SRP and (b) chlorophyll-*a* concentrations and total assemblage biovolume for Morland (n = 66), Pow (n = 66) and Dacre (n = 61) catchments from March 2011 to March 2013. SRP concentration is based on monthly spot sample data, Source: EdenDTC.

4.2.6.2 ISF spectral signature of diatoms, cyanobacteria and green algae in Morland, Pow and Dare catchments

This section explores patterns in the ISF spectral composition (diatom, cyanobacteria and green algae) of the benthic biofilm across the 10 km^2 catchments. Characterization of the spatial and temporal heterogeneity in ISF determination of chlorophyll-*a* concentration and spectral signature of the benthic biofilm at the 2 km^2 catchment scale are presented in appendix 1.3. A quasi-cyclic pattern was observed in the Morland catchment with highest chlorophyll-*a* in winter and summer months. Note, however, that highest chlorophyll-*a* concentrations were under winter conditions in both years (January 2011 and December 2012; Figure 4.17, a). In the Pow and Dacre catchments patterns in chlorophyll-*a* varied throughout the year. In the Pow catchment, highest concentrations were observed in 2011, reaching a maximum concentration of 7.91 µg/cm^2 in November 2011. Monthly chlorophyll-*a* concentration in the Dacre catchment through 2012 demonstrated higher chlorophyll-*a* concentrations under winter conditions and declined thereafter to 2.22 µg/cm^2 in October 2012. Lower chlorophyll-*a* concentrations in April compared to both March and May demonstrated intra-seasonal variability. Comparison across years demonstrated how patterns can vary among seasons. Highest chlorophyll-*a* concentrations were observed in August 2011 (7.07 µg/cm^2) and lowest in November 2011 (0.04 µg/cm^2). Conversely in 2012 chlorophyll-*a* concentration were low in August (2.37 µg/cm^2). Highest concentrations were observed in November 2012 (7.18 µg/cm^2) with maximum concentrations observed in December 2012 (9.71 µg/cm^2). In contrast within the Pow catchment, higher chlorophyll-*a* concentrations were observed in 2011 than 2012. Across both the Dacre and Pow catchments in 2012 trends chlorophyll-*a* concentrations were comparable across season. For example, chlorophyll-*a* concentrations increased from December 2011 to February 2012 with high chlorophyll-*a* concentrations in January 2013 across both catchments.

Spectral fingerprints demonstrated that diatoms were the dominant component of the benthic assemblage across all three catchments along with cyanobacteria, which were generally found in lower concentrations than diatoms (Figure 4.17). Green algae only made a minor contribution to total assemblage chlorophyll-*a* and was more evident in 2011 than 2012 and 2013. At Newby Beck, diatom chlorophyll-*a* concentrations ranged from 0.47 μg/cm^2 in December 2012 to 5.99 μg/cm^2 in August 2012. The spectral signature of diatoms comprised more than 50% of the benthic assemblage apart from December 2011 (27%), January 2012 (44%) and May 2012 (48 %). Similarly with both Pow and Dacre catchments diatoms occupied on average 60% and 65% respectively of the benthic assemblage. Greatest diversity in spectral signature was observed in July and August 2011 within the Pow catchment when green algae reached concentrations of 0.55 and 0.92 μg/cm^2 respectively. In Dacre catchment, green algae were present in October (0.84 μg/cm^2) and December (0.70 μg/cm^2) 2011 and thereafter, similar to both Morland and Pow catchments, diatoms in association with cyanobacteria dominated the spectral signature of chlorophyll-*a* concentration.

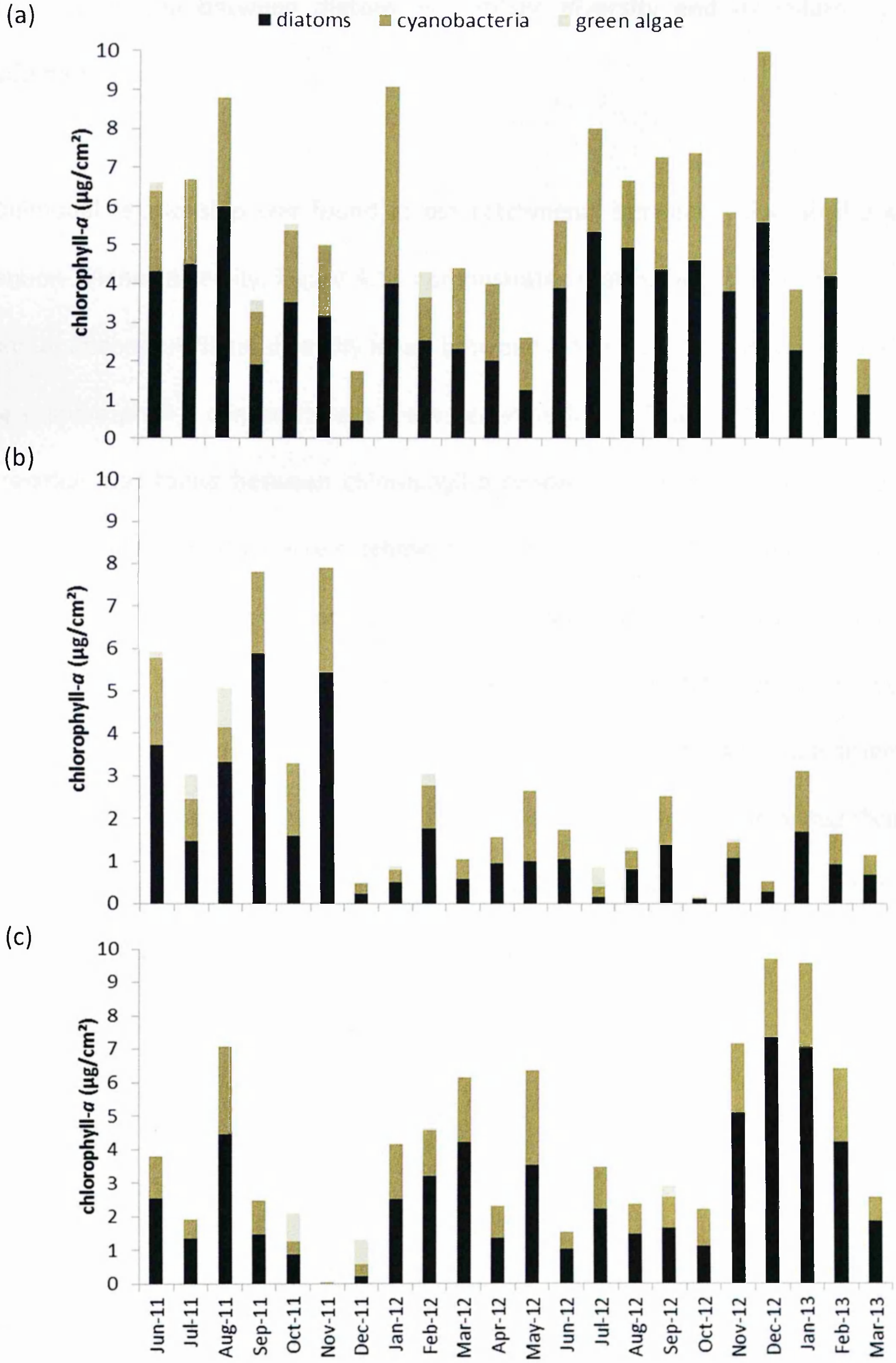


Figure 4.17: Relative contribution of diatoms, cyanobacteria and green algae to total benthic biofilm chlorophyll-*a* for (a) Newby Beck (Morland); (b) Pow Outlet; and (c) Thackthwaite Beck (Dacre) from June 2011 to March 2011.

4.2.7 Relationship between diatom assemblage diversity and its chlorophyll-*a* production

A unimodal relationship was found across catchments between chlorophyll-*a* and Shannon-Wiener diversity. Figure 4.18 demonstrates that highest chlorophyll-*a* was found at Shannon-Wiener diversity levels between 2 and 2.5. In the Pow catchment, where chlorophyll-*a* concentrations were generally below 5 µg/cm^2, no significant correlation was found between chlorophyll-*a* concentrations and Shannon-Wiener diversity. Similarly for the Dacre catchment, a non-significant relationship was found between Shannon-Wiener diversity and chlorophyll-*a*. This relationship although non-significant was negative, suggesting an inverse relation between both parameters. The Morland catchment, unlike both Pow and Dacre catchments, demonstrated higher and more variable chlorophyll-*a* values and a significant positive relationship between chlorophyll-*a* and diversity was observed ($r = 0.266$, $n = 64$, $p = 0.05$).

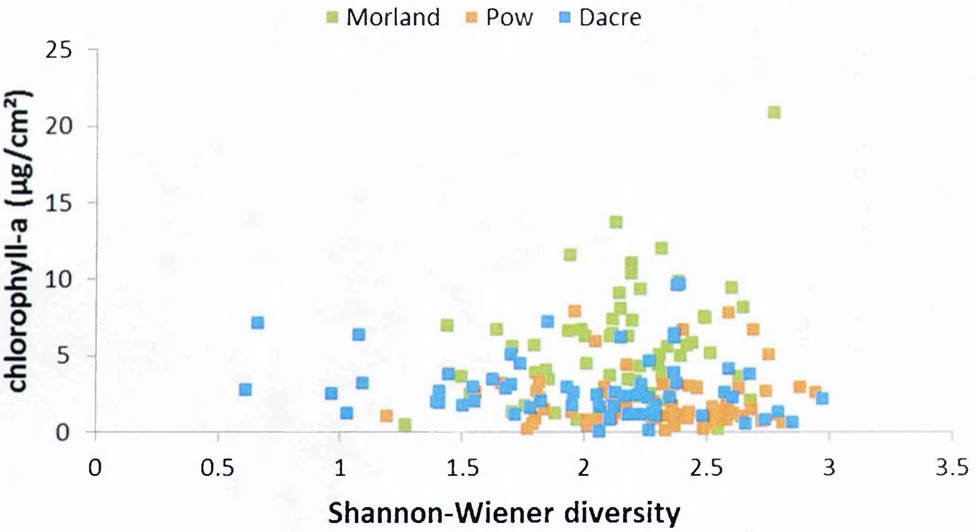


Figure 4.18: Diatom assemblage diversity (Shannon-Wiener diversity) and productivity (chlorophyll-*a*) for Morland (n= 64), Pow (n=66) and Dacre (n = 61) from March 2011 to March 2013.

4.2.8 Key environmental variables influencing diatom assemblage composition in Morland, Pow and Dacre catchments

Diatom assemblage data from March 2011 to March 2013 for all catchments are presented in figure 4.19. Axis 1 of the DCA, together with axis 2, explained 8.29% of variation in the data and clearly distinguished each of the three catchments.

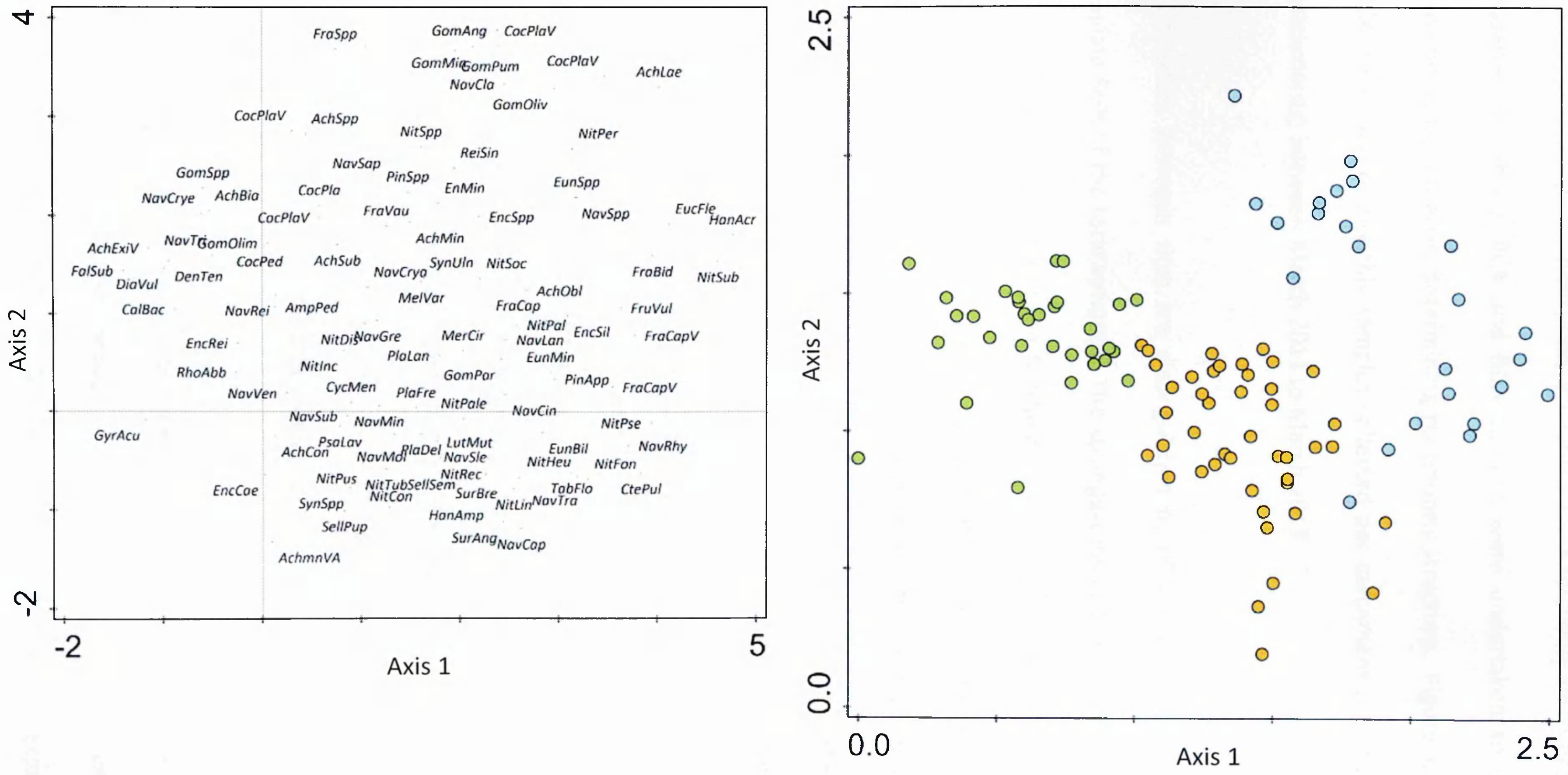


Figure 4.19: Detrended Correspondence Analysis (DCA) of monthly species composition among catchment (n = 3 sites per Morland, Pow and Dacre catchments) from March 2011 to March 2013. (Morland: Green, Dacre: Blue, Pow: Orange).

As spatial factor (catchment) only accounted for a relatively small proportion of the observed variability, PCA and RDA analysis were undertaken to determine key environmental variables determining community structure. Figure 4.20a presents a PCA ordination of monthly samples collected per catchment (n = 3 sites per n = 3 catchments) between March 2011 to March 2013.

Differences between sites are distinguished by PCA axis 1 and 2 which together explain 56% of the total variation. The strongest distinction in the data between the assemblage composition of the Morland and both Dacre and Pow catchments was provided by PCA axis 1 (Eigenvalue 0.30; Figure 4.20a). This component was made up primarily of variations in NO_3 (Factor loading = 0.79), NO_2 (Factor loading = 0.69), TP (Factor loading = 0.66), TRP (Factor loading = 0.68), conductivity (Factor loading = 0.71) and alkalinity (Factor loading = 0.67). Therefore, NO_3 explained most of the diatom community variation. Differences between Dacre and Pow catchments were determined by PCA axis 2 (eigenvalue 0.26) to which axis 1 species scores of the DCA was positively correlated (Factor loading = 0.597). Ordination analysis therefore demonstrates that assemblages within the Pow catchment were the most diverse of the three catchments with nutrients and DOC key drivers of assemblage composition whereas in the Morland catchment, catchment geology was a key driver of benthic diatom biofilm composition and chlorophyll-*a*.

RDA analysis suggested that measured environmental variables were constrained by 5 environmental variables: conductivity, NO_3, TRP, chlorophyll-*a* and DCA axis 1 species scores (Figure 4.20 b). Collectively RDA axis 1 and 2 explained 45% of observed variation. TRP, NO_3 and chlorophyll-*a* were correlated with axis 1. However, NO_3 and chlorophyll-*a* both only made a small contribution to the explained variation of 2.6 and 0.6% respectively. It was TRP which demonstrated the strongest correlation with axis 1, explaining 21% of variation. DCA species axis scores and conductivity were strongly correlated with RDA axis 2 and explained 3.4 and 17% of variation respectively. Therefore, this suggested that it was a gradient in TRP and differences in conductivity among catchment which were the key environmental drivers of differential assemblage composition.

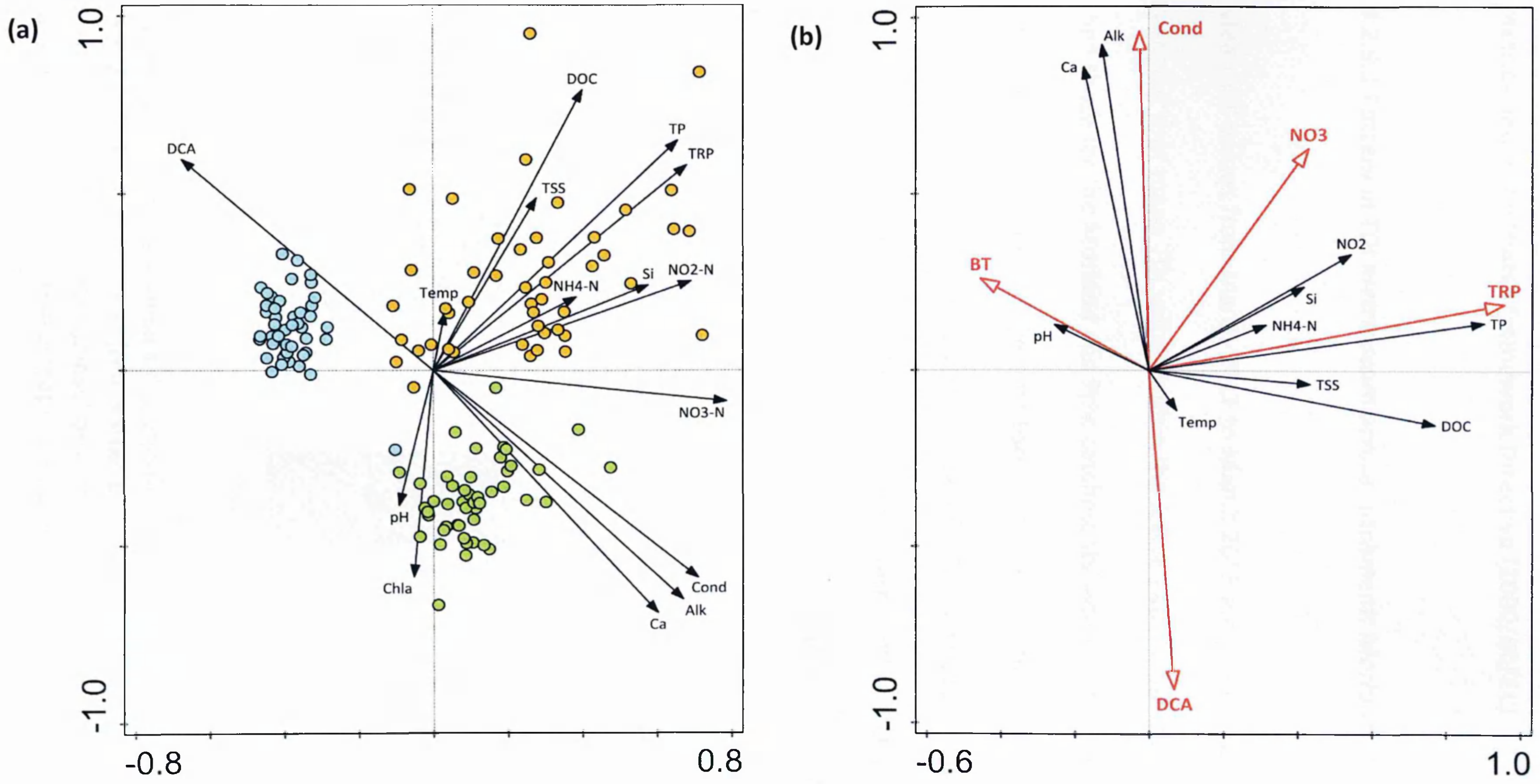


Figure 4.20: (a) Ordination of benthic diatom assemblages based on the PCA demonstration environment gradients of the measured environmental variables for each catchment (Morland: Green, Dacre: Blue, Pow: Orange); (b) RDA ordination graph for the first two axes with key drivers of catchment differences highlighted in red determined by forward selection. Chemical parameters based on spot sample data, Source: EdenDTC.

4.2.9 Diatoms as indicators of environmental status through the TDI and EQR metrics under the Water Framework Directive (2000/60/EC)

4.2.9.1 Pattern in TDI metric score across catchment: Morland, Pow and Dacre

Mean TDI values from March 2011 to March 2013 are presented in Figure 4.21. This revealed that mean TDI values within the Dacre catchment were significantly lower than those for the Morland and Pow catchments with values suggesting low levels of nutrient enrichment. While median TDI values differed significantly across catchments (Kruskal-wallis test: H = 36.34, df = 2, $p < 0.001$), no significant difference was observed between Morland and Pow, with both catchments indicative of moderate nutrient enrichment (Dunn's pairwise comparison: $p < 0.05$).

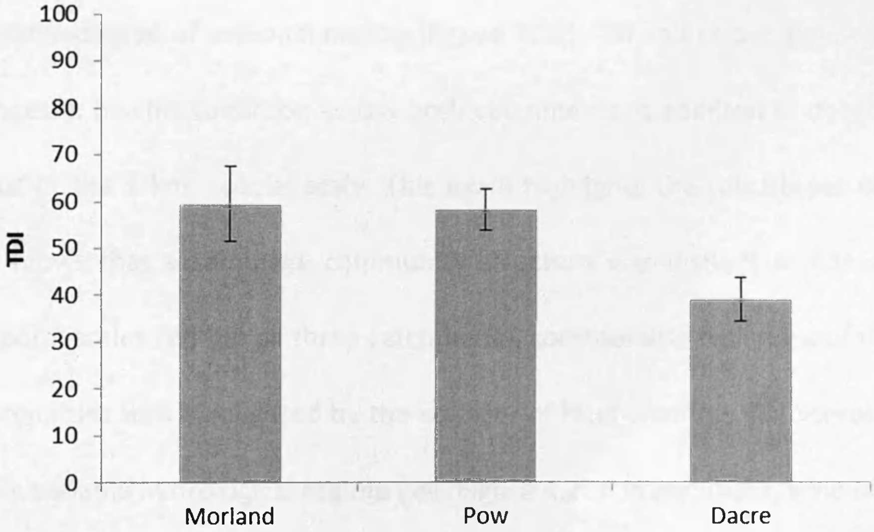


Figure 4.21: Mean (± standard deviation) monthly TDI values Morland (n = 73), Pow (n = 76) and Dacre (n = 67) (n = 3 sites per catchment). TDI values indicate trophic condition based on diatom phosphorus sensitivity. Values are expressed between 0 and 100 with 100 indicating eutrophic condition.

Mean monthly TDI values varied across the individual catchments. Evidence of a repeated cyclic seasonal pattern was greatest within the Morland catchment with higher trophic status classification in the spring and lowest in winter (Figure 4.22 a). For example at Newby Beck, TDI values were higher between September and February ($t = -16.07$, $df = 10$, $p < 0.05$), with a peak in December in both years, indicating a higher level of nutrient-tolerant taxa and thus, more nutrient-enriched conditions. Trends at the 10 km^2 scale were partly tracked by those at the 2 km^2 scale which revealed high annual repeatability, sensitivity and robustness of TDI scores at finer 2 km^2 scales. This demonstrates that annual mean TDI scores can conceal important catchment dynamics and cyclic seasonal pattern in trophic condition in headwater streams.

In comparison to the Morland catchment, Dacre and Pow catchments did not display the same degree of seasonal cycling (Figure 4.22). TDI values did, however, illustrate changes in trophic condition across both catchments in addition to detecting trophic status at the 2 km^2 spatial scale. This again highlights the robustness of TDI scores and shows that assemblage community structure was distinct at fine-spatial high-temporal scales. Within all three catchments, considerable resilience of these diatom communities was highlighted by the stability of inter-monthly TDI scores against the highly variable hydrological regime (see Figure 4.28). In summary, time of sampling is critical with month-on-month changes in TDI values evident (Figure 4.22), emphasising that both spatial and temporal factors can impact trophic classification.

Figure 4.22: Monthly TDI metric score within (a) Morland; (b) Pow; and (c) Dacre catchments from March 2011 to March 2013. TDI values indicate trophic condition based on diatom phosphorus sensitivity. Values are expressed between 0 and 100 with 100 indicating eutrophic condition.

4.2.9.2 EQR metric score across catchments: Morland, Pow and Dacre

Five biological quality elements were investigated per catchment comprising four specific elements under the WFD: phytoplankton, macrophytes and phytobenthos (diatoms), invertebrates and fish. A study on phytoplankton, from February 2012 to October 2012, demonstrated that there were negligible populations in these headwater streams (see chapter 3.4.3, Figure 3.6). Therefore, this discussion focuses on macrophytes, diatoms, macroinvertebrates and fish (Specific trends in macrophytes, and macroinvertebrate metrics along with a description of fish communities can be found in appendix 1.4).

Temporal trends in EQR reveals a quasi-stable cyclic seasonal pattern with higher ecological status observed in spring and summer compared to autumn and winter. There was considerable reproducibility in trends among Morland and Pow catchments ($r = 0.67$, $n = 25$, $p < 0.05$). However, Morland demonstrated the greatest range in EQR values (0.28 to 1), capturing the full range of WFD ecological status classification from "High" to "Poor". Overall Dacre Beck had the highest ecological status of which was sustained over considerably longer periods than in Morland and Pow catchments. EQR values only declined between October 2011 and January 2012 with values indicating at least good ecological status outside this window. Consequently, the Dacre catchment had the lowest annual range in EQR, spanning "High" to "Moderate" quality classes (Figure 4.23).

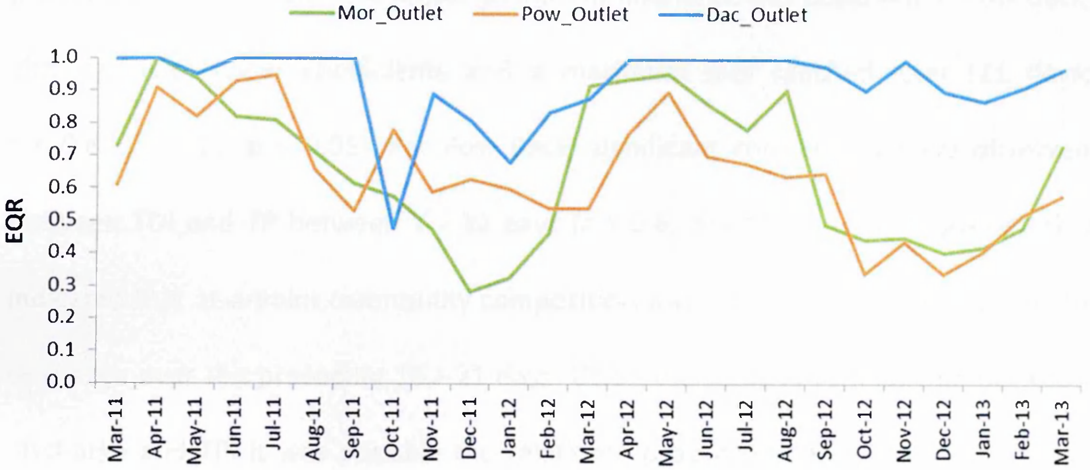


Figure 4.23: Monthly EQR values for Morland, Pow and Dacre from March 2011 to March 13 (n=25). Values from 0 – 100 indicate increasing ecological status, with a maximum of 100 indicating "High" ecological status. EQR WFD UK TAG classification boundaries: Bad (< 0.25), Poor/Bad (0.25), Poor/Moderate (0.50), Moderate/Good (0.75) and Good/High (1.00).

4.2.10 Response of diatom assemblage attributes (diversity and chlorophyll-*a*) to antecedent discharge and nutrient condition

High temporal variability in the benthic communities was attributed to their sensitivity of response to rainfall and associated discharge-nutrient characteristics. Therefore, the critical antecedent period determining the diatom community structure (using TDI as a surrogate) and ISF benthic chlorophyll-*a* was investigated (Figure 4.24). For Newby Beck an initial correlation between was found between TDI and mean discharge on the day of diatom sampling ($r = 0.54$, $n = 25$, $p < 0.05$), with the correlation strengthening to a maximum after 18 days ($r = 0.7$, $n = 25$, $p < 0.05$). Significant correlations were also observed between TDI and TP after 15 days

($r = 0.53$, $n = 25$, $p < 0.05$), but this increased further to a maximum after 21 days ($r = 0.66$, $n = 18$, $p < 0.05$). A similar pattern in discharge was observed in Pow Beck, although with lower coefficients and a maximum was reached later (21 days; $r = 0.63$, $n = 18$, $p < 0.05$). For Pow Beck, significant correlations were observed between TDI and TP between 7 - 12 days ($r = 0.6$, $n = 18$, $p < 0.05$). Overall, this indicated that at-a-point community composition was a product of factors related to discharge over the preceding 15 - 21 days. Given the positive relationship between discharge and TP, it was possible the relationship between TDI and discharge was partly mediated by nutrient concentration.

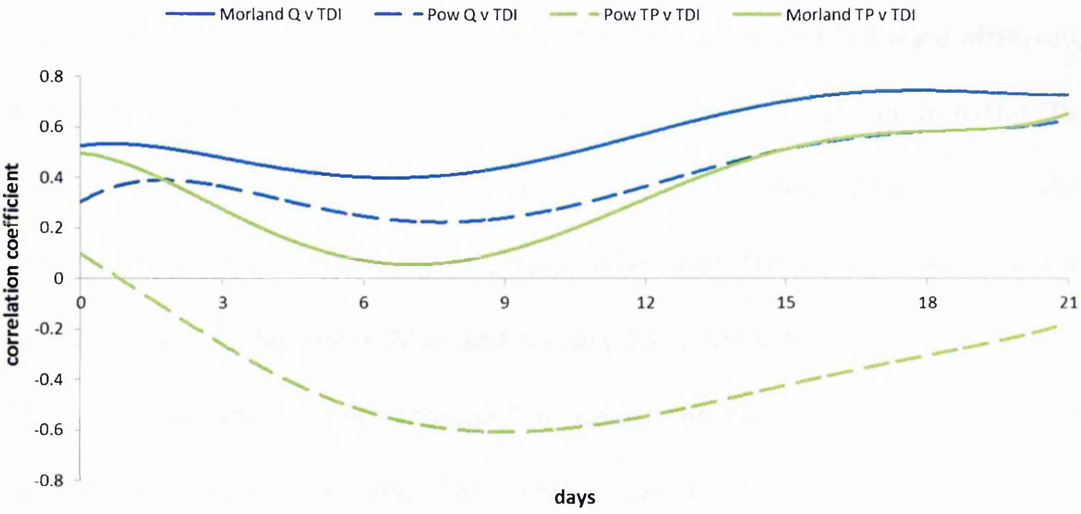


Figure 4.24: Antecedent forcing periods of TDI and ISF chlorophyll-*a*. Pearson's r is calculated between TDI and chlorophyll-*a* against mean discharge (Q) and TP for Pow Outlet and Newby Becks. The continuously sampled environmental data is averaged over periods from zero to 21 days (Source: EdenDTC). Curves are 3rd order polynomial regressions. The TDI and ISF are collected monthly over 25 months for Newby Beck (n = 25) and 18 months for Pow Beck (n = 18).

In Newby and Pow Becks, a non-significant relationship was found between benthic chlorophyll-*a* and antecedent discharge-TP conditions, thus indicating that antecedent conditions over the preceding 21 days were not a key determinant of benthic productivity. However, monthly development TDI and chlorophyll-*a* illustrated that chlorophyll-*a* concentration was sensitive to discharge-nutrient spikes in response to catchment rainfall (Figure 4.28).

For Newby Beck (Figure 4.25 a), two distinct quasi-cyclic periods were distinguished in the diatom community structure. Patterns in TDI, a proxy for community structure, were partly tracked by chlorophyll-*a*, which was used as a surrogate for benthic productivity. Within relatively quiescent hydrological periods, e.g. January to May 2012, broadly positive relationships between chlorophyll-*a* and TDI were observed, where lower TP concentrations and improved water quality, as inferred from the TDI, was matched by an increase in chlorophyll-*a*. However, Figure 4.28 also demonstrated near anti-phasing of chlorophyll-*a* with TDI during high discharge episodes, such as December 2012 and January 2013. Stability of the inter-monthly TDI scores and the benthic chlorophyll-*a* against the highly variable hydrological regime again illustrates the considerable resilience of headwater diatom communities.

Similar quasi-cyclic periods were observed in the Pow catchment for TDI (Figure 4.25 b) with inter-monthly variations in TDI again relatively small. Differences were also observed in chlorophyll-*a*. Values ranged from 0.14 to 7.92 $\mu g/cm^2$, generally lower than in Newby Beck. Unlike in Newby Beck, there was usually an inverse relationship between the TDI and benthic chlorophyll-*a*. When values of TDI were high in the Pow catchment from October to March in both years, benthic productivity was seen to be less than 1 μg/cm^2, which was lower than productivity in the Morland catchment. Similar to Newby Beck, there was non-significant relationship between water temperature and chlorophyll-*a* (Newby Beck: $r = 0.24$, $n = 25$, $p > 0.05$; Pow Beck: $r = 0.18$, $n = 18$, $p > 0.05$). Clusters of high rainfall events and associated high stream discharges correlated with high TDI values and low chlorophyll-*a*, suggesting that unlike in Newby Beck, physical rather than nutrient factors dominate. Extreme examples of this inverse response in the ecological community composition and chlorophyll-*a* to high discharge occurred in December 2011 and October 2012, and was attributed to a period of high rainfall. Furthermore, as for Newby Beck, the resilience of the communities in the Pow catchment was evidenced by their overall stability in month-on-month TDI scores and associated chlorophyll-*a*.

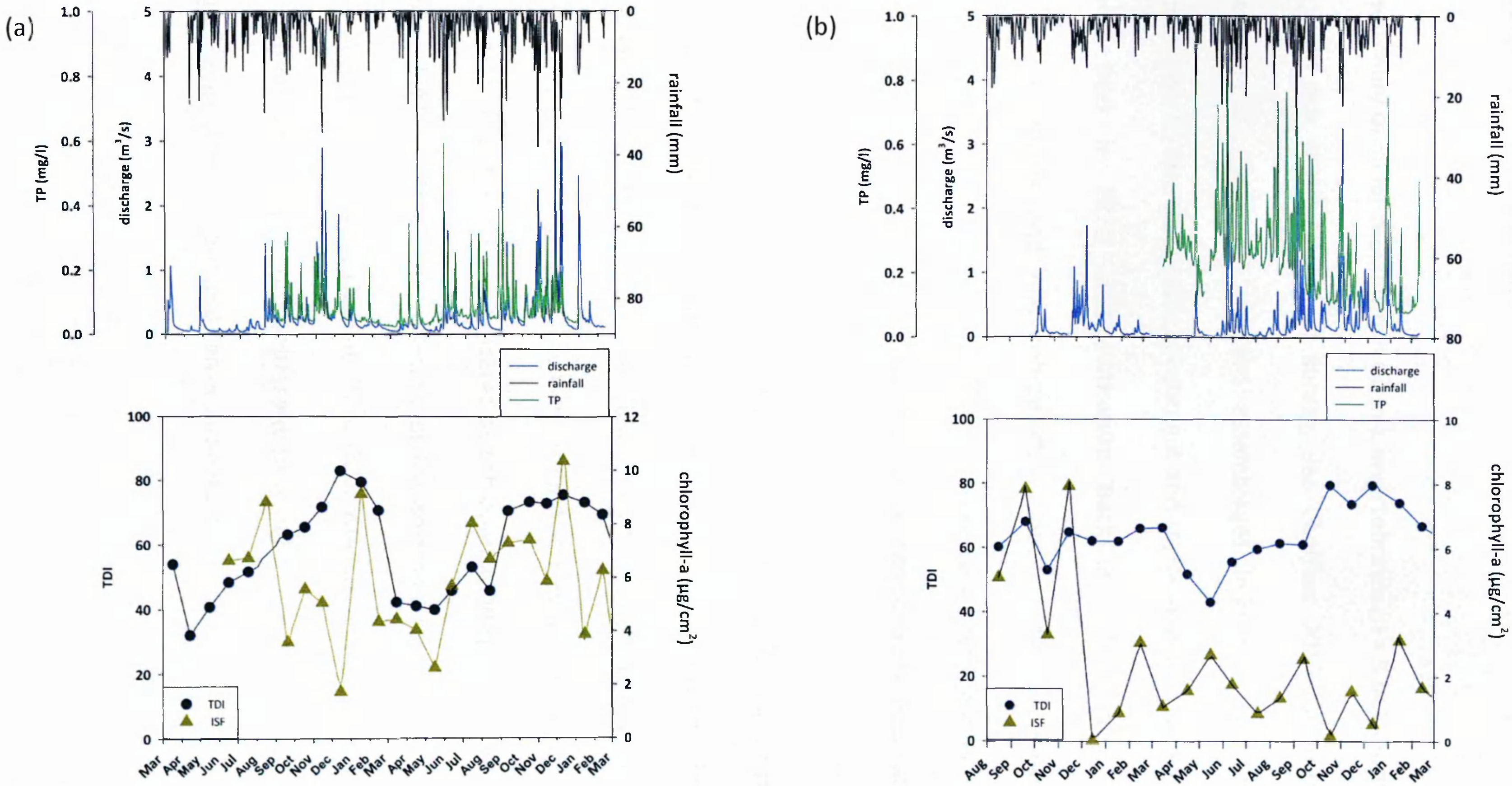


Figure 4.25: 15 minute rainfall, discharge and TP presented as daily averages (Source: EdenDTC) for (a) Newby Beck (Morland) and (b) Pow Outlet (Pow). Relationship between TDI and chlorophyll-*a* are based on monthly diatom sampling from March 2011 to March 2013 (and fitted with spline curve).

4.2.11 Ecological status signals across headwater biota: macrophyte, diatoms, macroinvertebrate and fish

A summary of ES for diatoms (n = 12 pa), invertebrates (n = 3 pa), macrophytes (n = 1 pa) and fish (2012 catchment survey, see Chaffeur 2013 for further details) is presented in Table 4.4. Diatom assemblages indicated that spatially within catchments ES was temporally concordant and stable year-on-year apart from Dedra Banks Beck in 2012 and Lowthwaite Beck in 2011, when ES decreased. Macroinvertebrate and macrophyte classification indicated good to high ES. Temporal consistency of ES classification was particularly evident in the Dacre catchment, with ES higher than that observed for Morland and Pow catchments.

The overall lower ES indicated by diatoms was likely a reflection of sample number with monthly diatom assemblages capturing discharge-nutrient dynamics within season (Table 4.4). Temporal variability is smaller for comparatively long lived taxonomic groups such as fish which respond to stress over extended time scales compared to diatoms. ES as indicated by fish population spanned the full range of ES from "High" to "Poor" and reflective of the environmental gradient in phosphorus concentration across catchment. This therefore suggested that while long lived taxonomic groups may miss represent short-term variability they can adequately signify general catchment-scale environmental gradients.

Table 4.4: Ecological Status for all sites within Morland, Pow and Dacre. Ecological status of Biological Quality Elements: H = High (blue), G = Good (green), M = Moderate (yellow), P = Poor (orange), B = Bad (red). EQR WFD UK TAG classification boundaries: Bad (< 0.25), Poor/Bad (0.25), Poor/Moderate (0.50), Moderate/Good (0.75) and Good/High (1.00).

		INVERTEBRATES			MACROPHYTES			DIATOMS			Fish		
Sub-catchment	**Site**	**2011**	**2012**	**2013**	**2011**	**2012**	**2013**	**2011**	**2012**	**2013**	**2011**	**2012**	**2013**
Morland	**Newby Beck**	H	H	H	G	H	G	M	M	M		M	
	Dedra Banks Beck							M	P	M			
	Sleagill Beck							M	M	M			
Pow	**Pow Outlet**	G	G	H	G	H	H	M	M	M		B	
	unnamed trib A							M	M	M			
	unnamed trib B							M	M	M			
Dacre	**Thackthwaite Beck**	H	H	H	H	H	H	G	G	G		H	
	Mellfell Beck								G	G			
	Lowthwaite Beck							M	G	G			

4.2.12 Conclusion

This chapter has demonstrated that phytobenthic communities in the three headwater streams studied were dominated by diatoms. Moreover, it illustrated the dynamic physical environment which ensured that nutrient concentrations and their availability to the benthic diatom community was temporally variable. However, despite this variabilty there was a distinct and reproducible seasonally-based pattern in benthic diatom assemblage richness, evenness, diversity and chlorophyll-*a* within and among the three catchments. *In situ* fluorometry proved a useful technique enabling the composition and biomass of the phytobenthic component of biofilms to be resolved. Moreover, it revealed a unimodal relationship across catchment between descriptors of diatom diversity and chlorophyll-*a.*

Ecological guilds provided critical information on structural diverstiy and functional development of these communties in the fluctuating, dynamic headwater environment. This variability in assemblage composition was captured using the nutrient-sensitive metric, TDI and classified under the WFD, thus highlighting metric robustness and distinctiveness of assemblage composition at high-temporal and fine-spatial scales. However, concordance among biotic assemblages in assessments of trophic condition and ecological status was poor. This was likely a result of dynamic discharge-nutrient regime and sampling resolution together with species life cycle and turnover.

Diatom communities can respond to physical and chemical parameters that can vary in magnitude within days, thus enhancing community stability (resistence and resilience). This was illustrated through the derived TDI which demonstrated that diatoms best reflected stream discharge conditions over the preceding 15 - 21 days and TP concentrations over a wider antecedent window of 7 – 21 days. TP, together with conductivity, was also shown to be a key environmental determinant of assemblage composition. Thus, the importance of hydrogeology and nutrient concentration on assemblage structure over high and fine spatial-temporal scales is emphasised.

In order to understand patterns in benthic biofilm diatom diversity and chlorophyll-*a* of headwater systems it is important to recognise that the natural flow regime of headwaters is dynamic. This heterogenity in flow is likely to generate considerable variability across diverse spatial scales in these systems from catchment to microhabitat. Therefore, the following chapter will explore key spatial determants of fine-spatial scale variability in benthic diatom diversity and chlorophyll-*a* at the reach scale.

Chapter 5: Reach-scale spatial variability in benthic diatom assemblages

5.1 Introduction

As diatoms are influenced by different factors at different scales within an hierarchical framework, and in particular the dynamic flow regime, spatial variance in physicochemical variables and the benthic biofilm community is a key attribute of headwater systems (Frissell *et al.*, 1986; Stevenson, 1997; Cohen *et al.*, 1998; Biggs *et al.*, 2005; Finn and Poff, 2005). Streams channels are longitudinally varied with fluvial features, including riffles, runs, glides and pools (Leopold, 1964). These fluvial features are a key determinant of the physical organisation and pattern in community composition (Hynes, 1970; Southwood, 1975; Meffe and Sheldon, 1988; Newson *et al.*, 1998; Maddock, 1999; Harvey and Clifford, 2009). The different flow types which occur in these morphologically heterogeneous channels, nested within the hierarchical framework of river organisation, are referred to as physical biotopes (Padmore, 1997; Padmore, 1998). Physical biotope units, therefore, form the basic unit of stream habitat, providing a micro- (within biotope unit) and meso-scale (biotope unit) assessment of stream ecological status (Jowett, 1993; Padmore, 1998; Newson and Newson, 2000; Harvey and Clifford, 2009).

