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Thesis title:	The Influence of Grazing Macroinvertebrates on the Structure of Benthic Diatom Assemblages: Implications for Biomonitoring
Qualification:	PhD
Date awarded:	19 March 2010

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THE INFLUENCE OF GRAZING MACROINVERTEBRATES ON THE STRUCTURE OF BENTHIC DIATOM ASSEMBLAGES: IMPLICATIONS FOR BIOMONITORING.

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Thesis submitted to the University of Sheffield for the degree of Doctor of Philosophy in the Department of Animal and Plant Sciences.

September 2009

Acknowledgements

Firstly, I wish to thank Professor Lorraine Maltby for her supervision, patience and support. I also wish to thank Dr Achim Paetzold for his advice, support and many useful discussions. This research was funded by NERC and CASE sponsorship from the Environment Agency. Facilities were provided by the department of Animal and Plant Sciences at the University of Sheffield, for which I thank Professor Malcolm Press and Professor Lorraine Maltby.

Numerous people within the department have helped with this work, both with Laboratory and fieldwork. I would like to thank Martin Kelly for advice on the TDI and Eileen Cox and Eliot Shubert for starting me off on identifying diatoms. Also I would like to thank Silke Skytte-Johannsen at the University of Bristol for teaching me to process diatoms. I extend thanks to all, to numerous to name, who assisted with fieldwork and with the construction of artificial streams.

Also thanks go to all who have read part or this entire thesis and all who have helped to proof read and improve drafts. I also thank the Freshwater group at the University of Sheffield for support and companionship. Lastly and by no means least I would also like to thank all my friends and family for encouragement and support throughout the course of this work.

THE INFLUENCE OF GRAZING MACROINVERTEBRATES ON THE STRUCTURE OF BENTHIC DIATOM ASSEMBLAGES: IMPLICATIONS FOR BIOMONITORING.

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Abstract

Macroinvertebrates and diatoms are involved in the provision of many ecosystem services and are frequently used for monitoring ecological quality; indices being primarily based on community structure. Macroinvertebrate-induced changes in the structure of diatom assemblages have the potential to result in erroneous assessments of ecological quality by altering the value of biotic indices (e.g. Trophic Diatom Index, TDI) in the absence of change in environmental quality.

The first aim of this thesis was therefore to determine how macroinvertebrate grazers, with different feeding modes, influence diatom assemblages. This was investigated in laboratory studies with artificial streams and via a field manipulation experiment. Mayfly grazers consistently decreased the relative abundance of high-profile diatoms, but the effect this had on the TDI was dependent on the relative sensitivity of the diatom species in the assemblage. Grazing induced changes in TDI, which were most pronounced when there was a significant difference in the average sensitivity of low-profile versus high-profile diatoms, has the potential to change the ecological quality assessment of a site, resulting in a possible misclassification.

The second aim was to explore the relationships between biomass, diversity and composition of the diatom and macroinvertebrate assemblages in minimally impacted sites. This was investigated by surveying minimally impacted streams and assessing the response of macroinvertebrate and diatom assemblages to a major disturbance event (flood). No correlation was found between the diversity or ecological quality indices of the two groups. A higher biomass of periphyton (as Chlorophyll *a*) was associated with greater macroinvertebrate abundance and high-profile diatoms in the assemblage were positively correlated with the abundance of mayfly grazers, indicating some trophic links. Macroinvertebrate diversity and abundance decreased due to the flood but there was no consistent response from diatoms. Seasonality appeared to be more important in determining changes in macroinvertebrate community than a one off flood event.

In conclusion, grazing macroinvertebrates (in particular mayflies) influence diatom assemblage structure by decreasing the relative abundance of high-profile diatoms, which can influence the TDI. Diatom and macroinvertebrate assemblages respond to different abiotic factors, as well as each other, and associations between them are not always detectable in the field (i.e. diversity). Assessments of ecological quality based on diatoms and macroinvertebrates were not concordant, meaning that one cannot be predicted from the other for monitoring purposes. Monitoring using both groups should provide better protection for the environment, as the lowest value can be taken, reducing the chance of false positives and provide greater understanding of how the system is functioning.

CONTENTS

1.0 INTRODUCTION

Freshwaters provide numerous ecosystem services that are essential for human health and wellbeing, such as water supplies: irrigation, waste disposal, leisure opportunities and many more (Malmqvist and Rundle 2002). In freshwater the biota of the ecosystem is a fundamental part of many of these services and needs to be protected and its status monitored (Heiskanen *et al* 2004). Cairns (1997) stated that "environmental monitoring is an activity that is essential to maintaining human quality-of-life", a sentiment reinforced in the European Water Framework Directive (WFD, directive 2000/60/EC), which sets goals of achieving good ecological status in European water courses by 2015 (Heiskanen *et al* 2004). The WFD defines good ecological status as "the biological quality elements should only indicate slight deviation from reference conditions, and the hydro-morphological, physico-chemical, and chemical quality elements should ensure ecosystem functioning" (Heiskanen *et al* 2004). Understanding how biological communities are structured should ensure robust biomonitoring approaches and will aide understanding of the underlying processes that occur in freshwaters and sustain ecosystem services (Petts *et al* 2006).

Traditionally, biomonitoring approaches in flowing water systems have focused on macroinvertebrate assemblages and have been designed and used successfully, to protect human health and regulate effluent rather than considering the needs of the freshwater system (Norris and Thoms 1999). However, the need to protect ecosystem services has lead to a focus on ecological quality and the view that a holistic approach to monitoring, involving more than one taxonomic group is required in order to track changes in ecosystem function and preserve fragile ecosystem services (Lancaster 2000). Using a small set of indicators to determine if an ecosystem is functioning well assumes that those few chosen measurements are relevant to other parts of the ecosystem (Norris and Hawkins 2000). Conversely, there is evidence that different taxonomic groups do not always respond in the same way to the same impact meaning that just monitoring one group could lead to sub-optimal management for many/ or all groups and the system as a whole (Hering *et al* 2006a). Different monitoring approaches and how different biological quality elements may influence each other in relation to their structure are

now discussed to raise environmental issues that should be addressed to improve the accuracy and efficacy of freshwater monitoring.

1.1 Biomonitoring approaches

Indicators for the status of a watercourse can be physical, chemical, biological or a suite of all of these (Boulton 1999). Biological status has historically been most commonly assessed using macroinvertebrates, as they are: ubiquitous, species rich, relatively sedentary in nature, relatively inexpensive to sample, possess a well described taxonomy, have established sensitivities to many disturbances, are suitable for experiments on pollution effects and are small enough to be useful for low-order streams (Bonada *et al* 2005, Herring *et al* 2006b). Using macroinvertebrates to assess an ecosystem is also helpful for management because they play important roles in many ecosystem services such as nutrient cycles, decomposition and translocation of materials (Malmqvist and Rundle 2002). They also act as an intermediate step in the food chain because they are the link between primary resources and higher consumers such as fish and riverine birds (Wallace and Webster 1996) making them a possible proxy for other organisms due to being connected to them. Macroinvertebrates are sensitive monitoring tools as they are influenced by a range of environmental factors including: water temperature, altitude, longitude, distance from source, stream size, channel width, conductivity, pH, substrate composition and many other factors (Turak *et al* 2001, Soininen and Kononen 2004, Heino *et al* 2002). However, they respond in characteristic ways to different disturbances and hence a shift in community structure can be used as a diagnostic tool. For example the macroinvertebrate assemblage has often been found to respond to impacts by certain aspects of their structure such as a decrease in total biomass, increase or decrease in total numbers of individuals, decrease in EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) and increases in the relative abundance of Chironomidae (Kiffney and Clements 1994). Different disturbances can have a different length and severity of effect, from a few weeks to decades if the impact fundamentally alters the food web (Wallace 1990). Therefore biological monitoring is needed alongside chemical and hydrological assessment as it can assess the effect of an impact after the initial stress has gone and determine if the system is impacted (Wallace 1990).

There are many different monitoring systems using macroinvertebrates throughout the world, an example being the system used in the UK called the RIVPACS (**R**iver **I**n**v**ertebrates **P**rediction **a**nd **C**lassification **S**ystem) method, which has been in use since 1990, and compares what is found at a site to what would be expected in a site with those physical conditions if it was in a pristine state (Wright *et al* 2000). This is important as the WFD requires sites to "only indicate slight variation from the reference conditions" to reach "good ecological quality" meaning that RIVPACS is already using a comparison with a reference state and similar schemes have recently been developed for other groups such as diatoms (Kelly *et al* 2008). This means that RIVPACS type systems are suitable for the monitoring required for the implementation of the WFD. The usefulness of these systems is demonstrated by their being developed for use in other countries such as AUSRIVAS (**Aus**tralia **Riv**ers **As**sessment scheme) in Australia and for other European countries most recently MEDPACS for use in Spain (**Med**iterranean **P**rediction **a**nd **C**lassification **S**ystem) (Simpson and Norris 2000, Poquet *et al* 2009).

RIVPACS was developed from existing systems that were established by the Biological Monitoring Working Party (BMWP), which was formed in the mid 1970s to classify running water sites and determine if biota could be predicted from chemical and physical attributes of a system (Hawkes 1998). The BMWP created an index that was calculated by different macroinvertebrate families being given different scores (From 1-10) depending on their sensitivity to pollution and these being added up to produce a score (the BMWP score) (Hawkes 1998). Due to the BMWP score being very much dependent on sample size and operator accuracy the Average Score Per Taxon (ASPT) is often the preferred method as this is an average of the BMWP scores (i.e. total BMWP score divided by total taxa) and is not so sensitive to missed taxa (Hawkes 1998). RIVPACS predicts what would be present in a site if it was in a pristine state and compares this to what is actually present to assess the quality (i.e. observed divided by expected) this allows different sites and levels of identification to be compared (Clarke *et al* 2003). RIVPACS initially only took into account the presence or absence of a macroinvertebrate family, as based on the BMWP, but more recent versions can use a

log abundance measure to take numbers into account and consider whether species are rare or common (Clarke *et al* 2003). Although used as the main measure of ecological quality, the scores used in RIVPACS mainly relate to their tolerance to pollution, especially organic rather than general degradation however they have been adapted to assess flow (Clarke *et al* 2003).