Physical biotope units illustrate the importance of stream bed morphology and the need for meaningful understanding of the spatial dynamics of stream flow which mediates the impact of physical and chemical parameters on benthic diatom structure, diversity and process (Harper *et al.*, 2000). While the concept of biotopes has received significant physical characterisation (Wadeson and Rowntree, 1998; Kemp *et al.*, 1999; Newson and Newson, 2000; Thomson *et al.*, 2001), there has been no study of the influence of physical flow biotopes units on benthic diatom biofilms. However, studies on diatom-physical habitat associations have suggested that greater community diversity and more rapid successional development of diatom communities is observed in slow current velocity habitats (Lamb *et al.*, 1987). This chapter aims to determine inter- physical flow biotope variation in benthic communities and relationships to in-stream physical habitat (velocity, shear stress and depth), to determine the appropriate resolution for biomonitoring. More specifically, this chapter aims to test the hypothesis that 1) diatom assemblage biodiversity is greater in pools than in riffles in headwater streams; 2) if temporal variation is held constant, the key physical variables determining benthic diatom community attributes (richness, evenness, diversity and chlorophyll-*a*) are flow velocity and habitat structure (depth), and 3) benthic diatom community attributes (diversity and chlorophyll-*a*) are linearly and inversely related.

To investigate these hypotheses this chapter will commence with a description of physiochemical characteristics of the physical biotope along the riffle-pool sequence studied. The structural composition of the riffle-pool units will then be presented, followed by reach ISF chlorophyll-*a* concentrations. The relationship between assemblage biodiversity and productivity will be explored and key environmental drivers of these parameters investigated. The chapter will then conclude with a discussion of variance in trophic and ecological status among biotope units.

5.2 Experimental design

5.2.1 Newby Beck: reach location

At Newby Outlet, four physical biotope units (2 riffles, 2 pools) were selected from a 50 m reach section approximately 3 m wide (Figure 5.1). Two sampling occasions, 25/10/2011 and 22/05/2012, were selected to replicate the experiment under potentially contrasting 'autumn' and 'spring' conditions respectively (see Chapter 6 for discussion on temporal variability). Experiment replication aimed to provide control against abiotic and biotic seasonal effects on benthic diatom assemblages, such as shading or grazing, that are difficult to quantify.

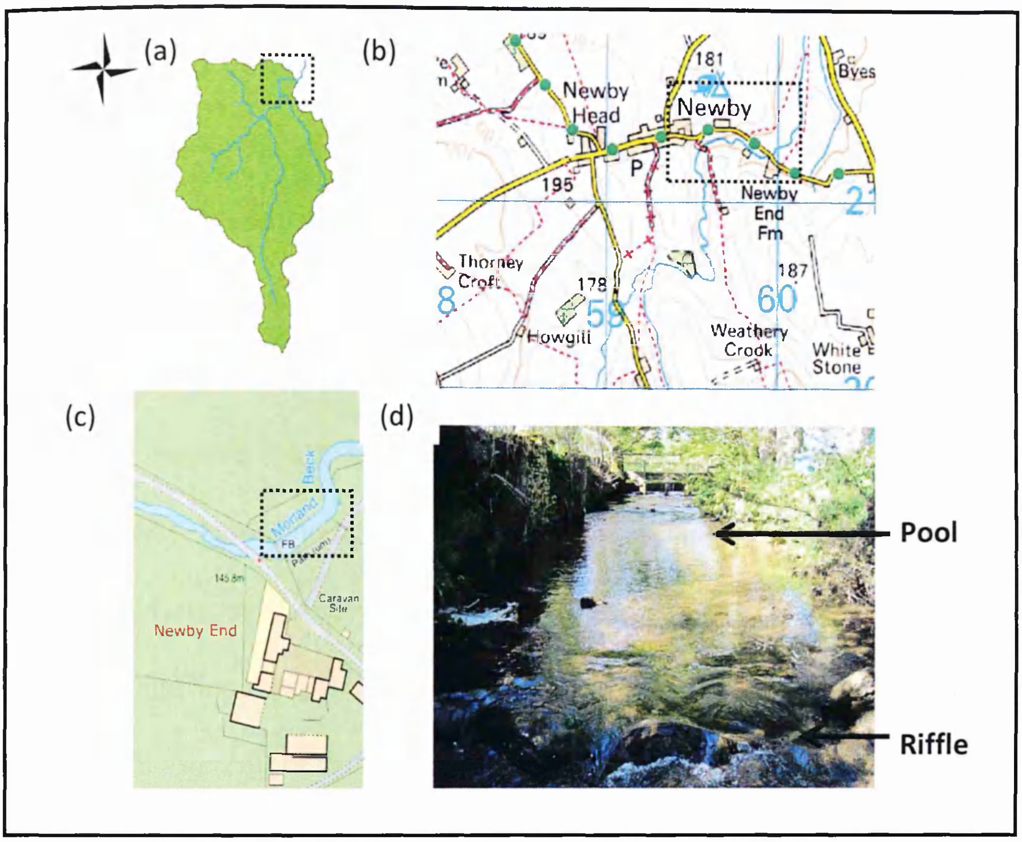


Figure 5.1: Newby Beck in Morland Catchment; (a) Morland 10 km^2 sub-catchment (b) Newby Beck location (c) reach section and (d) exemplar biotope units: riffle and pool.

5.2.2 Sampling framework for diatom composition and chlorophyll-*a* analysis

Four biotope units in a riffle-pool-riffle-pool sequence were investigated and are referred to throughout this chapter as riffle unit 1, pool unit 1, riffle unit 2 and pool unit 2 respectively. Figure 5.2 (a) shows riffle unit 1, pool unit 1, riffle unit 2 respectively with pool unit 2 found immediately following riffle unit 2 around the bend. Within each biotope unit, three 1 x 1 m quadrats (divided into 20 x 20 cm squares) were placed from right to left banks (in direction of flow) along a cross channel transect at the approximate mid-point of each biotope unit (e.g. Figure 5.2 b). Samples were taken from within quadrats from the top (sample 1), middle (sample 2) and bottom (sample 3) row of 20 x 20 cm quadrat divisions (in the direction of flow; Figure 5.2 c). Figure 5.2 b-d demonstrates the experimental set-up for a riffle biotope which was repeated for pool biotopes.

An ISF measurement was taken from one representative cobble from each 20 x 20 cm square across the top, middle and bottom row of each quadrat (Figure 5.2 d; see section 3.4.1). The same five cobbles from across each row where then scraped for diatom analysis (see section 3.3.1) and pooled to form a single composite sample. Diatom digestion, preparation and identification followed section 3.3.1. The diameter (b axis: cm) of each cobble sampled was measured.

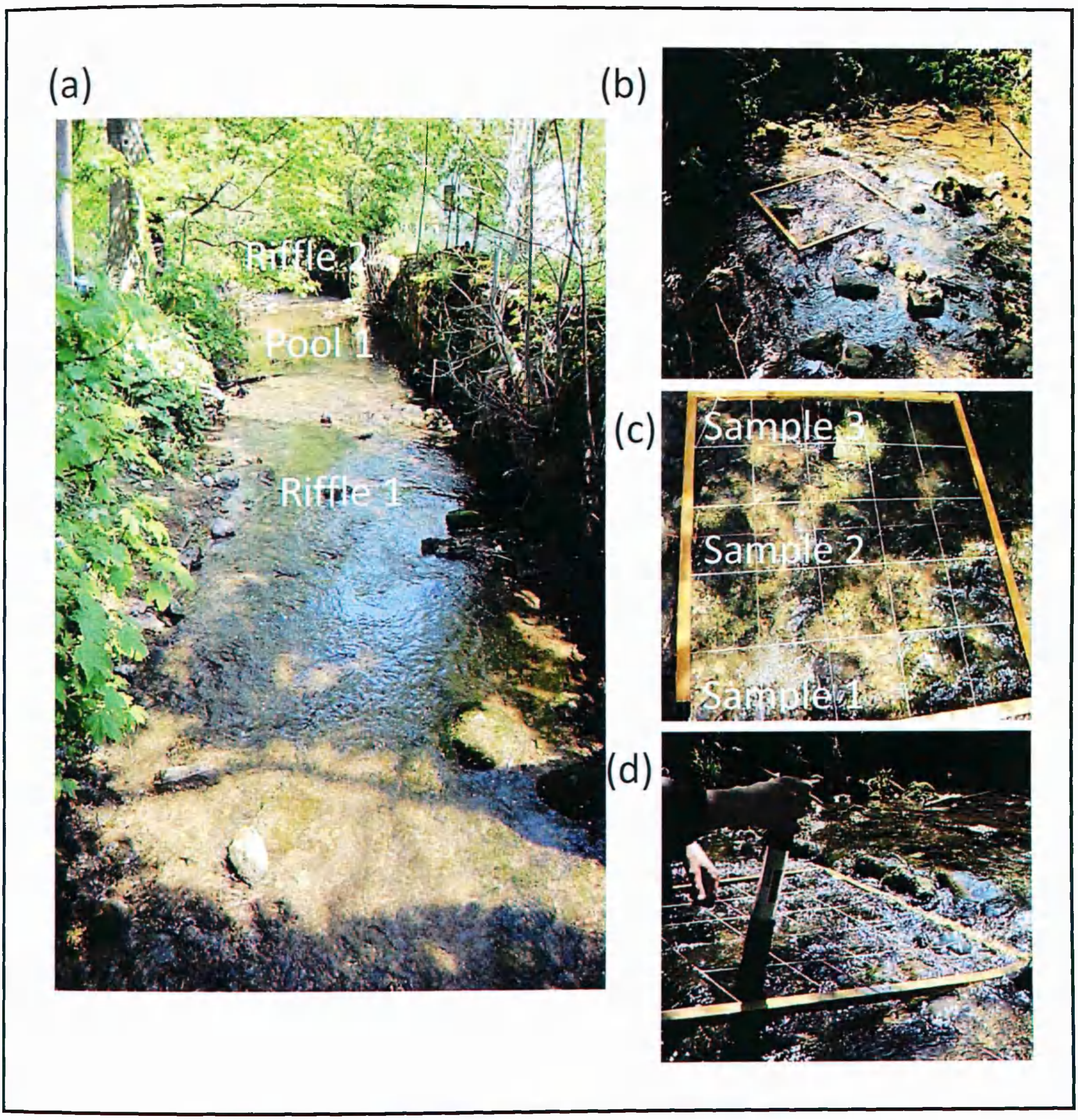


Figure 5.2: (a) Newby Beck showing riffle and pool habitats, (b) 1 x 1 m quadrat placed along mid-transect in riffle biotope, c) sample positions within the 1 x 1 m quadrat in a typical riffle zone and d) illustration of *in situ* ISF chlorophyll-*a* measurements obtained within the 1 x 1 m grid.

5.2.3 Physical and chemical characterisation of the reach

Stream bed elevation was mapped over the riffle-pool-riffle-pool sequence using a Leica TPS 1200 Digital Elevation Model (DEM; see Figure 5.1; Figure 5.8). An electromagnetic current-meter (Valeport Electromagnetic gauge, model 801) was used to obtain a velocity measurement, at 0.6 of the flow depth, from each 20 x 20 cm square across the top, middle and bottom row of each quadrat (as demonstrated in Figure 2.2). For each quadrat square sampled, depth (cm) was noted. Eight pH, conductivity, temperature, turbidity and %DO measurements were taken from each biotope unit at 1 m intervals along the top and bottom of the 1 x 1m quadrat transect using a HANNA HI 9828 multi-parameter meter with a HI 769828 multi-parameter probe. Turbidity was measured similarly, using a TurbiDirect portable turbidity meter. Shear stress was characterised per biotope unit by employing the Fliess Wasser Stammtisch (FST)-hemisphere method (Statzner and Muller, 1989). The FST-hemisphere method provides an indication of flow conditions close to the bed substrate by identifying the heaviest standard hemisphere of identical size and surface but difference density that is moved in prevailing flow conditions on a horizontal plane at the bottom of streams. A single shear stress measurement was taken within each of the twenty-five 20 x 20 cm squares per n = 3 quadrats per biotope unit, yielding a total of 75 measurements per biotope unit. On each sampling occasion a sample was collected from each physical biotope unit for water chemistry analysis by CEH Lancaster (see section 3.6.2). In the spring sampling, variation in light availability was estimated by taking a measurement every three hours between 0900 and 1800. The readings were taken at 50 cm intervals along a transect across the mid-point of each of the biotope units (equates to the middle row of 1 x 1 m quadrat) using a SCI-MART digital lux meter. This yielded n = 28 per biotope unit over the 9 hour period.

5.2.4 Meso- and micro-scale physical biotope variability in benthic biofilm composition and chlorophyll-*a*

At the meso-scale variability in benthic chlorophyll-*a* was investigated through biotope units defined by surface flow type (section 5.2.2). Lateral and longitudinal spatial variability in chlorophyll-*a* of benthic biofilms was investigated using cross-section transects along the reach section which incorporated the riffle-pool-riffle pool sequence understudy (section 5.2.4.1). To assess variability in micro-scale habitat hydraulics and its relationship to benthic biofilm chlorophyll-*a* the same 1 x 1 m quadrats referred to in section 5.2.2 were divided into twenty-five 20 x 20 cm cells and samples taken from within each cell (see section 5.2.4.2). A summary of the experimental design for meso-scale (biotope unit), micro-scale (within biotope unit) and lateral-longitudinal (transect) variability is presented in Table 5.1.

Table 5.1: Sample design for each of the three experiments under spring conditions assessing variability within and among the 4 physical biotope (B) units and transects (T) along riffle-pool-riffle-pool sequence.

Sampling unit	Diatom	Chlorophyll-*a*	Supporting data per sampling unit
Biotope unit	n = 9 per 4 B	n = 45 per 4 B	n = 45 for velocity, cobble size, depth
Quadrat cell	n/a	n = 75 per 3 B	n = 25 for shear stress
Transect	n/a	average n = 4 T*	n = 4 for shear stress and depth

*total along 40m section n = 37. n/a = diatom composition not analysed at this spatial resolution.

5.2.4.1 Meso-scale variability in benthic chlorophyll-a

Variability in chlorophyll-*a* along the reach was investigated to provide context for ISF measurements. Transects were taken across the channel over the approximately 40 m reach section at 1 m intervals. Along the transect an ISF (μg/cm^2) measurement was taken from a single cobble at 0.5 m intervals. A shear stress (N/m^2) and depth (cm) measurement was also taken, yielding n = 37 measurements per parameter; channel width was also noted.

5.2.4.2 Micro-scale variability in benthic chlorophyll-a

Within-biotope variability in chlorophyll-*a* was assessed for the same three 1 x 1 m quadrats divided into 20 x 20 cm squares that were placed along a cross channel transect at the approximate mid-point of each biotope unit. Due to sampling constraints (time and daylight), an estimation of chlorophyll-*a* variability was only obtained for riffle biotope unit 1 and 2 and the first pool unit (pool biotope unit 1). A single ISF measurement taken from each of the twenty-five 20 x 20 cm squares per quadrat, yielding a total of 75 measurements per biotope unit. A single shear stress measurement was also taken within each of the twenty-five 20 x 20 cm squares using the Fliess Wasser Stammtisch (FST)-hemisphere method (Statzner and Muller, 1989). This yielded a total of 75 measurements per biotope unit.

5.3: Results

5.3.1 Reach characterisation

Overall chemical condition was comparable among riffle and pool biotope units (Table 5.2). pH for pool biotopes in autumn were notably lower than for riffles and for both pools and riffles under spring conditions. This is attributed to pool unit 2, where a mean pH of 6.9 was observed. Furthermore, nutrient concentrations were also lower and only detected at the limit of detection (LOD) of 0.05 mg/l for TP, SRP, NO_3 and NH_4 (see Figure 5.12 for further exploration of the outlining chemical condition at pool 2 unit in relation to the benthic diatom community). Highest nutrient concentrations (TP, SRP and NO_3) were found in riffles units in the autumn sample. Riffle and pool biotope units therefore influenced in-stream chemical conditions in the autumn sampling. However, chemical condition along the riffle-pool-riffle-pool sequence appeared to be determined more by seasonal conditions than physical habitat in the spring sampling period (Table 5.2). However, light intensity was lower in riffle than pool units with maximum light intensity observed for pool unit 2 (Table 5.3). Along the riffle-pool sequence light intensity was greatest between 2 – 3.5 m across the channel.

Table 5.2: Mean and standard deviation (SD) of the physicochemical parameters measured for each biotope unit. n = 9 for each parameter in the spring and autumn sampling apart for the pool biotope unit in the autumn sampling (n = 4). Median values presented for pH.

	Riffle				**Pool**			
	autumn		**spring**		**autumn**		**spring**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
pH	8.19		8.29		7.52		8.28	
TP (mg/l)	0.04	0.00	0.02	0.01	0.02	0.02	0.03	0.00
PO_4-P (mg/l)	0.02	0.00	0.01	0.00	0.01	0.01	0.01	0.00
NO_2-N (mg/l)	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
NO_3-N (mg/l)	1.71	0.05	1.50	0.32	0.90	1.20	1.51	0.10
NH_4-N (mg/l)	0.03	0.01	0.02	0.00	0.03	0.02	0.01	0.00
DOC (mg/l)	2.63	0.01	1.66	0.11	2.05	2.19	1.64	0.05
TSS (mg/l)	7.80	7.35	2.85	0.07	2.00	1.70	3.60	0.57

Table 5.3: Light intensities measured in lux taken every 3 hours between 0900 and 1800 at 0.5 m intervals from right to left bank looking in the direction of flow across the mid-transect of each biotope unit under "spring" conditions.

	0.5m	**1m**	**1.5m**	**2m**	**2.5m**	**3m**	**3.5m**
riffle 1	348	402	589	660	313	464	442
pool 1	243	422	529	942	246	460	591
riffle 2	498	417	421	659	664	427	438
pool 2	380	269	289	470	999	799	670

5.3.2 Diatom assemblage composition of riffle and pool biotope units

Overall among riffle and pool units species richness was comparable with greater species number in riffles (S = 29) than pools (S = 26) in the autumn sampling but conversely higher, but not significant different, species number in pools (S = 30) than riffles in spring (S = 28). Total species richness concealed considerable dynamics within the diatom assemblage in terms of composition, with similar patterns of composition found to those in chapter 4. This section will focus on those common constituents of the benthic diatom assemblage (with relative abundance > 5 %) including key dominant pioneer species. A full community composition, which includes rare species (with relative abundance < 5 %), of riffle and pool biotope units across the two sampling periods is presented in appendix 1.2.

5.3.2.1 Pioneer species within assemblages of riffle and pool biotope units

Key early-successional pioneer diatom species influenced benthic assemblage composition across both riffle and pool units, similar to findings reported in the spatial-temporal studies in chapter 4. Key pioneer species included *Amphora pediculus* and *Achnanthidium minutissimum* under autumn conditions and *A. minutissimum* and *Achnanthidium biasolettiana* under spring conditions (Figure 5.3). *A. pediculus* and *A. minutissimum* collectively comprised 65% of assemblage composition in both riffle units under autumn conditions. However, their relative percentage contribution in pool units was more variable (Pool unit 1: 54%; Pool unit 2: 67%). The latter is due to colonisation ability which produces high resistance to scour and abrasion relative to other species within the diatom assemblage. Moreover, greater homogeneity in assemblage composition was observed in pool units, as demonstrated in pool unit 2, where *A. pediculus* alone occupied more than 50% of the relative assemblage contribution. This illustrated the control that a single pioneer species can have on assemblage composition.

Within the riffle-pool-riffle-pool sequence under spring condition *Achnanthidium* spp taxa dominated the species assemblage composition. *A. biasolettiana* had a higher relative abundance across both riffle and pool biotope units apart from in pool unit 1 when *A. minutissimum* displayed marginally higher relative abundance (Figure 5.3 b). Overall the influence of key pioneer species over the riffle-pool-riffle-pool sequence was greater under autumn conditions. Variability was higher in the spring composition of pool assemblages with the two dominant species collectively occupying 31% of the assemblage in pool unit 1 and 45% in pool unit 2 (Figure 5.3).

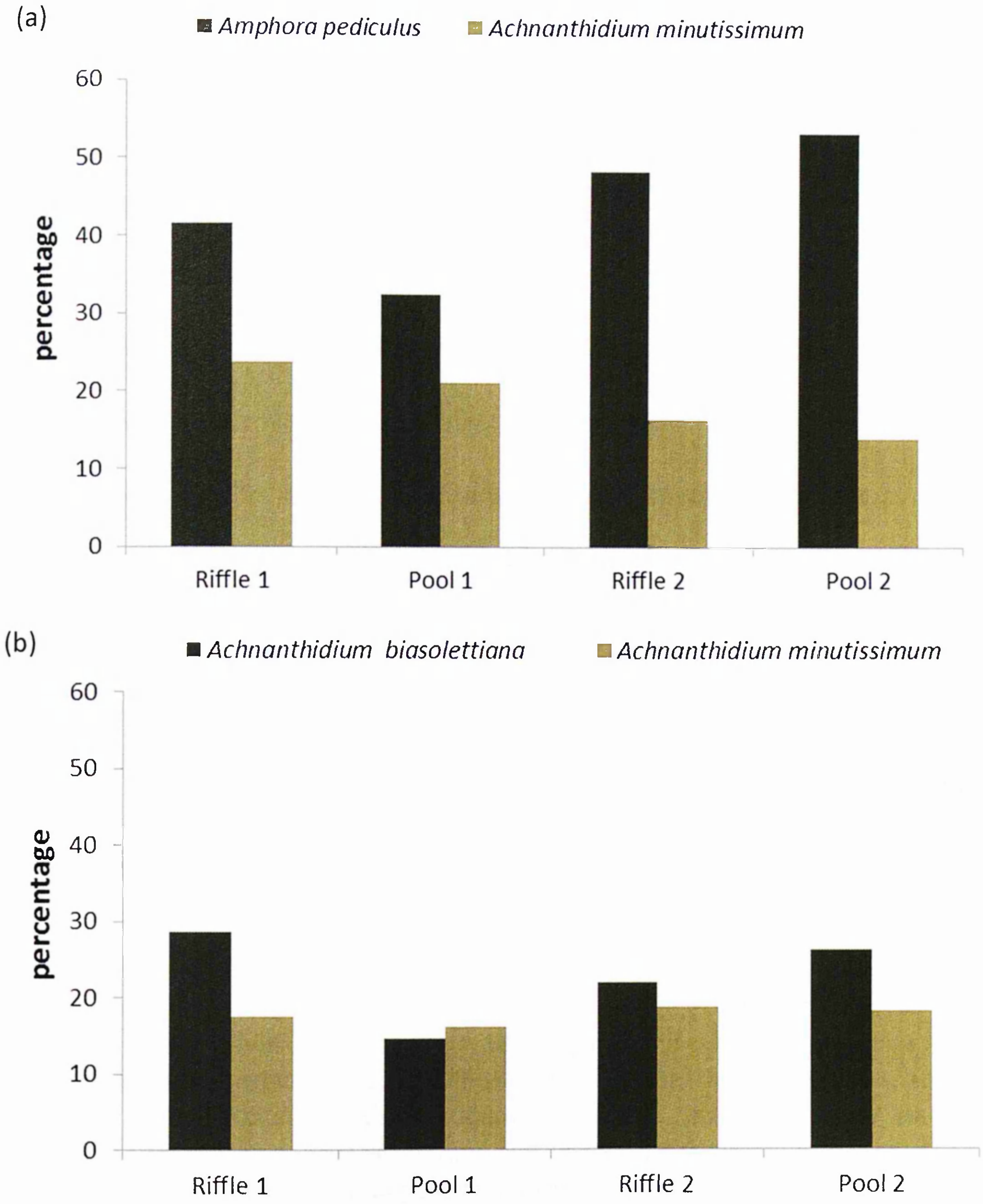


Figure 5.3: (a) Relative percentage abundance of *A. pediculus* and *A. minutissimum* along the riffle-pool-riffle-pool sequence in autumn (25/10/2011). (b) Relative percentage abundance of *A. biasolettiana* and *A. minutissimum* along the riffle-pool-riffle-pool sequence in spring (22/05/2012).

5.3.3 Diatom assemblage diversity among biotope units

5.3.3.1 Diatom assemblage diversity (richness and evenness) among riffle and pool biotope units

Mean Margalef richness index values indicated that richness was higher in pools than riffles and this pattern was repeated across season. Mean Pielou's evenness index demonstrated that the spread of individuals across species present was similar among riffle and pool biotope units and increased under spring sampling conditions (Table 5.4). However, no significant difference was observed in the richness and evenness of communities among riffle and pool biotope units (Margalef richness index; t-test: t = -1.734, df = 34, p = 0.092; Pielou's evenness index; Mann-Whitney test: U = 156, n = 18, p = 0.862).

Table 5.4: Mean and standard deviation (SD) for Margalef species richness index (n = 18) and Pielou's evenness index (n = 18) for riffle and pool biotope units across season. Margalef richness index is (S-1)/ln (n), where S is the number of taxa, and n is the number of individuals. Pielou's evenness index is Shannon-Wiener diversity divided by the logarithm of number of taxa providing a measure of the evenness with which individuals are divided among the taxa present.

		Riffle		**Pool**	
	Season	**Mean**	**SD**	**Mean**	**SD**
Margalef Richness	Autumn	2.98	0.45	3.62	0.00
	Spring	3.90	0.23	4.22	0.37
Pielou's Evenness	Autumn	0.61	0.05	0.64	0.09
	Spring	0.76	0.06	0.77	0.04

5.3.3.2 Diatom assemblage diversity (richness and evenness) within riffle and pool biotope units

Differences were observed in species richness and evenness of samples taken from within quadrats across the biotope unit between the two sampling periods (Figure 5.4). Within pool units under both autumn and spring conditions there was no significant difference in species richness (ANOVA: f = 2.88, df = 1, 26, p = 0.10) and evenness (ANOVA: f = 0.74, df = 1, 26, p = 0.39), contrasting with significant between pool differences in species richness (ANOVA: f = 10.97, df = 1, 26, p < 0.01) and evenness (ANOVA: f = 5.99, df = 1, 26, p = 0.02). For riffle units there was no significant difference in species richness within or among riffle units under autumn and spring sampling. However, species evenness did differ significantly within riffle units (ANOVA: f = 6.52, df = 1, 32, p = 0.01) but not among riffle biotope (ANOVA: f = 0.45, df = 1, 32, p = 0.10) across season (ANOVA: f = 0.23, df = 1, 32, p = 0.64). This emphasised that benthic diatom community attributes (richness and evenness) varied along the riffle-pool-riffle-pool sequence. Therefore, spatial position within biotope units from which a sample was taken is important and varied under different environmental (seasonal) conditions.

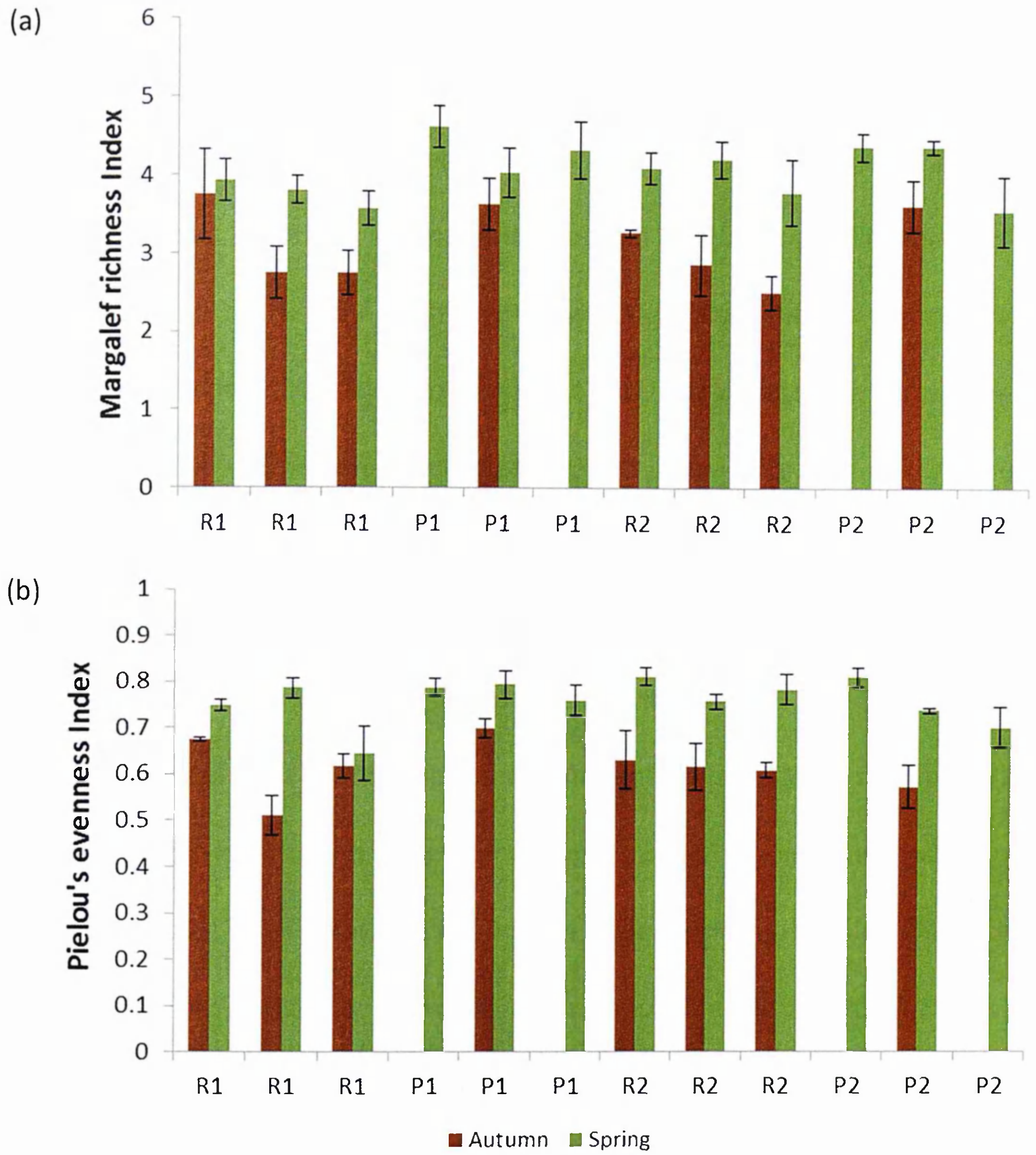


Figure 5.4: Patterns in (a) diatom richness (Margalef richness index; n = 3) and (b) evenness (Pielou's evenness index; n = 3) among replicate samples within riffle and pool biotope units under autumn (25/10/2011) and spring (22/05/2012) conditions. (R1 = riffle unit 1, R2 = riffle unit 2, P1 = pool unit 1, P2 = pool unit 2). Margalef richness index is (S-1)/ln (n), where S is the number of taxa, and n is the number of individuals. Pielou's evenness index is Shannon-Wiener diversity divided by the logarithm of number of taxa providing a measure of the evenness with which individuals are divided among the taxa present.

5.3.4 Spatial variability in diatom assemblage functional diversity among riffle and pool biotope units

Functional diversity among riffle-pool-riffle-pool sequence was explored in terms of "Ecological Guilds" (Figure 5.5; Rimet and Bouchez, 2012b). Both riffle and pool units were dominated by the low profile guild with significant differences observed in relative contribution of the low profile guild to assemblage composition between the two sampling periods (Mann-Whitney test: $U = 105.5$, $n = 24$, $p < 0.001$). This was consistent with previous findings in physiognomic patterns of diatom assemblage with high variable discharge and associated hydrological parameters such as velocity resulting in dominance of low profile species (Stevenson *et al.*, 1996; Passy, 2007b). The abundance of high profile species was higher in riffles (22%) than pools (16%) but not statistically different under spring condition (Mann-Whitney test: $U = 110$, $n = 18$, $p = 0.103$). Higher relative abundance under spring conditions illustrates the importance of temporal factors in diatoms and is explored in chapter 6. Riffles units, therefore, were more favourable for assemblages which can exploit nutrients but which are sensitive to variable discharge conditions (Rimet and Bouchez, 2012b). Different patterns in high profile species across riffle and pools biotope units also demonstrated how successional processes, three-dimensional development and nutrient acquisition of the biofilm vary spatially according to biotope. No significant difference was observed in motile species among riffle and pool biotope units (Mann-Whitney test: $U = 104.5$, $n = 18$, $p = 0.071$).

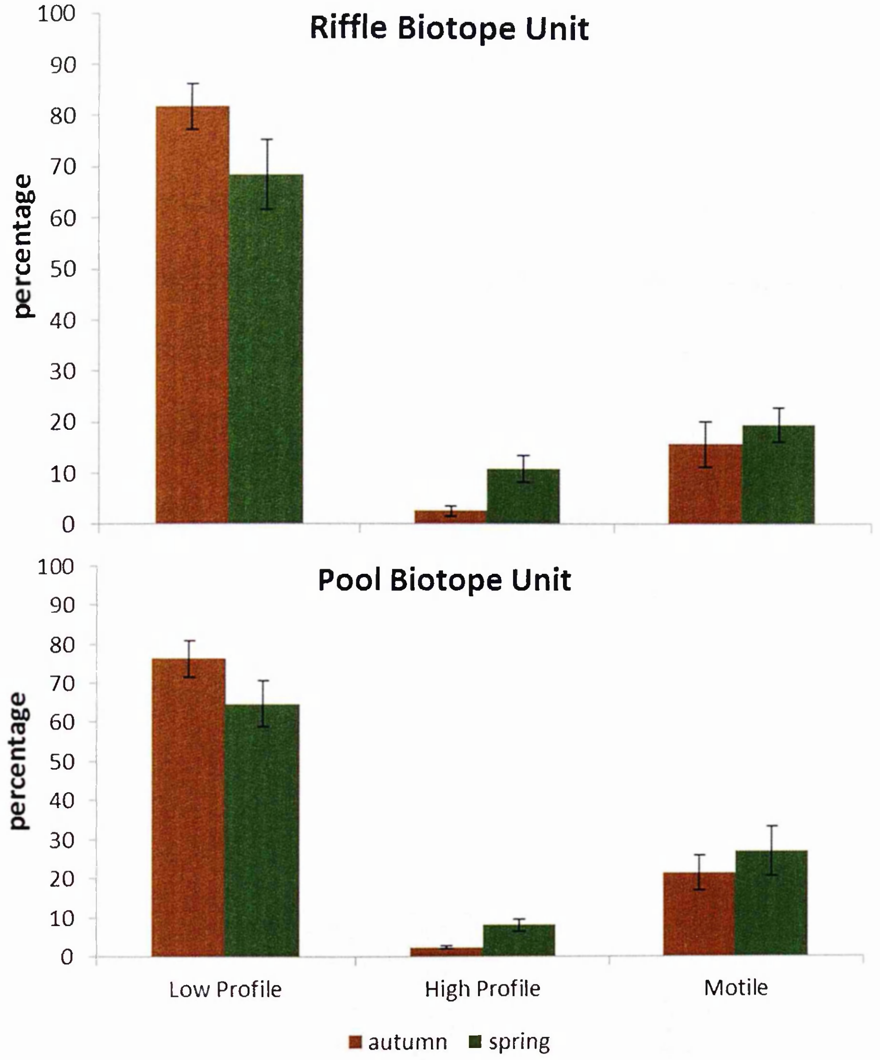


Figure 5.5: Relative abundance of ecological guilds; low profile, high profile and motile, across (a) riffle and (b) pool biotope units. (n = 18 per riffle and pool unit in the spring and riffle sample except autumn pool n = 6).

5.3.5 Chlorophyll-*a* among riffle and pool biotope units

5.3.5.1 Chlorophyll-a and spectral signatures among riffle and pool biotope units

Chlorophyll-*a* concentrations were significantly higher in riffles than pools (Mann-Whitney U = 70, n = 18, $p < 0.05$) and this pattern was repeated between sampling periods (25/10/2011 and 22/05/2012; Figure 5.6). Among biotope units, riffle unit 2 was more productive with a peak chlorophyll-*a* value of 7.11 μg/cm^2 in the spring sample. A lower peak of 6.41 μg/cm^2 was observed under autumn conditions. Within pool biotope units, chlorophyll-*a* values reached 3.99 μg/cm^2 in pool unit 2 in spring.

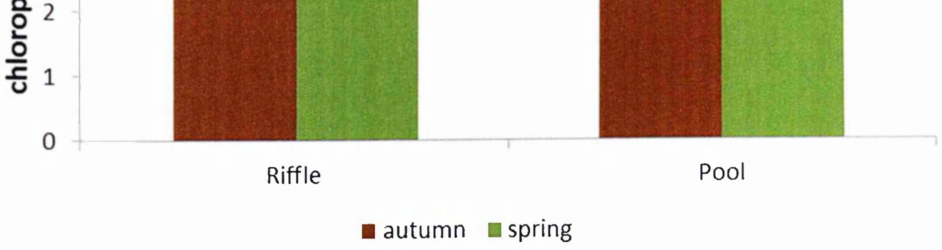


Figure 5.6: Mean chlorophyll-*a* concentration in riffle (n = 18) and pool (n = 18) biotope units under autumn (25/10/2011) and spring (22/05/2012). (n = 3 for the autumn pool sample).

5.3.5.2 Mico-scale variability in benthic chlorophyll-a and habitat hydraulics (depth, shear stress and depth) within biotope units

Variability in chlorophyll-*a* was investigated within riffle unit 1, riffle unit 2 and pool unit 1 under spring conditions (Figure 5.7). Highest mean chlorophyll-*a* concentration of 6.19 µg/cm^2 was observed in riffle unit 1, followed by riffle unit 2 (4.96 µg/cm^2) and pool unit 1 (1.63 µg/cm^2). Riffle biotope units also demonstrated greater heterogeneity in chlorophyll-*a* than pool biotope units (Range: Riffle unit 1 = 0 – 14.96 µg/cm^2; Pool unit 1: 0.34 – 6.12 µg/cm^2; Riffle unit 2: 0 – 13.29 µg/cm^2). Highest chlorophyll-*a* concentrations were observed between mid-reach and the left bank in riffle biotope units but for the pool biotope unit, highest concentrations were observed towards the right bank (Figure 5.7). Therefore, it is important to consider spatial resolution at micro-scales in experimental and sampling designs to capture the variability in chlorophyll-*a* concentration within riffle and pool biotope units.

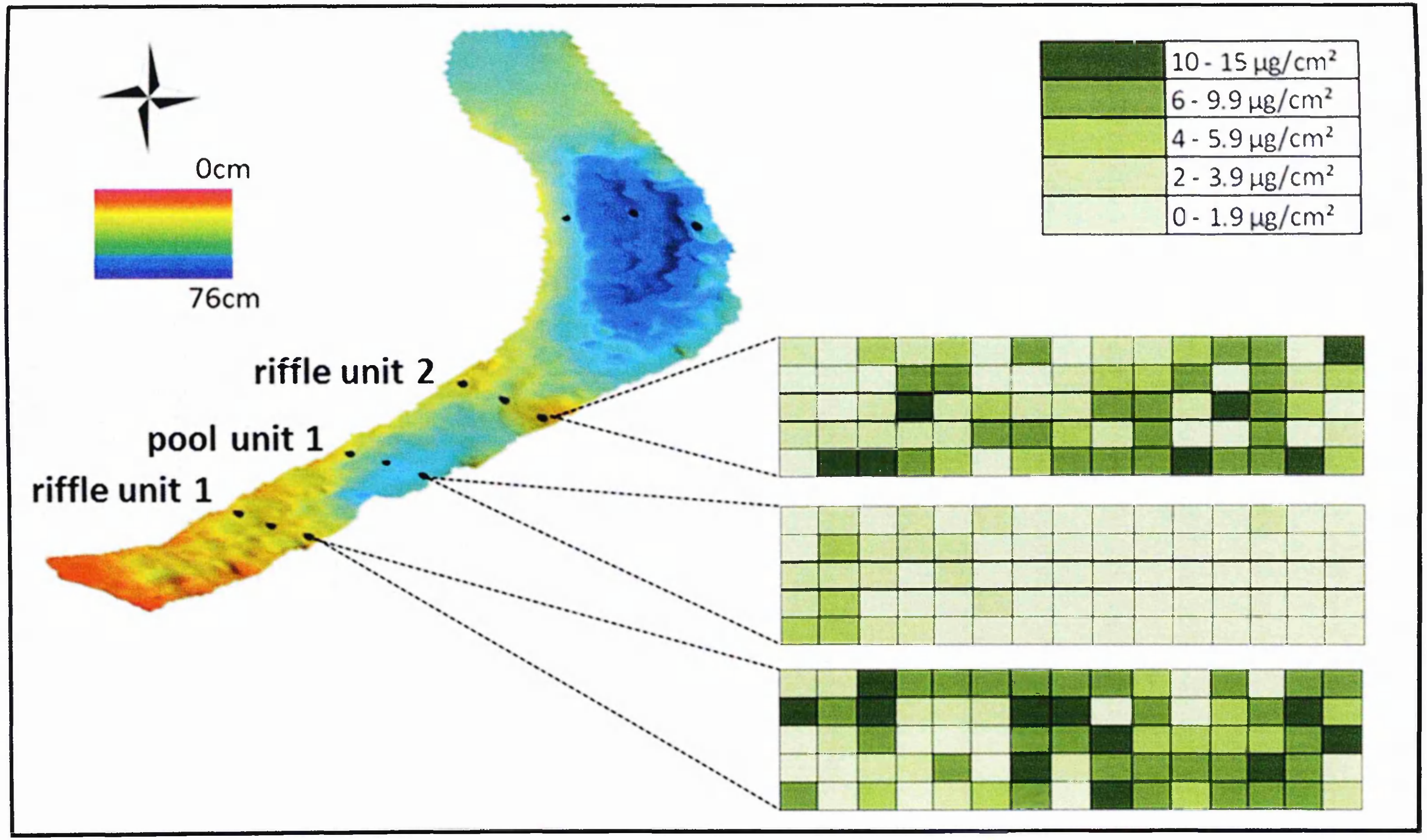


Figure 5.7: Reach characterisation of stream bed elevation using a DEM illustrating differences in depth along the riffle-pool-riffle-pool sequence. Differences in chlorophyll-*a* concentration, as detected within each cell (n = 25) in the three 1 x 1 m quadrats, yielding n = 75 measurements per biotope unit; riffle unit 1, riffle unit 2 and pool unit 1.

Figures 5.8 demonstrates how chlorophyll-*a* varied according to biotope properties of depth, velocity and shear stress. Significant differences in depth were observed between riffle and pool biotope units ($t = -12.31$, $df = 170$, $p < 0.05$) with lower depth (mean riffles = 10.43 cm; pools = 29.61 cm) and higher chlorophyll-*a* associated within riffle biotope units (mean riffle = 4.99 $\mu g/cm^2$; mean pool = 2.90 $\mu g/cm^2$). Figure 5.8 (a) demonstrated variability in shear stress among riffle units which was generally below 1 M/m^3. Near bed velocity was also higher in riffle than pool biotope units (Figure 5.8, b). However, only chlorophyll-*a* and both depth ($r = 0.22$, $n = 90$, $p < 0.05$,) and shear stress ($r = 0.31$, $n = 90$, $p < 0.05$) were significantly correlated for riffle biotope units. Therefore, this suggests that key drivers of chlorophyll-*a* are biotope defined hydro-morphological variables of shear stress and depth. Lower water depth and higher shear stress in riffle compared to pools is likely to contribute to higher riffle chlorophyll-*a* concentrations through greater sloughing of the benthic biofilm and recolonization by pioneer species which characteristically have higher rates of reproduction. However, differences in velocity among riffle and pool biotopes are likely to be important factors in chlorophyll-*a* determination (Figure 5.8). Higher velocity in riffle units can potentially have a stimulatory effect on the diatom assemblage through facilitation of diffusion of molecules into the biofilm (nitrogen and phosphorus for example) by reduction of stationary layer (Biggs *et al.*, 1998a). This was observed in particular in riffle unit 2 where higher velocities lead to higher chlorophyll-*a* concentrations (Figure 5.8 b, c). Furthermore, velocity, together with shear stress, was also likely to be an important factor in determining loss from the biofilm through mechanisms such as sloughing thickness (Steinman and McIntire, 1990; Lau and Liu, 1993). Hence, hydro-physical habitat heterogeneity and changes therein, as defined by biotope units, are likely to have significant impact on primary production through influencing factors that control diatom growth and removal.