Indices based on different taxonomic groups, such as macroinvertebrates, diatoms, macrophytes and fish, can be converted into an Ecological Quality Index, or ratio (EQI). EQI compares the observed taxa (i.e. observed/expected (the RIVPACS approach)) at a site with what would be expected in pristine conditions and has boundaries set for the ecological quality classes that can allow the status based on different groups to be compared directly (Furse *et al* 2006). However, there is little work done on whether different taxonomic groups are concordant in their monitoring results and what evidence there is suggests that different taxonomic groups usually give results that do not correlate, probably due to responding to different aspects of the environment (Paavola *et al* 2003, Heino *et al* 2005, Hering *et al* 2006a). For example there was no concordance found in the diversity of multiple assemblages in lakes or between macroinvertebrates, bryophytes and fish in streams (Allen *et al* 1999, Heino *et al* 2005). It would be advantageous if one group could act as a surrogate for others but from the current evidence this seems unlikely in most cases, although for a known specific impact it may be possible to use the most sensitive group (Hering *et al* 2006). Currently there is insufficient evidence to state whether macroinvertebrates (or any other group) can act as a proxy for others so it is increasingly recommended to use multiple groups, as good ecological status for one group does not necessarily mean the system is at good ecological status for all groups or as a whole (Heino *et al* 2005). Monitoring groups from different trophic levels (i.e. diatoms, macroinvertebrates and fish) is beneficial because it integrates conditions over multiple spatial and temporal scales and it may also be possible to work out the mechanism for any observed change both because of the different trophic levels being influenced by different factors and due to different taxa responding most strongly to different variables (Lancaster *et al* 1996). For example it has been found that different groups of aquatic organisms respond best to different aspects of the environment so for the most comprehensive assessment of general

degradation using multiple groups should be most successful (Heino 2009). Thus, using multiple groups could give a more accurate assessment of the system and therefore could be more useful for maintaining or achieving good ecological status than just monitoring a single group, however more still needs to be understood about different groups and what they show us in freshwaters (Heino 2009).

One taxonomic group that has been used in addition to macroinvertebrates are algae, in particular diatoms, which have been used to assess certain aspects of the ecology of freshwaters such as eutrophication and organic enrichment (i.e. the urban waste water treatment directive) (Kelly *et al* 1995). Algae, of which diatoms are one of the most important components, are involved in several important ecosystem functions, including the absorption of carbon dioxide and release of oxygen (Heino *et al* 2005). They are also an important element of the base of the aquatic food chain and provide food for consumers which, in turn provide other ecosystem services such as wildlife and fish production, making them a fundamental part of the riverine ecosystem (Power 1990). Many aspects of the ecology of diatoms make them ideal candidates for monitoring tools and they are becoming a more regular part of many monitoring programmes (e.g. Kelly 1998).

Diatoms are useful for monitoring due to their sensitivity to various environmental impacts, their ease of sampling and robust silicon exoskeleton – meaning they can be preserved and stored easily, and they have many forms that are characteristic of certain environmental conditions (Kelly 1998). Diatoms can be particularly useful for monitoring when chemical and macroinvertebrate assessments do not agree and they are particularly responsive to short term impacts due to their short life cycle and high turnover rate (Fore and Grafe 2002). Diatoms can be used to assess several different aspects of water-bodies, in particular nutrient status, and it has been recommended by one research group that for suspected organic pollution diatoms should be the first choice indicator (Hering *et al* 2006b). Diatom assemblages can be assessed using a number of attributes including; percentage diatom valves sensitive to disturbance, percentage valves tolerant to disturbance, eutrophic species richness, percentage of valves that are nitrogen heterotrophic, percentage of polysaprobic species, alkaphilic

species richness, percentage of valves belonging to species requiring high oxygen, percentage of very motile taxa and percentage of deformed valves (Hill *et al* 2000, Fore and Grafe 2002). The most common indices utilise the relative abundance of species ad their different sensitivities, and include the BDI (Biological Diatom Index), GDI (Generic Diatom Index) and TDI (Trophic Diatom Index) and these are used all over Europe, North America and many other parts of the world (Coste et al 2009). These indices are mainly based on the equation of Zelinka and Marvin (1961) and use scores that have been determined from individual species tolerances to pollution (mainly organic) meaning that they tend to correlate well with each other (Kelly 1998). This means that any factors that influence one diatom index are likely to be applicable to other diatom indices.