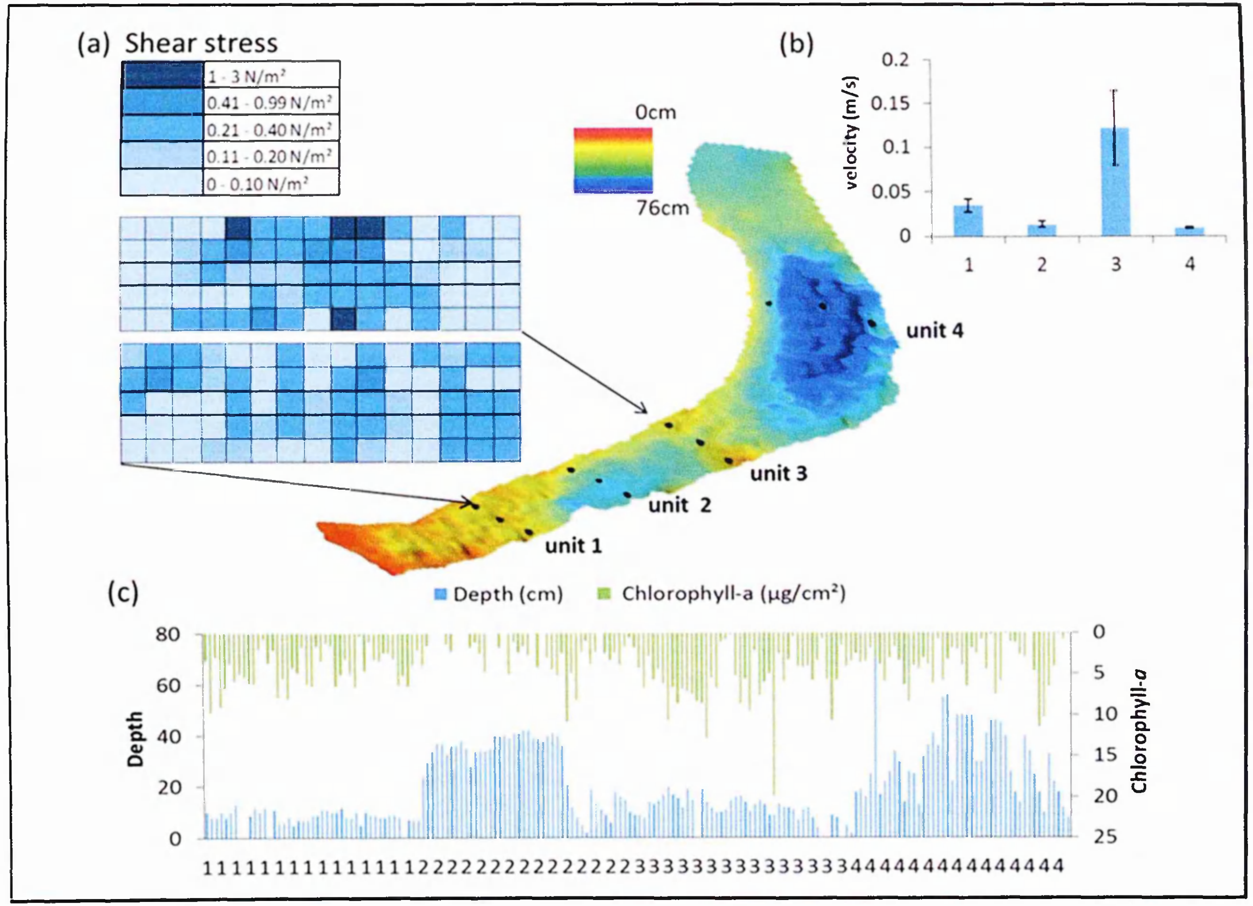


Figure 5.8: Reach characterisation of stream bed elevation using a DEM to illustrate differences in depth along the riffle-pool-riffle-pool sequence. (a) Differences in shear stress, as detected within each cell (n = 25) in the three 1 x 1 m quadrats with the riffle units. The FST-hemisphere did not detect shear stress in pool biotope units; (b) difference in velocity among biotope units (n = 45 per biotope unit); (c) relationship between variability in chlorophyll-*a* and depth (n = 34 per biotope unit). 1 = riffle unit 1; 2 = pool unit 1; 3 = riffle unit 2; 4 = pool unit 2.

5.3.5.3 Chlorophyll-a concentrations along reach transects

Lateral and longitudinal variability was observed in chlorophyll-*a* along the riffle-pool sequence from the footbridge to the bend in the stream (see Figure 5.1 c). Longitudinally along the riffle-pool sequence chlorophyll-*a* values varied from 1 – 7.01 µg/cm^2, while across the channel values varied from 0.04 to 10.43 µg/cm^2 (Figure 5.9). Lateral variability in mean chlorophyll-*a* was highest 1 m from the right bank (3.73 µg/cm^2) followed by 1.5 m (3.55 µg/cm^2), 2 m (3.44 µg/cm^2) and 0.5 m (3 µg/cm^2). Therefore, chlorophyll-*a* varied more along the reach section rather than across the channel and this is likely confined by biotope properties with highest chlorophyll-*a* approached in the vicinity of pool biotope unit 2. However, this does demonstrate concordance with patterns found along the riffle-pool sequence with higher chlorophyll-*a* found at 5 – 10 m from in the footbridge where riffle biotope unit 1 was located. Conversely, lowest chlorophyll-*a* concentrations were found in the deeper the pool unit 2 located approximately 35 m from the footbridge.

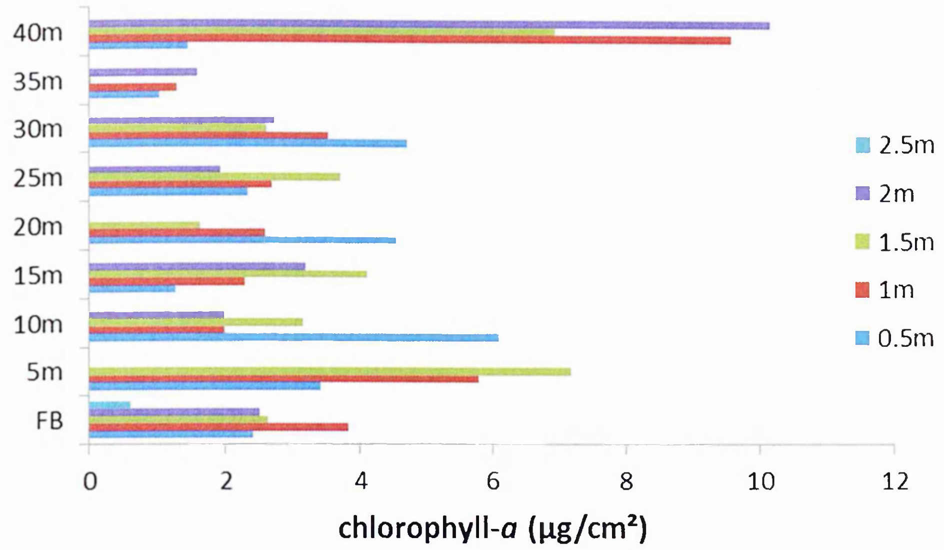


Figure 5.9: Lateral and longitudinal variability in chlorophyll-*a* concentration at intervals of 5 m along the reach commencing at the footbridge (FB; see Figure 5.1). At each 5 m interval, a chlorophyll-*a* measurements were taken at 0.5 m intervals across a channel transect.

5.3.6 Relationship between diatom assemblage diversity and its chlorophyll-*a* production

No significant relationship was found between Shannon-Wiener diversity and chlorophyll-*a* among riffle and pool biotope units (Figure 5.10). Overall, riffle units were more productive under both spring and autumn conditions, with highest diversity (2.53) observed in pool biotope unit 1 under spring conditions.

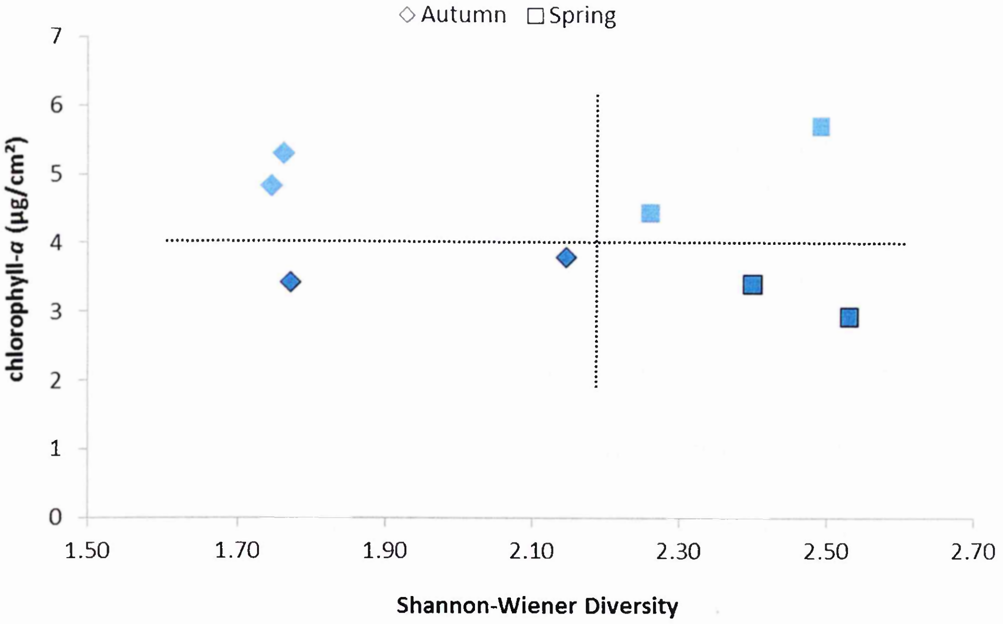


Figure 5.10: The relationship between Shannon-Wiener diversity and chlorophyll-*a* among riffle (light blue) and pool (dark blue) biotope units. (Horizontal and vertical lines provided to illustrate division between riffle and pool biotope units across spring and autumn).

5.3.6.1 Importance of assemblage composition and species biovolume in terming chlorophyll-a production

For both riffle and pool biotope units in the autumn sample, more than 75% of total assemblage biovolume was controlled by three diatom species. For riffle units, *Denticula tenuis, A. pediculus* and *C. placentula var. euglypta* contributed 50, 21 and 13% respectively to total assemblage biovolume but 1%, 45% and 7% respectively to mean percentage relative abundance. Similarly for pool biotope units both *Denticula tenuis* and *A. pediculus* contributed 61% and 15% respectively to total assemblage biovolume but 2 and 43% respectively to mean percentage relative abundance. Note, *A. pediculus* together with *A. minutissimum* contributed more than 50% to diatom assemblage abundance among both riffle and pool biotope units. Under autumn conditions no significant relationship was found between Shannon-Wiener diversity, biovolume and chlorophyll-*a* (Table 5.4).

Within pool biotope units in the spring sample a significant relationship was found between Shannon-Wiener diversity, biovolume and chlorophyll-*a* (Table 5.4). In pool biotope units *Surirella brebissimonii* along with *C. placentula var. euglypta* contributed significantly to total cell biovolume (16 and 13% respectively), while *A. pediculus, A. minutissimum* and *A. biasolettiana* comprised approximately 50% of the relative assemblage abundance. Similar to pool biotope units, under spring conditions, *A. pediculus, A. minutissimum* and *A. biasolettiana* comprised more than 50% of the relative assemblage abundance in the riffle biotope units. *C. placentula var. euglypta* and *Rhoicosphenia abbreviata* contributed 15 and 13% respectively to total assemblage biovolume. No one taxa contributed more than 15% to total assemblage biovolume, contributing to a significant relationship between Shannon-Wiener diversity and biovolume ($r = 0.84$, $n = 18$, $p < 0.05$). This demonstrates that, while some species may dominate assemblage composition, species with only a relative minor contribution to assemblage composition can contribute to a large component of total assemblage biovolume. This provides evidence of the importance of rare species within benthic diatom biofilms for understanding diatom productivity which varies according to biotope under seasonal condition.

Table 5.5: Pearson correlation coefficient between Shannon-Wiener diversity (n = 18), chlorophyll-*a* (n = 18) and cell biovolume (n = 18) for riffle and pool biotope units across season. Significant correlations ($p < 0.05$) are highlighted in red. Chlorophyll-*a* is based on an ISF based chlorophyll-a measurement. Cell biovolume is calculated following Rimet and Bouchez, 2012.

		Chlorophyll-*a*	**Biovolume**
Riffle Spring 2012	Biovolume	-0.227	1
	Shannon Wiener	0.142	0.84
Riffle Autumn 2011	Biovolume	-0.142	1
	Shannon Wiener	-0.168	0.207
Pool Spring 2012	Biovolume	-0.58	1
	Shannon Wiener	-0.616	0.809
Pool Autumn 2011	Biovolume	0.004	1
	Shannon Wiener	-0.735	0.597

5.3.7 Physical determinants: diatom assemblage diversity and chlorophyll-*a*

Diatom assemblages of samples taken within biotope units were distinct (Figure 5.11 a); however, when benthic diatom assemblages were aggregated to the riffle and pool scale, lower stress values indicated season had a greater influence on assemblage similarity than riffle and pool units (Figure 5.11 b). Pool unit 2, however, demonstrated a distinct community irrespective of biotope and season which may be associated with the chemical characteristics of this relatively deeper pool unit (Figure 5.11 b; see section 5.3.1). Differences in diatom assemblage composition among riffle and pool units, as observed in Figure 5.11, were explored in terms of hypothesised key physical parameters confined by biotope. The control of velocity, cobble size and depth on attributes of diatom assemblage composition (Shannon-Wiener Diversity and ISF chlorophyll-*a*) were investigated using PCA (Figure 5.12). Note, despite outliners in chemistry found within pool unit 2 (see Table 5.1) the PCA was based on the full dataset due to distinct community observed in pool unit 2 during autumn sampling (Figure 5.11 b). The strongest distinction in the dataset (PCA axis 1, eigenvalue = 0.35) was provided by depth (factor loading = 1.38) and Shannon-Wiener diversity (factor loading = 1.32). In particular, PCA 1 distinguished riffle and pool biotope units. This suggested that depth, was a key structuring variable which contributed to greater diversity in pool units. Depth is an important co-variable with other parameters impacting upon diatom assemblage composition, which were not investigated in detail within this thesis (i.e. light). Riffle units were best discerned by PCA axis 2 (eigenvalue = 0.22). This component primarily comprised of variations in ISF (factor loading = 1.10), cobble size (factor loading = 1.16) and velocity (factor loading = -0.82). Therefore, deeper pool biotope units were more diverse while velocity and cobble size within riffle units were important determinants of chlorophyll-*a*. This illustrates that physical biotope attributes (velocity, depth and substrate) provide strong distinction between diatom assemblage diversity and chlorophyll-*a* concentration.

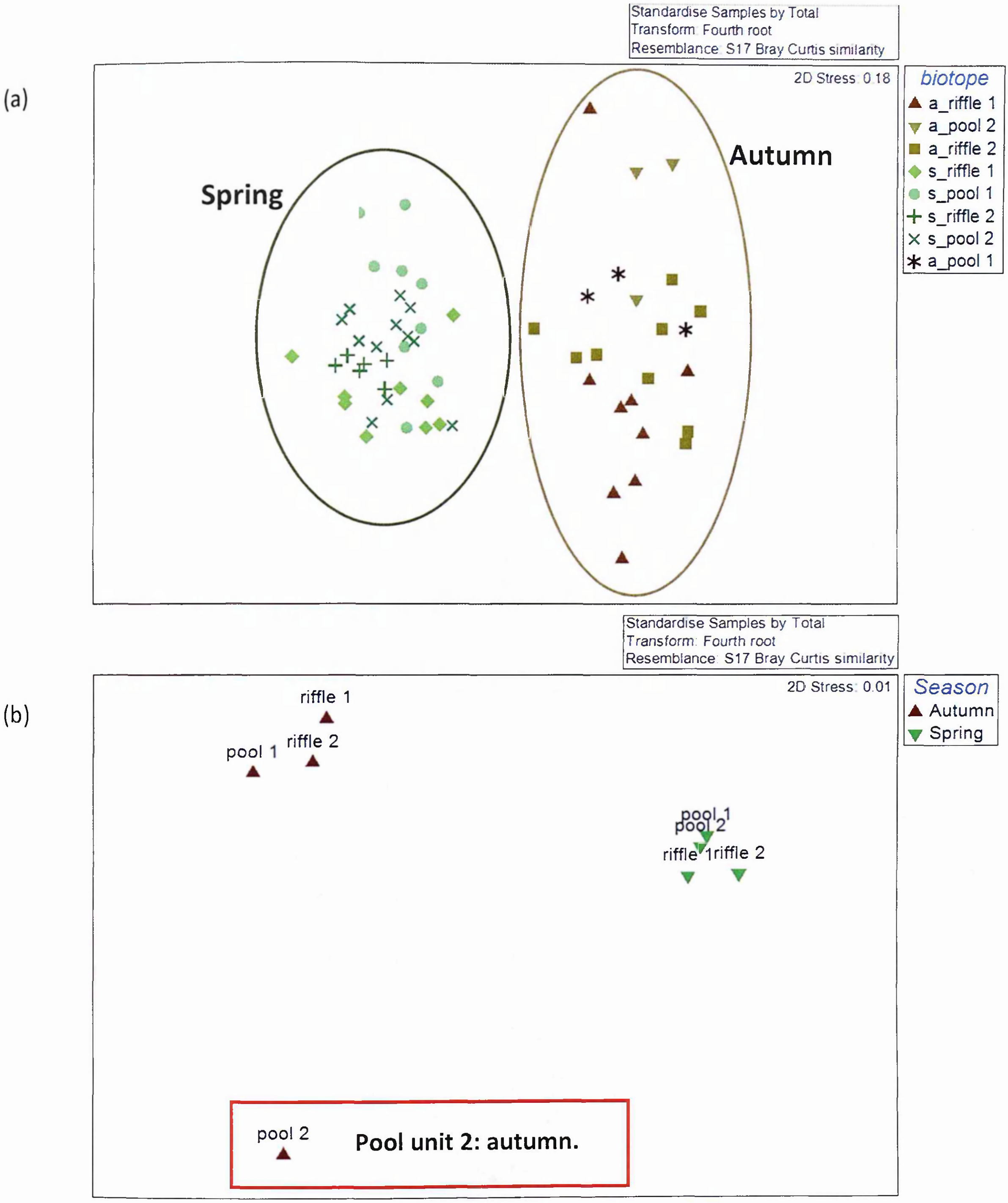


Figure 5.11: (a) NMDS of benthic diatom assemblages among riffle and pools in biotope units in autumn (n = 18 for riffle biotope units and n = 3 for pool biotope unit 1 and 2) and spring (n = 18 per riffle and pool biotope); (b) community assemblage similarly when aggregated to riffle and pool scale, demonstrating the diatom community at pool unit 2 under autumn conditions as an outlier.

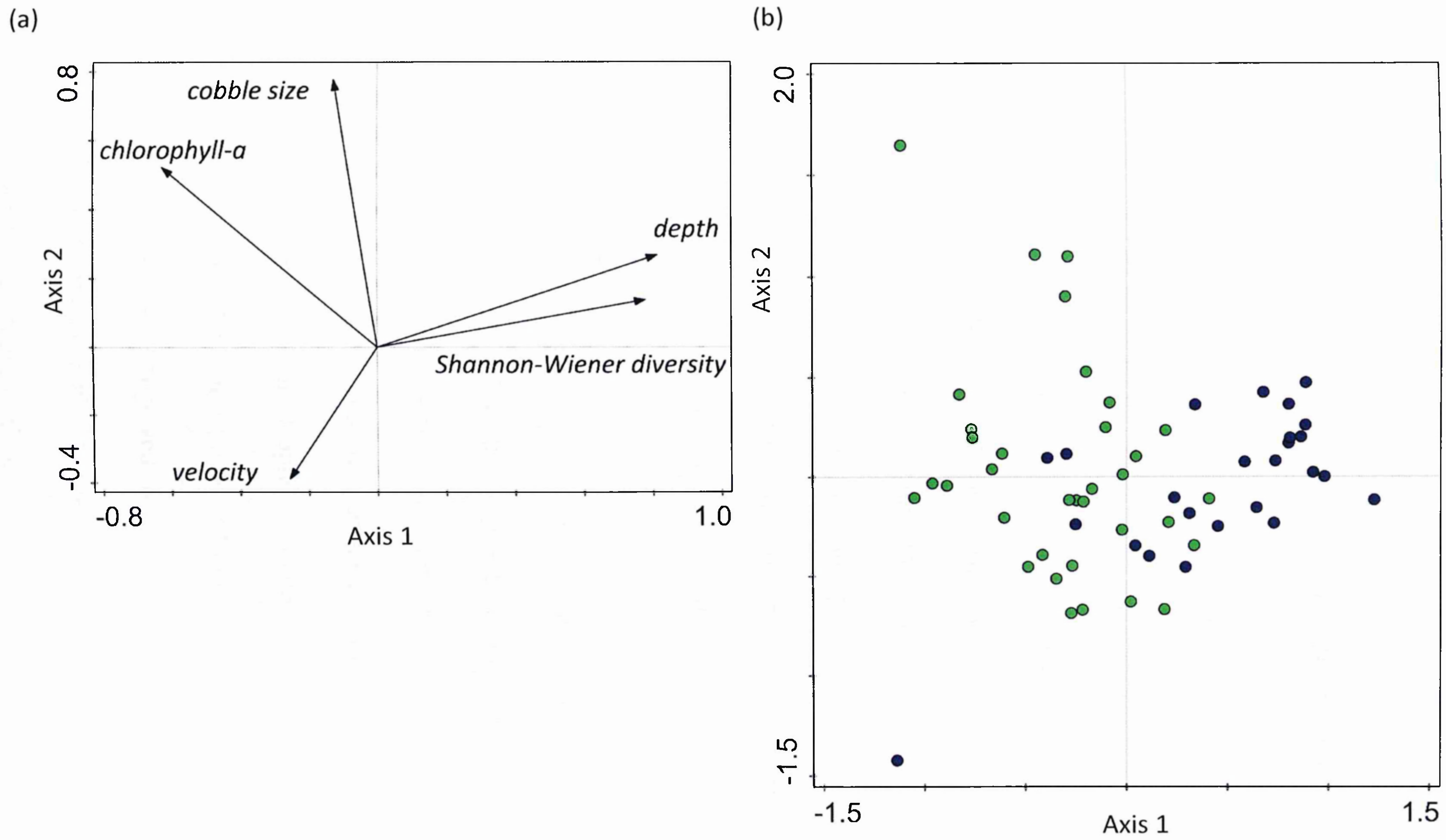


Figure 5.12: (a) PCA of key physical determinants (cobble size, depth, velocity; n = 60) on diatom assemblage diversity and chlorophyll-*a* across riffle and pool biotope units; (b) PCA of diatom community assemblage according to riffle (green) and pool (blue) biotope units.

5.3.8 Diatoms as indicators of environmental status through the TDI and EQR metrics under the Water Framework Directive (2000/60/EC)

5.3.8.1 Pattern in TDI metric score across riffle-pool-riffle-pool sequence

Trophic conditions, inferred from the TDI metric, were more variable in pool than riffle units (Figure 5.13). The greatest range in TDI was observed within pool units under autumn conditions (P1 = 65.6, P2 = 80.4). Under spring sampling conditions, it was pool unit 1 which had the highest mean TDI value (57.1). Mean TDI values between riffle and pools units were comparable between the two sampling periods with an average of 60.2 and 63 for riffle and pool units respectively. The small variation in mean TDI within riffle biotope units suggests these were more reliable in terms of reproducibility in estimates of trophic status.

Variability in TDI within biotope units was observed among samples taken from the cells of the three 1 x 1 m quadrats (see Figure 5.2). For example, within riffle unit 1 under autumn sampling conditions TDI values ranged from 37.2 to 82.5. This illustrates how samples taken within a single biotope can indicate trophic status ranging from "Good" to "Poor" trophic condition. The range of TDI values from samples taken within riffle unit 2 under autumn conditions was less (67.8 – 85.3). Greater variability was also observed in riffle unit 1 (33.7 – 57) than riffle unit 2 (42.3 - 55.7) under spring conditions. Therefore, greater variability was observed in riffle unit 1 than 2, irrespective of time or seasonal condition. Variability in TDI within pool biotope units under spring conditions also spanned from "High" to "Poor" trophic status. This was particularly evident in pool unit 1 (45.25 – 71.82) compared to pool unit 2 (36 – 58.58). Consideration of the position from which a sample is taken within biotope units is therefore an important consideration for assessment of trophic condition. Across both riffle and pool biotope unit's mid-reach conditions were most similar to mean trophic condition under spring conditions (e.g. riffle units mid-reach mean TDI: 47.58, overall mean TDI: 47.91; pool units mid-reach mean TDI: 53.01, overall mean TDI: 53.20). This suggests that variability in trophic status, mediated through differences in diatom assemblage composition, was best captured within samples taken from mid-reach and supports current sampling guidelines for routine assessments of water quality (Kelly *et al.*, 1998b).

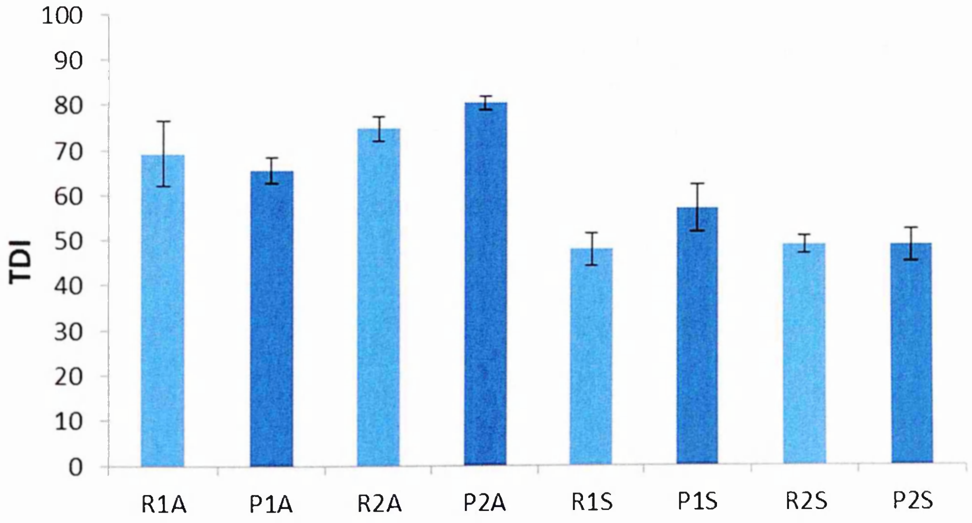


Figure 5.13: TDI values among riffle and pool biotope units across season (R1A = riffle 1 Autumn, P1A = pool 1 Autumn, R2A = riffle 2 Autumn, P2A = pool 2 Autumn, R1S = riffle 1 Spring, P1S = pool 1 Spring, R2S = riffle 2 Spring, P2S = pool 2 Spring; n = 9, for each biotope unit except for P1A and P2A where n = 3). TDI values indicate trophic condition based on diatom phosphorus sensitivity. Values are expressed between 0 and 100 with 100 indicating eutrophic condition

5.3.8.2 Diatom EQR values among riffle units

Mean EQR values remained constant among riffle units in spring (riffle unit 1: 0.82; riffle unit 2: 0.81), indicating "Good" ecological quality status across both riffle biotope units (Figure 5.14). For pool biotope units, contrary to riffle units, greater variability in EQR was observed within biotope units in spring than autumn. Mean EQR was higher in pool unit 2 compared to pool unit 1 (Pool 1: 0.68; Pool 2: 0.81) indicating "Moderate and "Good" conditions respectively (Figure 5.14). Median EQR among riffle and pool units did not differ significantly in the spring sample (Mann-Whitney Rank Sum test: $U = 125.5$, $n = 18$, $p > 0.05$). However, it was found that despite the observed variability in riffle and pool units, samples taken from mid-reach were the best approximate of within reach variability confined by biotope unit (e.g. riffle unit 1 in the autumn sample: mid-reach mean EQR: 0.57, overall mean EQR: 0.51; riffle unit 1 in the spring sample: mid-reach mean 0.83, overall mean 0.82) meaning overall riffle units provided a better indication of ecological status.

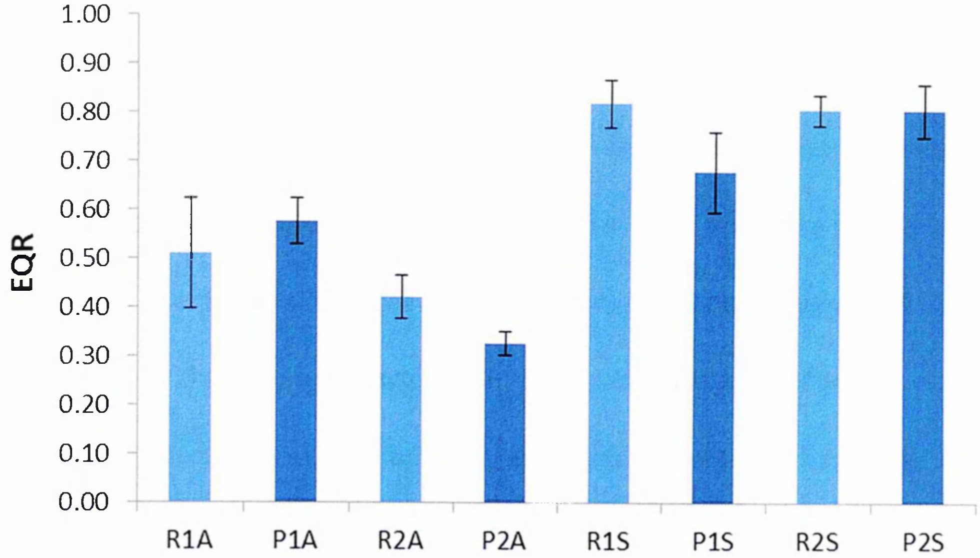


Figure 5.14: EQR values among riffle biotope units across season (R1A = riffle 1 Autumn, R1S = riffle 1 Spring, R2A = riffle 2 Autumn, R2S = riffle 2 Spring; n = 9, for each biotope unit except for P1A and P2A where n = 3). Class boundaries: High = 1.00, Good = 0.75, Moderate = 0.50, Poor = 0.25 and Bad = < 0.25).

5.3.9 Conclusion

Pool biotope units were more diverse in terms of diatom assemblage composition with both species richness and diversity higher under spring conditions. Riffle biotope units were more productive and demonstrated greater variability in chlorophyll-*a* concentrations than pools. Fundamental characteristics of the diatom assemblage, as demonstrated through ecological guilds, were related through biotope specific properties. The importance of hydro-physical complexity within and among biotope units was demonstrated through the spatial differences found in diatom assemblage productivity associated with depth and shear stress. Moreover, this was also emphasised through estimates of both trophic and ecological status which demonstrated that position within the biotope did matter and that mid-reach section of riffle biotope units best represented variability in trophic and ecological status. The implications of this are that while significant spatial differences were observed, temporal differences shown by the two sampling intervals and as presented in the previous chapter (see Chapter 4), were more important. Therefore, the following chapter will investigate fine-scale temporal changes in diatom assemblage diversity and chlorophyll-*a* and their hypothesised relation to event-based threshold changes in velocity and nutrient concentrations.

Chapter 6: Reach-scale temporal dynamics in benthic diatom assemblages

6.1 Introduction

Disturbance driven by fluctuations in discharge is an important determinant of benthic diatom diversity and productivity in headwater streams (Sousa, 1984; Resh *et al.*, 1988; Biggs and Close, 1989; Bunn and Arthington, 2002). As a result, ecological succession in headwater diatom communities is important but frequently truncated due to the dynamic flow environment. This leads to the benthic diatom community being predominantly pioneer in character (Biggs *et al.*, 1998b). Distinctive characteristics of the benthic community are rapid colonisation and growth rates, thus making accrual and loss through sloughing the dominant processes (Bouletreau *et al.*, 2006). Habitat characteristics, species assemblage composition and persistence of residues that withstand one disturbance event, can all contribute to the recovery of successional trajectories of diatom communities (Stevenson, 1990; Soininen and Eloranta, 2004). However, the influence of discharge disturbance of variable intensity, and especially antecedent discharge-nutrient conditions, on assemblage composition and chlorophyll-*a* is poorly understood.

A key intermediary influence between flow dynamics and the benthic assemblage composition and chlorophyll-*a* concentration is velocity and associated shear stress (Biggs *et al.*, 1998a; Biggs *et al.*, 2005). Benthic biofilms comprised of a dense mat of low profile species with adnate attachment may be resistant to dislodgement by shear stress and diffusion of nutrients through the biofilm (Douglas, 1958; Rounick and Gregory, 1981; Peterson and Stevenson, 1990; Biggs *et al.*, 1998a; Passy, 2007b). Increases in velocity during disturbance events may positively affect such diatom communities by enhancing biomass accrual through increasing rates of mass transfer without increased losses through scour and abrasion (Stevenson, 1990; Stevenson, 1996; Biggs *et al.*, 1998a). Fluctuations in discharge contribute to variable short-term changes in nutrient concentrations (Johnson *et al.*, 1969b; Hinton *et al.*, 1997). The diatom matrix composed of high profile species, and facilitated by high rates of diffusion into the biofilm at low velocity, can exploit these available nutrients (Biggs *et al.*, 1998a; Passy, 2007b). Benthic diatom assemblages which consist of both low and high profile species have a more open matrix. Consequently, such three-dimensional architecture of biofilms is more sensitive to increases in velocity as high skin friction and drag contributes to higher rates of sloughing (Biggs *et al.*, 1998a; Hart and Finelli, 1999; Passy, 2007b). Understanding the interactive effects of discharge, and associated changes in hydraulic conditions and nutrient supply, on diatom community composition and productivity is essential to determine if the effects of frequent, stochastic disturbance on headwater benthic diatom assemblages.

Temporal and spatial heterogeneity in reach-scale composition and productivity of benthic algal communities can be explained by the interactions between the timing of disturbance, successional state in taxonomic composition of benthic assemblage and physical biotope (Peterson and Stevenson, 1990; Newson and Newson, 2000; Sekar *et al.*, 2004). This aspect of work in the River Eden aims to better understand how reach-scale temporal dynamics in stream discharge, velocity, shear stress and nutrient concentration (TP and NO_3) may determine benthic composition and chlorophyll-*a* production in riffle biotope units over a short time period (28 days). More specifically, the experiment design outlined below tests the hypotheses that (1) there is a threshold relationship between change in benthic diatom composition and chlorophyll-*a* production, and (2) that benthic biofilm composition changes as a result of discharge, velocity and nutrient pulses (TP and NO_3).

To address these hypotheses this chapter will first characterise the physical and chemical attributes of the reach. Species composition, structural biodiversity and chlorophyll-*a* production of diatom communities on artificial substrate from each riffle will then be presented and compared to cobble substrate to provide an estimate of the representativeness of artificial substrate to streambed substrate. Function diversity of these assemblages is then investigated through life-form and relation to environmental conditions is explored. Key relationships between benthic diatom diversity and chlorophyll-*a* will then be presented in the context of velocity and nutrient condition (TP and NO_3), parameters proposed as key determinants of assemblage diversity and chlorophyll-*a*. The chapter will conclude with the importance of considering reach-scale temporal variability in discharge for diatom assessment under WFD classification.

6.2 Experimental Design

Benthic diatoms were sampled from three riffle zones within a 50 m reach section of Newby Beck (Morland outlet; Figure 6.1) to investigate the impact of discharge-nutrient relationships on benthic diatom assemblages under meso-trophic condition within headwater grassland systems (Owen *et al.*, 2012).

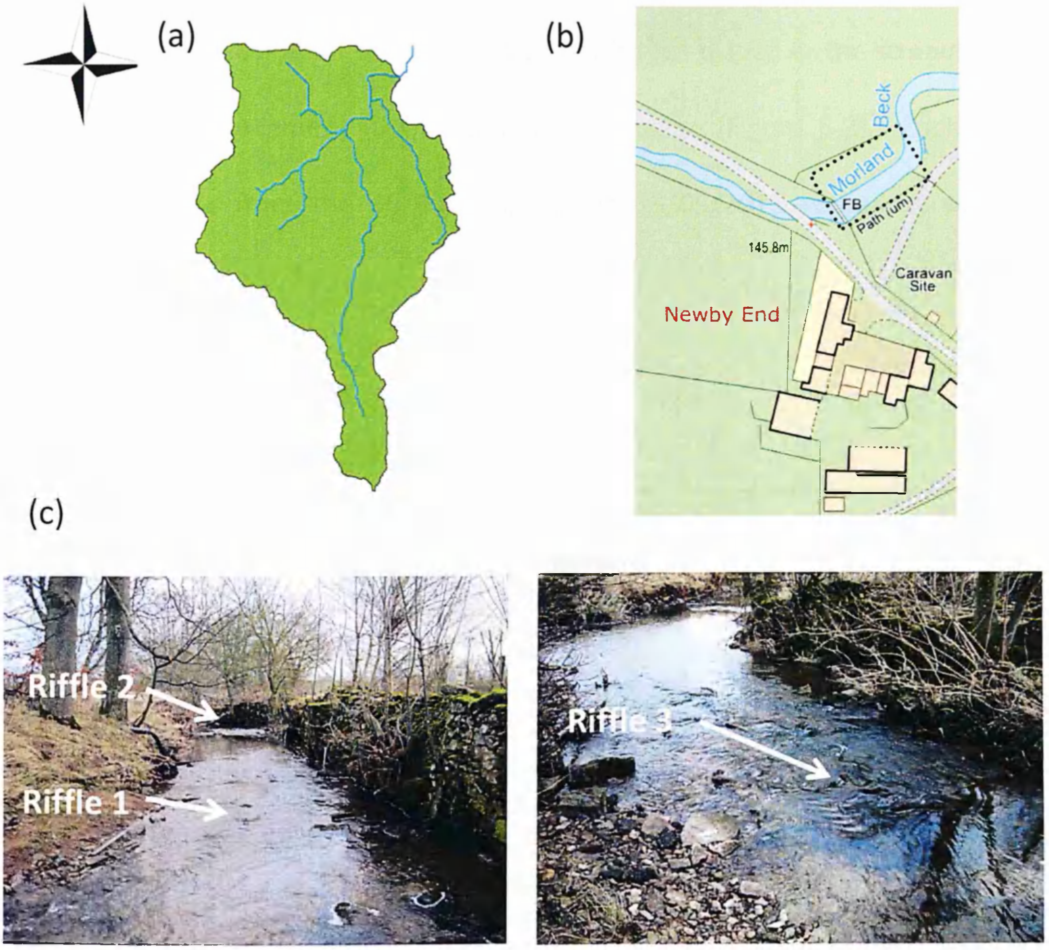


Figure 6.1: Newby Beck in Morland Catchment; (a) Morland 10km^2 sub-catchment; (b) Newby Beck reach section studied (dotted rectangle); and (c) 3 riffle units sampled.

Three glazed clay tiles 30 x 60 cm were placed on the stream bed across each of the three riffle units. These were attached to an upper wooden frame via cable ties. To minimise effects of nutrient release from the artificial substrate, the tiles were exposed to 0.5 M HCl for 24 hours followed by deionised water for 48 hours (Niederhauser and Schanz, 1993). Diatom community composition and chlorophyll-*a* field sampling occurred at a minimum of every 72 hours (16th April, 19th April, 21st April, 22nd April, 25th April, 26th April, 28th April, 30th April, 1st May, 3rd May, 4th May, 5th May, 6th May, 7th May, 10th May, 11th May, 12th May and 13th May) over a four week period commencing on the 16/04/2013, with tiles placed in the stream 7 days prior to the commencement of the sampling campaign (Figure 6.2). Each of the dates listed above from the 16/04/2013 to the 13/05/2013 represent sampling events 1-18 respectfully.

Figure 6.2: (a) Newby Beck reach section investigated; (b) tile installation 7 days prior to the commencement of the sampling campaign; and (c) biofilm development after 20 days.

On each sampling occasion tiles were sequentially removed from each of the riffle zones and a single ISF chlorophyll-*a* measurement and diatom composition sample taken (see chapter 3) from a single 3 x 3 cm area. Figure 6.3 demonstrates the position on the tile that each sample was taken from with numbers 1 - 18 corresponding to the 18 sampling events respectively. In addition to this single ISF chlorophyll-*a* measurement and diatom composition sample, a further three samples were taken from the same 3 x 3 cm areas positioned across each tile on each sampling of the 18 sampling events to account for variability and edge effects (Figure 6.3; red squares). Tiles were returned to the same position as collected by gently gliding them back to the bed downstream to minimise sudden exposure to current. For control purposes, on the 22/04/2013, 28/04/2013, 03/05/2013, 04/05/2013 and 10/05/2013 an ISF measurement was taken from three representative cobbles per riffle unit, and subsequently scraped for diatom analysis and processed as per section 3.3.

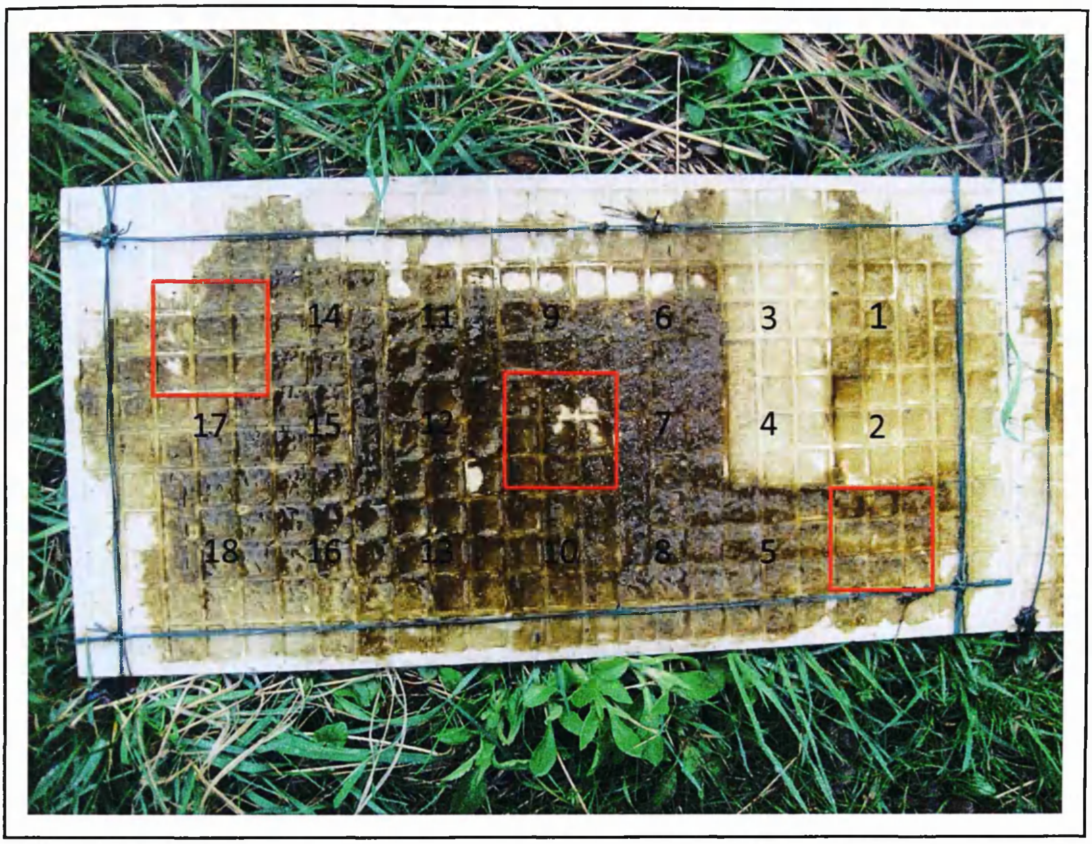


Figure 6.3: Artificial tile substrate, 30 x 60 cm, with 3 x 3 cm areas for ISF chlorophyll-*a* and diatom composition analysis represented by numbers 1-18. Each number 1-18 equates to sampling events 1-18. The 3 x 3 cm red squares represent the repeated ISF analysis on each sampling occasion in addition to individual analysis on positions 1-18 on particular sampling events.

6.3 Results

6.3.1 Reach Characterisation

Hourly mean rainfall and associated discharge-nutrient conditions, based on 15 minute data, from 16/04/2013 to 13/05/2013 are presented in Figure 6.4. Rainfall reached maximum of 3.61 mm/h on the 18/04/2013 at 0200. This increase in rainfall was reflected in discharge, turbidity, TP and NO_3. There was strong significant relationship between discharge and both turbidity ($r = 0.91$, $n = 673$, $p < 0.01$) and TP ($r = 0.83$, $n = 673$, $p < 0.01$). Throughout the experiment mean discharge, turbidity, TP and NO_3 were 0.28 m^3/s, 4.99 NTU, 0.49 mg/l and 9.06 mg/l respectively. A greater range of values were observed in TP than NO_3 (NO_3: minimum: 5.52 mg/l, maximum: 20.87 mg/l; TP: minimum: 0.16, maximum 3 mg/l). Mean water temperature over this time period was 9.21 °C, ranging from 5.7 – 15.7 °C. %DO was supersaturated with an average value of 108.9% and it was significantly correlated to pH demonstrating a diurnal pattern ($r = 0.84$, $n = 673$, $p < 0.01$). Mean conductivity was 483 µScm.

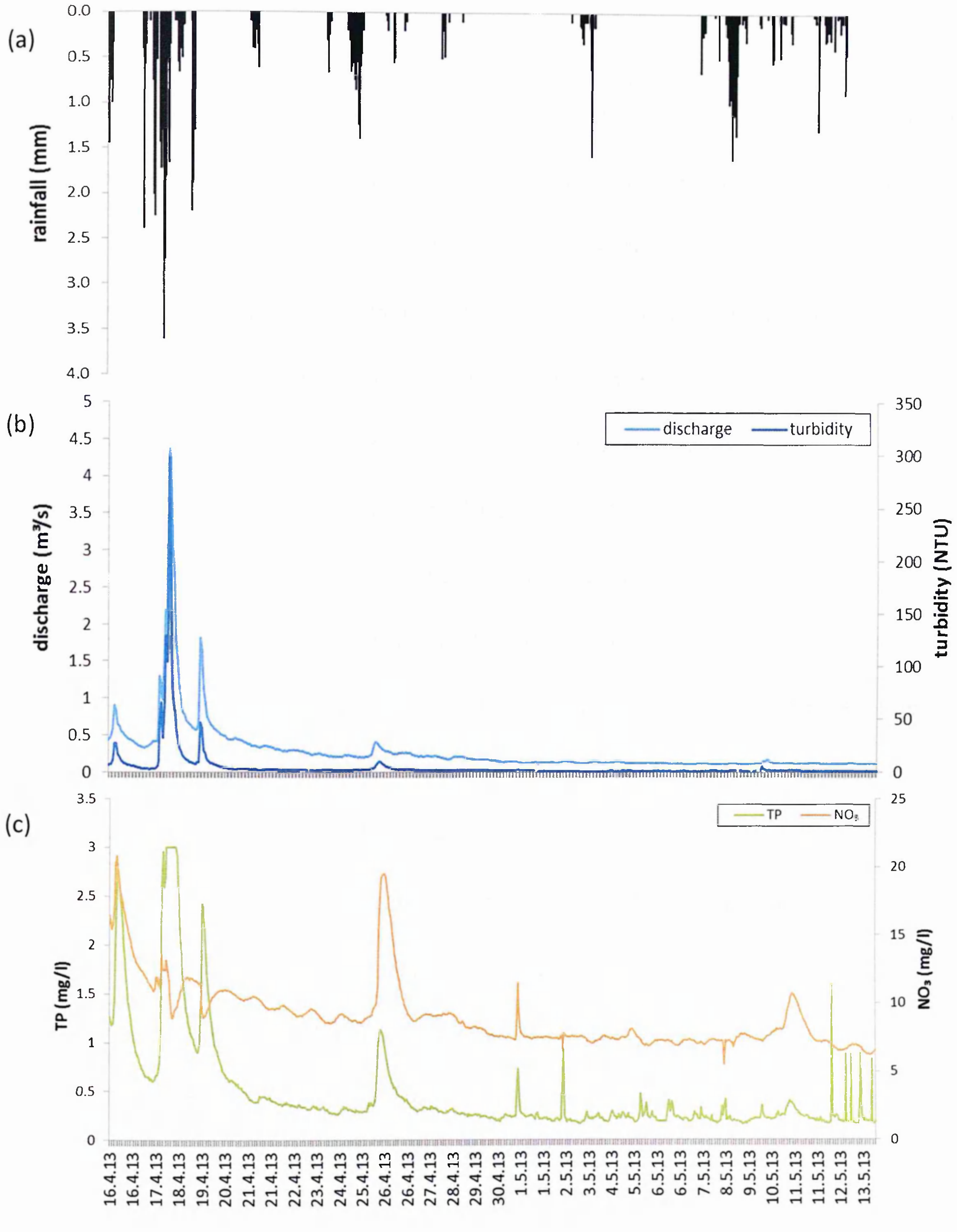


Figure 6.4: Hourly averaged 15 minute data for (a) rainfall and water level; (b) discharge and turbidity; and (c) TP and NO_3 from the 16/04/2013 to the 13/05/2013. (Source: EdenDTC).

6.3.2 Hydrological events and associated discharge and nutrient conditions

Between the 16/04/2013 and the 13/05/2013 five rainfall events divided into seven in-stream response periods were identified (Table 6.1 and see appendix 1.5). A key feature of the rainfall events was variable in-stream response in discharge, TP and NO_3 (Table 6.2), thus highlighting the dynamism of these systems. Differences in total rainfall duration were an important characteristic in determining change in discharge and associated nutrient condition (TP and NO_3). For rainfall event 1, 4 and 5 increases were observed in discharge and nutrient condition (TP, NO_3). The most rapid in-stream response of longest duration was observed during rainfall event 1 during which 4.34 mm of rain fell within 5 hours (Table 6.1). This resulted in an immediate increase in NO_3 concentration. Discharge and TP, in response to the same rainfall event, increased in concentration after 5 hours and remained elevated for 17h and 13h respectively. Rainfall event 2 and 3 demonstrated an increased in TP and a decreased in NO_3 concentration. Therefore, while TP concentration increased in response to all rainfall events, dilution and elevation in NO_3 concentrations was dependant on rainfall event characteristics (see Table 6.2). Lag time in response to all rainfall events was evident in discharge, TP and NO_3 but duration of in-stream response varied.

Within this experimental period, for interpretation of response in benthic diatom communities to rainfall, rainfall event 1 and 2 are considered high rainfall events and 3 and 4 are low rainfall events. Rainfall event 5 is termed an intermediate rainfall event in terms of impact on benthic biofilm communities. Both intermediate and high rainfall events demonstrated elevation in discharge, TP and NO_3 condition. The intermediate event demonstrated a greater lag response than the high rainfall event. Furthermore, the in-stream response in discharge and TP was most pronounced for discharge and TP after the high rainfall event (Table 6.2).

Table 6.1: Seven main rainfall events across each of the hydrological periods, outlining their duration, mean, peak and accumulated rainfall.

Rainfall Event (RE)	Date	Duration (h)	Mean rainfall rate (mm/h)	Peak rainfall (mm)	Total rainfall (mm)
RE 1	16th April	5h	0.87	1.45	4.335
RE 2 Response A	17 - 18th April	25h	1.03	3.61	25.70
RE 2 Response B	19th April	5h	1.33	2.20	6.63
RE 3	21st April	8h	0.30	0.62	2.40
RE 4 Response A	25th April	15h	0.59	1.4	8.82
RE 4 Response B	3rd - 4th May	14h	0.25	1.59	3.51
RE 5	9th - 10th May	26h	0.36	1.62	9.36

Table 6.2: Discharge, TP and NO_3 response characteristics for each of the 7 hydrological periods across seven rainfall events. Elevation or dilution in discharge, TP and NO_3 is based change from period mean which refers to the mean over that particular hydrological period as defined in Table 6.1. (*changes in parameters based on that preceding the event; ** as for * and incomplete data series).

Rainfall Event (RE)	**Parameter**	**Period mean**	**Lag response**	**Duration Elevated/Diluted**	**Mean over Duration Elevated/Diluted**	**Peak & Lag Time**	**Dilution/ Elevation**
RE 1	**Discharge**	0.50 m^3/s	5h	17h	0.59 m^3/s	0.91 m^3/s; 1h after event	Elevation
	TP	1.48 mg/l	5h	13h	2.09 mg/l	2.77 mg/l; 3h after event	Elevation
	NO_3	15.59 mg/l	0h	16h	17.51 mg/l	20.87 mg/l; 2h after event	Elevation
RE 2 Response A	**Discharge**	1.02 m^3/s	14h	18h	2.20 m^3/s	4.36 mg/l; 22h into event	Elevation
	TP	1.47 mg/l	15h	20h	2.65 mg/l	3 mg/l; 19h into event	Elevation
	NO_3	11.05 mg/l	22h	9h	9.70 mg/l	8.91mg/l; 24 into event	Dilution
RE 2 Response B	**Discharge**	1.02 m^3/s	4h	11h	1.06 m^3/s	1.81 m^3/s; 1h after event	Elevation
	TP	1.47 mg/l	5h	7h	1.97 mg/l	2.42 mg/l; 2h after event	Elevation
	NO_3	11.05 mg/l	6h	9h	9.55 mg/l	8.96 mg/l; 3h after event	Dilution
RE 3	**Discharge**	0.30 m^3/s	5h	7h	0.35 m^3/s	0.35 m^3/s; 5h into event	Elevation
	TP	0.38 mg/l	2h	4h	0.44 mg/l	0.45 mg/l; 2h into event	Elevation
	NO_3	9.67 mg/l	10h	7h	9.61 mg/l	9.56 mg/l; 2h after event	Dilution
RE 4 Response A	**Discharge**	0.16 m^3/s	13h	20h	0.31 m^3/s	0.41 m^3/s; 1h after event	Elevation
	TP	0.29 mg/l	10h	37h	0.6 mg/l	1.13 mg/l; 5h after event	Elevation
	NO_3 *	8.39 mg/l*	6h	42h	12.60 mg/l	19.52 mg/l; 8h after event	Elevation
RE 4 Response B	**Discharge***	0.16 m^3/s	16h	2h	0.15 m^3/s	0.15 m^3/s; 2h after event	Elevation
	TP *	0.29 mg/l	12h	3h	0.28 mg/l	0.29 mg/l; 12h into event	Elevation
	NO_3 *	8.39 mg/l	22h	15h	7.72 mg/l	8.12 mg/l; 30h after event	Elevation
RE 5	**Discharge***	0.01 m^3/s	12h	32h	0.13 m^3/s	0.16 m^3/s; 21h into event	Elevation
	TP**	0.04 mg/l	30h	23h	0.30 mg/l	0.39 mg/l; 15h after event	Elevation
	NO_3	0.79 mg/l	41h	6h	10.49 mg/l	10.70 mg/l; 18h after event	Elevation

6.3.3 Diatom assemblage composition

Overall, 56 diatom species were found within 24 genera on the tiles compared to 46 species belonging to 23 genera on neighbouring cobble substrates. The dominant species were *Gomphonema olivaceum* (38%) followed by *Achnanthidium minutissimum* (21%) and *Meridion circulare* (16%). Collectively they accounted for 75% of the diatom assemblage and indicated a different trophic status. *G. olivaceum* is commonly found in the benthos of eutrophic rivers and shallow lake systems (Ács and Buczko, 1995; Kelly and Whitton, 1995a; Sekar *et al.*, 2004; Plenkovic-Moraj *et al.*, 2008). *A. minutissimum* is also a common feature of benthic assemblages in streams with low to moderate levels of nutrient and organic pollution. Similar to *A. minutissimum, M. circulare* is an early successional species typical of initial spring assemblages (Keithan and Lowe, 1985; Áce *et al.*, 2000; Plenkovic-Moraj *et al.*, 2008). The structure of this assemblage is notable due to the presence of both lower tier species, *A. minutissimum,* which tightly adhere to the substrate surface, and *G. olivaceum* which is an upper tier stalked species. The presence of *G. olivaceum* stalked species indicated a more established biofilm structure and greater exploitation of elevated nutrient conditions.

Figure 6.5 presents the relative percentage contribution of *G. olivaceum, A. minutissimum* and *M. circulare* to assemblage composition. Together these three species occupied at least 50% of assemblage composition throughout the experimental period. Differences in the relative abundance of these species was observed among riffle units in response to rainfall events 1 to 5 (Figure 6.5, Table 6.2, 6.3) and highlighted the ability of diatom communities to respond within hours to discharge-nutrient conditions. Across all three riffle units, and in particular riffle unit 1, a clear response was evident in response to Rainfall Event 1 with *M. circulare* reaching a maximum abundance of 46%. During the same period, lower relative abundance of *A. minutissimum* and *G. olivaceum* was observed.

Differences in biofilm architecture can also be discerned among the three riffle biotopes through rainfall event 3 – 4. During these rainfall events, the relative proportion of *G. olivaceum* was higher in riffle unit 1 than both riffle unit 2 and 3. *M. circulare* was a prominent feature of the diatom assemblage during rainfall event 4 in riffle unit 2 and 3, suggesting greater discharge disturbance on riffle unit 2 and 3 than riffle unit 1. Moreover, the presence of fast-growing, pioneer species such *A. minutissimum,* that are resistant disturbance (discharge and grazing), together *G. olivaceum* and *M. circulare* which can exploit nutrients, demonstrates that low rainfall events (Rainfall Event 3 - 4) can promote three-dimensional development of benthic diatom assemblages within headwater streams.

During intermediate rainfall event at the end of the experimental period (Rainfall Event 5) similar responses were observed in the relative percentage contribution of *A. minutissimum* which increased among all three riffle units. As for the high rainfall event (Rainfall Event 1 - 2), highest sensitivity in diatom response was observed in riffle 1. For example on the 07/05/2013 *A. minutissimum* compromised 7% increasing to 44% on the 12/05/2013. This demonstrates how benthic diatom biofilms develop according to discharge, TP and NO_3 conditions with intermediate events allowing for greater species richness and diversity through nutrient delivery and stimulatory, rather inhibitory, sloughing of the benthic biofilm.

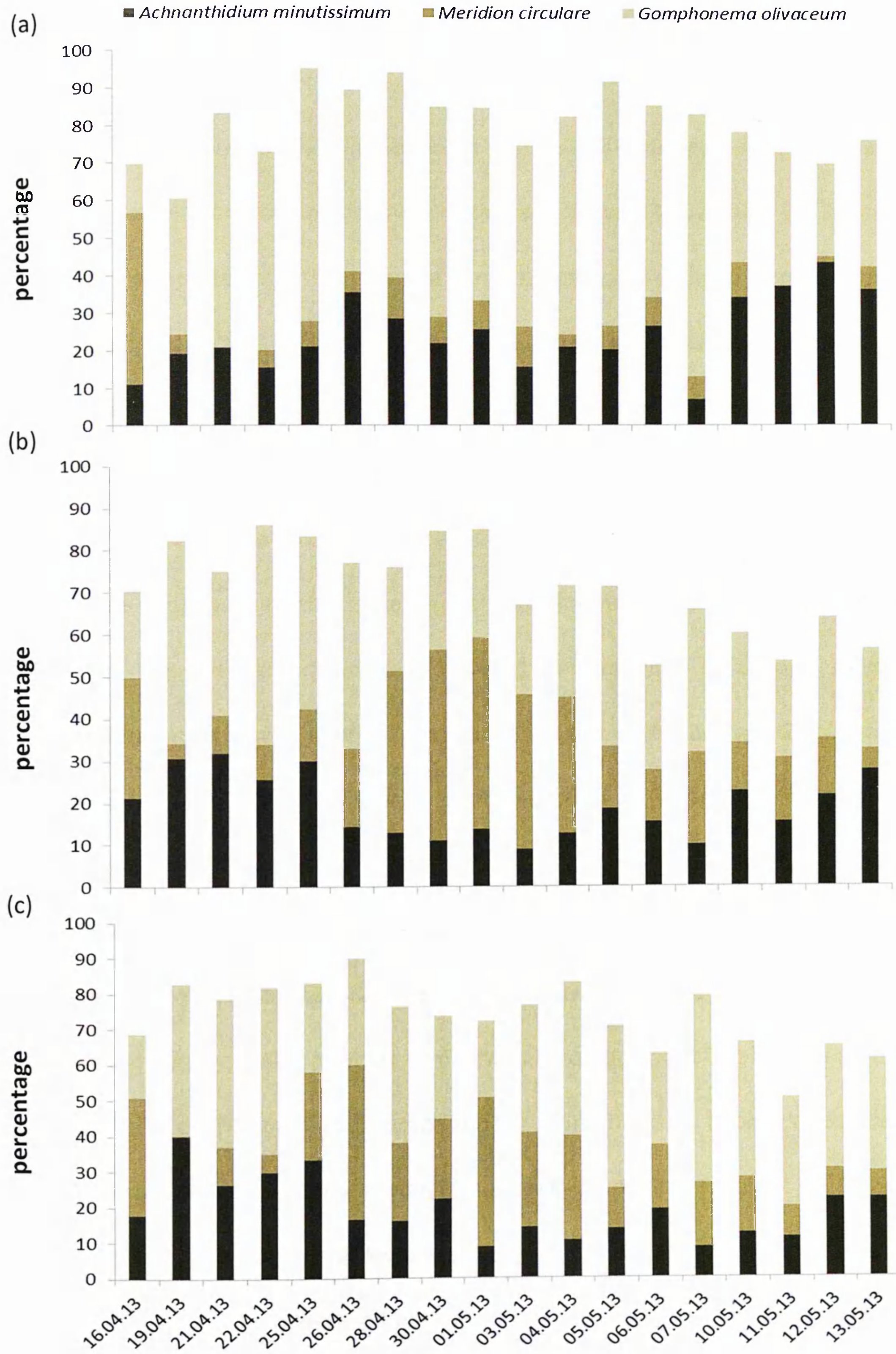


Figure 6.5: Relative percentage contribution of *G. olivaceum*, *A. minutissimum* and *M. circulare* to the total assemblage composition in riffle (a) unit 1; (b) unit 2; and (c) unit 3 from 16/04/2013 to 13/05/2013.

6.3.3.1 Comparison of diatom assemblage composition on cobble and tile substratum

Key differences were observed in dominant species and their relative contribution to total assemblage composition between cobble and tile substrate. Table 6.3 illustrates the top three species and their relative contribution to assemblage composition for both substrates. Across riffle unit 1, the two key species on cobble substrate were *A. minutissimum* and *A. biasolettiana.* In riffle unit 2 and 3, *A. minutissimum* and *A. biasolettiana* featured prominently but *G. olivaceum* also had a key presence. This demonstrates that the microhabitat architecture of biofilms on cobble and tile substratum differed in the successional development. Succession on cobble substrates appeared truncated with reduced development of canopy structure evident through a lower relative abundance of the stalk species, *G. olivaceum,* compared to tiles. Differential species performance of these pedunculate and stalk forms is mediated through their ecophysiology, disturbance and resource availability. Tile substrate may have heightened these differences by reducing substrate heterogeneity offered by naturally occurring substrata. Cobble substrata provide a more varied range of microhabitats through their compositional make up and highly textured surfaces (Murdock and Dodds, 2007). Communities on cobble substrate are highly prone to disturbance through substrate mobility (Tett *et al.*, 1978). Fluctuations in discharge, water level and turbidity can result in abrasion, movement of bed substrate and variance in light incidence reaching the bed (Figure 6.4). This can reduce the ability of species, such as *G. olivaceum*, to exploit nutrient resource through scour and thus, contributing to the dominance of *Achnanthidium* spp. The dominance of stalk species on tile substrate may be as a result of the standardisation provided though protection from substrate movement and micro-hydrological changes along the bed-biofilm interface, therefore allowing successional process to exist through the exploitation of elevated nutrient concentration. Note however, that on the 04/05/2013 which corresponds with Rainfall Event 4 (Rainfall Event 4; Response B; Table 6.2) *G. olivaceum* became dominant on both cobble and tile substrate. This may be a function of change in biotope discharge characteristics. As discharge increased throughout this rainfall event habitat conditions may become increasingly homogeneous and thus, more "tile-like". This emphasises the importance of rainfall event characteristics; intensity, rate and duration, in understanding the structural composition of the diatom biofilm assemblage.

Table 6.3: Comparison of the relative percentage contribution of the three most abundant species to diatom assemblage composition on cobble and tile substrate.

	Cobble substrate	**Tile substrate**
22.04.13	*Achnanthidium minutissima* (37%)	*Gomphonema olivaceum* (53%)
	Achnanthidium biasdettiana (12%)	*Achnanthidium minutissima* (15%)
	Amphora pediculus (11%)	*Achnanthidium biasdettiana* (12%)
28.04.13	*Achnanthidium biasdettiana* (20%)	*Gomphonema olivaceum* (55%)
	Gomphonema olivaceum (15%)	*Achnanthidium minutissima* (29%)
	Meridion circulare (11%)	*Meridion circulare* (11%)
03.05.13	*Achnanthidium minutissima* (41%)	*Gomphonema olivaceum* (48%)
	Achnanthidium biasdettiana (22%)	*Achnanthidium minutissima* (16%)
	Gomphonema olivaceum (10%)	*Meridion circulare* (11%)
04.05.13	*Achnanthidium minutissima* (35%)	*Gomphonema olivaceum* (58%)
	Gomphonema olivaceum (20%)	*Achnanthidium minutissima* (21%)
	Achnanthidium biasdettiana (14%)	*Diatoma vulgare* (7%)
10.05.13	*Gomphonema olivaceum* (20%)	*Gomphonema olivaceum* (35%)
	Rhoicosphenia abbreviata (13%)	*Achnanthidium minutissima* (34%)
	Achnanthidium minutissima (11%)	*Meridion circulare* (9%)
22.04.13	*Achnanthidium biasdettiana* (29%)	*Gomphonema olivaceum* (52%)
	Achnanthidium minutissima (26%)	*Achnanthidium minutissima* (26%)
	Gomphonema olivaceum (18%)	*Meridion circulare* (8%)
28.04.13	*Gomphonema olivaceum* (26%)	*Meridion circulare* (38%)
	Achnanthidium minutissima (19%)	*Gomphonema olivaceum* (25%)
	Cocconeis placentula var euglypta (7%)	*Achnanthidium minutissima* (13%)
03.05.13	*Achnanthidium minutissima* (50%)	*Meridion circulare* (37%)
	Achnanthidium biasdettiana (25%)	*Gomphonema olivaceum* (21%)
	Gomphonema olivaceum (6%)	*Achnanthidium minutissima* (9%)
04.05.13	*Gomphonema olivaceum* (24%)	*Meridion circulare* (32%)
	Achnanthidium minutissima (15%)	*Gomphonema olivaceum* (27%)
	Achnanthidium biasdettiana (12%)	*Achnanthidium minutissima* (13%)
10.05.13	*Amphora pediculus* (27%)	*Gomphonema olivaceum* (26%)
	Rhoicosphenia abbreviata (13%)	*Achnanthidium minutissima* (23%)
	Reimeria sinuata (13%)	*Meridion circulare* (12%)
22.04.13	*Achnanthidium minutissima* (29%)	*Gomphonema olivaceum* (47%)
	Gomphonema olivaceum (21%)	*Achnanthidium minutissima* (30%)
	Meridion circulare (9%)	*Meridion circulare* (5%)
28.04.13	*Achnanthidium biasdettiana* (37%)	*Gomphonema olivaceum* (38%)
	Gomphonema olivaceum (19%)	*Meridion circulare* (22%)
	Achnanthidium subatomus (9%)	*Achnanthidium minutissima* (16%)
03.05.13	*Achnanthidium minutissima* (49%)	*Gomphonema olivaceum* (36%)
	Gomphonema olivaceum (24%)	*Meridion circulare* (27%)
	Achnanthidium biasdettiana (12%)	*Achnanthidium minutissima* (14%)
04.05.13	*Gomphonema olivaceum* (29%)	*Gomphonema olivaceum* (43%)
	Rhoicosphenia abbreviata (11%)	*Meridion circulare* (29%)
	Achnanthidium minutissima (11%)	*Achnanthidium minutissima* (10%)
10.05.13	*Achnanthidium minutissima* (17%)	*Gomphonema olivaceum* (38%)
	Achnanthidium biasdettiana (16%)	*Diatoma vulgare* (16%)
	Cocconeis placentula var *euglypta* (13%)	*Meridion circulare* (16%)

6.3.4 Diatom assemblage diversity (richness and evenness)

6.3.4.1 Response of diatom assemblage richness and evenness to changes in discharge, TP and NO_3 through the 5 rainfall events over the experimental period

Trends in Margalef richness index and Pielou's evenness index in response to change in discharge-nutrient condition among the individual riffle units over the experimental period are presented in Figure 6.6. In riffle unit 1, Margalef richness declined following the high intensity discharge events on 16/04/2013 and 19/04/2013 (Rainfall Event 1 - 2) and then recovered towards the intermediate rainfall event at the end of experimental period (Rainfall Event 5; Figure 6.6). In riffle unit 2, both Margalef richness and Pielou's evenness index reached a peak during the intermediate rainfall event of 4.38 and 0.76 respectively. However, in riffle unit 1 and 3, both Margalef richness and Pielou's evenness index was highest during Rainfall Event 1 but did reach comparable values during the intermediate rainfall (Rainfall Event 5). Diatom assemblage richness was high and most comparable among all three riffle units during the intermediate rainfall event (Rainfall event 5), suggesting that there was an even spread in number of individuals among species.

6.3.4.2 Comparison between diatom assemblage diversity on tile and cobble substratum

There was a significant difference between Margalef richness index for cobbles and tiles ($t = -4.55$, $df = 12$, $p < 0.05$). Mean Margalef richness index for tile substrate was 2.31 compared to 3.68 for cobble substrate in riffle unit 1, indicating greater diversity on cobble substrate. The range of values was greater for tiles rather than cobbles (tile 1.23 – 3.51, cobble 3.16 – 4.56). Similarly, for Pielou's evenness index average values were lower for tile (0.59) than cobble substrate (0.72), with a greater range in tile (0.42 – 0.71) than cobble substrate (0.63 – 0.83). This suggested a more even distribution of species on cobble compared to tile substratum. There was a non-significant difference between Pielou's evenness for cobble and tile substrate ($t = -3.11$, $df = 12$, $p > 0.05$), with values similar or higher on cobbles than tiles. Therefore, assemblage diversity was significantly different between tile and cobble substrate with cobbles more diverse. However, there was no significant difference in the variation in communities between species.

In riffle unit 2, there was a greater range of Margalef richness index values observed on tiles than cobbles but values for cobbles were higher, reaching a maximum of 4.56 on 04/05/2013 (Figure 6.6). Analysis of variance showed no significant difference in values for Margalef richness index on tile and cobble substrate ($t = -2.02$, $df = 8$, $p > 0.05$). There was a greater range of values for Pielou's evenness index on cobbles (0.55 – 0.80) than tiles (0.57 – 0.76). A significant difference was observed between tiles and cobble substrate ($t = -11.16$, $df = 8$, $p < 0.05$), with an average value of 0.67 on tiles and 0.72 on cobbles.

There was a significant difference in Margalef richness index observed in riffle unit 3 on cobbles and tiles ($t = -3.2$, $df = 8$, $p < 0.05$) but not for Pielou's evenness index ($t = -1.77$, $df = 8$, $p > 0.05$). Contrary to riffle unit 1 and 2, in riffle unit 3 a greater range of values for Margalef richness index was observed on cobble than tile substratum but similar to riffle unit 1 and 2 a lower maximum of 3.51 was observed for tiles compared to 4.56 for cobbles. Furthermore, Pielou's evenness index was also lower on tile substrate than cobbles (Tiles: 0.66; Cobbles: 0.72). Therefore, examination of the compositional complexity of diatom communities through richness and evenness measures demonstrated their inter-dependence and how this relationship varied according to biotope unit and substrate type.

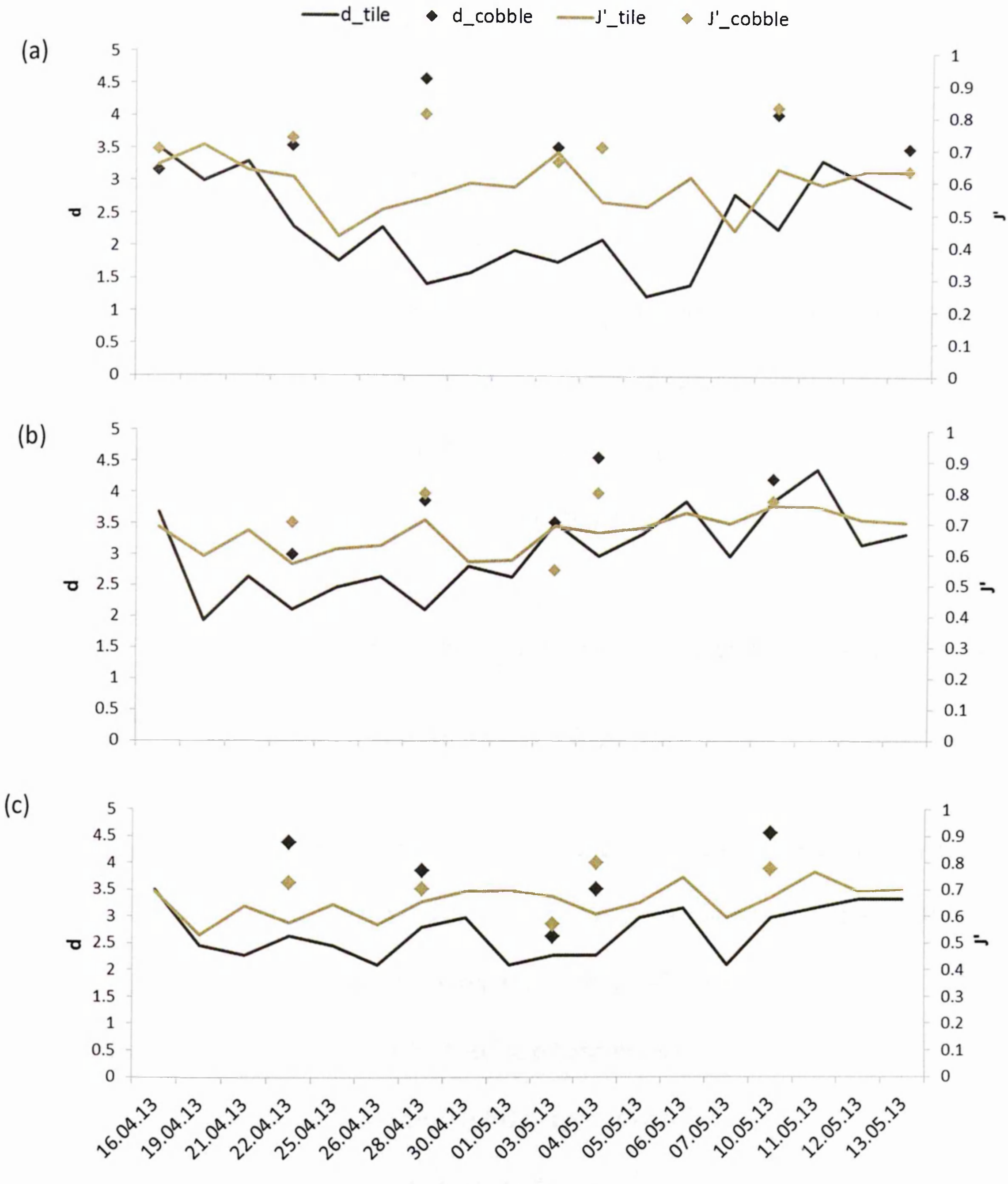


Figure 6.6: Margalef richness index (d) (y axis 1) and Pielou's evenness index (J') (y axis 2) for (a) riffle unit 1; (b) riffle unit 2; and (c) riffle unit 3, on tile and cobble substrates. Margalef richness index is (S-1)/ln (n), where S is the number of taxa, and n is the number of individuals. Pielou's evenness index is Shannon-Wiener diversity divided by the logarithm of number of taxa providing a measure of the evenness with which individuals are divided among the taxa present.

6.3.5 Response of diatom assemblage functional diversity to variability in discharge-nutrient (TP, NO_3) condition

The functional diversity of the diatom assemblage is explored within this section with reference to life-forms which are re-organised into larger ecological groups termed "Ecological Guilds" (Rimet and Bouchez, 2012). Mean ecological guild diversity across the three riffle units is examined in relation to hourly averaged discharge and nutrient concentration (TP and NO_3) based on 15 minute data over the experimental period. Ecological guild diversity of each individual riffle unit is also presented.

6.3.5.1 Ecological guild diversity in relation to discharge-nutrient (TP, NO_3) condition

The most prominent guild across all three riffle biotope units was the low profile guild (48%), followed by the high profile guild (44%) and the motile guild (8%). Throughout the experimental period the relative abundance of ecological guilds changed in response to the 'high', 'low' and 'intermediate' rainfall events (Figure 6.7; see section 6.3.2). On the 16/04/2013 high rainfall and discharge conditions corresponded with a high relative proportion of low profile species (58%; Rainfall Event 1). Immediately after the high intensity rainfall period, from the 19/04/2013 to 22/04/2013, the relative abundance of low profile guild remained constant at 41% but the contribution from the high profile guild steadily increased, reaching a maximum of 54% on the 22/04/2013. The relative abundance of the motile guild was highest immediately after the high rainfall event with an average contribution of 12%

between 16/04/2013 and 21/04/2013. The presence of both motile and high profile species illustrated the high sensitivity of these communities to prevailing environmental conditions and their ability to change their functional composition to exploit increased resource availability. Note, that during the low intensity rainfall period (Rainfall Event 3 - 4) the contribution from the motile guild decreased and the relative contribution from the high profile guild increased. Given the ability of both of these guilds to exploit prevailing nutrient concentration, this suggest that facilitative and competition interactions are present in headwater diatom communities and are dependent on rainfall event characteristics.

The low profile guild reached a similar relative proportion on the 07/05/2013 (Rainfall Event 4 to 5) to the high rainfall event (Rainfall event 1). In addition, there was a significant contribution from the high profile guild with motile species only providing a minor contribution to assemblage composition (16/04/2013: High profile guild = 25%, Motile guild = 17%; 07/05/2013: High profile guild 34%: Motile guild = 6%). Thereafter the relative percentage contribution of the motile guild increased to 13% on the 13/10/2013 (Rainfall Event 5) and approached a similar relative abundance as observed on the 16/04/2013 (Rainfall Event 1). The motile guild therefore demonstrated similar patterns to the low profile species, which increased in relative abundance during the high and intermediate rainfall events.

Comparison between ecological guilds on tile and cobble substrate showed higher uniformity on cobble substratum in the relative contribution from the four ecological guilds across the three riffle units. The low profile guild demonstrated the greatest relative contribution to assemblage composition (Riffle unit 1: 69%; Riffle unit 2: 65%; Riffle unit 3: 61 %) followed by high profile (Riffle unit 1: 20%; Riffle unit 2: 23%; Riffle unit 3: 30 %) and motile guild (Riffle unit 1: 11%; Riffle unit 2: 12%; Riffle unit 3: 9%). Therefore, this reiterates earlier findings (see section 6.3.3.1) of the importance of substratum heterogeneity and mobility for diatom successional processes.

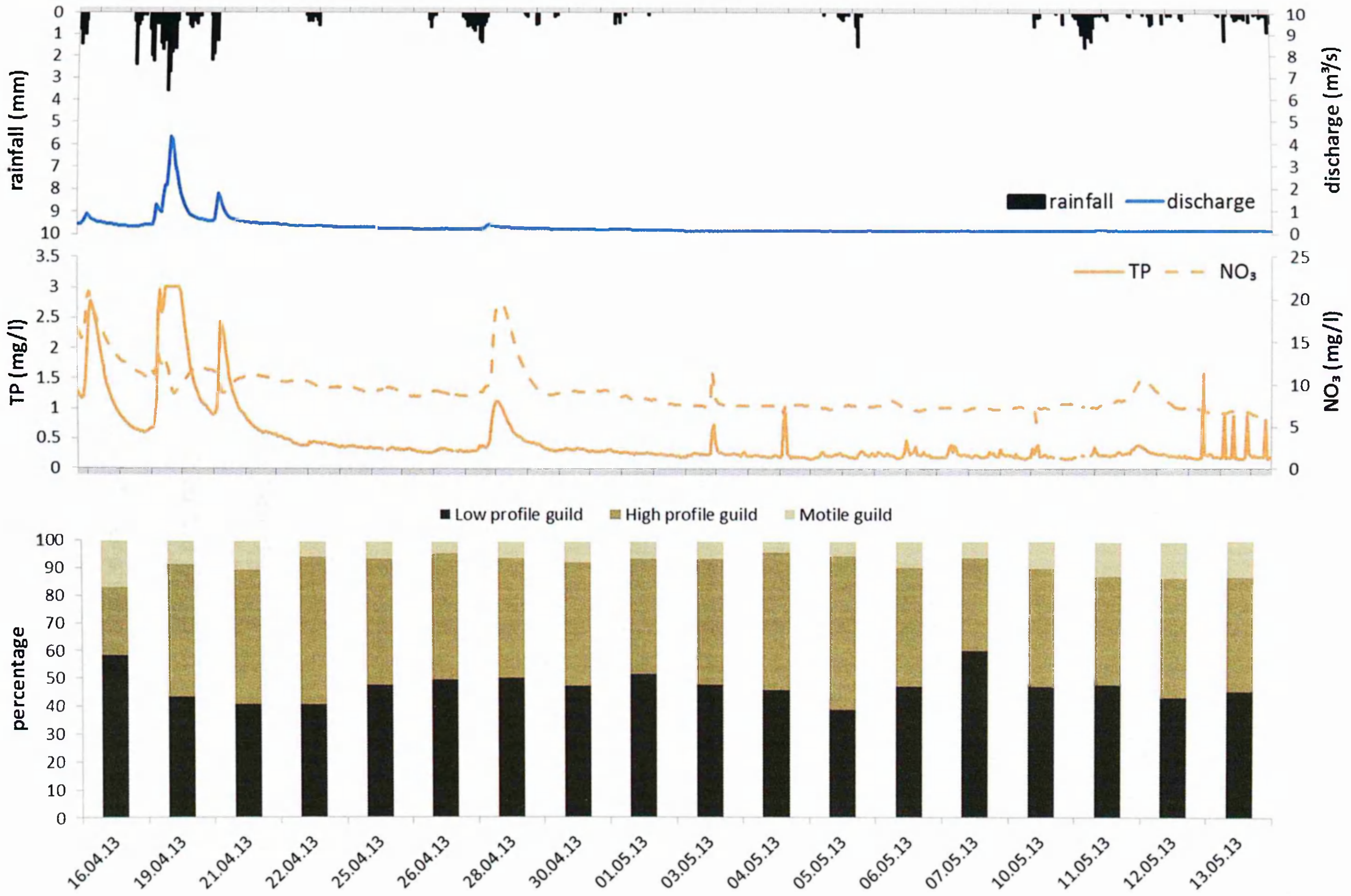


Figure 6.7: Response of diatom functional composition (Ecological Guilds) averaged across the three riffle units (n = 9 per 18 sampling events) to 15 minute rainfall, discharge, TP and NO_3 averaged over 60 minutes from the 16/04/2013 to 13/05/2013.

6.3.5.2 Ecological guild assemblage composition among riffle units

Pattern in the relative percentage contribution of ecological guilds among riffle units are presented in Figure 6.8. Within each of the riffle units, a clear response was observed in response to the high rainfall event (Rainfall Event 1 - 2) at the beginning of the experiment. The relative contribution of low profile and motile species was high during this period (Riffle 1: low profile guild 65%; motile guild 15%; Riffle 2: low profile guild 54%; motile guild 18%; Riffle 3: low profile guild 56%; motile guild 18%). During the low intensity rainfall events (Rainfall Event 3 - 4), the relative contribution of low profile and motile guilds decreased, while the high profile guild increased. In riffle unit 1, from the 21/04/2013 to the 07/05/2013, the mean relative contribution from the high profile guild to assemblage composition was notably higher (62%) than observed over the rest of the experimental period. In riffle unit 2 and 3, the low profile guild showed a higher mean contribution of 56% in both riffle units through the low intensity rainfall events from 25/04/2013 to the 04/05/2013 (Rainfall Event 3 - 4). The relative contribution of the low profile guild then decreased towards intermediate rainfall event (Rainfall Event 5) to a minimum over the whole time series of 39% on the 13/05/2013 for riffle unit 2 and 33% on the 11/05/2013 in riffle unit 3. Between the 25/04/2013 to the 04/05/2013 (Rainfall Event 3 - 4) when the relative percentage contribution of low profile guild to assemblage composition was high, the mean percentage of high profile species was 37% and 39% respectively compared to 44% outside this period for riffle unit 2 and 3. Therefore, across all riffle units the motile guild responded to high rainfall events and associated discharge-nutrient conditions and, as demonstrated in section 6.3.5.1, an inverse relationship was observed between low and high profile guilds.

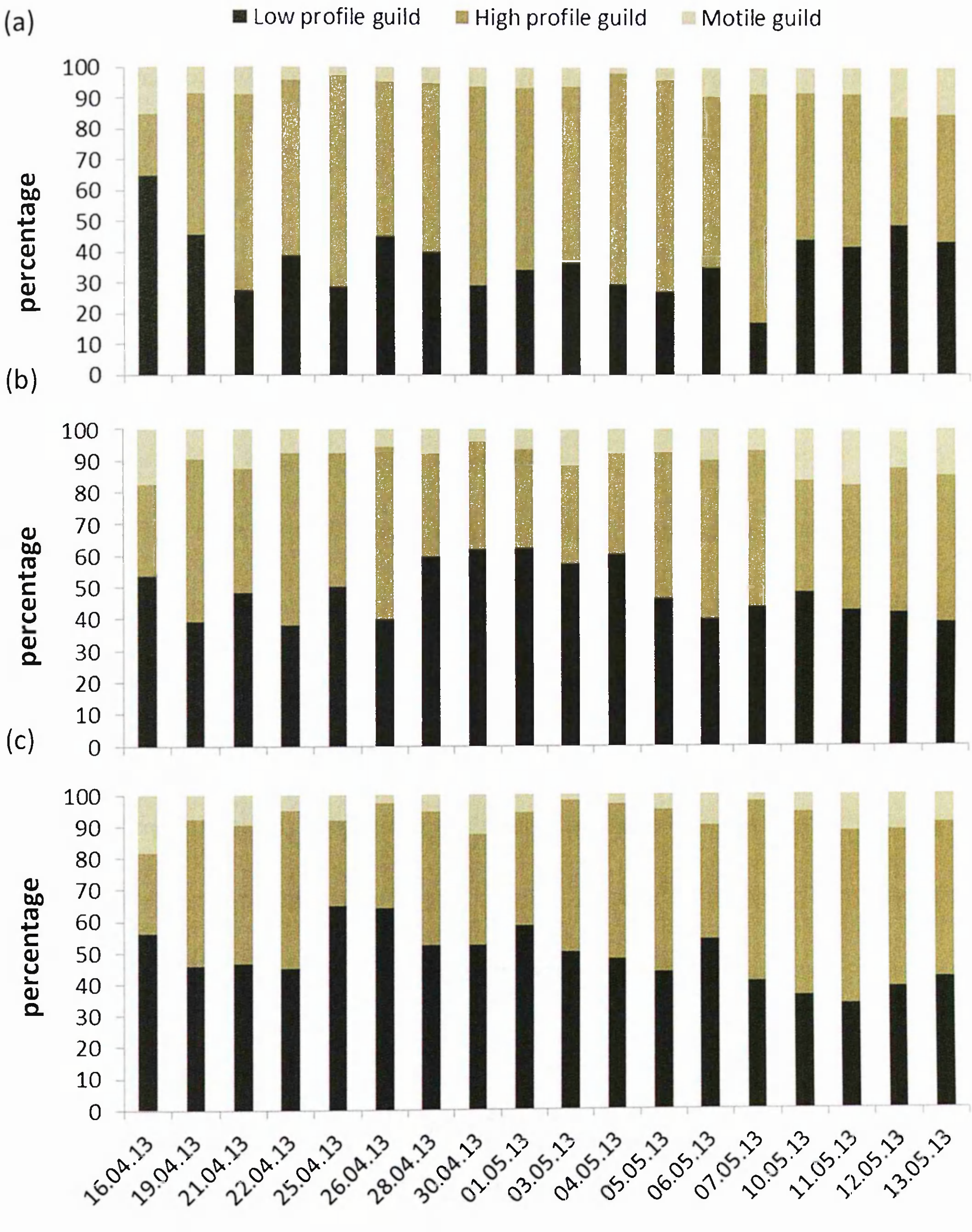


Figure 6.8: Relative percentage contribution of each ecological guild: Low profile, high profile, motile and planktonic, for (a) riffle unit 1; (b) riffle unit 2; and (c) riffle unit 3, over the time series from 16/04/2013 to 13/05/2013.