Differences in the composition of diatom assemblages result from inter-specific variation in sensitivities to environmental factors (Kelly 1998, Medley and Clements 1998). The Trophic Diatom Index (TDI) is the most commonly used index for diatom monitoring in the UK and was developed in response to the need for new and improved ways for monitoring freshwaters in particular eutrophication (Kelly and Whitton 1995). This was specifically driven by the Urban Waste Water Treatment directive of 1991 and resulted in a workable index in 1995 that has since been developed to be suitable for the WFD (Kelly and Whitton 1995, Kelly *et al* 2008). The TDI works on the principle that species-specific optima for nutrient concentrations lead to shifts in relative abundances and hence assemblages that are characteristic of different nutrient states (Kelly and Whitton 1995). Diatom species are given a score (between 1-5 with 1 being high sensitivity and 5 low sensitivity) depending on their sensitivity to Phosphorous (as Filtered reactive Phosphorous - FRP), established by the concentration of FRP that species were associated with at their maximum abundance, these scores are used in the calculation of the TDI (Kelly and Whitton 1995, Kelly *et al* 2008). This was done by examining the relationship of each diatom taxon to FRP using graphs summarising FRP versus percentage of that taxon present and the taxa in question being assigned its sensitivity value depending on what level of Phosphorous it was most abundant at (Kelly and Whitton 1995). The TDI can be calculated as follows by the revised methods of Kelly *et al* (2008):

$$TDI = (WMS \times 25) + 25 \qquad \text{Equation 1.1}$$

Where WMS, the working mean score, has a value between 0-100) – based on the weighted average equation of Zelinka and Marvan 1961 (Kelly *et al* 2008):

$$\text{WMS} = \frac{\sum a_j s_j}{\sum a_j} \qquad \text{Equation 1.2}$$

Where a_j is abundance of species j in the sample, s_j is pollution sensitivity (1-5 obtained from Kelly *et al* 2007) of species j.

The TDI ranges from 0-100 with lower values indicating high water quality and higher values indicating higher levels of eutrophication and nutrient enrichment. A high score for the TDI indicates high levels of nutrient impact and a low score indicates low levels of impact (Kelly and Whitton 1995). The TDI has recently been adapted for use in the WFD by utilising an observed and expected ratio similar to that used by RIVPACS, thus allowing a score based on diatoms to be compared to other groups (this also means that a score close to 1 equals good quality and a lower score worse quality) (Kelly *et al* 2007, Kelly *et al* 2008).

All biotic assemblages have a certain amount of spatial and temporal variation meaning that indices based on these elements are liable to have some uncertainty (Kelly *et al* 2009). There is generally found to be more variation in the diatom assemblage than the macroinvertebrate assemblage, possibly due to the greater temporal variation in diatom assemblages and the different variables they respond to (Chessman *et al* 1999) meaning that determining reasons for this variability will be beneficial for ecological understanding and monitoring. Another reason for the greater observed variability in diatom indices could be due to diatoms having been less thoroughly studied than macroinvertebrates. In the UK diatoms have only been widely employed for monitoring since the TDI came into use in 1995 (Kelly and Whitton 1995) whereas the macroinvertebrate based methods have been in use since the early 1970s and early versions of RIVPACS have been in development since the early 1980s (Wright *et al*

2000). Another possible cause of variation in diatom assemblage structure is interactions between them and other biota through food chain interactions. How interactions between diatoms and other biotic groups influence relative abundance based biotic indices are have not been studied in detail and are not well understood.

1.2 Influence of abiotic and biotic factors in structuring communities.

Biomonitoring approaches assume that changes in assemblage are primarily driven by, and hence can be related to, changes in abiotic (environmental) factors, whilst biotic factors have been largely ignored (MacNeil *et al* 2000). Considerable evidence suggests that abiotic factors, in particular disturbance, (defined as "any relatively discrete event in time that disrupts ecosystem, community, or population structure, and that changes resources, availability of substructure, or the physical environment" (Resh *et al* 1988)) are extremely important for structuring the aquatic biota (Peckarsky *et al* 1990) as illustrated by the intermediate disturbance hypothesis, which predicts that the most diverse assemblages are found at intermediate levels of disturbance (Connell 1979). The highest diversity is predicted to occur at intermediate levels of disturbance due to the creation of more niches by disturbance which decreases the chance of dominance and of ecological processes being performed by any one taxon (Connell 1979). Disturbance increases diversity up to a point where the impact would become to great for many organisms to cope with it and/or benefiting highly adapted organisms that can cope with it and thus become dominant (Connell 1979). Some studies have found the predictions of the intermediate disturbance hypothesis to be correct, for example stream algae and macroinvertebrates in small New Zealand streams, have been found to have the highest diversity at an intermediate levels of hydrological disturbance (Biggs and Smith 2002, Townsend *et al* 1997) whereas others studies have disagreed (Eckert and Walz 1998).

Despite the importance of abiotic factors, biotic interactions should not be ignored when considering how assemblages are structured and when evaluating biomonitoring results that are based on the relative abundance of a group of organisms. Biotic interactions can furthermore play an important role in structuring aquatic systems by influencing the assemblage structure of both macroinvertebrates and diatoms and these two groups can

particularly influence each other (MacNeil *et al* 2000). Macroinvertebrates and diatoms can influence aspects of each other's ecology due to food-chain interactions additional to water and habitat quality *per se* (Wallace and Webster 1996). For example, theory suggests that the highest species diversity of macroinvertebrates should be found at an intermediate level of primary productivity, of which diatoms are a part (Waide *et al* 1999). Grazing by macroinvertebrates can act as a disturbance to diatom assemblages and can result in greater biomass of primary producers, due to possible removal of senescent cells, meaning that some stress will result in a greater abundance of diatoms (as in the intermediate disturbance hypothesis) (Niyogi *et al* 2002). Indirect responses to abiotic factors can occur mediated by biotic interactions such as increases in nutrients being found to increase grazer biomass as a consequence of increasing periphyton biomass, as opposed to the macroinvertebrates actually responding to the nutrients (Roll *et al* 2005). Evidence for grazing influencing the diatom assemblage as much or more than abiotic factors is shown by Hillebrand and Kahlert (2001) finding that when the effect of nutrients and grazing on periphyton taxonomic composition was compared, grazing had the greater influence.

The harsh-benign hypothesis highlights the importance of abiotic factors in structuring communities in combination with biotic factors (Menge and Sutherland 1976). A harsh regime is one where the biota is faced with unfavourable conditions, often hydrological in nature, whereas a benign environment has favourable conditions for the biota (Peckarsky *et al* 1990). It suggests that in ecosystems that have a harsh abiotic regime communities will be structured mainly by abiotic factors, whereas biotic interactions such as predation and competition will be more important in structuring communities in benign environments (Menge and Sutherland 1976). This hypothesis was developed for a marine environment where the prey were sessile and the predators mobile, meaning that the prey had evolved better strategies to cope with harsh conditions, thus when conditions were harsh predation pressure was decreased (Menge and Sutherland 1976). Often stream systems can demonstrate both harsh and benign conditions either over time or in different patches. The predictions of the harsh- benign hypothesis have been corroborated by predatory stoneflies being found to have a greater influence on their mayfly prey when conditions are more benign (Peckarsky *et al* 1990). The way that

predator-prey interactions are influenced by disturbance can depend on whether the prey or the predator is most stressed. If the predator is more affected (i.e. decreased) when the system is impacted this leads to a release of the prey from predation pressure allowing an increase in prey numbers, this is a "consumer stress model" (Thompson *et al* 2002). If the prey is more stressed by a pressure than the predator then their defences may be weakened allowing them to be easier prey, this is a "prey stress model" (Thompson *et al* 2002).

Theory thus indicates that the outcomes of predator-prey interactions depend on what disturbances are present and how individual taxa respond to them, demonstrating that the forces structuring a system are multi-faceted and not likely to be just abiotic factors or just biotic factors, in reality a combination of the two is most likely. For example a study on the effect of flooding on predation found that some predator-prey interactions were unchanged and some increased due to the changed hydrological conditions (Thompson *et al* 2002). Hydraulic factors are one of the strongest sources of disturbance on aquatic organisms having been shown to influence how grazing macroinvertebrates behave and how they are distributed in streams thus effecting how much they predate on their algal (i.e. diatom) food source (Rempel *et al* 2000, Hoffman *et al* 2006). The food sources present in a system also strongly influence what organisms can exist there, as has been demonstrated for rivers using the River Continuum Concept (Vannote *et al* 1980) but can also be more specific with the exact type of food influencing what does best at a patch scale. For example movement of a grazing caddisfly was strongly influenced by algal density and taxonomic composition (Poff and Ward 1995).

1.3 Diatom traits and grazing

Species traits, which are aspects of their ecology such as body size and body form of different groups or organisms that can be put into different categories, are an important factor in structuring the biotic assemblages and influencing their interactions (Statzner *et al* 2005, Ilg and Castella 2006). The diatom assemblage is made up of many genera that have evolved different traits in order to be competitive in a multi-species complex (Table 1.1).

Table 1.1: Growth-form traits of diatoms. Adapted from Molloy (1992), Wellnitz and Ward (2000) and Tall *et al* (2006).

Type	High or Low profile	Characteristics	Examples
Prostrate	Low	Small, mono-raphid or bi-raphid	*Achnanthes spp., Cocconeis spp*.
Adnate	Low	Adjacent to surface without being prostrate or erect	*Surirella, Amphora*
Erect	High	Perpendicular to substrate without stalks	*Fragilaria, Diatoma*
Stalked	High	Arborescent or stalk forming genera	*Encyonema, Gomphonema*
Motile	Usually low, can move	Prostrate-motile	*Navicula spp, Nitzschia spp*

The dominant growth form of the diatoms present in an assemblage can be determined by the environmental conditions and the biotic (grazer-diatom) interactions. For example, low-profile taxa (prostrate, adnate) are often dominant in nutrient-poor conditions with high disturbance, high-profile taxa are found at nutrient-rich low disturbance sites and taxa with motility are found at sites with increased nutrients and increased disturbance (Passy 2007). Diatoms that are competitive for light have developed erect or stalked characteristics (i.e. high-profile), but this makes them more vulnerable to grazing, whereas low-profile diatoms without these adaptations can be out-competed for light, but are less vulnerable to predation (Wellnitz and Ward 2000). Grazing is thought to be one of the most important causes of variation in diatom assemblages in flowing waters and needs to be investigated thoroughly both for its influence on the ecology of the system and its possible influence on monitoring results. It is reasonably well established that some algal taxa are more successfully grazed than others (Steinman 1996). This has been found to be largely due to their growth form as opposed to any active choice by grazers, with large high-profile taxa such as *Cymbella spp, Gomphonema spp* and *Rhoicosphenia abbreviata* being more readily consumed than small prostrate taxa such as *Achnanthes spp* and *Cocconeis spp* (Jacoby 1987, Peterson 1987, McCormack and Stevenson 1989, Wellnitz and Ward 1998). Different

diatom species therefore have different grazer sensitivities as well as different sensitivities to nutrients, metals and other factors (Colletti *et al* 1987).