6.3.6 ISF spectral signature of diatoms, cyanobacteria and green algae chlorophyll-*a* in response to discharge and nutrient condition

6.3.6.1 ISF chlorophyll-a among riffle units in response to rainfall, discharge, TP and NO_3

Variation in benthic chlorophyll-*a* was observed over the 28 day period with values of chlorophyll-*a* ranging from 0.14 to 3.66 µg/cm^2 with an average of 1.28 µg/cm^2. This observed variation was explored in relation to discharge-nutrient relationships (Figure 6.9). The high rainfall event (Rainfall Event 1), on the 16/04/2013, resulted in low chlorophyll-*a* concentration with values less than 1 µg/cm^2 until 22/04/2013. Despite elevated TP and NO_3 concentrations, benthic diatom chlorophyll-*a* concentration was low, suggesting that chlorophyll-*a* concentration is primarily controlled by physical conditions. More specifically, increased discharge and consequent scour and abrasion of the biofilm, as well as reduced light penetration due to increased depth and turbidity may have contributed to lower chlorophyll-*a* concentrations (see section 6.3.1). Low rainfall events, for example on the 25/04/2013 (Rainfall Event 4, Response A), which result in sub-scour discharge conditions stimulated chlorophyll-*a* production through nutrient delivery into the biofilm mat. From the 08/05/2013 to the 13/05/2013 (Rainfall Event 5) maximum chlorophyll-*a* concentrations were observed at 3.66 µg/cm^2 with a mean of 2.08 µg/cm^2. Hence, rainfall characteristics; intensity, rate and duration are also important determinants of chlorophyll-*a* concentration.

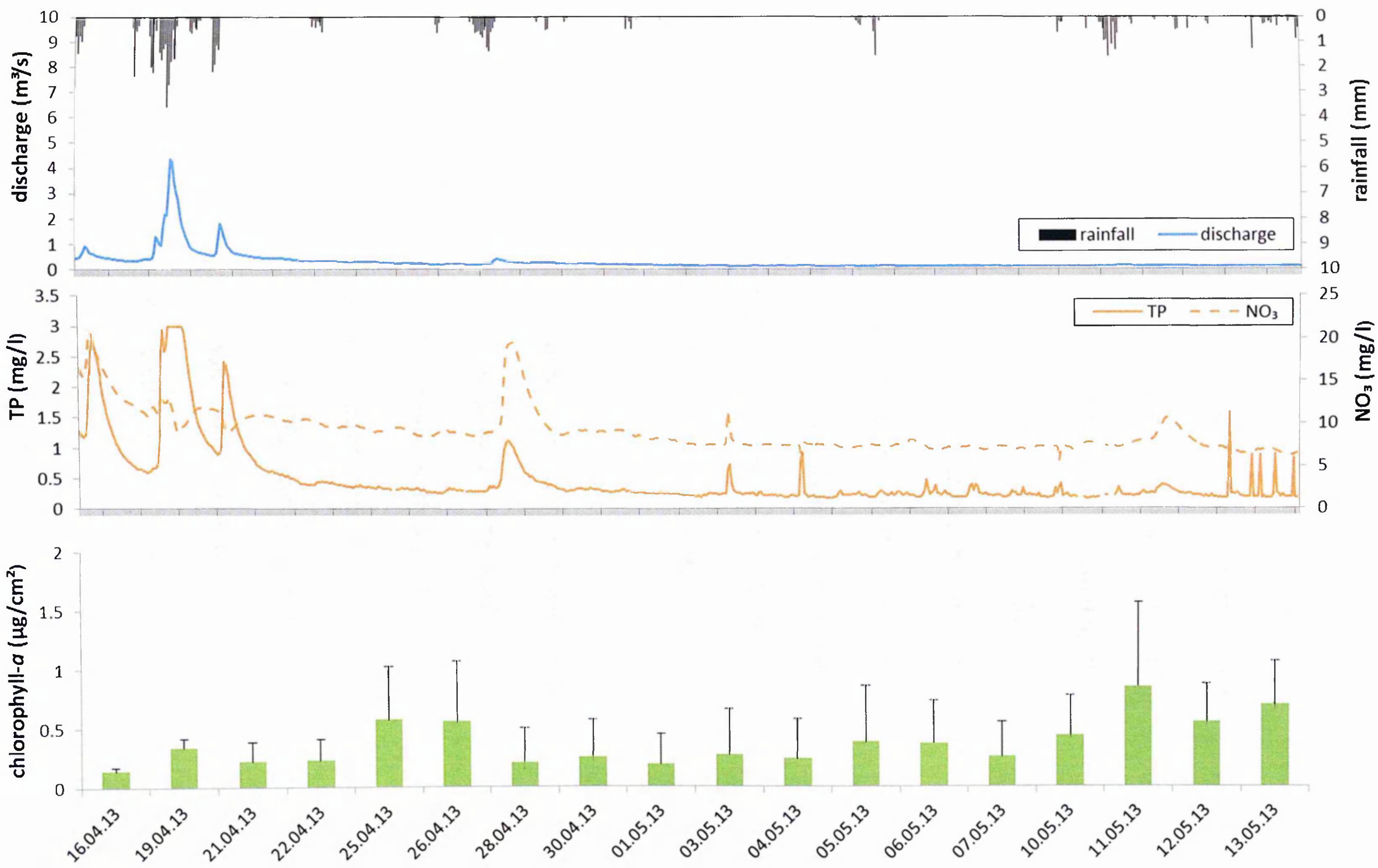


Figure 6.9: (a) 15 minute rainfall and discharge, TP and NO_3 averaged over 60 minutes; (b) 15 minute TP and NO_3 averaged over 60 minutes; and (c) chlorophyll-*a* (n = 36 per n = 18 sampling event) from 16/04/2013 to 13/05/2013.

6.3.6.2 ISF spectral signature of diatoms, cyanobacteria and green algae among riffle units

Exploration of chlorophyll-*a* concentration, alongside ISF spectral signatures, revealed that differences in the relative contribution of diatoms, cyanobacteria and green algae to assemblage composition were evident and that diatoms were the key contributor to benthic biofilm chlorophyll-*a*. Among the three riffle units, the concentration of total chlorophyll-*a* was higher in riffle unit 1 compared to unit 2 and 3 (Figure 6.10). Mean concentration of chlorophyll-*a* in riffle unit 1 was 1.28 μg/cm^2 compared to 0.13 μg/cm^2 riffle unit 2 and 0.09 μg/cm^2 riffle unit 3. There was also a greater range of values observed in riffle unit 1, with values increasing toward the end of the series and reaching a maximum of 3.66 μg/cm^2 on the 11/05/2013 from 0.14 μg/cm^2 at the start of the series on the 16/04/2013.

Compared to riffle unit 1, cyanobacteria were the major contributors to chlorophyll-*a* concentration in riffle unit 2 and 3 during the period 28/05/2013 to the 05/05/2013 comprising 100% of the ISF signature with a mean concentration of 0.02 μg/cm^2. Cyanobacteria remained key contributors to assemblage composition on the 06/05/2013 and 07/05/2013 in riffle unit 2 (Rainfall Event 4 - 5) but thereafter diatoms became the dominant component of the assemblage with an average contribution 65% followed by cyanobacteria (24%) and green algae (11%). Between the 16/04/2013 to the 26/04/2013 diatoms were dominant (48%) followed by cyanobacteria (37%) and green algae (14%). In riffle unit 3 cyanobacteria (54%) were the greatest contributor to average total chlorophyll-*a* with 36% from diatoms and

11% from green algae. Between the 16/04/2013 to the 26/04/2013 and 06/05/2013 to the 13/05/2013, diatoms had a relative contribution of 41% and 54% respectively to total ISF chlorophyll-*a*. However, during the period 16/04/2013 to the 26/04/2013 cyanobacteria dominated with a relative contribution of 54% which decreased to 36% during the period the 06/05/2013 to the 13/05/2013. Green algae only made a substantial average contribution at the beginning of the time series on the 16/04/2013 and again between 07/05/2013 and 13/05/2013 with an average of 11%, peaking at 29% on the 12/05/2013. This dynamics in the relative contribution of diatoms and cyanobacteria to total chlorophyll-*a* illustrates how successional trajectories can vary within and between biotope units leading to variable chlorophyll-*a* concentrations. Moreover, this is comparable to other studies, such as those of Sekar *et al.*, (2004), who also found that initial colonisation is dominated by key pioneer species *Achnanthidium* spp., *Cocconeis* spp. and *Gomphonema* spp. with cyanobacteria reaching high relative abundance 10 days following events. This thesis therefore supports these findings, demonstrating that for headwater systems early stages of biofilm development and its composition (diatom, cyanobacteria and green algae) varied according to two successional phases; early dominance by pioneer diatom species following by high relative concentration of cyanobacteria. Differences in the relative contribution of diatoms and cyanobacteria among the riffle 1 and riffle unit 2 and 3 are likely a function of physical habitat characteristics and biofilm thickness and density prior to the high intensity rainfall event (Rainfall Event 1 - 2; Besemer *et al.,* 2013).

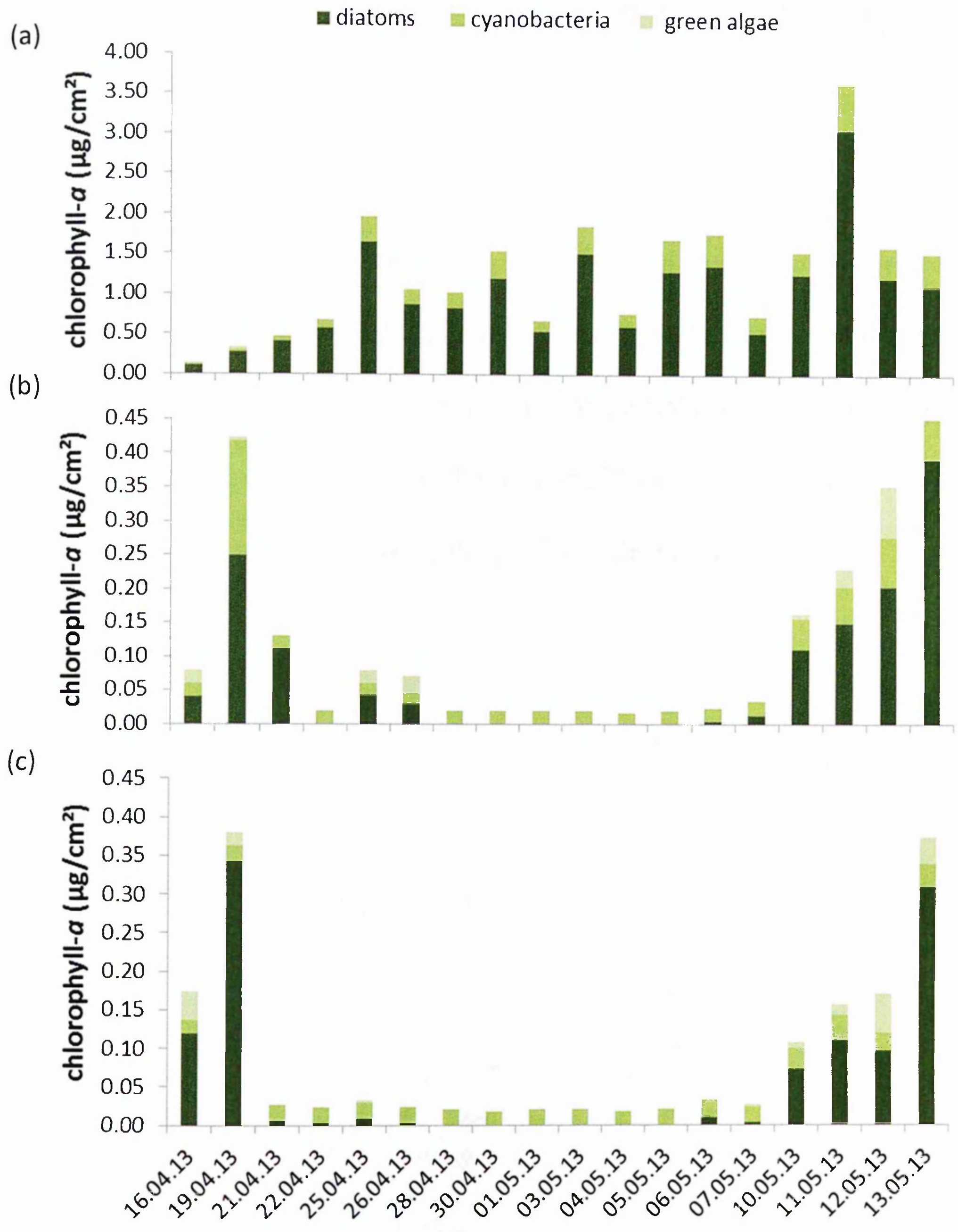


Figure 6.10: ISF chlorophyll-*a* concentrations for the spectral groups; diatoms, cyanobacteria and green algae for (a) riffle unit 1; (b) riffle unit 2 and (c) riffle unit 3, over the time series from 16/04/2013 to 13/05/2013.

6.3.7 Relationship between diatom assemblage diversity and its chlorophyll-*a* production

Shannon-Wiener diversity ranged from 1.48 – 2.16 and diatom-spectral ISF chlorophyll-*a* from 0.09 to 1.11 µg/cm^2. Overall, there was a non-significant relationship between the diversity of the diatom assemblage and its chlorophyll-*a* concentration. However, in general higher values of Shannon-Weaver diversity led to higher and more variable ISF diatom-spectral chlorophyll-*a* values (Figure 6.11).

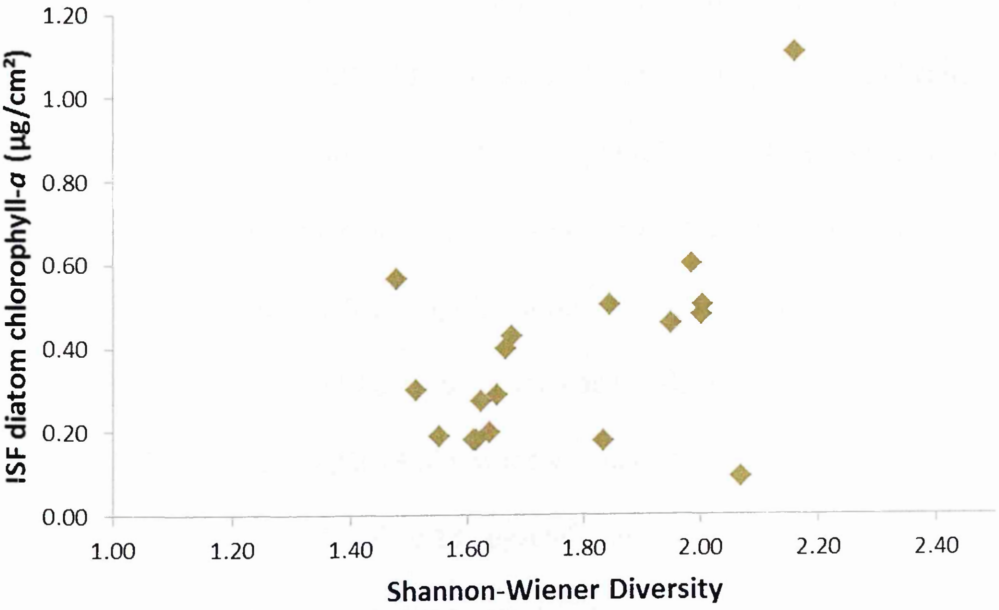


Figure 6.11: ISF chlorophyll-*a* (diatom spectral chlorophyll-*a*) and Shannon-Wiener diversity from the 16/04/2013 to 13/05/2013.

6.3.8 Velocity-Nutrient Condition: Key determinants of assemblage diversity and chlorophyll-*a*

Over the experimental period environmental parameters (nutrient condition; TP and NO_3, shear stress and velocity) were highly correlated. Shannon-Wiener diversity was also significantly correlated with TP (Table 6.4). From the 16/04/2013 to the 21/04/2013 (Rainfall Event 1 - 3), mean velocity, shear stress, NO_3 and TP were all higher than the mean recorded throughout the experimental period (Figure 6.12). Mean velocity was 76.6 cm/s, and reached a peak of 121.9 cm/s on the 18/04/2013. This was higher than the mean for the experimental period of 42.26 cm/s. Similarly mean shear stress over the experimental period was 21.82 n/m^2, and increased to 39.88 n/m^2 between the 16/04/2013 and 21/04/2013 (Table 6.4; Rainfall Event 1 - 3). Mean NO_3 and TP concentrations over this time period were 14.08 mg/l and 1.5 mg/l respectively; higher than concentrations observed over the whole time series (Mean NO_3: 9.65 mg/l; Mean TP: 0.55 mg/l). Moreover, between the 16/04/2013 and 21/04/2013, mean Shannon-Wiener diversity was consistently high at 1.99, reaching a peak of 2.05 on the 19/04/2013. Conversely, mean chlorophyll-*a* was 0.23 $\mu g/cm^2$ decreasing to a minimum of 0.13 $\mu g/cm^2$ on the 16/04/2013, the lowest concentration recorded over the time series (Figure 6.12). Therefore, high nutrient condition (TP and NO_3), velocity and shear stress promoted diversity in assemblage composition but led to low chlorophyll-*a* concentrations.

Intermediate events (Rainfall event 5) provided optimum velocity, shear stress, TP and NO_3 conditions which promoted diversity in benthic diatom assemblage composition and contributed to high chlorophyll-*a* production. Between 11/05/2013 and 13/05/2013 (Rainfall event 5) values of Shannon-Wiener diversity approached similar mean values of 1.78 to that observed during rainfall events 1 to 3. Mean chlorophyll-*a* values were higher (0.96 µg/cm^2) reaching a maximum of 1.35 µg/cm^2 on the 11/05/2013 (Figure 6.12). Over this period, 11/05/2013 and 13/05/2013, despite mean rainfall of 0.11 mm/h and accumulated rainfall of 7.77 mm, mean NO_3 (7.94 mg/l), TP (0.26 mg/l), velocity (25.94 cm/s) and shear stress (13.32 n/m^2) were all below the average recorded throughout the experimental period; 9.65 mg/l, 0.55 mg/l, 42.26 cm/s and 21.82 n/m^2 respectively. This demonstrates that during 'intermediate events', sub-scouring discharge is evident throughout, having a stimulatory effect on the benthic biofilm through resource delivery and contributing to higher chlorophyll-*a* concentration (Figure 6.12).

Table 6.4: Pearson's correlation values between average velocity (cm/s), shear stress (n/m^2), TP (mg/l), NO_3 (mg/l), chlorophyll-*a* (µg/cm^2) and Shannon-Wiener diversity. Velocity (cm/s), shear stress (n/m^2), TP (mg/l) and NO_3 (mg/l) are based on daily averages (n = 18). (Critical r = 0.497 at p = 0.05).

	Shear Stress	Velocity	TP	NO_3	Chl-*a*
Shear Stress	1				
Velocity	0.999	1			
TP	0.907	0.903	1		
NO3	0.676	0.648	0.814	1	
Chl-*a*	-0.438	-0.426	-0.359	-0.385	1
Diversity	0.466	0.474	0.571	0.415	0.023

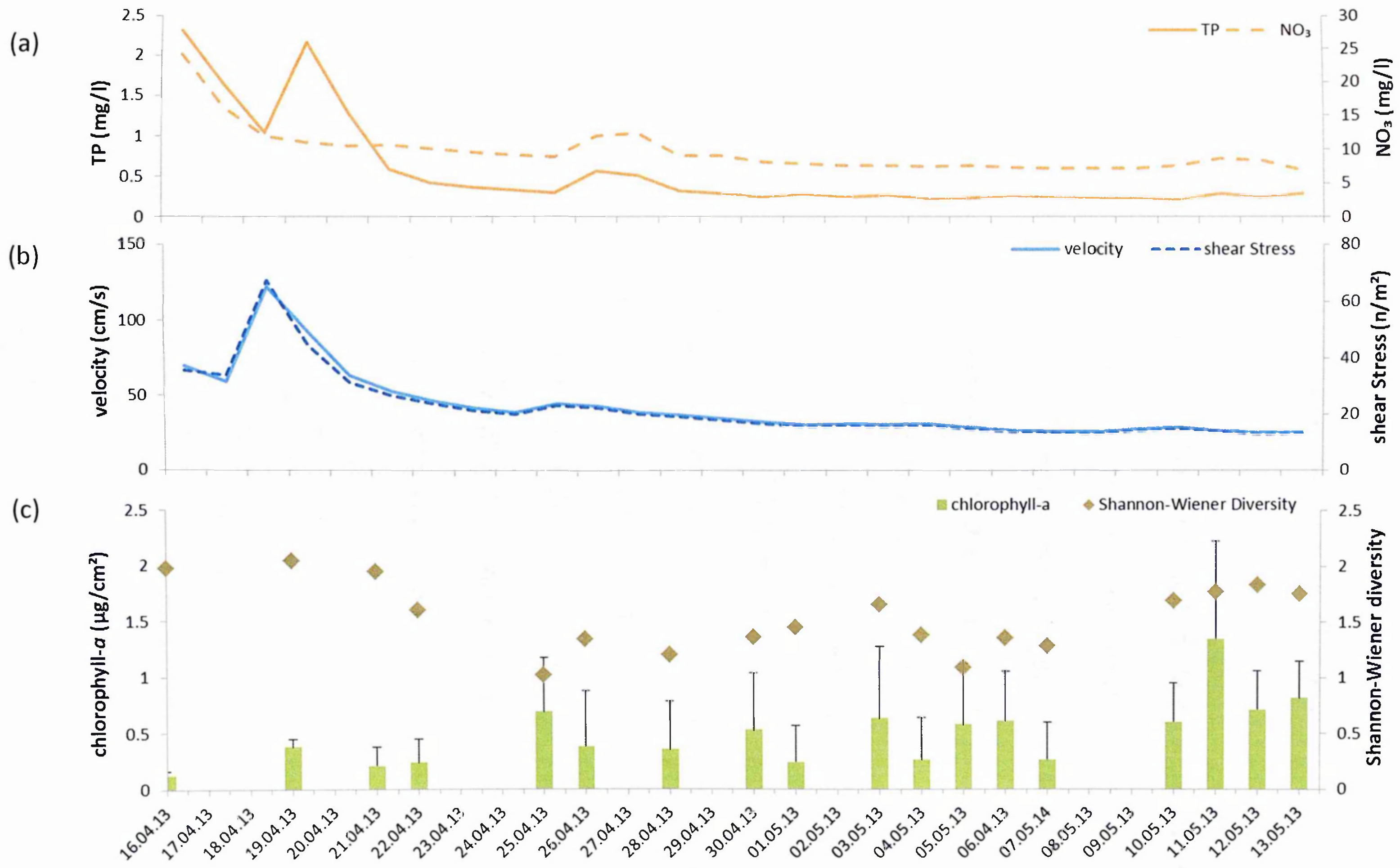


Figure 6.12: (a) 15 minute TP and NO_3 averaged over 60 minutes; (b) 15 minute velocity and shear stress averaged over 60 minutes; and (c) Shannon-Wiener (n = 9 per sampling event) diversity and chlorophyll-*a* (n = 9 per sampling event) between the 16/4/2013 and 13/05/2013.

6.3.9 Diatoms as indicators of environmental status through the TDI and EQR metrics under the Water Framework Directive (2000/60/EC)

6.3.9.1: Comparison of TDI and EQR values among riffle units

Mean TDI values across the riffle zones over the experimental period ranged from 41.4 to 44.1, thus indicating good trophic status (Figure 6.13 a). Mean EQR indicated "Good" ecological status over the same period (Figure 6.13 b). However, EQR demonstrated considerable variability over the experimental period (Riffle 1: average 0.93, range 0.83 – 1; Riffle 2: average 0.90, range 0.75 – 1; Riffle 3: 0.89, range 0.70 – 1). Within riffle unit 1 and 2, EQR values were within the range of "Good" ecological status but for riffle unit 3 EQR values declined to "Moderate" ecological status (Table 6.5). This emphasised the importance of reach scale variability and how ecological status can cross critical ecological status class boundaries over a 28 day period. Moreover, this demonstrated the need for both uniformity in sampling procedure and for appropriate sampling design that considers the EQR of at least one biotope unit in order to reduce uncertainty and bias in assessment of ecological status.

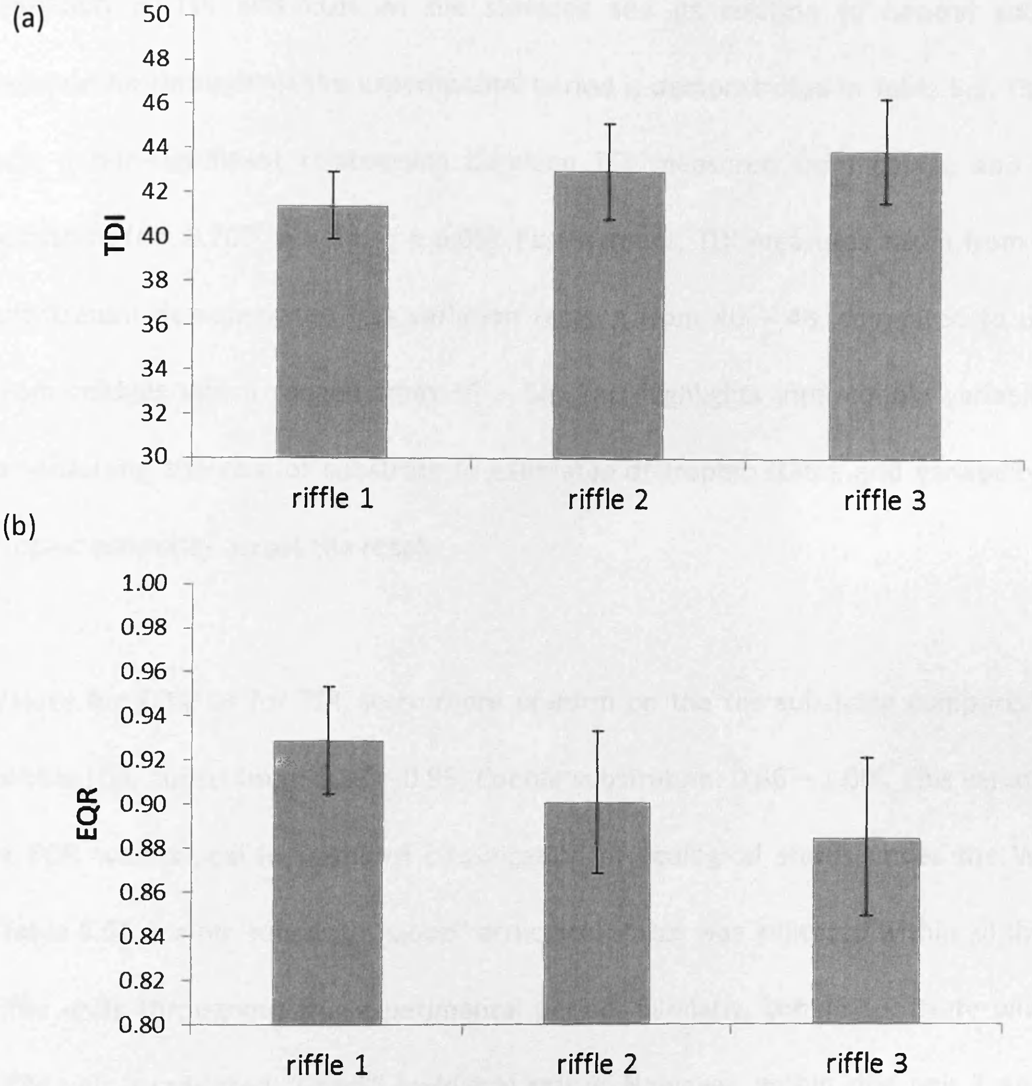


Figure 6.13: Mean (a) TDI and (b) EQR values per riffle unit (n = 18) between 16/04/2013 and 13/05/2013. For TDI values range from 0 - 100 with 100 indicating poor trophic status and 0 indicating high trophic status. For EQR values from 0 – 100 indicate increasing ecological status, with a maximum of 100 indicating "High" ecological status. EQR WFD UK TAG classification boundaries: Bad (< 0.25), Poor/Bad (0.25), Poor/Moderate (0.50), Moderate/Good (0.75) and Good/High (1.00).

6.3.9.1: Comparison of TDI and EQR values between cobble and tile substratum

Variation in TDI and EQR on tile surfaces and its relation to natural cobble substratum throughout the experimental period is demonstrated in Table 6.5. There was a non-significant relationship between TDI measured from cobble and tile substrate ($r = 0.709$, $n = 54$, $p < 0.05$). Furthermore, TDI measures taken from tile substratum demonstrated less variation ranging from 40 – 48, compared to data from cobbles which ranged from 36 – 58. This highlights inter-cobble variability, emphasising the role of substrate in estimates of trophic status and variability in trophic estimates across the reach.

Values for EQR, as for TDI, were more uniform on the tile substrate compared to cobble (Tile substratum: 0.83 – 0.95; Cobble substratum: 0.66 – 1.00). This variation in EQR was critical in terms of classification of ecological status under the WFD (Table 6.5). For tile substrate "Good" ecological status was indicated within all three riffle units throughout the experimental period. Similarly, cobble substrate within riffle unit 3 indicated "Good" ecological status. However, within rifle unit 1 and 2 ecological status ranged from "Good" to "Moderate" and from "High" to "Poor" respectively. This highlights, therefore, that there can be a difference of four quality classes for samples taken on the same day but from different substrates within the same biotope unit, reiterating, the findings in chapter 5, of the importance of substrate and implying the need for consistent methodology.

Table 6.5: TDI and EQR metric values and EQR class across each riffle unit between tile and cobble substrata between 16/04/2013 and 13/05/2013. For TDI values range from 0-100 with 100 indicating poor trophic status and 0 indicating high trophic status. For EQR values from 0 – 100 indicate increasing ecological status, with a maximum of 100 indicating "High" ecological status. EQR WFD UK TAG classification boundaries: Bad (< 0.25), Poor/Bad (0.25), Poor/Moderate (0.50), Moderate/Good (0.75) and Good/High (1.00).

	TDI cobble	TDI tile	EQR cobble	EQR tile	Class cobble	Class tile
riffle1_22.04.13	44.42	43.25	0.88	0.90	Good	Good
riffle1_28.04.13	49.25	41.08	0.80	0.93	Good	Good
riffle1_03.05.13	36.92	39.58	1.00	0.96	Good	Good
riffle1_04.05.13	43.08	47.08	0.90	0.84	Good	Good
riffle1_10.05.13	55.00	37.42	0.71	0.99	Moderate	Good
riffle2_22.04.13	38.83	42.67	0.97	0.91	Good	Good
riffle2_28.04.13	49.00	40.33	0.81	0.95	Good	Good
riffle2_03.05.13	31.75	44.97	1.00	0.87	High	Good
riffle2_04.05.13	48.75	41.42	0.81	0.93	Good	Good
riffle2_10.05.13	70.15	47.00	0.47	0.84	Poor	Good
riffle3_22.04.13	45.65	41.75	0.86	0.92	Good	Good
riffle3_28.04.13	41.58	44.33	0.93	0.88	Good	Good
riffle3_03.05.13	37.83	44.83	0.99	0.87	Good	Good
riffle3_04.05.13	51.50	43.00	0.77	0.90	Good	Good
riffle3_10.05.13	50.25	52.42	0.79	0.75	Good	Good

6.3.10 Response of diatom assemblage composition to changes in discharge and nutrient condition

Potential critical threshold relationships observed in this chapter between benthic diatom composition and discharge were explored. Discharge was used as a surrogate for hydrological factors and TDI was selected to reflect change in diatom assemblage composition mediated through changes in nutrients. Figure 6.14 presents a conceptual model illustrating how both increases and decreases in discharge from average conditions can lead to a decrease in TDI values, thus suggesting higher trophic status. This conceptual model and dataset demonstrated that decreases in discharge less than -30% from average and greater than -49% provide result in lower TDI values. It is important to note that in instances where sampling occurs immediately after a high discharge event, the TDI metric may give a false positive as TDI may be controlled more by discharge rather than nutrient condition. Below average discharge conditions contributed to both higher and lower TDI values. For this dataset average discharge conditions were 0.22 m^3/s with below average discharge conditions ranging from 0.11 – 0.19 m^3/s leading to more favourable trophic condition. Such changes in discharge and TDI are referred to as 'optimum conditions' and are highlighted in red in Figure 6.14a.

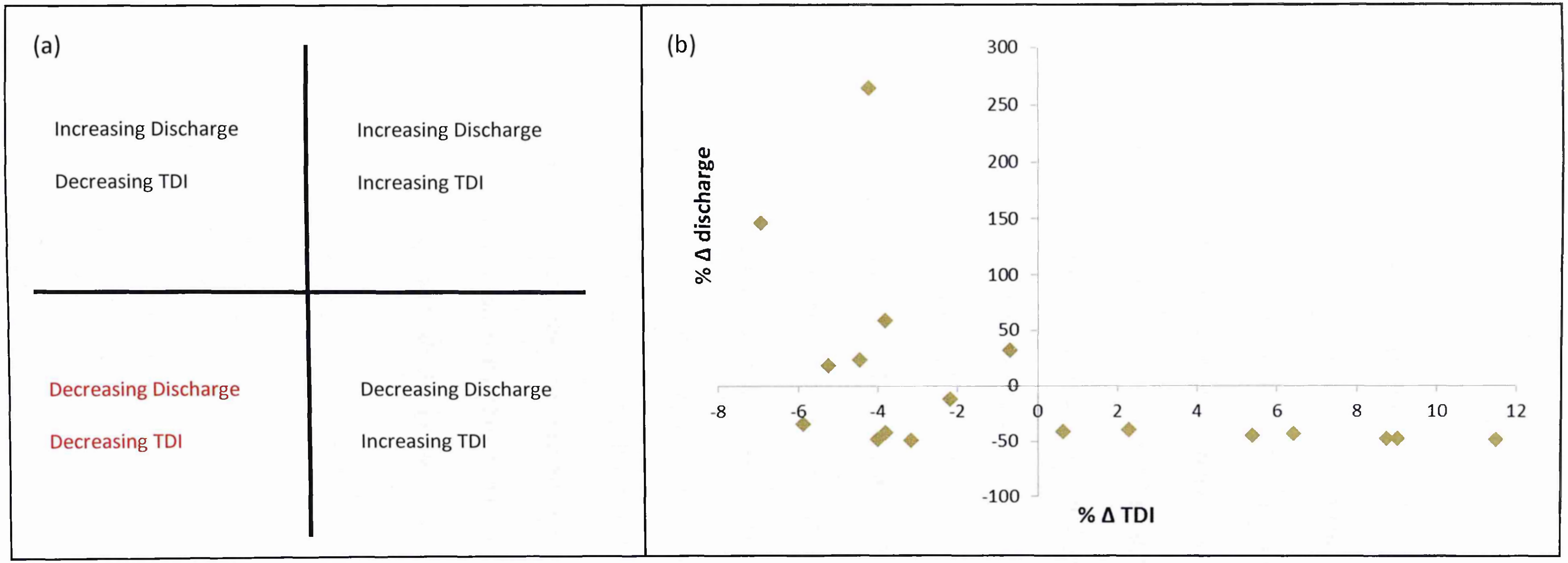


Figure 6.14: Relationship between percentage change in discharge and TDI metric score from average conditions over the experimental period with (a) presenting a conceptual model of optimum conditions; and (b) based 15 minute discharge data averaged to 60 minutes and average TDI across all three riffles on tile substrata (n = 3 per 18 sampling events), from 16/04/2013 to the 13/05/2014. Over the experimental period, mean discharge conditions were 0.22 m^3/s and TDI scores were 42.9. Below average discharge conditions (0.11 – 0.19 m^3/s) which contribute to higher trophic condition independent of discharge are referred to as 'optimum conditions' (highlighted red text Figure a).

6.3.11 Conclusion

Through this chapter it has been suggested that there is a threshold relationship between change in benthic diatom composition and chlorophyll-*a* production. Key species controlled assemblage composition on the tile substratum. These included *A. minitissimium*, *G. olivaceum* and *M. circulare.* Furthermore, there was considerable variability in benthic productivity and the relative contribution from the different spectral groups; diatoms, cyanobacteria and green algae. Despite this, diatoms provided the greatest contribution to assemblage productivity. Periods of highest productivity were associated with periods of high diatom diversity and this relationhip was mediated by changes in discharge and nutrient condition. Periods of high assemblage diversity and productivity were associated with a medium intensity rainfall event relative to catchment rainfall with a duration of three days. Key characteristics of events, therefore, are their frequency, intensity and duration and, when combined, these are key in terms of predicting benthic assemblage response. In conclusion, intermediate discharge and nutrient concentrations levels support high assemblage diversity and productivity, thus providing support for the hypothesis that communities actively respond to discharge, velocity and nutrient pulses.

Chapter 7: Discussion

7.1 Spatial and temporal pattern in benthic diatom assemblages

Headwater streams within the River Eden catchment demonstrated distinct communities at the catchment scale with differences detected in the key communities attributes of richness, evenness, diversity and chlorophyll-*a* within and among the three 10 km^2 catchments; Morland, Dacre and Pow. However, while differences were observed there was no evidence of discrete community types (*sensu stricto* Clements 1916), such as those hypothesised within chapter 4 (hypothesis 1) and presented under research aim 1 (section 1.3). Similarities amongst communities across catchments were constrained by the dynamic discharge regime and associated nutrient delivery (Biggs, 2000; Biggs and Smith, 2002; Biggs *et al.*, 2005). The high relative abundance of pioneer species; *Achnanthidium minutissimum* and *Amphora pediculus* was attributed to the dynamic hydrologic regime (Stevenson and Bahls, 1999; Cardinale, 2011) and was particularly evident in the Dacre catchment where *A. minutissimum* occupied 75% of the assemblage composition through the sampling period March 2011 to March 2013.

Catchment-scale factors, such as land use, together with differential run-off patterns are an important determinant of in-stream hydrological and chemical condition to which benthic communities respond (Johnson *et al.*, 1997b; Pan *et al.*, 2004). Through aim 2 of this thesis (section 1.3), this research demonstrated that benthic diatoms are responsive to ultimate and intermediate factors of rainfall (Figure 2.6 and 7.1) which can change over hours within the dynamic 10 km^2 headwater catchments (e.g. see section 6.3.2). The Dacre catchment, with high rainfall (Table 4.1) and flashy hydrological regime (Figure 4.2), is representative of upland-type conditions, and drains the surrounding fells of Great Mell Fell (altitude 537 m) and Little Mell Fell (altitude 505 m; Allen *et al.*, 2010). The resultant dynamic hydrological regime contributes to the notably high abundance of *A. minutissimum.* Greatest evidence of near cyclic patterns in diatom assemblage richness and evenness was present in the Dacre catchment (Figure 4.10 - 12), suggesting that rare species, through exhibiting differential tolerance to seasonal microhabitat differences, were important determinants of pattern in diatom assemblage structure (Cao *et al.*, 1998; Cao *et al.*, 2001; Lenat and Resh, 2001; Nijboer and Schmidt-Kloiber, 2004). Within the Pow catchment water chemistry was closely related to land use patterns within the catchment rather than wider biogeochemical processes. The most prominent common species within the diatom assemblage of the Pow catchment was *Cocconeis placentula.* These species demonstrated alternative cyclic patterns with *A. minutissimum.* Intensive agricultural practice within the Pow catchment underpins its eutrophic condition (Owen *et al.*, 2012). It is likely that *C. placentula* var. *euglypta* featured prominently due to its higher efficiencies for obtaining limiting nitrogen resources (Marcus, 1980). As widely demonstrated within the literature environmental gradients, especially in N and P, are important in determining community composition with composition itself reflecting the degree to which species tolerances overlapped (Cox, 1990; Kelly and Whitton, 1995a; Biggs, 1996b;

Battarbee *et al.*, 1999; Francoeur *et al.*, 1999; Kelly *et al.*, 2008a; Jarvie *et al.*, 2010) and this is applicable to small headwater catchments with dynamic flow regimes.

Patterns in the structural and functional composition of diatom assemblage investigated over a 28 day period highlighted the importance of temporal patterns in both delivery and timing of nutrients reaching headwater streams (section 1.3, aim 4). Rainfall to runoff patterns varied among the catchments (Table 4.1). Within the Pow catchment, rapid nutrient delivery path-ways were facilitated by ultimate factors, such as precipitation and clay base soils, and intermediate factors including the seasonality of arable farming practice (see Figure 7.1; Mainstone and Parr, 2002; Owen *et al.*, 2012). Within the Morland catchment, a cyclic seasonal pattern in *A. minutissimum,* which prefers circumneutral water of moderate enrichment, and *A. pediculus,* which becomes abundant in more nutrient enriched waters with pH > 7, was evident (Figure 4.6). This demonstrates that both land use and geological characteristics can impact upon assemblage composition through species sensitivity to changes in alkalinity and P (Johnson *et al.*, 1997b). In turn, this can directly influence EQR under the WFD ecological status classification (Figure 4.27; WFD-UKTAG, 2014).

Catchment specific differences are, therefore, important in understanding diatom community composition with sensitivity of response to terrestrial – aquatic linkages contributing to fine-scale differences in the relative abundances of catchment specific assemblages, as supported by Gothe *et al.* (2014). Headwater diatom assemblage composition can be explained within the hierarchical framework presented in Figure 7.1 with ultimate and intermediate factors important determinants of spatial and temporal patterns in benthic diatom assemblage composition (Stevenson, 1997; Habersack, 2000; Parsons and Thoms, 2007). Within the headwaters of the River Eden, the dominance of key pioneer species which differed among the sub-catchments of Morland, Pow and Dacre due to catchment specific discharge-nutrient regimes and trophic state (see section 4.2.3.1), prevented the formation of truly discrete communities. As a result, patterns in diatom communities in headwater streams can be interpreted within Gleasonian gradients and nestedness (Gleason, 1926; Heino and Soininen, 2005).

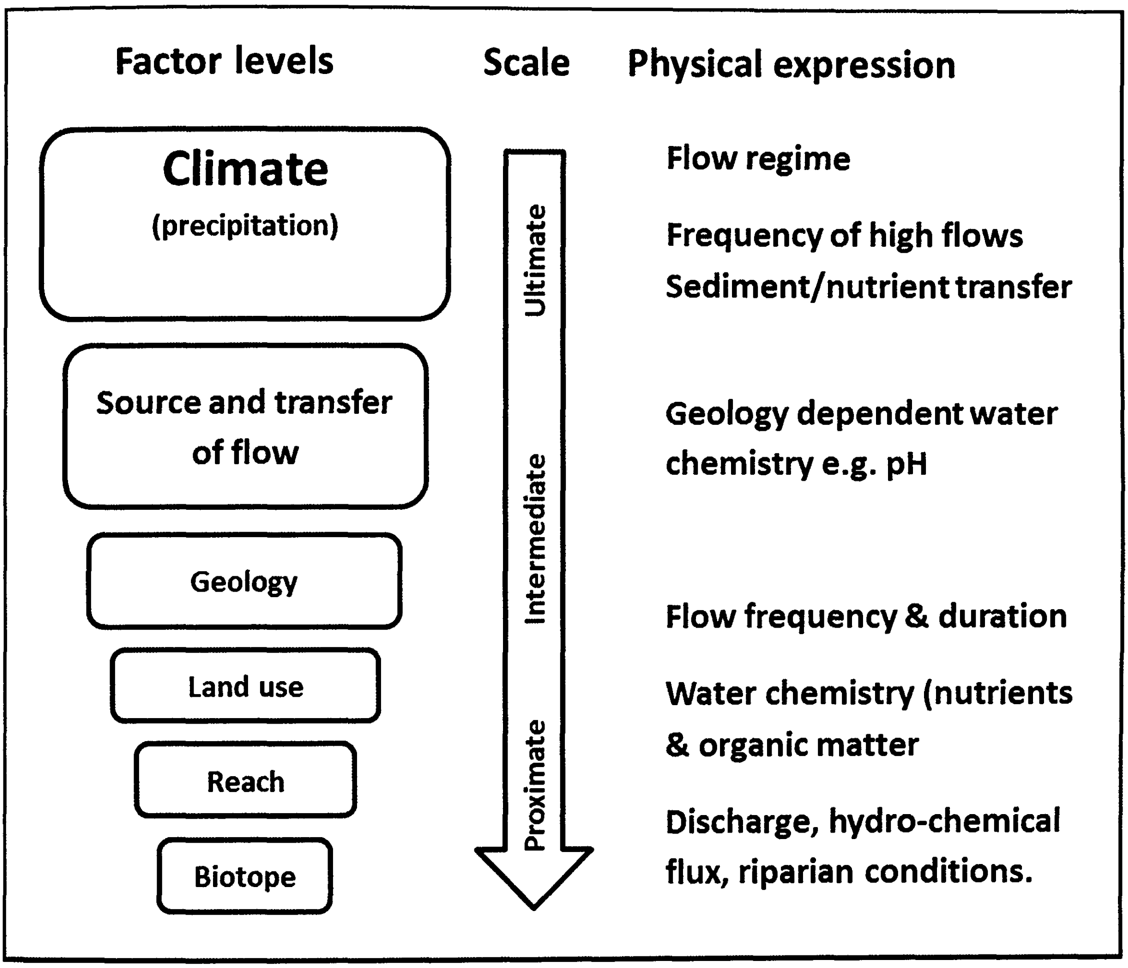


Figure 7.1: The hierarchy of factor levels impacting upon benthic diatom biofilm communities and the spatial scale at which various physical characteristics of headwater catchments are expressed. (Adapted from Stevenson 1997; Snelder and Biggs 2002).

Within Newby Beck, in the catchment of Morland, species richness varied according to the physical biotope unit, but evenness was influenced more by seasonal condition than spatial variability in habitat. As hypothesised in chapter 5 (section 5.1), pools were more diverse than riffle habitats with these trends increasing under spring conditions (section 5.2.4.1, Table 5.3). However, only statistically significant differences in productivity were observed between riffles and pools with riffles demonstrating higher and more heterogeneous productivity, as supported by Cardinale *et al.* (2002) (section 5.2.6.1, Figure 5.8). Higher chlorophyll-*a* concentrations can be attributed to greater hydraulic dynamism (velocity and shear stress; Figure 5.7; Hart *et al.,* 1999) and the associated rapid accumulation rates by pioneer species, *A. minutissimum and A. pediculus,* during the early colonisation phase of succession (Oemke and Burton, 1986). Differences in depth between riffle and pool units was also a key determinant of community composition and chlorophyll-*a* (section 5.2.6.2, Figure 5.8). Note, however, that depth is a co-variable for important factors influencing benthic biofilm growth and loss such as light, sedimentation, temperature, turbidity and velocity (Eros *et al.*, 2003; Abdul-Aziz *et al.*, 2010; Law, 2011). These findings support the hypothesis (section 6.1, hypothesis 2) that if temporal variation is held constant, physical variables of velocity, shear stress and depth, which are confined by physical biotope, determine benthic diatom composition and chlorophyll-*a* production. Therefore, differences in benthic biofilm productivity among the riffle and pools units support previously expressed views on the importance of biotope diversity for system functional diversity, processing and buffering against downstream transport (Vannote *et al.*, 1980; Newbold *et al.*, 1982; Newson and Newson, 2000; Fisher *et al.*, 2004; Demars *et al.*, 2012). Moreover, the ability of the physical biotope assessment approach to capture temporal dynamics in headwater stream biofilm dynamics, as demonstrated through chapter 6, makes this research highly relevant to exploring the spatial and temporal variability of habitat providing support for patch dynamics and the habitat template theory (Poff and Ward, 1990; Townsend and Hildrew, 1994; Newson and Newson, 2000; Biggs *et al.*, 2005; Clifford *et al.*, 2006; Harvey *et al.*, 2008; Harvey and Clifford, 2009).

Morphological traits provide a useful way of exploring these differences in composition and susceptibility to both abiotic and biotic disturbance (e.g. grazing and discharge-nutrient condition). Within riffle habitats high profile species were most prominent with motile species reaching higher relative abundance in pool biotope units (section 5.2.5). This suggests that spatial variations in hydrodynamic conditions are key contributing factors to variability in species composition through immigration and growth in riffle units. In pools the ability to migrate and exploit nutrients confers a competitive advantage. Therefore ecological guilds are useful for the exploration of the importance of riffle-pool sequences in determining community composition (research aim 3 and 4, section 1.3).

Across both the riffle and pool biotopes units' low profile diatoms provided the greatest contribution to assemblage composition (section 5.2.5). While the dominant presence of low profile species is predominantly due to their resistance to discharge events or resilience in terms of rapid immigration and colonisation rates, biotic factors may also be important. In headwater streams with their characteristically simple food web structure due to dynamic hydrologic regime (Marks *et al.*, 2000), the food web primarily consists of the benthic diatom biofilm which provides a food source for many invertebrate and fish (Minshall, 1978; Lamberti and Resh, 1983). The grazing ability of macroinvertebrates can change according to ecological guild type (Steinman *et al.*, 1991; Feminella and Hawkins, 1995; Passy, 2007b). For example, low profile species with adnate attachment to benthic substrate are resistant to grazing by macroinvertebrates (Steinman *et al.*, 1991; Steinman, 1996; McCollum *et al.*, 1998; Holomuzki and Biggs, 2006). The increase in abundance of low profile species in spring and autumn compared to high profile species may therefore be attributed to susceptibility to grazing. However, it is hypothesized that grazing is a significant controlling factor on community composition in this research due to the dynamic flow environment which together with substrate texture can be an important control on grazing (Dudley and Dantonio, 1991). It is also important to recognise that periods of quiescent discharge are also central to community dynamics with a higher proportion of high profile and motile species observed following a spring or autumn events. This may be attributed to the fact that recovery of macroinvertebrates following spates is slower compared to diatom growth rate (Biggs and Close, 1989; Mulholland *et al.*, 1991a). As benthic biofilms provide the basis for trophic structure in headwater streams an understanding of functional diversity, as determined by life-form, is important in determining their ability to support macroinvertebrate and fish communities particularly in the face of climate change (Rounick and Winterbourn, 1983; Mayer and Liken, 1987; Lamberti, 1996).

Morphological traits, through ecological guilds, provide an alternative way of exploring patterns in benthic diatom communities across catchments and their relationship to discharge-nutrient condition (research aim 1 and 2, section 1.3), demonstrating simple dynamics to change in environment condition (section 4.2.5). Moreover, results presented in chapter 6 demonstrate how rainfall characteristics (size, intensity and duration) can accentuate differences in community composition and chlorophyll-*a* within and among riffle units (Figure 6.10). However, during high rainfall-discharge events (as defined in section 6.3.2, Table 6.10) high discharge, velocity and shear stress combined with mobilisation of fine sediment can result in abrasion and sloughing of the benthic biofilm (Biggs and Close, 1989; Biggs and Smith, 2002) where the stationary layer about the bed becomes thinner and a larger area becomes exposed to turbulent flow. In such instances, shear forces and abrasion from suspended material can cause dislodgement and significantly reduce the height of the biofilm (Reiter, 1986; Power and Stewart, 1987; Resh *et al.*, 1988; Biggs and Hickey, 1994; Whiting *et al.*, 1999; Biggs *et al.*, 2005; Bouletreau *et al.*, 2006; Graba *et al.*, 2013). Such occurrences contribute to an increase in low profile species which can with-stand such pressure through their adnate attachment (Passy, 2007b; Rimet and Bouchez, 2012b). These effects are likely to be greater in riffles than pool biotope units (Robert, 1997; Wilkinson *et al.*, 2004), providing further empirical evidence for differences observed in diatom chlorophyll-*a* production between riffle and pool units. From a management and legislative perspective, these flow related habitat characteristics can be significant in terms of re-setting successional processes (Peterson and Stevenson, 1992; Biggs and Thomsen, 1995; Luttenton and Baisden, 2006), with successional state having a direct impact on response to future events. Moreover, as demonstrated with this study (section 6.3.9) and supported by Mykra *et al.* (2012) successional state can also impact upon metric scores and WFD classification.

Comparison of diatom assemblages on artificial and cobble substrate presented in section 6.3.3.1 showed that *Gomphonema olivaceum* was the most dominant species on tile substrate followed by *A. minutissimum* and *Meridion circulare*. This is an interesting assembly of dominant species as is suggestive of an advanced colonisation sequence and thus, contradicts successional theory (Hudon and Bourget, 1981; Hoagland *et al.*, 1982; Korte and Blinn, 1983; Stock and Ward, 1989). However, similar observations were found in other studies of diatom colonisation on artificial substrata (e.g. Plenkovic-Moraj *et al.*, 2008). *A. minutissium* has a relative small cell size with shallow valves and girdle. They are bent about the median transapical plane, allowing them to adhere tightly to a substrate, in particular curved substrata, via their raphid valve (Round *et al.*, 1990; Barbiero, 2000; Plenkovic-Moraj *et al.*, 2008). *A. minutissium* are early successional species which can also reproduce at relatively high growth rates enabling them to populate the surface before their competitors (Korte and Blinn, 1983; Peterson and Hoagland, 1990; McCormick, 1996). *M. circulare* is also an early succession coloniser with attachment via an aphical pad (Keithan and Lowe, 1985; Áce *et al.*, 2000; Plenkovic-Moraj *et al.*, 2008). However, unlike these two species *G. olivaceum* is typical of species associated with later successional stages due to its production of longer stalks. Note, this species can also produce shorter stalks depending on the flow environment and resulting colonisation time (Plenkovic-Moraj *et al.*, 2008). The stability offered by attached artificial tile substrate is likely to favour later successional species which are sensitive to physical perturbation but able to exploit available nutrient resources (Passy, 2007b; Rimet and Bouchez, 2012b). In comparison, on cobble substrate the community was comprised primarily of *A. minutissimum* and *Achnanthidium biasolettiana*. Similar to tile substrata *G. olivaceum* was prominent within the cobble assemblages. The dominant presence of *A. minutissimum* and *A. biasolettiana* is likely a direct result of exposure to increased substrate heterogeneity in terms of surface texture and bed load movement offered by natural occurring substrata (Tett *et al.*, 1978; Murdock and Dodds, 2007). Therefore, the comparison of community composition on cobble and artificial tile substrata emphasises the role of physical habitat in determining assemblage composition and provides support for previous studies illustrating the importance of substratum (Murdock and Dodds, 2007) and influence of bed load movement in these dynamic streams (Biggs and Smith, 2002).

Through addressing aim 3 of this thesis (section 1.3), it has been demonstrated that the physical flow biotope unit is an important determinant of benthic biofilm diversity and productivity. These findings also suggest that short term dynamics in biofilm diversity and chlorophyll-*a* dynamics can be concealed by variability if the spatial resolution of sampling is either too fine or too coarse. For example, quantifying dynamics at cobble scale within biotope units will reveal much greater variability in chlorophyll-*a* as result of micro-scale biofilm processes (e.g. nutrient cycles, light attenuation) and habitat (e.g. shear stress, velocity). Conversely, benthic biofilms sampled at larger spatial scales may incorporate several different biotope units (e.g., riffles, runs and pools) among reaches. This will reflect elements of community stability (resistance, resilience, recovery) incorporating variable successional stages and species growth rates. Consequently, as a result of investigation into spatial variance though biotope units, findings of this research suggest that habitat heterogeneity should be considered as an important characteristic of biotope units and that biotope units present an appropriate unit of ecological assessment.

7.2 Response of benthic diatom biofilm to antecedent conditions; discharge and nutrients

Increases in discharge in headwater catchments can occur rapidly within timescales of hours to days with equally rapid recovery from peaks to baseline conditions (section 6.3.2). Within the Morland catchment, steepness of the terrain and shallow soils overlying bedrock are attributed to the flashy hydrograph observed within the catchment. Similarly as clay-rich glacial till is widespread in the Pow catchment, surface runoff can be generated quickly following rainfall (Mainstone and Parr, 2002). More widely in other catchments this flashy hydrological response has been shown to contribute to extremely variable nutrient concentrations (Johnson *et al.*, 1969b; Hinton *et al.*, 1997; Bowes *et al.*, 2012), which benthic communities, with their longer regeneration times, must respond to (Peterson and Stevenson, 1990; Stevenson, 1990). Hence, as demonstrated in Figure 7.1, ultimate hierarchical factors of geology, soil type and rainfall offer the primary control to benthic diatom assemblage structure and productivity which is coherent between these geographically related sites. This is also evidenced by species life-form and the relative abundance of ecological guilds (see chapter 4).

Despite the dynamic nature of the physical environment, strong similarities were observed in the overall structural and functional benthic diatom biofilm composition. Supporting hypothesis 3 present in chapter 4, it was found that at any point in time the benthic diatom community (using TDI as a surrogate) represented a critical time period which was reflective of accumulative antecedent temporal dynamics in discharge-nutrient condition. In Morland, a catchment with moderate nutrient and agricultural pressure, TDI reflected discharge and TP conditions over the preceding 18 and 15-21 days respectively. For the Pow catchment, which has a higher relative nutrient and agricultural pressure, TDI reflected conditions over a broader antecedent window of 21 days for discharge and TP conditions between 7-12 days. These findings indicated that at-a-point, community composition is a product of factors related to discharge over the preceding 15 - 21 days. Moreover, given the positive relationship between discharge and TP, it is possible that the relationship between TDI and discharge is partly mediated by nutrient concentration.

In Newby Beck and Pow Beck, a non-significant relationship was found between benthic chlorophyll-*a* and antecedent discharge-TP conditions, thus indicating that antecedent conditions over the preceding 21 days is not a key determinant of benthic productivity which may be due to disturbance frequency (Lohman *et al.*, 1992). This is consistent with community structure being defined by nutrient supply and retention within benthic biofilms (Findlay and Sinsabaugh, 2006), whereas physical controls on productivity, especially damage to biofilms through scouring, may be expected to have a more immediate influence (Biggs and Close, 1989). Therefore, this analysis demonstrated that aspects of community structure and ecological functional processes, such as chlorophyll-*a* production, respond differently to antecedent conditions, and that this may be dependent on catchment specific factors such as geology and land use which may be equally important determinants of benthic community composition as climate.

7.3 Trade-off and thresholds for understanding and predication of diatom assemblage diversity and productivity

Environmental gradients in discharge and nutrients (both in-stream and within the catchment) condition, confined through the hierarchical framework in Figure 7.1, have been found to be key determinants of biodiversity and influence productivity (chapter 4). Gradients in environment characteristics are a major source of temporal and spatial heterogeneity in headwater communities and constitute a key driver of natural selection and critical trade-offs in biological processes (Roxburgh *et al.*, 2004; Fraterrigo and Rusak, 2008; Pathikonda *et al.*, 2009). Within this thesis a unimodal relationship was found between catchments. A unimodal relationship was first proposed by Grimes (1973) to describe herbaceous vegetation in Britain and subsequently has been acknowledged as a general empirical relationship (Begon *et al.*, 1996), frequently proposed for other systems within the literature although it remains controversial (Mittelbach *et al.*, 2001). However, few such relationships have presented for headwater systems despite the long standing recognition of their importance of catchment functioning.

7.3.1 Critical trade-offs in biological attributes of diatoms determining biofilm compositional diversity

Figure 7.2 presents an intentionally simple conceptual model which is based on the finding of a unimodal relationship between benthic biofilm diversity and productivity. It proposed that critical trade-offs between key species traits (e.g. colonisation, competition and facilitation) found within assemblages of the River Eden sub-catchments are both the cause and consequence of the unimodal productivity relationship that is underpinned by the critical threshold relationship between change in discharge-nutrient condition and species diversity. Trade-offs exist as diatoms have the ability to respond within short times frames (15 - 21 days; section 4.2.10) to temporally rapid changes in discharge and nutrients (TP and NO_3). Moreover, differential ecological strategies among benthic diatom species to environment stress (discharge) and resource limitation (TP, NO_3) provides further resilience (Elmqvist *et al.*, 2003; Mori *et al.*, 2013). It should be stressed that this model necessitates interpretation within the context of the hierarchical framework proposed in Figure 7.1. This is important in order to understand the mechanisms underpinning the relationship between diversity and environmental variation. For example, processes occurring at the ultimate and intermediate factor levels, such as precipitation, land use and flow patterns, impact upon aspects of ecological stability (resistance, resilience and persistence). These, together with proximate factors and biotic interactions (e.g. competition and grazing) influence the fine scale structural and functional response of the diversity of the benthic diatom biofilm to environment gradients.

As suggested by the conceptual model presented in Figure 7.2, both positive and negative trade-offs occur in community traits across a discharge-nutrient disturbance gradient resulting in peak diversity. Within this thesis, positive traits-off are observed in terms of the species pool and colonisation. Such periods of rapid species colonisation from the available species pool correspond with high discharge disturbance. The immigration of species from the available species pool is critical for system stability within the dynamic headwater environment (Lake, 2000) and highlights the importance of species diversity in imparting resilience to the benthic assemblage. Positive correlation is observed between colonisation rates and increasing discharge. This is because small adnate diatom species, typified through pioneer species including *Achnanthidium, Amphora* and *Cocconeis* sp., present within the headwaters of the River Eden, can colonize bare cobble substrate and undergo exponential growth (Korte and Blinn, 1983). This exponential growth is mediated by the availability of resources mediated through current velocity and the cell mode of reproduction (Horner and Welch, 1981; Oemke and Burton, 1986; Stevenson, 1996). More specifically, the small cell size and morphology of these pioneer species contributes to their dispersal and settlement on cobble substrata (Jones, 1978; Kaufman, 1980; Osborne, 1983; Stevenson, 1983; Bothwell, 1989; Peterson and Stevenson, 1990) facilitating rapid linear immigration and colonization processes (Stevenson *et al.*, 1991). Following establishment, the small adnate species become resistant to the variable discharge regimes due to their cell morphology and attachment. Therefore, once established on the cobble substrate they can also withstand changes in discharge in particular when persistence times increase allowing for reproduction (Steinman and McIntire, 1990; Passy, 2007b). Periods of lower discharge and higher nutrient condition, provide opportunities for community succession and for later colonisation species to form the three-dimensional biofilm structure. Development of the three-dimensional biofilm structure gives rise to competitive interactions within the biofilm initially for nutrients between the high profile and motile species but later for space between high and low profile species (McCormick and Stevenson, 1991; McCormick, 1996). Declines in diversity are observed along such a successional gradient as the three-dimensional canopy is more susceptible to grazing and scour (Pan and Lowe, 1994; Bouletreau *et al.*, 2006; Passy, 2007b).

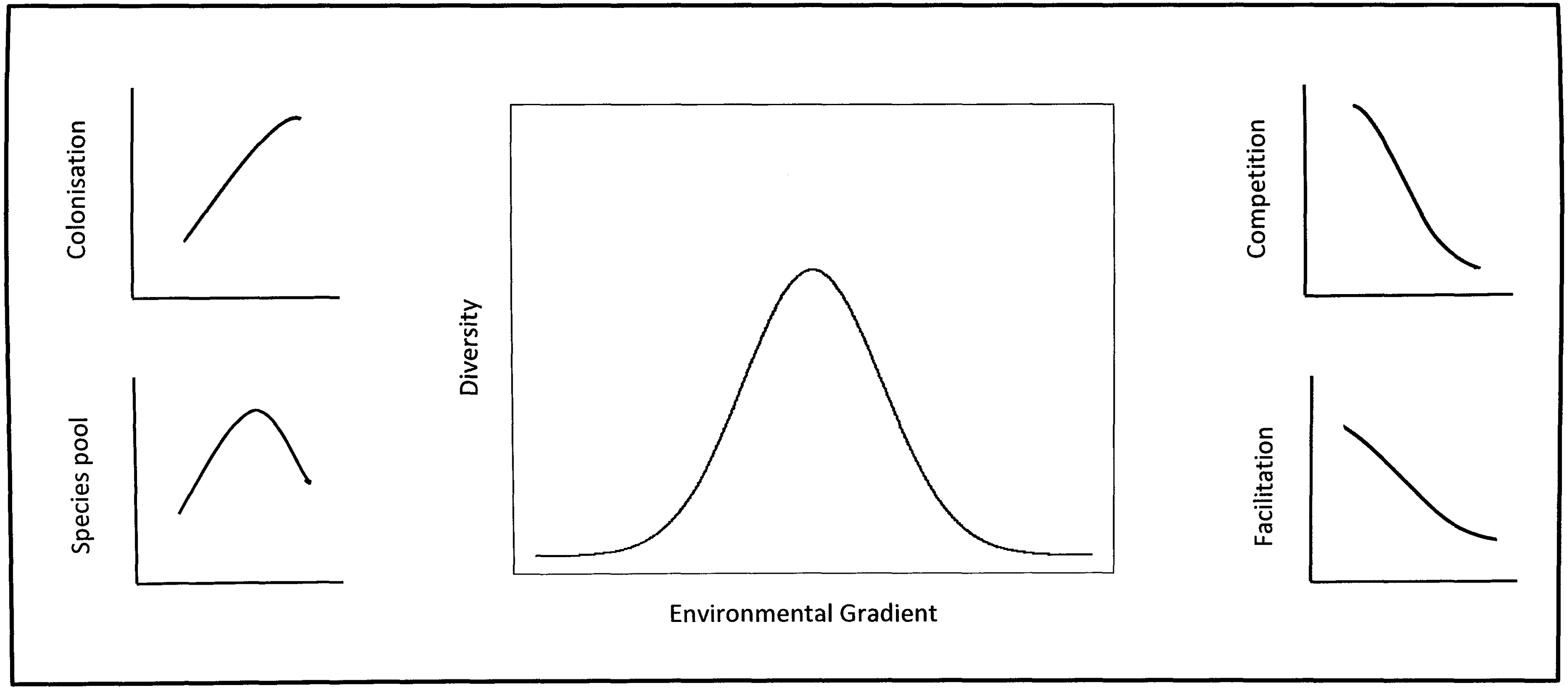


Figure 7.2: Conceptual model of the critical trade-offs in biological traits and processes underpinning diversity and its unimodal relationship to environmental gradients (discharge and nutrients (TP and NO_3).

7.3.2 Critical thresholds in disturbance characteristics determining biofilm community diversity and productivity.

Based on the principles of the Intermediate Disturbance Hypothesis (IDH; Connell, 1978) a conceptual model (Figure 7.4) has been proposed for headwater streams based on the findings of this thesis. This model demonstrates the relationship between the rate of disturbance and species diversity along with the correspondent relationship with chlorophyll-*a* production. This is based on the findings of chapter 6 which demonstrated the threshold relationship between change in benthic diatom composition and chlorophyll-*a* production that occurs as a result of discharge-nutrient pulses (Figure 7.3).

Based on the data presented in chapter 6 and summarised in Figure 7.3, a unimodal relationship, which is composed of two peaks in diversity represented by assemblage type 'A' and 'B', is proposed for headwater streams (Figure 7.4). The critical biological processes which determine assemblage diversity are presented in Figure 7.2 and are used to inform understanding of the community composition of assemblage type 'A' and 'B'. The high rainfall event (section 6.3.2, Table 6.2, Rainfall Event 1 - 2) at the start of the experimental period from 16/04/2013 to the 19/04/2013 (see Figure 7.3) contributes to high discharge disturbance but also high diversity and is represented by assemblage type 'A'. This community is composed primarily of low profile pioneer species such as *A. minutissimum, A. pediculus* and *C. placentula* var. *euglypta*. Low profile and common pioneer species out-compete species for available space through their rapid colonization (McCormick and

Stevenson, 1991). Therefore, high diversity during the high intensity event may be a function of space available for colonisation (Petraitis *et al.*, 1989). Moreover, Figure 7.3 demonstrated that there was a higher relative proportion of motile species during the period of high disturbance. The ability of mobile species to move from disturbance and exploit the delivery of nutrients (TP and NO_3) to the stream makes them competitively superior to the high profile species which are excluded through scour and abrasion (McCormick and Stevenson, 1991; Biggs *et al.*, 1998b; Davie *et al.*, 2012). Therefore, this highlights the importance of trade-offs in species morphological traits at different threshold levels in environmental factors (i.e. discharge and nutrients). As conceptualised in Figure 7.4, during periods of high disturbance benthic biofilm chlorophyll-*a* concentration was low (see Figure 7.3) and this was attributed to scour and abrasion of the biofilm.

The second peak in the unimodal relationship between diversity and disturbance, as evidenced through assemblage type 'B', represents the response of the benthic diatom biofilm and its production to critical threshold in discharge characteristics and associated nutrient condition as identified in chapter 6 (see section 6.3.2 Rainfall Event 5). During periods of lower disturbance, competitive species exploit available nutrient resources but are limited by discharge through scouring. This also favours low profile species through increasing light and nutrient availability with the biofilm. Moreover, removal of dead and senescent material will increase the availability of open area for colonisation. Delivery of TP and NO_3 to the stream during Rainfall Event 5 (09/01/2013 – 13/05/2013; Figure 7.3) also facilitated an increase in abundance of motile species. This illustrated that relative contribution of ecological guilds to assemblage composition increased during periods of low flow (Figure 7.4). Moreover, this demonstrated the occurrence of trade-offs in biological traits such as facilitative processes between high profile and motile species for nutrients and competitive interactions between high and low profile species for space and light (competitive species; Grimes, 1979, Passy, 2007).

Unlike the patterns between diversity and disturbance which are determined through critical thresholds, patterns in chlorophyll-*a* reflect a trade-off between accrual through immigration, colonization and exponential growth and loss due to processes of abrasion, scour and sloughing as the successional sequence develops (Biggs, 1996b). The trade-off between these two processes (accrual and loss) is dependent on critical threshold in flow regime and associated nutrient condition (TP and NO_3; Biggs and Close, 1989). During such critical threshold periods in discharge-nutrient condition, water velocity and elevated nutrient concentrations, are likely to maintain higher diffusion gradients within the biofilm (Biggs *et al.*, 1998a). This results in thicker biofilm structures and consequently, increased productivity (Horner *et al.*, 1990; Biggs and Gerbeaux, 1993). Therefore, high intensity events (Table 6.1, RF 1 - 2) can lead to the sloughing of the biofilm while critical threshold conditions in discharge-nutrient condition as evidenced through Rainfall Event 5 (09/05/2013 – 13/05/2013; Table 6.1, Figure 7.3) events stimulate biomass production. This latter scenario represents peak chlorophyll-*a* concentration for dynamic headwater streams (Figure 7.4, assemblage type 'B').

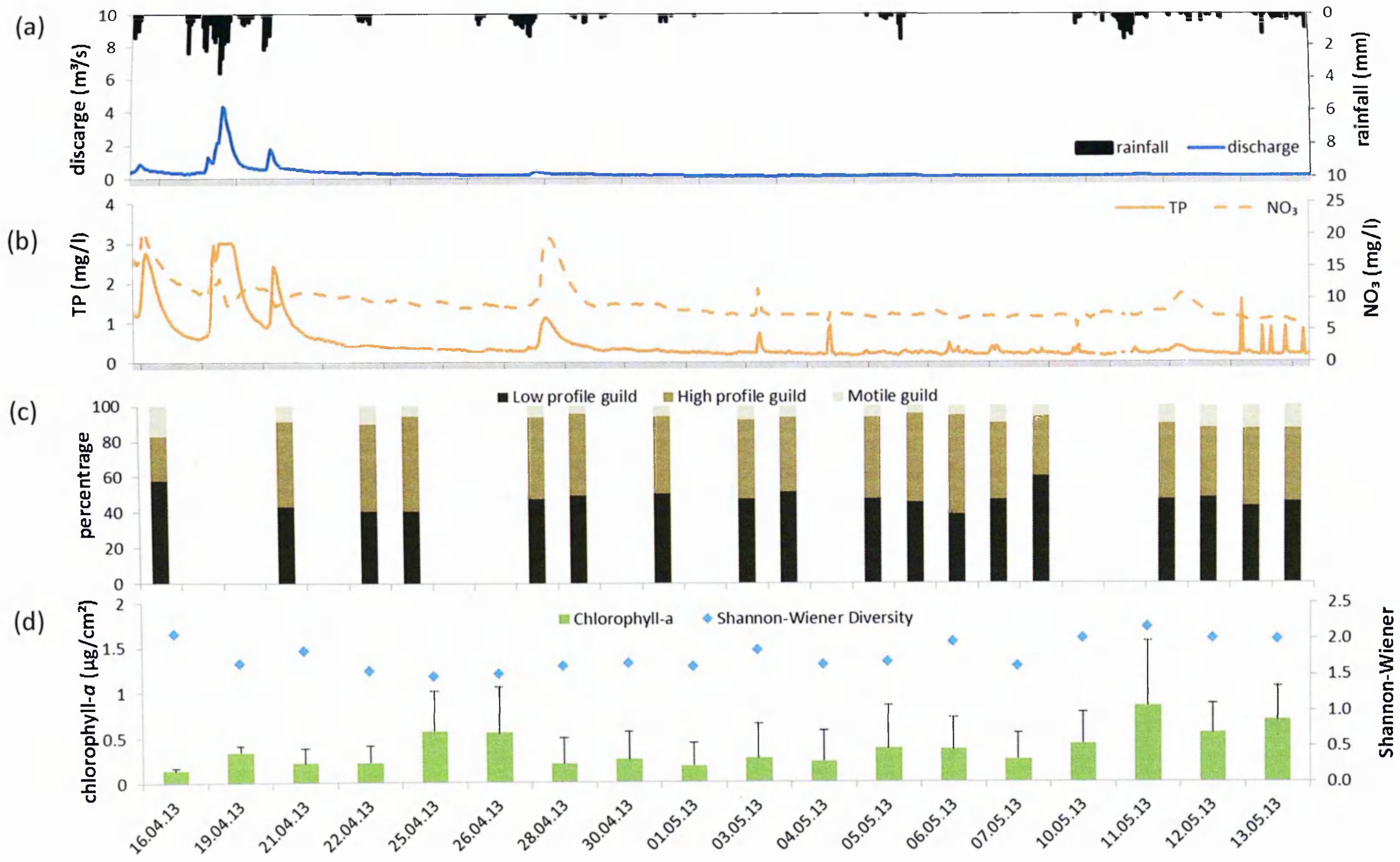


Figure 7.3: (a) 15 minute rainfall and discharge averaged over 60 minutes, (b) 15 minute TP and NO_3 averaged over 60 minutes, (c) relative percentage abundance of ecological guilds; low, high and motile. (d) Shannon-Wiener (n = 9 per sampling event) diversity and chlorophyll-*a* (n = 9 per sampling event 1 - 18) between the 16/4/2013 and 13/05/2013.

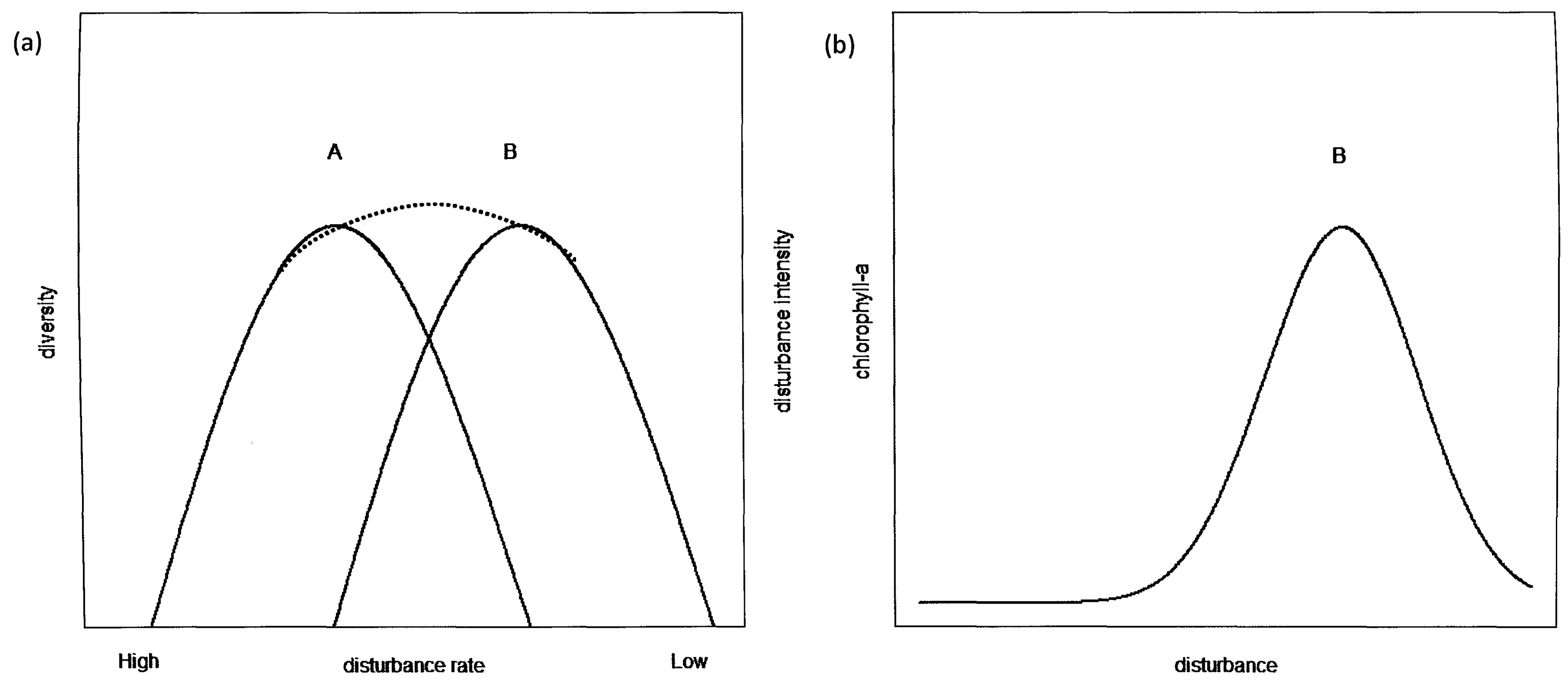


Figure 7.4: (a) The influence of disturbance rate on species diversity. Peak A represents assemblage type 'A' corresponding with an high intensity rainfall event while peak B represents assemblage type 'B' associated with critical threshold discharge-nutrient conditions which facilitate high benthic biofilm diversity and chlorophyll-*a* production. High intensity and rainfall events at critical threshold generate a peak in diversity at intermediate rate of disturbance for that event. (b) The effect of disturbance on chlorophyll-*a* accrual and loss with B representing assemblage type 'B' and peak chlorophyll-*a* concentrations. (Adapted from Miller 1981, Biggs 1996).

7.3.3 Relationship between benthic diatom biofilm diversity and productivity.

Productivity – diversity relationships were investigated over two scales; catchment and reach to address the hypothesis that these descriptors of diatom community attributes are linearly or inversely related (chapter 4, hypothesis 2 and chapter 5, hypothesis 3 respectively). At both scales variability in both time and space was investigated. Shannon-Wiener diversity was used as a proxy for diversity and ISF chlorophyll-*a* as a proxy for productivity. Many studies measure static algae on natural substrate as surrogate of primary production in streams (e.g. Morin *et al.*, 1999). However measuring productivity through surrogates may weaken observed relationships (Mittelbach *et al.*, 2001). Furthermore, it is important to stress that chlorophyll-*a* concentration may not directly relate to the rate of metabolism because it is the product of the interaction between net primary production, activity of grazing invertebrates, the physical disturbance regime and associated changes in water chemistry (Fellows *et al.*, 2006).

A unimodal relationship was observed between diversity and productivity across catchments within this thesis. Much of the literature on diversity-productivity relationship supports this observation (Grime, 1979; Rosenzweig and Abramsky, 1993; Leibold, 1999; Waide *et al.*, 1999; Mittelbach *et al.*, 2001; Chase and Leibold, 2002). However, considerable theoretical debate exists within the literature on the form of the species richness-productivity relationship and its scalar dependency (Gross *et al.*, 2000; Chase and Leibold, 2002; Foody, 2004; Whittaker, 2010). As proposed by the conceptual model in Figure 7.2, the unimodal relationship requires trade-offs in species characteristics with respect to environmental gradients (primarily colonizing versus competitive ability in headwater streams). The trophic gradient at the catchment scale influences the productive capacity of the stream (Biggs, 2000). In the Dacre catchment under oligotrophic conditions, the greatest range in diversity was observed compared to both the Morland and Pow catchments. However, higher productivity was observed in the Morland catchment under mesotrophic conditions. Eutrophic conditions in Pow contributed to relatively high diversity values but low productivity (section 4.2.6; Figure 7.5). Common species, especially pioneer species, tolerant of dynamic flow condition within each catchment are likely to contribute to the unimodal relationship, while rare species with more stringent requirements are restricted to catchment specific factors, thus providing spread in the data.

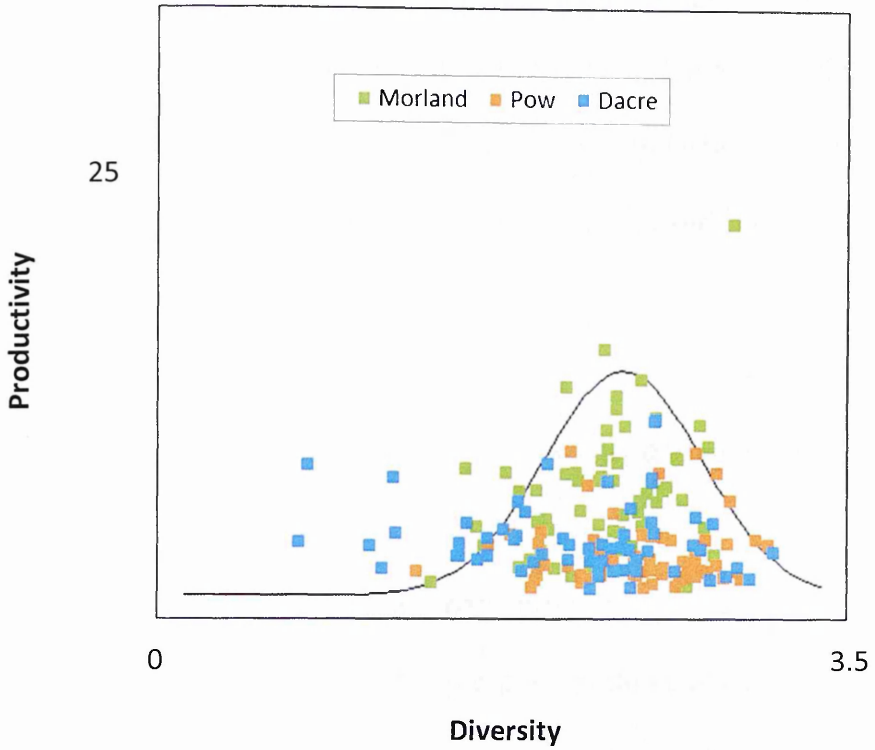


Figure 7.5: Unimodal relationship between diversity (Shannon-Wiener diversity) and productivity (chlorophyll-*a*) across catchment from March 2011 to March 2013. The unimodal curve is for illustrative purposes only.

A challenge facing the advancement of the understanding of diversity–productivity relationships is the transferability of observed patterns among scales given environmental heterogeneity and spatial-temporal variation (Kominoski *et al.*, 2010). Within this research no relationship between diversity and productivity was found at the reach scale. This may be attributed to the absence of gradient in species richness over space and time at this scale. More specifically, the combination of dynamic near-bed, substrate heterogeneity and the relatively uniform chemical environment found within biotope units observed in the absent of time, may contribute to increased species diversity per fixed area. When diversity of diatom species is large, comprising species of different physiology or life history a single dominant trade-off may not exist, thus reducing the likelihood of detecting a unimodal pattern (Scheiner and Willig, 2005). Consequently, this study clearly demonstrated the importance of considering the multifactorial mechanisms maintaining biodiversity and that the shape of the productivity-diversity relationship depends on the spatial scale under consideration.

7.3.4 Application of the Multivariate Productivity Diversity Hypothesis

Within this research the multivariate productivity – diversity (MPD) hypothesis (Cardinale *et al.*, 2009) was used to explore patterns of co-variance in richness and chlorophyll-*a* at Newby Beck (Morland) mediated by the sensitivity of the benthic assemblage to resource supply (TP). Application of the MPD demonstrated that daily averaged TP conditions over the previous 21 days had a negative but non-significant relationship with species richness (Figure 7.6). Increasing concentrations of TP lead to a decline in species richness, thus suggesting that higher TP concentration contributed to a more homogeneous assemblage composition. An important co-variable was discharge to which TP was significantly correlated ($r = 0.817$, $n = 13$, $p < 0.05$). This again re-iterated that discharge-TP were important intermediary determinants of the functioning of these headwater systems with regards to biomass production.

Within the context of this research there was an apparent "optimum" richness at approximately 3.5 which allowed for a relatively constant chlorophyll-*a* concentration of 2 - 4 μg/cm^2 (Figure 7.6). The inverse relationship between species richness and TP suggested that values of Margalef richness index which approach the optimum of 3.5 reflected low TP conditions resulting in lower chlorophyll-*a* values. This suggests that resource supply to these streams may actually set an upper limit to both the richness and evenness of the assemblage providing support for the work of Cardinale *et al.* (2006) and Passy (2010). Conversely, values which fall outside this optimum and declining from Margalef richness index value of 4, suggest that higher TP conditions and lower species richness contributed to concentrations of chlorophyll-*a* greater than 4 μg/cm^2. Therefore, resource supply mediated through the variable headwater discharge regime limited the rate of new production by influencing the richness of the species assemblage that can both withstand discharge disturbance and compete locally for resources. Through a resource delivery effect productivity was influenced by species richness, thus suggesting that chlorophyll-*a* is the consequence rather than cause of biodiversity.