Traits of diatoms are also important for determining what species are present at sites with different anthropogenic impacts, for example erect diatoms are generally more sensitive to metals than low-profile diatoms, due to their greater surface area (Medley and Clements 1998, Hirst *et al* 2002). This means that taxonomic changes in the diatom assemblage due to an impact could be mediated by the traits of diatoms and how vulnerable they are to different impacts (Medley and Clements 1998). The section of river being examined also influences what diatom assemblage is present with structure tending to change downstream from prostrate diatoms (low-profile) in the headwaters to erect diatoms in the lowlands (Medley and Clements 1998). The maturity of a diatom assemblage is another aspect that influences what species and traits are present at a site at the time of sampling, as diatom assemblages develop from low-profile adnate and prostrate pioneer species, such as *Achnanthes spp.* and *Epithemia spp,* to erect, stalked and filamentous species, such as *Fragilaria spp.* and *Gomphonema spp.*, this is often related to the time since the last disturbance event that scoured the stone surfaces (Hoagland *et al* 1982, Pan and Lowe 1994).

Heavy macroinvertebrate grazing can cause assemblages to remain predominantly low-profile like a pioneer assemblage due to grazing pressure on the more available high-profile taxa (Pan and Lowe 1994). The diatom taxa present do not appear to influence their removal rate (only their growth form) by macroinvertebrates, suggesting that grazers will feed at the same rate on those species that their mouthparts are adapted to graze (Cattaneo and Mousseau 1995). Grazers in lakes have been found to shift the dominant growth form of the diatom assemblage towards being less edible, thus influencing what species persist and in what percentages (Hodgsen and Vinebrook 2006). Macroinvertebrate grazers are also influenced by the environment and anthropogenic impacts, meaning that even if diatoms are not directly affected by an environmental change they could be influenced indirectly by a change in the macroinvertebrate community if it results in change in the amount and/or type of grazing pressure (Wallace and Webster 1996).

Additional to the traits of diatoms determining how vulnerable they are to grazing, the type of grazing macroinvertebrate present can influence the make-up of the diatom assemblage due to different grazers varying in their ability to graze low-growing parts of the diatom assemblage (Lamberti *et al* 1987). The extent to which a grazer is able to consume the diatom assemblage depends on their foraging behaviour, mobility, head orientation and mouthpart morphology (Tall *et al* 2006). The most important factor is thought to be mouthpart morphology, for example mayflies are generally only able to consume the upper surface of the diatom assemblage, with their brushing mouthparts, whereas snails have scraping radula mouthparts that are able to graze down to the base (Tall *et al* 2006). This suggests that the type of grazer(s) present will determine the magnitude of impact on the diatom assemblage and whether there is any change in the relative abundance of different species. An example of this is that Karouna and Fuller (1992) observed that in laboratory trials differences in the effect of three mayfly species and a caddisfly species on the periphyton community was due to their different mouthpart morphologies indicating that they were not actively choosing their food but it was dictated by what they were able to consume. Other examples from artificial streams are that, Hill and Knight (1987) and Colletti *et al* (1987) found that mayfly grazers reduced large over-story components of the community and decreased overall standing crop, but did not decrease the low-profile taxa or increased them respectively. In some environments macroinvertebrate grazers may have spatially segregated feeding within the periphyton biofilm due to the different species abilities to graze at different levels allowing more grazing taxa to co-exist and utilising more of the diatom assemblage (Figure 1.1) (Tall *et al* 2006).

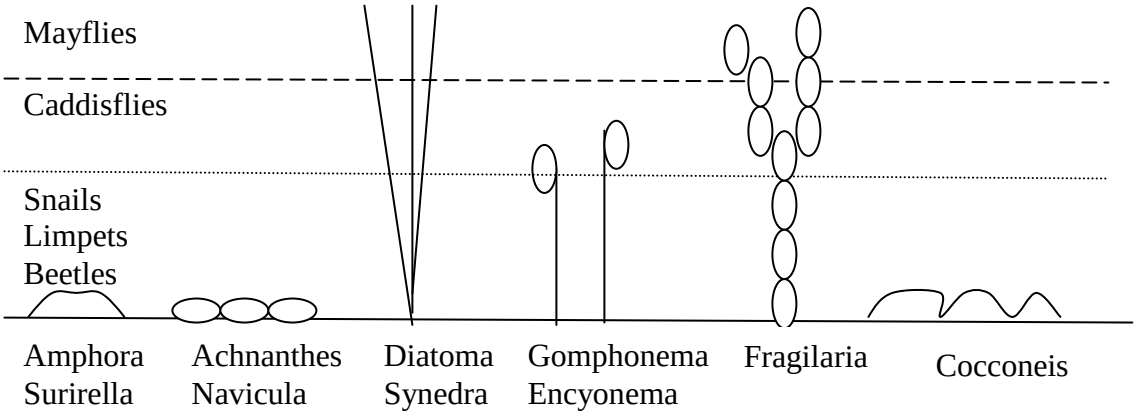

Figure 1.1: Physiognomies of diatoms with examples of families belonging to that group and the macroinvertebrates that can feed on them. Adapted from Tall *et al* 2006.