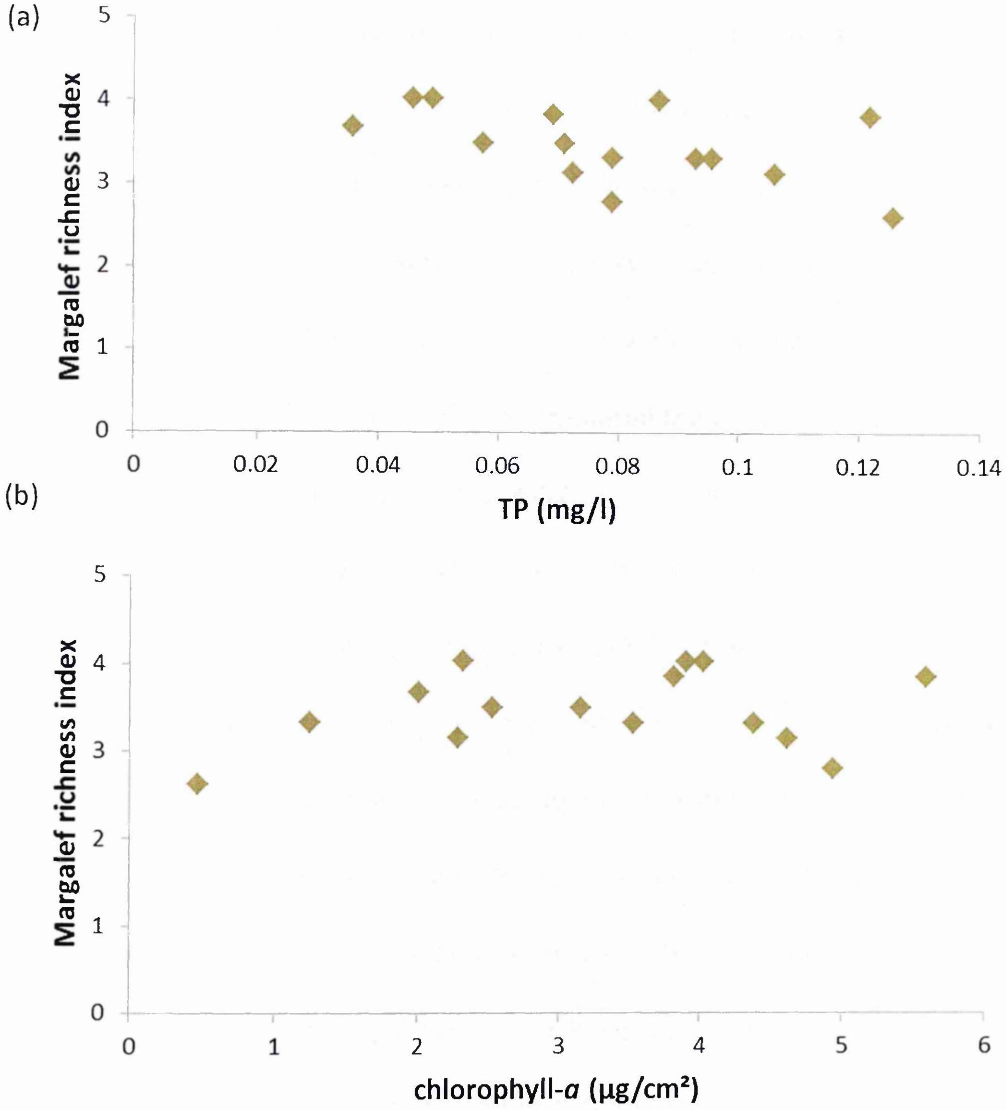


Figure 7.6: (a) species richness (Margalef richness index) (n = 15) and mean antecedent TP condition over previous 21 days (n = 15) and (b) species richness (Margalef richness index) and chlorophyll-*a* (n = 15) from October 2011 to January 2013.

7.4 Predicting chlorophyll-*a* concentrations from trophic condition

In-stream benthic biofilm structure and production can be limited by N, P or co-limited by both N and P (Tilman, 1977; Tilman, 1982; Bothwell, 1989; Pringle, 1990; Stelzer and Lamberti, 2001; Dodds *et al.*, 2002; Luttenton and Baisden, 2006). Note, that Si is also important for diatom growth (Lewin and Reimann, 1969; Martin-Jezequel *et al.*, 2000) but not considered limiting within the context of this research (Allan and Castillo, 2007). For the UK, P is considered the principal limiting nutrient in aquatic systems (Mainstone and Parr, 2002; Withers and Lord, 2002). However, findings of this research have demonstrated that the variable hydrological regime within these predominately agricultural headwater streams, high rainfall:runoff ratios and associated nutrient delivery to steams, can cause changes in nutrient and discharge conditions over time scale of hours. Consequently, NO_3 may become limiting (Gburek and Sharpley, 1998; Mainstone and Parr, 2002; Sharpley *et al.*, 2008). Also higher relative inputs of TP into the system and initial dilution of NO_3 immediately after events may also be associated with potential N limitation (see section 4.2.2, Table 6.2). In addition to the synchronicity of TP and NO_3 delivery to the stream, periods of biological demand may be important determinants of the impact of N and P on stream biota under varying flow conditions (Jarvie *et al.*, 2006; Edwards and Withers, 2007). Hence, the availability of these key nutrients can vary temporally and over short time frames due to the timing of certain hydrological events and their frequency and intensity which benthic biofilms must respond to.

Differential chlorophyll-*a* concentrations observed across the 10 km^2 sub-catchments of the River Eden of different trophic status provides insight into the relationship between chlorophyll-*a* production and nutrient concentration. The Morland catchment, with intermediate-level agricultural pressures and nutrient concentration had the highest chlorophyll-*a* concentration, the Pow catchment with intensive agricultural practice demonstrated the lowest chlorophyll-*a* concentration but highest nutrient concentration, while the upland, low pressure catchment of Dacre demonstrated intermediate chlorophyll-*a* concentrations and very low nutrient concentration (Table 7.1). Within the Pow catchment an apparent paradox was evident with eutrophic conditions coinciding with low chlorophyll-*a* production. This may be due to nutrient limitation in terms of limiting concentrations of N for optimum N:P ratios which, therefore, limit diatom accrual and productivity within the biofilm matrix (Luttenton and Baisden, 2006; Elser *et al.*, 2009). Moreover, shallow depth and higher sedimentation rather than self-shading within the biofilm (see section 7.6.2 for further discussion of ISF measurements and caveats) may contribute to the lower primary productivity.

This research illustrated that a key factor controlling differences in productivity across the headwater catchments of the River Eden was agricultural determined trophic condition. Therefore consideration of catchment specific run-off patterns and discharge-nutrient regimes attributed to climate (e.g. precipitation) and the seasonal farming calendar is important for identification of periods of high sensitivity of diatom assemblage composition and productivity to nuisance levels of algae growth. Across the headwater catchments of Morland, Pow and Dacre, chlorophyll-*a* concentrations ranged from 0.04 to 20.89 μg/cm^2, thus within the range of what is considered nuisance algae growth (Dodds *et al.*, 1997) and comparable to other studies of headwater streams (Brown *et al.*, 2008). The overall low level of chlorophyll-*a* may be attributed to community succession which is primarily confined to early successional stages due to the dynamic flow regime (Fisher *et al.*, 1982a). Therefore, it should be noted that overall biofilm proliferation is not of concern in result interpretation of headwater streams biofilm communities within this study. These findings provide an important evidence base from which to inform discussion within the literature on the ability to control biomass production through nutrient criteria and management (Biggs, 2000; Dodds and Welch, 2000; Royer *et al.*, 2008).

Table 7.1: Trophic classification of Eden DTC headwater streams based on UK Environment Agency standards for freshwater (EA, 1998) with trophic categories based on SRP. Mean, standard deviation (SD) and range based on monthly samples from March 2011 to March 2013. Dacre (n = 25 for nutrient concentration, n = 61 for chlorophyll-*a* concentration), Morland (n = 25 for nutrient concentration, n = 66 for chlorophyll-*a* concentration) and Pow (n = 25 for nutrient concentration, n = 66 for chlorophyll-*a* concentration).

	Dacre (oligotrophic)		**Morland (mesotrophic)**		**Pow (eutrotrophic)**	
	Range	**mean (SD)**	**range**	**mean (SD)**	**range**	**mean (SD)**
Nutrient concentration						
TP (mg/l)	0.1 - 0.43	0.04 (0.06)	0.01 - 9.33	0.18 (1.07)	0.04 - 1.37	0.28 (0.26)
PO_4-P (mg/l)	0.01 - 0.02	0.01 (0.00)	0.01 - 0.2	0.03 (0.03)	0.02 - 0.95	0.18 (0.18)
NO_3-N (mg/l)	0.2 - 0.75	0.32 (0.14)	1.06 - 3.74	2.01 (0.67)	0.25 -17.26	3.22 (3.17)
Chlorophyll-a concentration						
Chlorophyll-*a*	0.04-9.72	2.88 (2.11)	0.2 - 20.89	5.43 (3.60)	0.12-7.92	1.98 (1.78)

7.5 River Health Diagnostics

7.5.1 Application of metrics in headwater catchments.

This study demonstrated that monitoring protocols and tools developed for and applied in downstream sections of river networks can successfully be employed in dynamic headwater environments. Commonly used metrics employed within this research were the Shannon-Wiener diversity metric and TDI. Moreover, the novel technique, ISF, for the assessment of chlorophyll-*a* concentration was also employed throughout. Similar to both Shannon-Wiener diversity index and TDI, sensitivity in the ISF technique was detected in the benthic biofilm community (diatom, cyanobacteria and green algae) in response to dynamic discharge-nutrient regimes across the 10 km^2 catchments of the River Eden and nested 2 km^2 sub-catchments.

The Shannon-Wiener diversity metric detected differences within and among biotope units across all three catchments of Dacre, Morland and Pow. TDI was also effective in detecting the changes in species composition at monthly temporal resolution to discharge and nutrient condition. It was demonstrated for both Newby and Pow Becks that TDI increased as discharge increased indicating the delivery of nutrients to the stream during high rainfall and associated scouring effects related high discharge (section 4.2.10, Figure 4.29). Conversely, ISF chlorophyll-*a* values tended to be lower during high discharge events corresponding with an increased dominance of low profile and motile species guild (Figure 7.3). As suggested from the literature, this is most likely a combination of high near-bed shear stress scouring the biofilms enhanced by sediment abrasion and lower light levels restricting photosynthesis under deep water with high turbidity levels (Smith *et al.*, 2003; Besemer *et al.*, 2009; Lange *et al.*, 2011). ISF implied that yearly chlorophyll-*a* concentration of the community can change 10-fold, whereas month-on-month community composition remained relatively stable within the annual cycle (section 4.2.10, Figure 4.29). This highlights the ability of benthic algae communities to recover quickly in response to events as supported by previous studies (see Fisher *et al.*, 1982a; Peterson and Stevenson, 1990; Stevenson, 1990). Examination of diatom assemblage composition alongside the TDI metric, demonstrated that the TDI does mask some internal variation in changes of assemblage diversity of more specialist species. Furthermore, the value of TDI is largely controlled by the ratio of the aforementioned key pioneer species that are both present and abundant all year round in the benthic assemblage (refer to sections 4.2.3.1, 5.3.2.1, 6.3.3), and have the ability to withstand changes in their habitat associated with flow including shear stress, light and nutrient concentration.

All three metrics, Shannon-Wiener diversity, TDI and ISF demonstrated the ability of the community to recover from event-driven disturbances to their underlying equilibrium with water quality. Therefore, these metrics can capture aspects of benthic biofilm stability, in particular their considerable resilience (Connell and Sousa, 1983). Moreover, sustained differences in the magnitude of the TDI and chlorophyll-*a* levels between Newby and Pow Becks highlights the importance of catchment specific factors in assessment, as well as temporal changes in physical and chemical variables. These two similarly sized headwater catchments have comparable rainfall and discharge characteristics (section 4.2.1.1, Figure 4.2), yet ultimate, intermediate and proximate hierarchical influences on the stream ecology can be discerned using these metrics, including geology, flow paths, residence times and farming practices which have been depicted in other relative larger systems (Soininen and Weckstrom, 2009; Brown *et al.*, 2011; Smucker and Vis, 2011; Heino, 2013).

7.5.2 Classification of ecological status of headwater streams under the WFD

Classification of ecological status under the WFD is composed of biological, physicochemical and hydromorphological quality elements (European Council Directive, 2000/60/EC). There are five biological quality elements of which diatoms, though the TDI metric, have been chosen as a proxy for the phytobenthos (King *et al.*, 2000). Little empirical justification within the literature for adopting diatoms as a representative organism for the wider phytobenthos has contributed to various approaches for the assessment of "macrophytes and phytobenthos" within Europe by MS (Rimet *et al.*, 2004; Schaumburg *et al.*, 2004a; Schaumburg *et al.*, 2004b; Tison *et al.*, 2005b; Kelly, 2007a). This research illustrated that diatoms are an abundant and integral part of trophic web in headwater streams of the River Eden. This supports findings of Biggs *et al.* (1998b), Mosisch *et al.* (1999) and Greenwood and Rosemond (2006) on the dominance of diatoms within phytobenthic communities. Within the catchments of Morland, Dacre and Pow diatom assemblages were strongly controlled by pioneer and common species within the diatom assemblage (section 4.2.3), yet spatial and temporal differences were discerned in assemblage composition (chapter 4 and 5). The prominence of a few diatom species with the assemblages is similar to findings for macroinvertebrate and fish populations in headwater systems where populations are dominated by a few key species (Mykra *et al.*, 2012). Moreover, trends in ISF diatom chlorophyll-*a* concentrations generally follow, and thus represent, that of the wider assemblage of cyanobacteria and green algae. Therefore, this provides validity for using diatoms as a proxy for the complete phytobenthic assemblage and is supported by Kelly *et al.* (2008b).

This research has provided evidence that headwater communities may be responding to stochastic events and associate discharge-nutrient pulses more than dominant anthropogenic pressures, such as nutrients, which may be more spatially extensive. Therefore, appreciation of differences in the biological characteristics of different ecological groups present in headwater streams is important in terms of understanding and developing more representative concepts, especially for headwaters, than those such as the "one out all principle" which is commonly employed to estimate ecological status (Moss *et al.*, 2003; Everard, 2008; Moss, 2008). Failure to obtain a coherent ecological status classification through the EQR metric among benthic diatoms, macrophytes, macroinvertebrate and fish, with different life history strategies and sensitivity to environment perturbations (Dworak *et al.*, 2005; Johnson *et al.*, 2006; Carstensen, 2007) may be a more realistic expectation as demonstrated within section 4.2.11. However, assessment of congruency across biotic groups is important for a holistic understanding of how the ecosystem is functioning and should not be overlooked (Soininen and Kononen, 2004; Hughes *et al.*, 2009).

As the next phase of the WFD is approached, it is important that legislation is based on an appreciation of the response of benthic communities to dynamic environments and their recovery over relevant time scales and appropriate spatial extent. Traditional sampling protocols based on the hypothesis of a homogeneous distribution at reach scale implicitly ignore environmental and associated biological heterogeneity (Chapman *et al.*, 2010; Spilmont *et al.*, 2011). Within this research estimates of EQR varied among biotope but considerable variability within individual biotope units was also demonstrated through space and time. More specifically, it was observed that even within a single biotope unit different locations can give different estimates of ES which cross important boundaries of high/good and good/moderate ecological status. Therefore, reach characterisation based on a limited number of replicates from a single biotope unit type may be biased. Such characterisations are erroneously based on the assumption of homogeneity, an assumption which can be disregarded due to the findings of this study. Note, that a further confounding factor is that spatial heterogeneity can increase with increased sampling resolution (Levin, 1992; Seuront and Spilmont, 2002). Studies investigating temporal patterns and dynamics based on a limited number of replicates per sampling occasion over time are also typically biased (Brito *et al.*, 2009). As demonstrated in Figure 7.7, temporal resolution larger or smaller than the community process scale may cause excessive smoothing and misrepresent of temporal dynamics. Therefore, for routine analysis, the biotope unit needs to be defined and both the relevance and representativeness of performance characteristics, such as number of samples and replicates, as well as extent of spatial and temporal dynamics, should be considered.

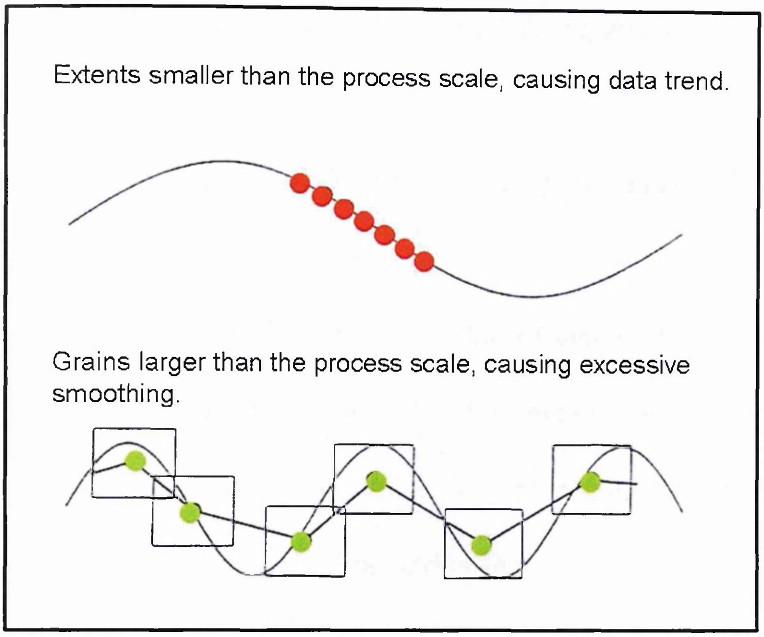


Figure 7.7: Demonstration of the importance of spatial resolution in capturing system dynamics. (Large, pers. comm., 2013).

7.6 Towards a toolbox for Headwater Assessment

Based on the results of this research, a monitoring framework is proposed incorporating existing tools and rapid assessment techniques (RAT; e.g. ISF, biotope unit) to better understand dynamics in headwater systems. Developing a better understanding of a system and using existing tools for the right reason rather than developing new tools is important, as developing more ways to monitor or assess the system from the same perspective will not necessarily yield a better understanding of the ecosystem. Therefore, while it is important to build on existing metrics and develop new concepts to meet the needs of specific legislation, it is often not the tools but rather the philosophy surrounding their use which requires improvement (Moss, 2008; Kelly *et al.*, 2009c; Everard, 2012).

7.6.1 Trait based assessment of diatom assemblage composition

Using biotope units nested within a hierarchical framework, this research explored the potential of ecological guilds (as defined in section 3.9.1) for the assessment of the impacts of variable discharge-nutrient regime on the structure and function of the diatom assemblage. Classification within the ecological guild metric is based on four divisions (High profile, low profile, motile and planktonic: Rimet and Bouchez, 2012b), three of which are common (High profile, low profile, motile) within headwater benthic diatom biofilms of the River Eden. This approach to assessment of the structural and functional diversity of the diatom assemblage provides many advantages over the traditional employment of metrics based on diatom counts. Due to the diverse taxonomy of diatoms and frequent revisions, difficulties can arise when identifying species and uncertainties are introduced. Therefore, assessment of environmental condition based on ecological guilds provides a more robust assessment of ecological status (Prygiel *et al.*, 2002; Besse-Lototskaya *et al.*, 2006; Kahlert *et al.*, 2009; Rimet and Bouchez, 2012b). Ecological guilds also overcome difficulties arising from ecological assessments based on the pollution sensitivities of datasets dominated by a few pioneer species through inclusion of both rare and common species (Archaimbault *et al.*, 2010). A further potential advantage of assessment based on a species-trait approach is their general applicability and predictability of response to anthropogenic disturbance (Doledec *et al.*, 1999;

Statzner *et al.*, 2001a; Berthon *et al.*, 2011). Metrics based on life-form are useful at genus level and therefore, are more time and cost effective for routine monitoring purposes (Rimet, 2011; Rimet and Bouchez, 2012b). Ecological guilds simplify the signal and reduce noise in the dataset, providing ease of comparison between sites (Statzner *et al.*, 2001a; Fore and Grafe, 2002).

Diatom species composition varied across the catchments of Morland, Dacre and Pow in response to gradients in environmental factors (section 4.2.8, Figure 4.20-21) in resources (TP and NO_3) or some environmental condition such as disturbance (e.g. grazing, discharge; Scheiner and Willig, 2005). These taxonomy based responses to environmental condition were also captured through changes in the relative abundance of ecological guilds across catchments over the 25 month study (section 4.2.5; Passy, 2007). Examination of changes in the composition of ecological guilds within the benthic diatom assemblage is particularly informative, providing information on the biofilm architecture, successional stage and sensitivity to disturbance (discharge, grazing) and nutrients (Willby, 2011). Given the differential sensitivity of each guild to perturbation and nutrient stress (Hoagland *et al.*, 1982; Pringle, 1990; Passy, 2007b; Berthon *et al.*, 2011; Rimet, 2011; Rimet and Bouchez, 2012b), examination of community dynamics through ecological guilds builds on, and adds to, assessment based on species specific tolerance of a single dimension anthropogenic pressure typically eutrophication and saprobity (Prygiel and Coste, 1993; Kelly *et al.*, 1995b; Kelly and Whitton, 1995a; Reid *et al.*, 1995; Walley *et al.*, 2001; Potapova and Charles, 2003), allowing for inclusion of assessment of physical, chemical and biological pressures to the biofilm (e.g. Passy, 2007).

7.6.2 RAT: *In situ* fluorometry

The often-observed heterogeneity in chlorophyll-*a,* as demonstrated within chapter 5 and illustrated in Figure 5.8, emphasizes the importance of fine-spatial and high-temporal *in situ* ISF monitoring which the Benthotorch© can facilitate. Application of ISF demonstrated the usefulness of this approach in dynamic headwater streams to understanding the impact of reach-scale discharge-nutrient thresholds on spatial patchiness, patterns in spatial synchrony of biomass and their interaction with environmental condition through time. ISF provides a time sensitive sampling method with a single measurement obtained in 10 seconds. A large number of samples can be taken over micro- and meso-scales, facilitating repeatability in investigation. Consequently, ISF is particularly advantageous at assessing scalar spatial heterogeneity and the representativeness of reach-scale monitoring strategies in chlorophyll-*a* estimations (Cooper *et al.*, 1997; Stevenson, 1997). Furthermore, the rapidity of analysis facilitates temporally intensive sampling capturing natural as well as anthropogenic driven variation in chlorophyll-*a* concentrations, thus eliminating previous issues of bias at seasonal and annual scales. ISF, therefore, allows for previously unquantified estimates of uncertainty in ecological assessment of benthic diatoms communities at the biotope scale to be examined. ISF also facilitates first attempts in the ecological validation of biotope units for benthic biofilm composition and biomass (Newson and Newson, 2000; Harvey and Clifford, 2009; Kelly *et al.*, 2009b).

While reach scale remains the predominant scale of assessment in river ecology, results of this research have shown that habitat characterisation based on only a few replicates over a reach can lead to bias. This is due to the considerable patchiness in chlorophyll-*a* within and among hydraulically-defined biotopes (section 5.2.6) which is supported by the more traditional diatom counts and community composition assessments (section 5.24.2). Consequently, ISF provides a means of better informing monitoring strategies in terms of sampling location. It also provides a more accurate estimation of the number of samples needed within a particular geographical location for reliable estimates of chlorophyll-*a*. A key novel element to ISF is its ability to discriminate between the relative proportion of diatoms, cyanobacteria and green algae within the measured biofilm biomass (Aberle *et al.*, 2006). This permits a more accurate temporal assessment in relation to seasonal changes or the monthly shifts in composition. Understanding of algal community development provides a vital new quantitative component to biological assessment to accompany traditional diatom counts and compositional surveys of wider algae assemblage. This is important as it overcomes previous difficulties in obtaining estimates of green algae and cyanobacteria due to problems surrounding cell definition, size and subsequent unit of measurement (King *et al.*, 2000).

Despite the many advantages of the ISF technique certain considerations should be borne in mind when using this technique. Firstly, ISF methods may be subject to interference from other algae groups. Examples include red algae which may have the same spectral signal as blue-greens and quantification of filamentous greens due to structural properties (King *et al.*, 2000; Samsonoff and MacColl, 2001; Kiang *et al.*, 2007; Kelly *et al.*, 2008b). Secondly, substrate characteristics may also be important in terms of background reflection and subsequent interference in the florescence response. However, reflection from the substrate is corrected for within the Benthotorch© method (Carpentier *et al.*, 2013). Thirdly, when assessing biofilm succession and key chemical drivers of TP and NO_3 it is important to consider the impact of nutrient saturation and biofilm age on ISF determined chlorophyll-*a*. Through the process of succession, species composition of the biofilm approaches a climax where there will be a canopy layer with sub-layers underneath (Kelly *et al.*, 2009c). Saturation due to biofilm thickness, and associated self-shading within the biofilm, can influence fluorescence intensity due to changes in the peripheral antenna (Beutler *et al.*, 2002b; Carpentier *et al.*, 2013). Within the Morland, Pow and Dacre sub-catchments of the River Eden, estimates of ISF chlorophyll-*a* were within the range of the Benthotorch© probe, which can facilitate assessment of 0 - 20 µg/cm^2 chlorophyll-*a* for each spectral fingerprint of; diatoms, cyanobacteria and green algae (BBE, pers. comm., 2012). To facilitate application of this technique to other systems, further work is needed to address questions relating to the reliability of ISF assessment in terms of biofilm structure, self-shading and thickness.

Patterns between catchments are consistent in chlorophyll-*a* concentration determined by both ISF and traditional non-discriminatory spectrophotometric methods, but the absolute magnitude differs (Figure 3.4; Snell *et al.* in prep). This can be attributed to a number of key characteristics of the biofilm, including composition and thickness, and fundamental methodological differences, as discussed in Whiltshire (2000), which make these measures not directly comparable.

However, it does serve to highlight the key advantages and limitations of both ISF and traditional methods of chlorophyll-*a* determination. ISF methods provide an areally-defined, rapid *in situ* multi-replicate assessment of the photosynthetically active layer which can yield specific spectral fingerprint of benthic chlorophyll-*a* (Aberle *et al.*, 2006). This is in contrast to spectrophotometric determination which is performed on the full assemblage and is subject to errors related to measuring non-photosynthetically active layers within the biofilm. The latter arise as the phytobenthic mat constitutes a sink for dead algae cells from *in situ* growth and upstream deposition of terrestrially-derived organic debris (Garcia-Robledo *et al.*, 2008). Decay of this organic matter results in organic decay products with similar fluorescent properties as the living benthic algal assemblage (Biggs and Kilroy, 2000). This can confound analysis making it difficult to determine what is actually extracted due to possible misinterpretation arising from phaeopigment corrections and unit area calculations.

Difficulties in the comparison between methods also arise given that ISF is based on single measurements subject to significant intra-cobble variability, which is homogenised in scraping assemblage for the laboratory determinations (Snell *et al.* in prep). Most effective comparisons will be based on the full characterization of the cobble surface area, capturing structural differences across the cobble and differential pressures on the cobble such as grazing which may lead to patchiness at the cobble scale. However, higher ISF chlorophyll-*a* estimates can be attributed to the fact that the physical scraping of cobbles is unable to remove all of the chlorophyll-*a* for laboratory determination (Carpentier *et al.*, 2013).

In summary, ISF techniques are critical for understanding the scalar issues in assessments of chlorophyll-*a* and benthic productivity. ISF offers a new functional ecological understanding which builds upon fundamental and well established principles surrounding spatial heterogeneity in benthic diatom community response to environmental condition and pattern in riffle-pool productivity, all of which could not be previously measured due to the limitation of *ex situ* approaches.

7.6.3 Physical biotope: research strategy and assessment unit

Based on this research, physical flow biotope units and associated benthic diatom biofilm assemblages present a useful and meaningful way of integrating ecology and hydro-morphology, thus fulfilling obligations presented under the WFD (Udvardy, 1959; Newson *et al.*, 1998; Harvey *et al.*, 2008). Biotope units have potential as indicators of ecosystem structure and function in headwater catchments incorporating both taxonomy-based and trait-based assessment (Townsend and Hildrew, 1994). This is useful for investigation of key biological processes such as dispersal, colonisation, competition and succession (Townsend *et al.*, 1997; Passy, 2007b; Berthon *et al.*, 2011). For example, within this research it allowed for the demonstration of how colonisation processes can determine the taxonomical composition of diatom assemblages and how its interaction with environmental pressures can determine key ecological-environmental thresholds (see chapter 6). Moreover, physical biotopes provide a unit of assessment which is at a scale relevant to what diatoms "experience" in terms of hydrological pressures within the boundary layer including velocity and shear stress (Crowder and Diplas, 2000).

Understanding of the physical environment is important due to the control it exerts on the benthic response to nutrients through diffusion limited solute retention and uptake by the biofilm (Gantzer *et al.*, 1988; Aumen, 1990; Sabater *et al.*, 2002; Battin *et al.*, 2003b) as has been demonstrated through this research (e.g. see section 5.2.6.2) and more broadly by Biggs and Thomsen (1995) and Biggs *et al.* (2005). This is important as it is diatom sensitivity to nutrients, in particular TP, which underpins the metric based assessment including those presented under the WFD (WFD-UKTAG, 2014).

Biotope units allow for better predictability of the influence of interactive abiotic and biotic environment pressures and scale-appropriate impacts within the confinement of the hierarchical framework (Newson and Newson, 2000; Clarke *et al.*, 2003; Harvey *et al.*, 2008). The biotope scale of investigation is more informative providing an integrated, holistic and practical assessment of both the structure and function of benthic communities linking taxonomy with biological traits at the relevant hydromorphological scales. It also provides an overview of the trophic interactions amongst biotic groups and the connectivity between terrestrial and stream compartments while reflecting local habitat conditions (Thomson *et al.*, 2001; Barili *et al.*, 2011). Previously the use of benthic diversity as a key buffer to pollution has been discussed (Cardinale, 2011). Analysis of spatial variability in benthic diatom assemblages and their productivity through biotope units has shown that productivity does vary according to biotope type and therefore, this study presents a potentially invaluable tool in the conservation of biodiversity and nutrient management.

In conclusion, physical biotope units have the potential to be used as a more informative (traits and taxonomy), holistic (biota and environment) and cost-effective (rapid) assessment within headwater streams (Newson *et al.*, 1998; Harper *et al.*, 2000; Harvey *et al.*, 2008; Demars *et al.*, 2012). Moreover, findings of this research suggest that the biotope or habitat scale of assessment can be used as a surrogate for biodiversity and system functioning, building on a research concept that has formulated over five decades (Hynes, 1970; Meffe and Sheldon, 1988; Harper *et al.*, 1992; Harper and Everard, 1998; Wadeson and Rowntree, 1998; Newson and Newson, 2000).

Chapter 8: Conclusion

8.1 Key Findings

The importance of fine-spatial and high-temporal investigations to assess system dynamics in headwater streams was identified as a significant research gap in the literature and evaluated within this research. High connectivity between land and the stream ecosystem was observed with an average rainfall-runoff ratio of 0.63 across all three catchments; Morland, Dacre and Pow. This contributed to variability in the hydraulic regime (velocity, shear stress and discharge) and associated resource supply (especially TP and NO_3). Despite the observed dynamism in flow, distinct and reproducible catchment-specific patterns were observed amongst benthic diatom communities. This supports the current study of Gothe *et al.* (2014) which states that environmental control on the biodiversity is greatest within small headwater catchments. Monthly developments, from March 2011 to March 2013, in two community measures, namely the calculated TDI and ISF benthic chlorophyll-*a,* demonstrated a quasi-seasonal pattern. This seasonal pattern was strongly dominated by the distribution of rainfall rather than other seasonally-related climate, light, or ecological variables. TDI values, a proxy for the sensitivity of diatom community structure to phosphorus availability, were generally higher in autumn and winter than in spring or summer, indicating a higher level of nutrient tolerant taxa and thus, more enriched conditions (Kelly and Whitton, 1995a). This was supported by higher TP concentrations in autumn and winter. Considerable resilience of these diatom communities was highlighted by the stability of the inter-monthly

TDI scores against the highly variable hydrological regime. Trends in TDI at the 10 km^2 scale, similar to other community metrics (Margalef richness index, Pielou's evenness index and Shannon-Wiener diversity), were also observed within the 2 km^2 sub-catchments. This demonstrates that fine-spatial and high-temporal patterns in benthic diatom community attributes are robust to changes in spatial scale. This was an important finding for water quality mitigation efforts as it suggests measures employed within catchment and at the localised farm-yard scale can be detected at larger catchment outflows. Even within these small catchments the annual range of TDI was high, spanning from 'High' to 'Poor' ecological status classification under the WFD. This illustrates that biomonitoring efforts need to be assessed against a dynamic catchment specific baseline that reflects the intra-annual variability of headwater catchments.

Patterns in TDI were partly tracked in Newby Beck (Morland) by benthic chlorophyll-*a*, which is used as a surrogate for benthic productivity. Within relatively quiescent hydrological periods, e.g. January to May 2012, broadly positive relationships between benthic productivity and community structure were evident. Lower TP concentrations and improved water quality, as inferred by the TDI, were matched by increased chlorophyll-*a*. Clusters of high rainfall events in the Pow catchment and associated TP delivery correlated with high TDI values and low chlorophyll-*a* values. High rainfall:runoff ratios within Pow, coupled with intensive arable production on clay based soil, can lead to rapid transfer of sediment-bound P to the stream (Haygarth *et al.*, 1997; Mainstone and Parr, 2002; Haygarth *et al.*, 2005, Heathwaite et al., 2005). This suggests that even if resource supply external to the biofilm mat is high, physical factors such as discharge and sedimentation may restrict chlorophyll-*a* production. Within headwater streams, therefore, physical factors rather than nutrient concentrations may determine productivity.

Catchment specific differences in trophic state and the hydrological regime is an important determinant of chlorophyll-*a* concentration (Biggs, 2000). The Pow catchment, which demonstrates eutrophic conditions, supported the lowest productivity. Oligotrophic conditions observed in the Dacre catchment demonstrated intermediate chlorophyll-*a* concentration to that observed with the Morland and Pow catchments. This is important in the context of studies which aim to manage biomass production through setting nutrient criteria (e.g. Biggs, 2000; Dodds and Welch, 2000; Royer *et al.*, 2008; Miltner, 2010), emphasising the critical role of a dynamic hydrological regime in catchment productivity and water quality.

Community structure across the individual catchments reflected ultimate and intermediate hierarchical determinants of geology and land use, as supported by Biggs (1995). The strongest seasonal pattern in the TDI metric was observed in the high alkalinity catchment of Morland. A key pioneer species, *Achnanthidium minutissium,* dominated community structure on an annual cycle between spring and early summer. *Amphora pediculus* was dominant through autumn and winter reaching maximum percentage abundance in December 2012 and 2013. Therefore, periods of higher TP and alkalinity corresponded with peak percentage abundance in *A. pediculus* and associated productivity. Community composition, and the resultant TDI metric score within the Dacre catchment, reflected less enriched upland flashy conditions. Similar to other studies on the response of *A. minutissium* to variable hydraulic regimes (e.g. Peterson and Stevenson, 1990, Biggs and Smith, 2002), *A. minutissium* was particularly noticeable within the Dacre catchment, comprising up to 90% of the assemblage composition. Unlike both Morland and Dacre catchments, *Cocconeis placentula* var. *euglypta* featured prominently in the diatom assemblages of the Pow catchment. Higher TP concentration associated with intensive agricultural practice (Owen 2012) and the potential of *C. placentula* var. *euglypta* to fix nitrogen (Marcus, 1980) demonstrated the importance of N and the potential for N:P limitation, which has been previously suggested by Luttenton and Lowe (2006). As evidenced throughout this thesis and supported by Camacho and de Wit (2003), differences in stream enrichment and N:P availability to the biofilm can contribute to differences in community composition and therefore, presents an important area for future research.

Benthic diatom biofilm composition and chlorophyll-*a* production varied according to physical biotope properties of depth, velocity and shear stress. Chlorophyll-*a* concentrations were higher in riffles than pools and demonstrated greater heterogeneity (see Figure 5.6). Benthic diatom richness was higher in pools than riffles and this pattern was observed across season (Table 5.3). Previous studies have also reported greater variability in riffle units but higher diversity in community composition in pools (Korte and Blinn, 1983). However, high diversity was observed in riffle units (see Figure 6.12) in response to threshold rainfall and associated discharge-nutrient conditions as presented in section 6.3.2. This can be attributed to flow dynamism and associated rapid accumulation rates by pioneer species during the early colonisation phase (Oemke and Burton, 1986). Reach-scale differences in biodiversity and chlorophyll-*a* in riffle biotope units were also observed over time in response to temporal dynamics in velocity, shear stress and nutrient availability (TP and NO_3). This data builds on the work of Harper *et al.* (1998), Kemp *et al.* (1999), Newson and Newson (2000), Harvey *et al.* (2008) and Demars *et al.* (2012) among others in demonstrating the applicability of biotope-scale assessment of benthic diatom communities at fine-spatial and high-temporal scales.

Investigation of diatom composition and chlorophyll-*a* at biotope scales also emphasised the importance of incorporating functional processes in definitions of biodiversity. These include assembly history, interspecific interactions, hydrological disturbances and nutrient condition (TP and NO_3) which are fundamental to maintaining biodiversity. Benthic algae assemblage structure is dependent upon mechanisms of accrual-loss and colonisation-competition including species membership and their abundance (Figure 7.2). Such critical trade-offs, underpinned by the Intermediate Disturbance Hypothesis (IDH) and confined by the physical biotope, directly impact upon successional stage, diversity and response to future events (Connell and Sousa, 1983, Steinman and McIntire, 1990, Peterson and Stevenson, 1992, Biggs, 1996, Biggs and Smith, 2002). Highest diversity was observed at- and above- threshold discharge conditions. This reflects species-specific abilities to resist competition or disturbance. At high levels of disturbance, fast reproducing pioneer species can colonise the available substrate, thus contributing to high diversity (Oemke and Burton, 1986). Highest level of diversity was observed at intermediate levels of disturbance. This is as a consequence to a trade-off in environment conditions, allowing for 'understory' low profile species to exist with high profile species which form a 'canopy' layer (Connell and Sousa, 1983; Petraitis *et al.*, 1989; Passy, 2007). However, optimum chlorophyll-*a* production occurs at threshold conditions. The benthic diatom biofilm is therefore positively affected by discharge levels just below sub-scouring conditions through nutrient and oxygen delivery into the biofilm and autogenic sloughing of dead and senescent material (Lock *et al.*, 1984; Stevenson, 1990; Biggs *et al.*, 1998; Hondzo and Wang, 2002; Abdul-Aziz *et al.*, 2010; Figure 7.5).

An important finding of this research was the observed unimodal relationship between benthic diatom diversity and chlorophyll-*a* at the 10 km^2 catchment scale. This pattern has been found by others but is widely debated and seldom investigated within headwater systems (Rosenzweig, 1992; Rosenzweig, 1995; Waide *et al.*, 1999; Mittelbach *et al.*, 2001; Partel *et al.*, 2010). However, Cardinale *et al.* (2005) has demonstrated that the relationship between diversity and productivity may vary as a function of the natural disturbance regime. An equally important finding was the usefulness of functional diversity, more specifically ecological guilds (Passy, 2007; Rimet and Bouchez, 2012), in exploring the relationship between biodiversity and ecosystem functioning in terms of nutrients (TP and NO_3) and productivity (chlorophyll-*a*). Clear responses were observed in the functional diversity of the benthic diatom assemblage at below-threshold conditions in rainfall intensity and associated discharge. In such circumstances, high profile species which can withstand low current velocities and exploit available nutrient resources became dominant (Passy, 2007; Rimet and Bouchez, 2012). This highlights the importance of species rarity and the uniqueness of combinations of traits on ecological process and thus builds on the work of Gillett *et al.* (2011) on the importance of rarity in bioassessment.

8.2 Recommendations and critique of assessment protocols

Due to the inherent variability of headwater streams it is important that ecological monitoring is conducted at an appropriate spatial-temporal resolution, and employs the appropriate community measures. Outcomes of this research suggest that benthic diatoms should be sampled at a minimum of every 21 days from mid reach conditions in riffle biotope units. This finding is particularly beneficial to studies of baseline water quality conditions highlighting the time-integrating property of water quality assessments based on benthic community composition. For chlorophyll-*a* assessment, high resolution and appropriate replication are vital for meaningful assessment given inherent intra-cobble variability within riffle and pool biotope units. Combining taxonomy and trait based assessments with these recommendations for capturing spatial and temporal variability in benthic diatom communities presents a useful and meaningful way of integrating diatom community structure with functional diversity. This is important as examination of benthic diatom functional diversity through species traits will allow for greater understanding of resource availability (e.g. light, nutrients) within the biofilm which may directly influence chlorophyll-*a* concentration and interpretation of system integrity (Rhee and Gotham, 1981; Paul *et al.*, 1991; Johnson *et al.*, 1997b).

In headwater systems, and wider catchment monitoring, the scale of assessment is critical. Studies have previously examined how macroinvertebrates vary with biotope (e.g. Demars *et al.*, 2012). With further development and application, biotope-based studies provide an appropriate and informative scale of ecological assessment which incorporates bottom-up and top-down trophic links to the benthic diatom assemblage. Assessment at this scale over an appropriate temporal resolution of multi-annual duration, that encompasses both short term events and seasonal variability, will provide particular value in improving understanding of benthic community composition and productivity.

The implication of such findings outlined above is that current monitoring programmes as conducted by regulatory authorities are unlikely to be representative of annual spatial and temporal dynamics in headwater catchments (WFD-UKTAG, 2014). Results of this research imply that single-seasonal sampling frequency, such as suggested under the WFD, are inadequate and are unlikely to give results representative of the full annual cycle. At the other extreme, the benthic diatom community structure will not reflect single events, but rather an accumulated average, calculated with this research to be the preceding two to three weeks.

8.3 Research limitations and future directions

This research demonstrates, as suggested by Stevenson (1997) and Osmond *et al.* (2010), that there are several simultaneous and co-occurring pressures from different sources that may act in an hierarchical fashion contributing to spatial variance in diatom composition and chlorophyll-*a*. Results of this thesis support this and highlight the importance of understanding biological inertia in dynamic headwater environments. A key finding suggests that diatom communities, and the derived TDI, best reflect steam discharge condition over the preceding 18 - 21 days and TP over a wider antecedent window of 7 - 21 days. Note, however, this is based on the assumption of a direct relationship between water column nutrient concentration and availability to species within biofilm (Bernhardt and Likens, 2004). Moreover, these relationships have only been applied to two 10 km^2 catchments within the Northwest region of England and therefore, require validation in other headwater streams. Similar testing of the robustness of the observed unimodal relationship of diversity to chlorophyll-*a*, used as a proxy for productivity, across the three 10 km^2 sub-catchments of the River Eden is required. Particular emphasis should be placed on the scale-dependency of productivity-diversity relationships and the reliability of scaling-up results of meso-scale experiments to the wider catchment (Chase and Leibold, 2002; Foody, 2004; Gross *et al.*, 2000). In addition, employment of a greater range of indices and metrics is required for both biodiversity and, in particular, productivity, in order to provide a less biased and proxy-independent assessment of the relationship between diversity and productivity.

Through assessment of the monthly development of benthic diatom assemblage attributes (richness, evenness, diversity and chlorophyll-*a*), this research has highlighted that the concepts of succession and biodiversity in the context of headwater streams requires careful consideration and development. Successional state comprises both direction of change and species turnover in response to prevailing discharge-nutrient conditions. Thus, quantification of components of diatom ecological stability (resistance and resilience) and their impact upon critical environmental (e.g. discharge-nutrient) and biological (e.g. colonisation-competition, diversity-productivity) thresholds is important. Critically, the concept of biodiversity needs clear definition and should include mechanistic comprehension of its multidimensionality given the observed population dynamics of species in headwater streams.