Current knowledge is that different species of grazer have different effects and magnitudes of effect on the diatom assemblage and this can vary between studies, species and situations, meaning that more detailed, specific information is needed to inform management (Colletti *et al* 1987, Hill and Knight 1987 and Tall *et al* 2006). There are also very few studies performed in U.K. rivers and most information on grazer-diatom interactions is from artificial stream experiments that can be limited in their applicability to the field. Field studies have mostly used sedentary grazers, such as snails and caddisflies, meaning that the influence of mobile grazers in natural conditions has not been assessed and can only be predicted from laboratory assessments.

Grazing macroinvertebrates can reduce periphyton biomass (McAuliffe 1984) and a greater numerical abundance of grazers will result in greater decreases (Hill and Knight 1987). Grazer density also can have a strong influence on the taxa of diatoms present with the accrual of grazer sensitive taxa decreasing with increased grazer densities (Peterson *et al* 2001). The mobility of grazers also influences how they may impact the algal assemblage with mobile grazers such as mayflies tending to feed on patches of abundant periphyton abandoning them at much higher densities than less mobile grazers like snails and caddisflies would (Bergey 1995, McKenny 2005). Diatom densities have been found to be higher when macroinvertebrates are excluded, suggesting that the more competitive species will do better in these conditions than the less competitive but

grazer-resistant taxa (Jordan and Lake 1996). In some circumstances grazing can increase the diversity of the diatom assemblage, if the type of grazing results in a decrease in highly competitive species and gives other species an opportunity for resources (DeNicola *et al* 1990). However, a study in lake enclosures found that snail grazing decreased the diversity of total species and the amount of high-profile taxa, with the assemblage being dominated by small, grazer resistant, prostrate taxa, demonstrating the complicated nature of interactions between the two groups (Lowe and Hunter 1988). The whole macroinvertebrate assemblage could influence diatom assemblage structure as most aquatic-macroinvertebrates are considered generalists to a certain extent (Mihuc 1997). The aims and objective of this PhD relate to the interactions between macroinvertebrates and diatoms (both with and without disturbance), the importance of this in dictating their assemblage structure and the implications of this for biomonitoring.

1.4 AIMS AND OBJECTIVES

Previously, abiotic factors have been thought to be the most important factors in structuring riverine biota and thus indices are based on this assumption. However, evidence suggests that interactions between groups of organisms, in particular diatoms and macroinvertebrates, are important in structuring their assemblages with both groups having the potential to play an important role in each others structure and function (Wallace and Webster 1996). How different grazers influence diatom assemblage structure (particularly in relation to indices) and in different combinations has not been fully investigated meaning that the influence of the two groups on each other and their indices is not fully established. Indices based on diatoms use assemblage structure, in particular relative abundance, to assess ecological quality. Therefore the interaction between diatoms and macroinvertebrate grazers has the potential to influence assemblage structure independently from the environment and could influence index results but whether this occurs significantly in reality is unknown. It is also not fully established whether there is concordance between aspects of these two groups, especially in relatively undisturbed systems or when faced with unpredictable disturbance events, as studies that have found concordance have often been associated

with strong environmental gradients that could be misleading (Heino 2009). There is potential for grazing macroinvertebrates to influence diatom biomass, assemblage structure, relative abundance of trait groups and species, meaning that there is potential for this interaction to be significant in structuring the diatom assemblage and influence indices and for the two groups to have correlations in the field. The above issues will be investigated in this thesis with the aim being to provide an enhanced understanding of the links between macroinvertebrates and diatoms that underpin the aquatic environment and the monitoring of ecological quality, and thus provide better protection of ecosystem services.

The influence of grazing macroinvertebrates on the structure of benthic diatom assemblages: implications for biomonitoring.

This thesis aims to determine whether grazing macroinvertebrates can significantly influence the structure of the diatom assemblage and whether this can influence the results of monitoring that is based on relative abundance of diatom species. It also investigates the affect of different grazers on different diatoms assemblages, addressing the issue of under what circumstances grazers influence the diatom assemblage, whether this is predictable and what the consequences are for monitoring of ecosystem quality. The general hypothesis tested is that grazing-macroinvertebrates and diatoms are linked through the food chain and that grazers will therefore influence diatom structure and thus indices based on this. The thesis addresses four specific questions.

1. **How do grazers with different feeding modes influence the structure of diatom assemblages made up of different species and how does this influence diatom biomonitoring results?**

   The aim of this part of the thesis **(Chapter 2)** was to investigate if grazers with scraping mouthparts and those with brushing mouthparts, snails and mayflies respectively would result in different affects on diatom assemblage structure through selective grazing. This was investigated using artificial streams, three different mayfly families, one snail family and two different diatom assemblages with the

biomass of grazers controlled so any difference in grazer treatment was due to grazing ability not grazer biomass. The hypothesis tested was that mayflies should cause a greater change in structure of the diatom assemblage due to mouthpart morphology and this would occur as a decrease in all high-profile diatom relative abundance. This is predicted to result in a change in index mediated by the change in relative abundance for mayfly grazing but not for snails.