For the UK, the impact of nutrients on benthic diatom communities has focused on P (Kelly and Whitton, 1995a; Kelly *et al.*, 2008a). However, more emphasis needs to be placed on N and the control of lag times of N and P delivery through seasonal rainfall and farming activity in agricultural dominated headwater catchments. When N:P ratios, based on daily averaged concentration over the preceding 21 days, were explored in relation to EQR, a significant correlation was observed ($r = 0.653$, $n = 16$, $p < 0.05$) with higher EQR corresponding with periods of higher N:P ratios. Therefore, an important potential area for further research is to explore the potential for N and P limitation in headwater streams and impact upon monitoring frameworks such as the WFD. This will facilitate a more accurate and representative illustration of nutrient condition which benthic assemblages are responding to given diatom sensitivity to antecedent discharge – nutrient condition. Moreover, this would add to the work of Luttenton and Lowe (2006), Meals (2010) and Jarvie *et al.* (2012) to better inform understanding of the processes relating pressure to impact on the phytobenthos and related assessment of water quality. For example, do peaks in summer / winter have a greater or lesser interaction with temperature, farming activity, terrestrial runoff and N and P variability, leading to nutrient enrichment or limitation as indicated through N:P and diatom assemblage composition? Given the quasi-seasonal patterns in diatom communities and high rainfall-runoff ratio, quantification of temporal inputs from agriculture is important in order to facilitate real changes in terms of catchment management plans. In particular this would help inform nutrient budgets, as required under the Nitrates Directive (91/676/EEC), and consequently achievement of "good ecological status" as stipulated under the WFD. Understanding of how different biological quality elements (i.e. diatoms, macrophytes, macroinvertebrates, diatoms and fish) interact in space and time with in-stream and catchment variables will greatly enhance assessment of how changes in environmental pressures contribute to the assessment of 'ecological status' under the WFD.

In conclusion, this study is one of the first quantitative assessments of long-term diatom community development in response to near-continuous *in situ* measured stream nutrient concentration and discharge fluctuation. It highlights the importance of the determination of critical thresholds in discharge-nutrient regimes and antecedent response through critical trade-offs in diatom community attributes, which presents a key emerging area for future research. Further understanding of the role of antecedent conditions and the quantitative estimation of critical thresholds in the multiple environmental stresses influencing diatom community structure and function will help to advance ecological theory and better inform the assessment of the ecological status of headwater streams.

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Appendix

Appendix 1.1: Comparison of CEH and EA laboratory methods for spot samples including limit of detection (LOD; Source: EdenDTC).

Determinand	Unit	LOD	CEH: method	EA: method	Method Comparison
Suspended solids	mg/l	3	Suspended matter is removed from a measured volume of sample by filtration under reduced pressure through a pre-washed, pre-weighed, glass fibre filter paper and determined gravimetrically after washing and drying at 105°C.	Suspended matter is removed from a measured volume of sample by filtration under reduced pressure through a pre-washed, pre-weighed, pre-dried glass-fibre filter paper and determined gravimetrically after washing and drying at 105°C to constant weight.	$R^2 = 0.992$ $p = 0.001$ $n = 6$
Alkalinity (methyl orange)	mg/l $CaCO_3$		Not analysed.	The reagent used is methyl orange buffered with potassium hydrogen phthalate. Reduction in the red acid component of the indicator by carbonate/bicarbonates present in the sample is measured as a decrease in absorbance at 550nm.	

Nitrate -N	mg/l	0.2	The sample is treated with sulphanilamide and N-1-naphthylethylene diamine dihydrochloride under acidic conditions to form a pink azo-dye. Nitrate was measured by ion chromotography using a Dionex ICS2500	Nitrate is determined by subtracting nitrite from TON. The calculation is performed by StarLims	$R^2 = 0.584$ $p = 0.001$ $n = 6$
Nitrite -N	mg/l	0.005	The sample is treated with sulphanilamide and N-1-naphthylethylene diamine dihydrochloride under acidic conditions to form a pink azo-dye. The intensity of this azo-dye is measured spectrophotometrically and is related to the nitrite concentration by means of a calibration curve.	Nitrite ions, when reacted with a reagent containing sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride, in the presence of acid, produce a highly coloured azo dye that is measured photometrically at 540nm.	$R^2 = 0.990$ $p = 0.001$ $n = 6$

TP	mg/l	0.005	The condensed phosphates require hydrolysis by boiling in the presence of dilute sulphuric acid to convert them to orthophosphate. The orthophosphate ion reacts with ammonium molybdate under acidic conditions to form a 12-molybdophosphoric acid complex. Antimony (III) is added as a catalyst. The complex is reduced with ascorbic acid to form a blue heteropoly compound (phosphomolybdenum blue). The absorbance of this blue compound is proportional to the concentration of phosphate in the sample.	Condensed phosphates are hydrolysed by boiling in the presence of dilute sulphuric acid to convert them to orthophosphate. Organic forms are broken down by suitable techniques such as oxidation by persulphate. The orthophosphate ion reacts with ammonium molybdate under acidic conditions to form a 12-molybdophosphoric acid complex. Antimony (III) is added as a catalyst. The complex is reduced with ascorbic acid to form a blue heteropoly compound (phosphomolybdenum blue). The absorbance of this blue compound is proportional to the concentration of phosphate in the sample.	$R^2 = 0.591$ $p = 0.001$ $n = 6$

TRP	mg/l	0.005	The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate under acidic conditions to forma 12-molybdophosphoric acid complex. Antimony (III) is added as a catalyst. The complex is reduced with ascorbic acid to form a blue heteropoly compound (phosphomolybdenum blue). The absorbance of this blue compound is proportional to the concentration of orthophosphate in the sample. The acid conditions used may cause partial hydrolysis of condensed phosphates and some of the more labile organic phosphates, if present.	Orthophosphate reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex which, when reduced with ascorbic acid produces an intense blue colour, the absorbance of which is measured at 880nm.	$R^2 = 0.952$ $p = 0.001$ $n = 6$

DOC	mg/l	0.2	An aliquot is re-sampled for analysis. Buffered persulphate is added, the sample is irradiated in the UV digester, and hydroxylamine is added to convert all the carbon to carbon dioxide. The carbon dioxide produced is re-sampled into a weakly buffered phenolphthalein indicator solution, either by using dialysis or a gas/liquid separator, and the colour intensity of this solution decreases proportionately to the change in pH caused by the absorbed carbon dioxide gas. The colour intensity is measured at 550 nm.	The automated pre-treatment of the sample with acid and the entraining of the acidified liquid with a high velocity stream of nitrogen results in the removal of any inorganic carbon. The carbonate free sample is mixed with an oxidising agent and subjected to UV radiation. The CO_2 generated reacts with a weakly buffered phenolphthalein indicator and the decrease in colour is proportional to the organic carbon concentration Measured spectrophotometrically at 550 nm.	$R^2 = 0.976$ $p = 0.001$ $n = 6$

Appendix 1.2: Diatom assemblage composition

Please consult accompanying CD for full list of species found across the three experimental studies undertaken within this thesis. For further information on water chemistry consult: http://www.edendtc.org.uk/category/live-data/

Appendix: 1.3 Spatial-temporal differences in total chlorophyll-a concentration and spectral group composition: diatom, green algae and cyanobacteria

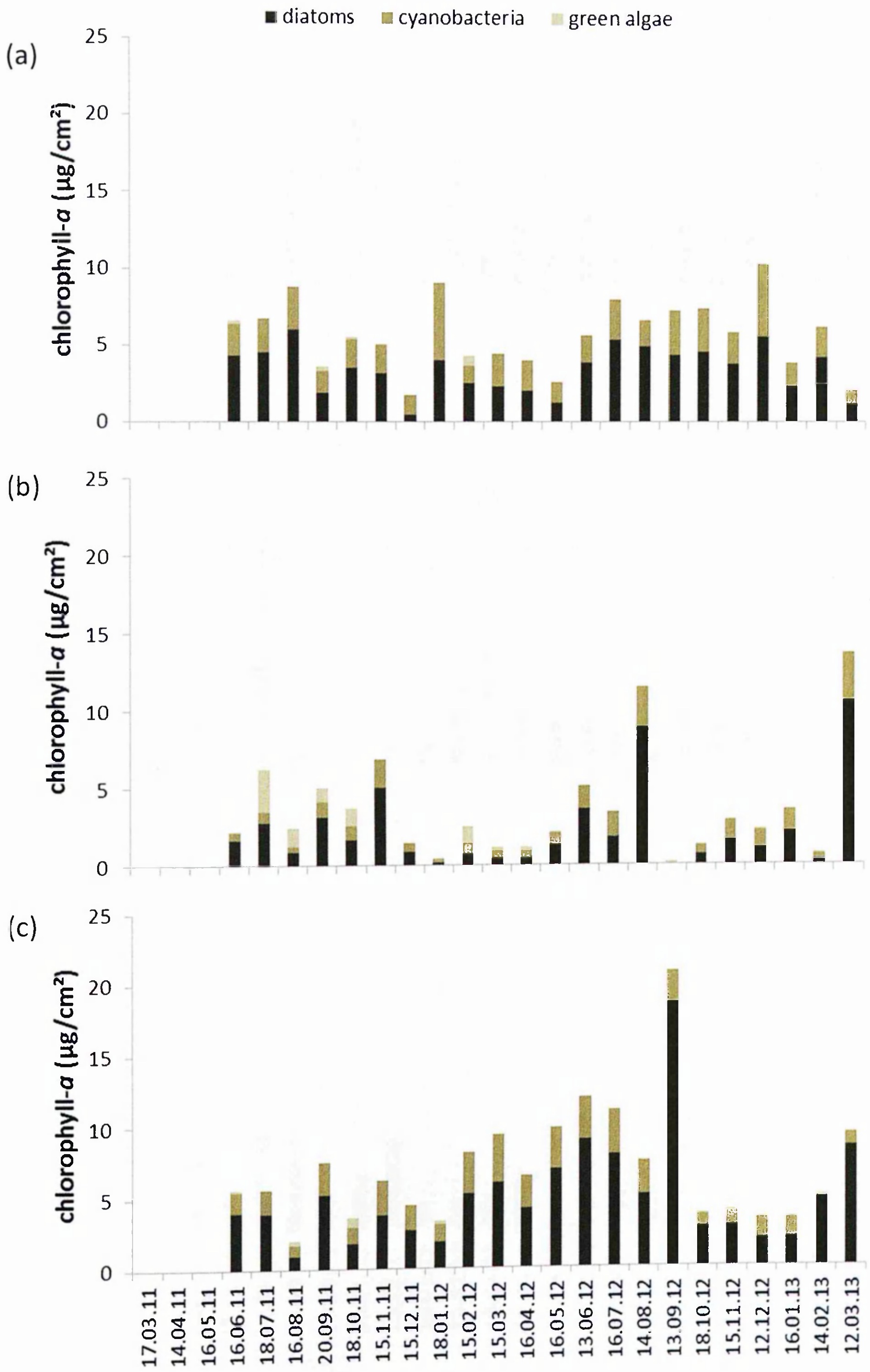

Figure 1.3.1: Relative contribution of diatom, cyanobacteria and green algae to total chlorophyll-*a* concentration in the Morland catchment (a = Newby Beck, b = Dedra Banks Beck, c = Sleagill Beck) from March 2011 to March 2013.

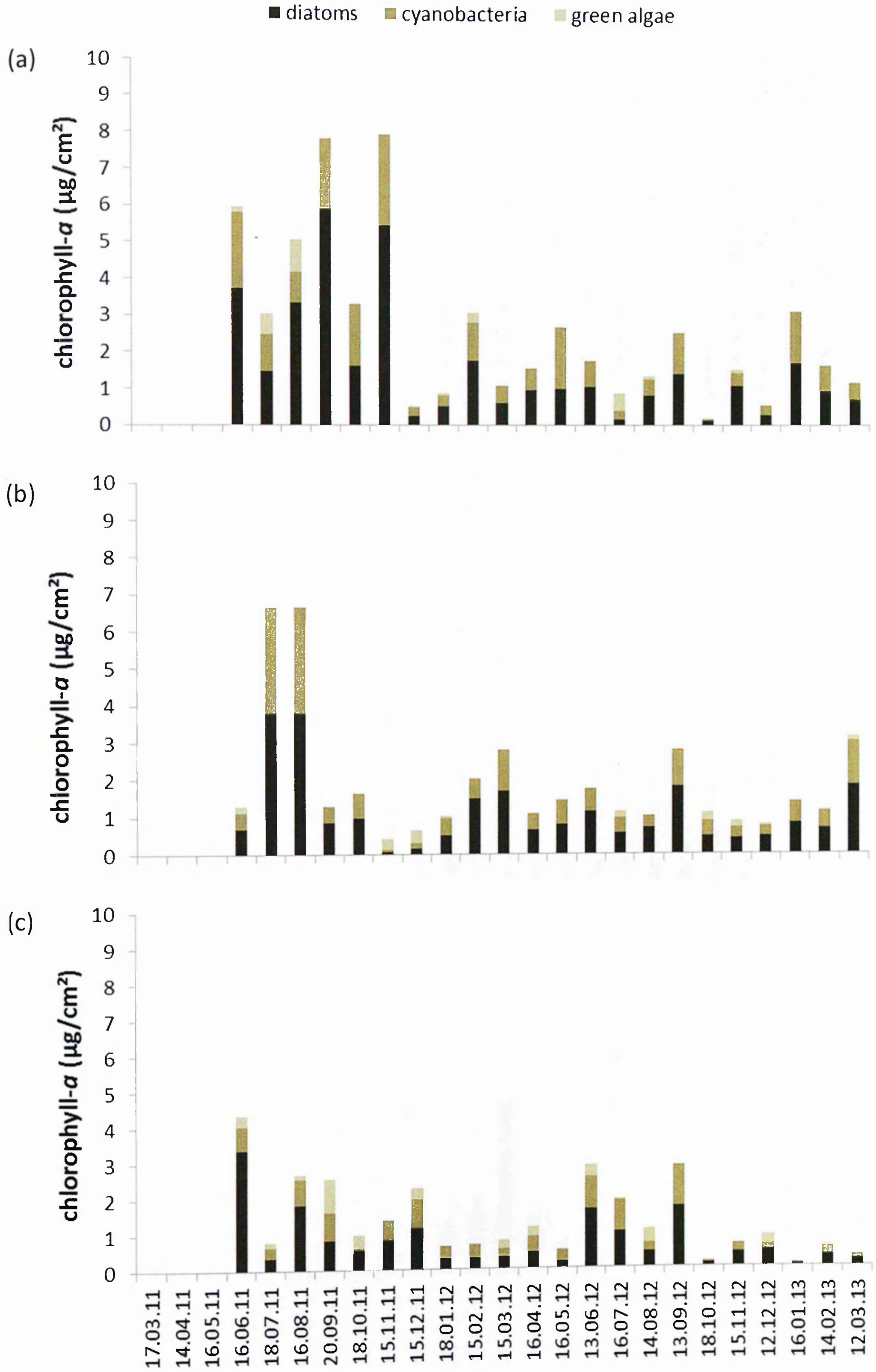

Figure 1.3.2: Relative contribution of diatom, cyanobacteria and green algae to total chlorophyll-*a* concentration in the Pow catchment (a = Pow outlet, b = unnamed Tributary A, c = unnamed Tributary B) from March 2011 to March 2013.

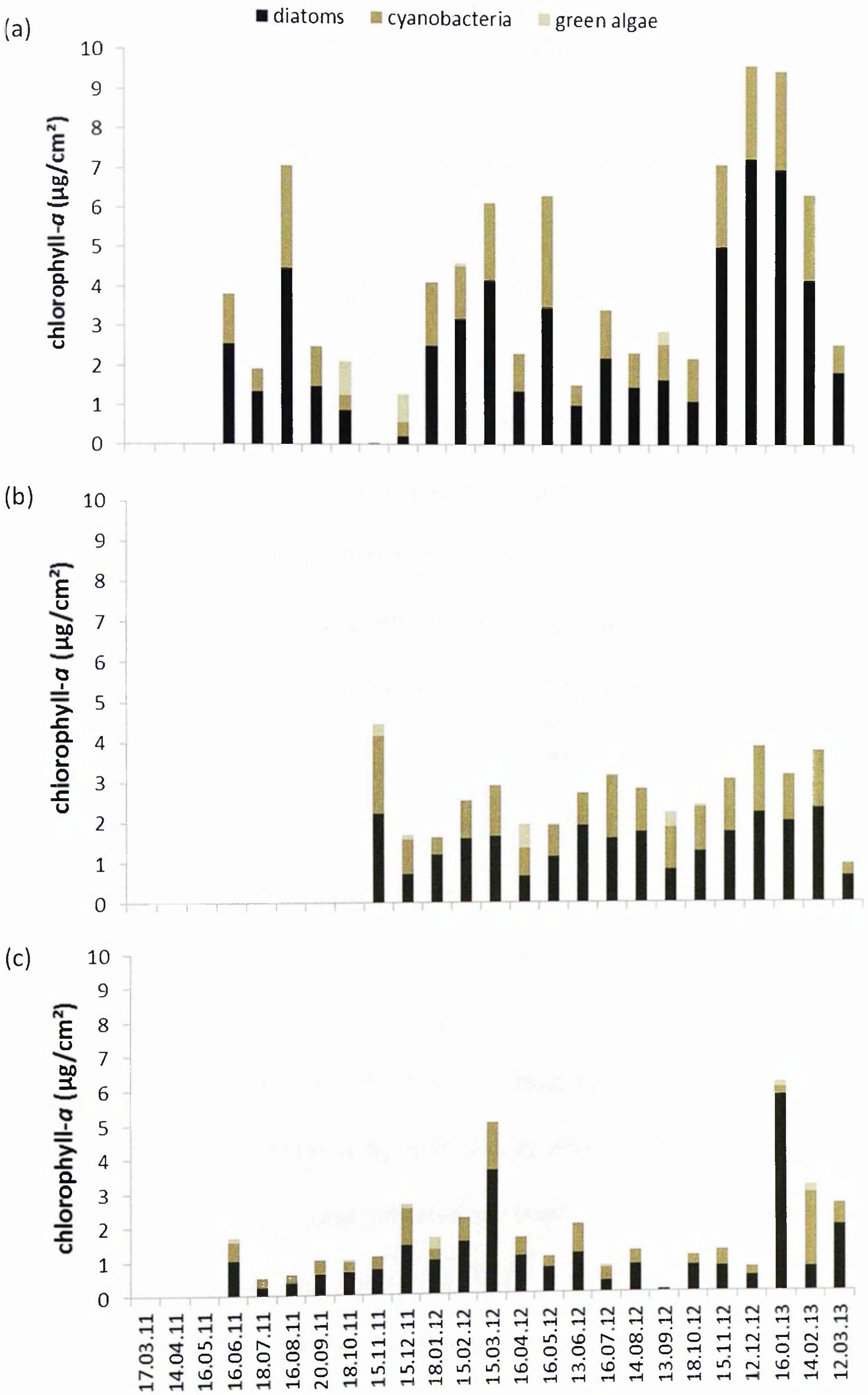

Figure 1.3.3: Relative contribution of diatom, cyanobacteria and green algae to total chlorophyll-*a* concentration in the Dacre catchment (a = Thackthwaite Beck, b = Mellfell Beck, c = Lowthwaite Beck) from March 2011 to March 2013.

Appendix 1.4: Description of diatoms, macrophytes and macroinvertebrate and fish assemblage of the Morland, Dacre and Pow catchments

1.4.1 Concordance between diatoms, macrophytes and macroinvertebrate metrics

Metric comparison among TDI (diatoms), BMWP (macroinvertebrates) and MTR (macrophytes) demonstrated that each biological assemblage provided a different catchment specific perspective of system trophy that varied temporally (Figure 1.4.1). For Newby Beck, TDI demonstrated that nutrient enrichment was lowest in spring and that trophic status declined through summer and autumn, with this pattern repeated in both years. BMWP indicted the trophic status at Newby Beck was high, only decreasing slightly in Autumn 2012 to suggest slight impact from nitrogen and phosphorus. Across season MTR was relatively stable indicating eutrophic conditions (Figure 1.4.1 a). TDI scores illustrated seasonal consistency in trends of trophic status in Pow Beck through 2011. Between spring and summer TDI indicated moderate trophic status within the Pow catchment, which increased into autumn. In 2012, a pattern similar in TDI to Morland was observed indicating intermediate nutrient status as in 2011 but this then increased across summer and into winter. This was supported by MTR values which also indicated eutrophic condition. However, BMWP scores indicated low trophic status across season in both years apart from summer 2011 and spring 2012 which suggest slightly impacted in nutrient condition (Figure 1.4.1 c). In contrast to Newby and Pow Beck, TDI values were lower in Dacre Beck, indicating low trophic enrichment, reaching a maximum of 47 in Autumn 2011 which approximated the best trophic status in Newby Beck where a minimum of 41 was reach in spring 2012 (Figure 1.4.1 b). Similarly for Pow, which had a minimum TDI value of 48 in summer 2011. In both years, trophic status was good in both spring and autumn with poorest trophic conditions observed in autumn. The low trophic status as indicated through the TDI was supported by the BMWP metric which indicated oligotrophic condition. Moreover, MTR values were highest for Dacre Beck compared to Newby and Pow Beck with values approaching an indication of low trophy.

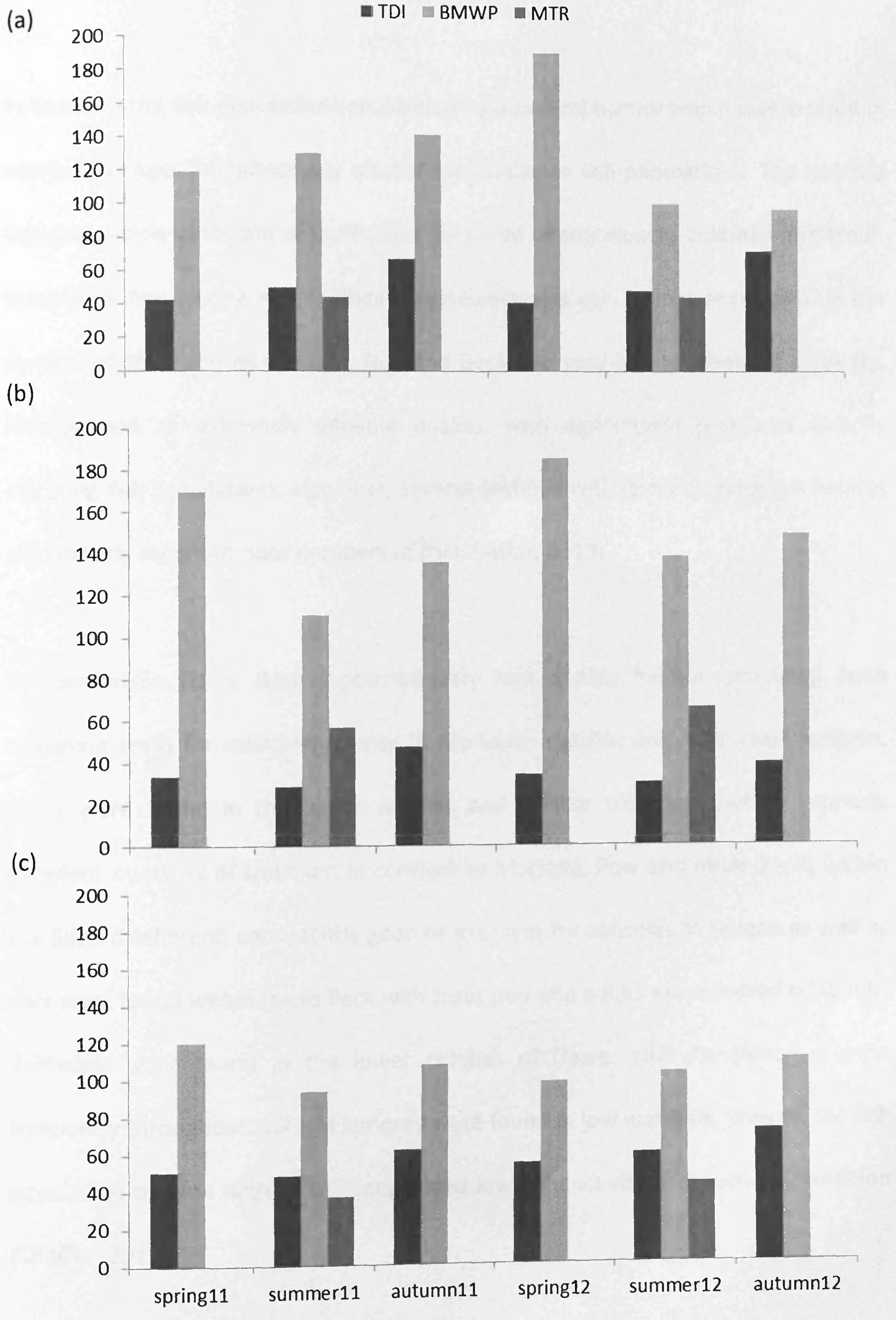

Figure 1.4.1: Seasonal metric comparison of TDI (n = 3 for diatoms), BMWP (n = 1 macroinvertebrate) and macrophyte (n = 1) for (a) Morland; (b) Dacre; and (c) Pow.

1.4.2 Fish assemblage of the Morland, Dacre and Pow catchments

In Morland the fish population was divided by a natural barrier which was located in Morland village. This effectively created two separate fish populations. The isolated fish population upstream of the barrier consisted of stoneloach, bullhead and trout. Elsewhere, trout along with bullhead, stonelaoch and eels were present. Within the context of the Eden as a whole, Morland Beck had very low numbers of trout fry. Habitat was of extremely variable quality, with agricultural pressures directly affecting fish populations. However, several sections with good or excellent habitat consistently recorded poor numbers of fry (Chaffer, 2013).

In comparison Dacre Beck supported very high quality habitat, providing good spawning areas for salmonid species in the lower reaches and main river sections. Trout were found in the upper reaches and smaller tributaries which supports excellent numbers of trout fry. In contrast to Morland, Pow and other Becks within the Eden catchment, consistently good or excellent fry densities of salmon as well as parr were found within Dacre Beck with trout parr and adults encountered relatively. Bullheads were found in the lower reaches of Dacre with stoneloach present frequently throughout. Eel and lamprey were found in low numbers. Overall, the fish population present within Dacre suggested low conductivity and nutrient condition (Chaffer, 2013).

Pow Beck was very different in nature to both Morland (Newby) and Dacre Becks, due to its relatively small size and lack of tributaries. Species found within Pow included stoneloach, minnow, sticklebacks and eels. Upstream sites in Pow with suitable habitat for salmonids, bullhead and stoneloach had no species present. Barriers presented in Pow were man-made weirs as opposed to the natural barriers present in Morland. These weirs are impassable, preventing natural re-colonisation by salmonids and minor species. In 2013 a survey was conducted between the confluence with the Caldew and the weirs. Low numbers of trout of several age classes along with bullhead, stoneloach and minnows were present. Thus, the fish assemblage suggested good water quality. However, this was likely a function of the oxygenating effect of the weirs (Chaffer, 2013).

Appendix 1.5: Graphical representation of Rainfall Event (RE) characteristics

1.5.1 RE 1 and RE 2 Response A (consult section 6.3.2, Table 6.1 -2)

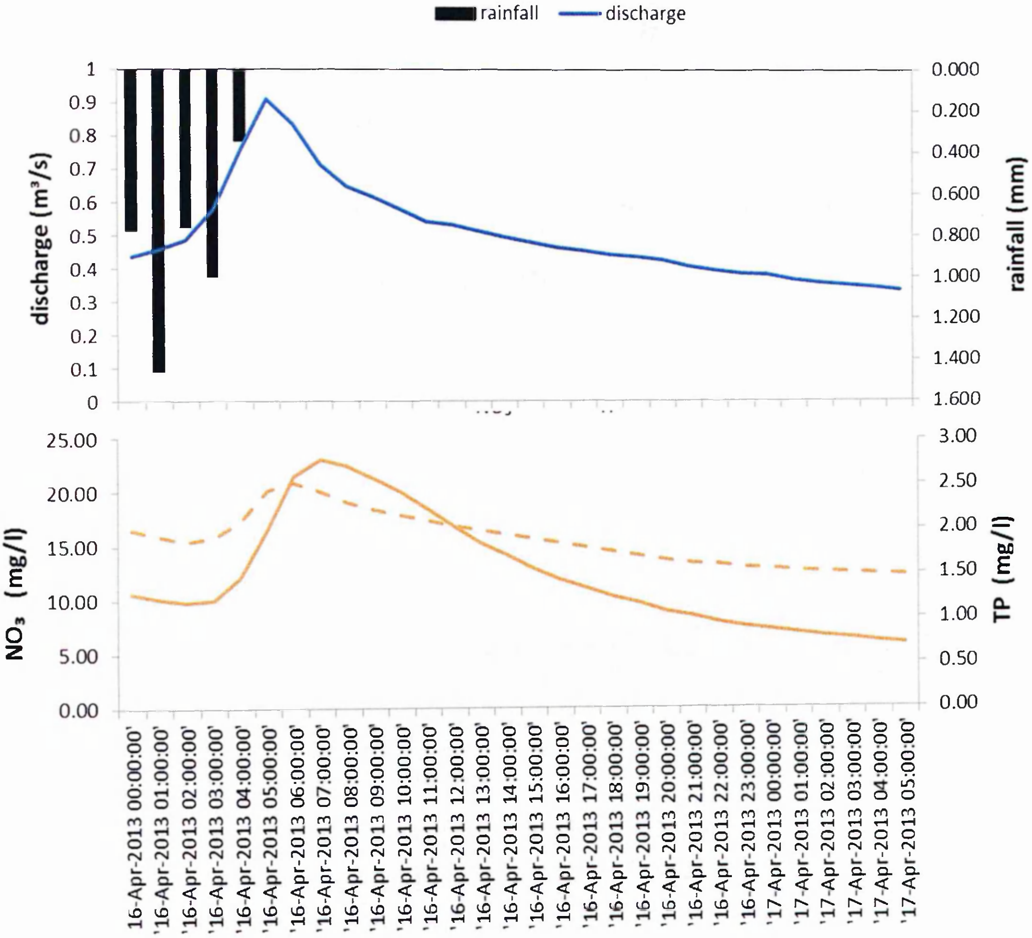

Figure 1.5.1: Mean hourly rainfall, discharge, TP and NO_3 based on 15 minute data between the 1200 on the 16/04/2013 and the 17/04/2013 at 0500. Source: EdenDTC.

1.5.2: RE 2 Response A and B(consult section 6.3.2, Table 6.1 -2)

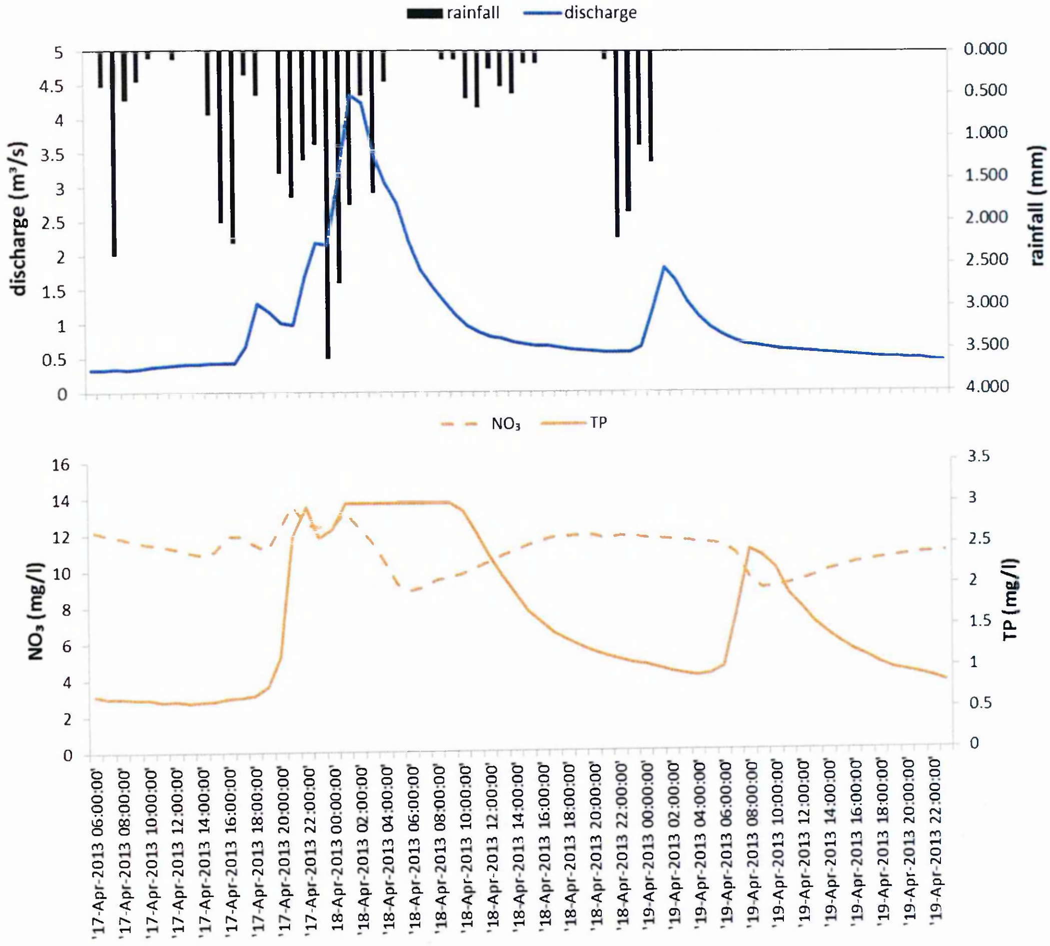

Figure 1.5.2: Mean hourly rainfall, discharge, TP and NO_3 based on 15 minute data between 0600 on the 17/04/2013 and the 20/04/2013 at 0700. Source: EdenDTC.

1.5.3: RE 3 (consult section 6.3.2, Table 6.1 -2)

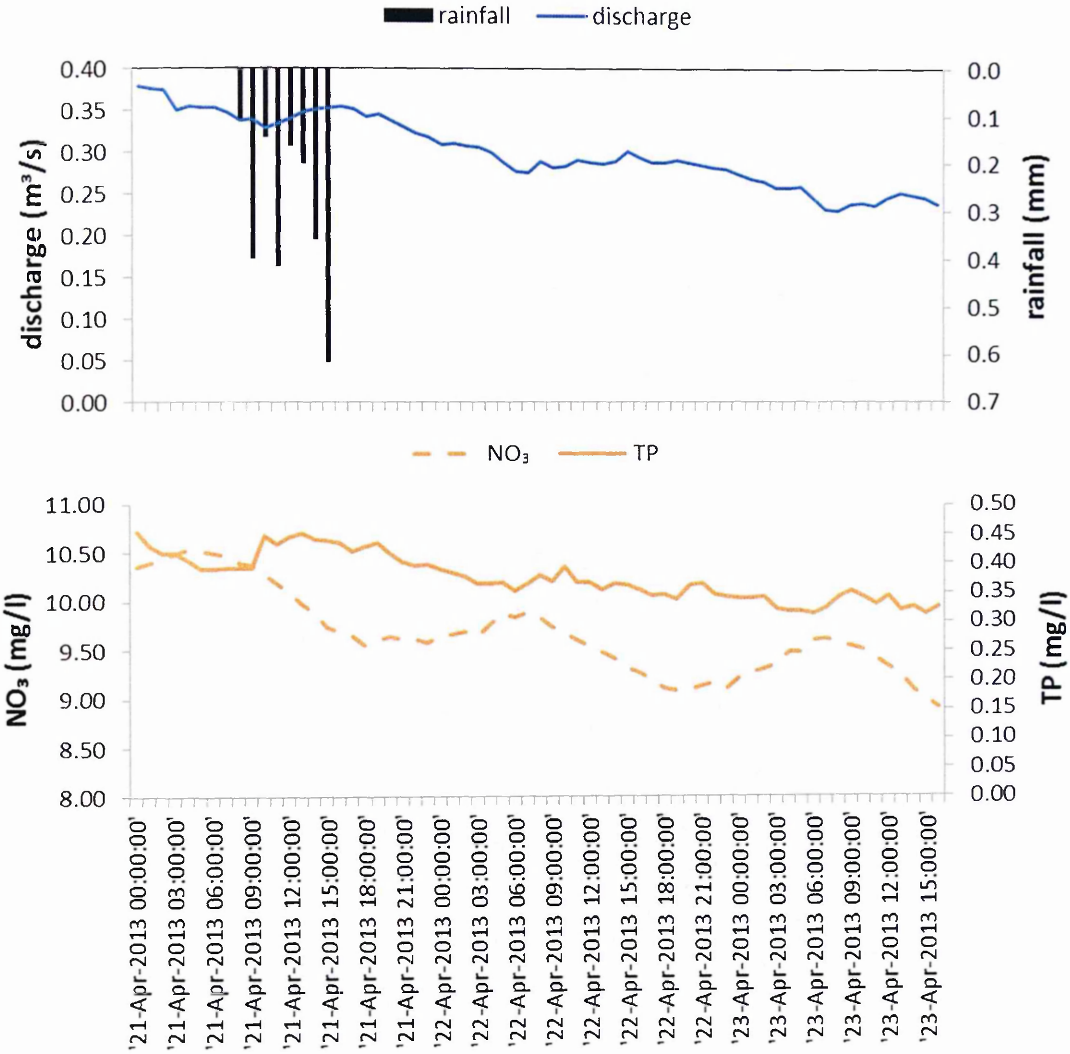

Figure 1.5.3: Mean hourly rainfall, discharge, TP and NO_3 based on 15 minute data between 0000 on the 21/04/2013 and the 23/04/2013 at 1600. Source: EdenDTC.

1.5.4: RE 4 Response A and B (consult section 6.3.2, Table 6.1 -2)

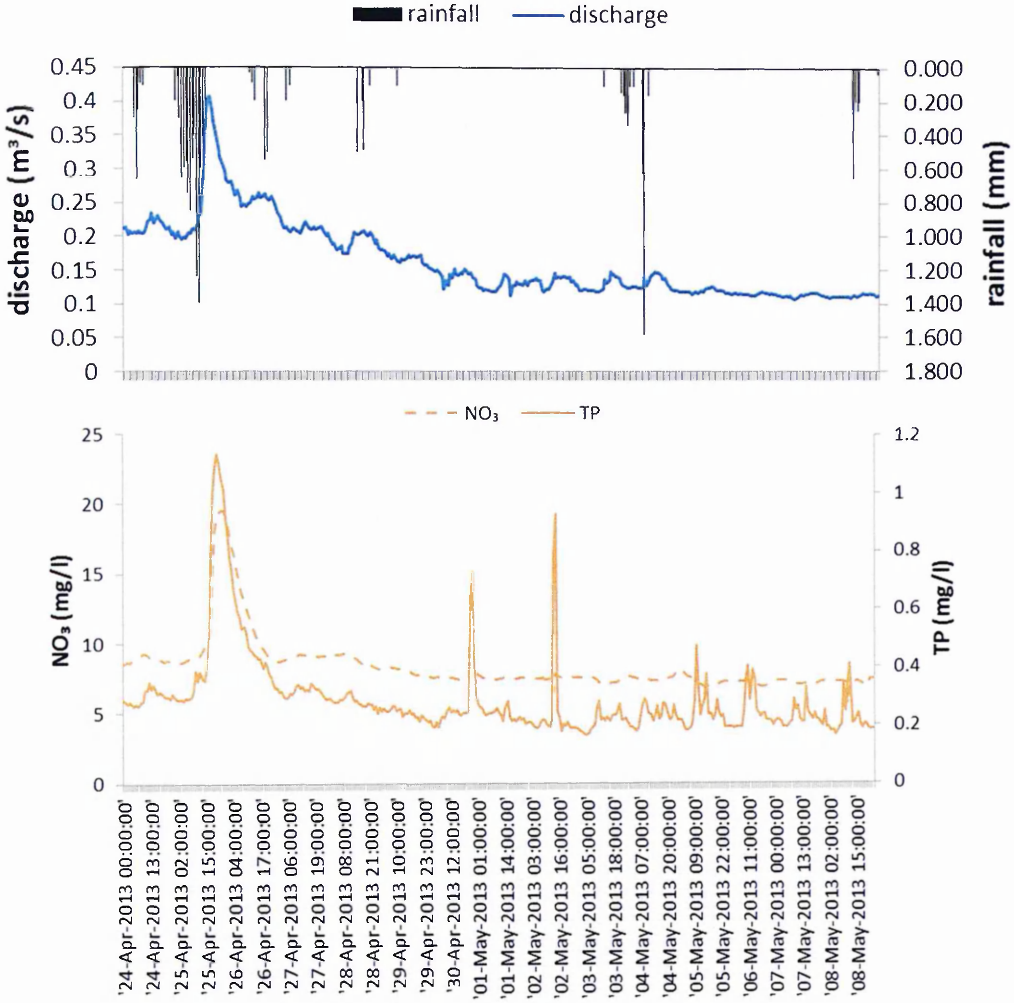

Figure 1.5.4: Mean hourly rainfall, discharge, TP and NO_3 based on 15 minute data between 0000 on the 24/04/2013 and the 08/05/2013 at 0600. Source: EdenDTC.

1.5.5: RE 5 (consult section 6.3.2, Table 6.1 -2)

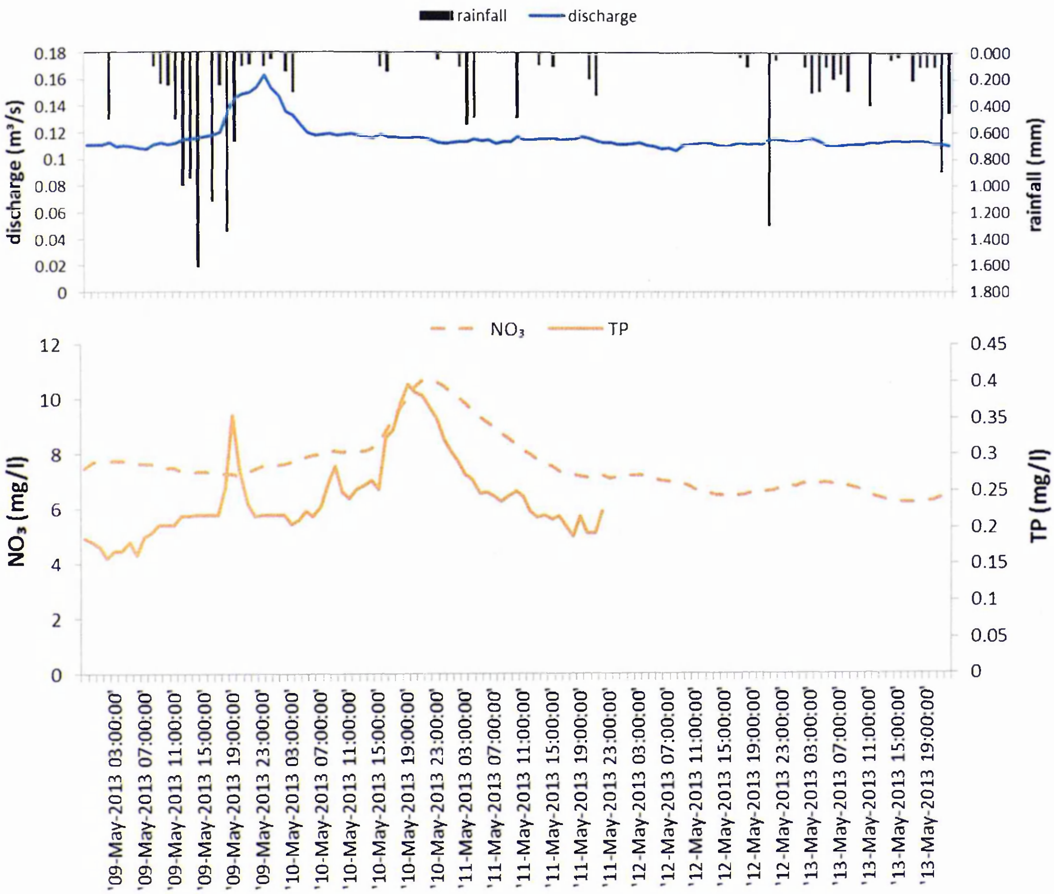

Figure 1.5.5: Mean hourly rainfall, discharge, TP and NO_3 based on 15 minute data between 0300 on the 09/05/2013 and the 13/05/2013 at 0000. Source: EdenDTC.