2. **How do mayfly-dominated grazing assemblages influence the structure of diatom assemblages in streams?**

The aim of this section of the investigation was to find out if in natural field conditions a mayfly-dominated macroinvertebrate assemblage had a significant effect on the diatom assemblage structure by decreasing the high-profile diatom relative abundance and whether this has consequences for the diatom index **(Chapter 3).** The abundance of mayflies was also taken into account to assess if abundance and not just the presence of grazers can influence the diatom assemblage structure. This study was carried out in 10 streams using an exclosure/enclosure design. The hypothesis investigated was that grazed treatments would have less relative abundance of high-profile diatoms and the difference between grazed and ungrazed treatments would be greater with a higher abundance of mayfly grazers.

3. **What is the relationship between structure, biomass, composition and indices of the diatom and the macroinvertebrate assemblages in minimally impacted streams?**

The aim of the above question was to determine if the correlations that are likely to be mediated through the food chain between diatoms and macroinvertebrates can be observed in natural condition through a survey, in relatively unimpacted sites **(Chapter 4).** This was in order to determine if the effects that grazers can have on diatom assemblage structure are observable in the field and if they are predictable. This aimed to determine if monitoring effort could be decreased or more understanding gained by making monitoring more robust. Surveys of 24 North of

England streams for both diatoms and macroinvertebrates was carried out to investigate this with the hypothesis investigated being that aspects of the two assemblages should be correlated due to food-chain interactions i.e. a greater species richness of diatoms should result in greater richness of macroinvertebrates due to more different food sources so more scope for different niches.

4. **How do diatoms and macroinvertebrates respond to a significant disturbance event?**

This part of the thesis investigated diatom and macroinvertebrate assemblages when responding to a disturbance namely the significant flood event of June 2007 **(Chapter 5).** It is thought that systems when highly disturbed are more influenced by abiotic than biotic factors so it was expected that links would not be observed between the two groups and that they would respond to the flood in a way consistent to their traits. How the groups respond to a natural disturbance event is important to be understood for monitoring purposes, as hydrology is one of the most important factors in structuring the biota of the ecosystem. It was hypothesised that macroinvertebrates would be more affected than diatoms due to their larger size and longer life cycles and the diatoms ability to re-colonise rapidly. The flood was assessed by samples being taken a month before the flood and a few days afterwards (2007) and this compared to the next year which was not hit by severe flooding (2008).

2.0 INFLUENCE OF GRAZING MACROINVERTEBRATES ON DIATOM ASSEMBLAGES IN ARTIFICIAL STREAMS: IMPLICATIONS FOR BIOMONITORING

2.1 Introduction

Diatoms contribute significantly to the primary production of rivers and streams and provide an important food source for grazing macroinvertebrates (Ferminella and Hawkins 1995). Diatom species differ in their relative sensitivities to nutrients and organic pollution making them valuable for ecological monitoring (Kelly and Whitton 1995). Diatom assemblages are increasingly used in biomonitoring and the inclusion of diatoms in the assessment of ecological status is a legislative requirement in some parts of the world (Hering *et al* 2006).

Diatom indices have been independently developed for various purposes in several different countries resulting in many indexes existing that have subtle variations (Kelly *et al* 2005). Some of the diatom indices most commonly used are: Descy's Index

(Lecointe *et al* 1993), the Generic Diatom Index (GDI) (Lecointe *et al* 1993), the Specific Pollution Sensitivity Index (SPI) (Lecointe *et al* 1993), the Biological Diatom Index (BDI-2006) (Coste *et al* 2009), the Practical Diatom Index (Prygiel *et al* 1996), the Trophic Diatom Index of Schiefele and Kohmann (TDI-S) (Kelly *et al* 1995) and the Trophic Diatom Index (TDI) (Kelly and Whitton 1995). Diatom indices tend to correlate well with each other due to being based on the relative abundance of a similar selection of indicator taxa and their response to impacts; also all of the European indices (GDI, SPI, TDI-S, TDI) are based on the same equation – the weighted averaging equation of Zelinka and Marvan 1961 (Kelly *et al* 1995, Coste *et al* 2009). Most diatom indices operate by different scores being allocated to taxa depending on their sensitivity to the stressor of interest, usually eutrophication or nutrient enrichment (Kelly and Whitton 1995, Coste *et al* 2009). Sensitivity to different stressors is usually assessed in the same way for the same species used in the different indices, so those that are sensitive in one index are likely to be sensitive in another, again leading to a high similarity in the results of the indices. In this study the TDI is used as an example of a relative abundance based diatom index.

Diatoms exhibit various growth forms (traits) that have been classified in different ways by different authors and at different times (Yallop and Kelly 2006), but for the purpose of this study four major growth forms are considered, the reason being that these traits are likely to lead to a difference in vulnerability to grazing based on Wellnitz and Ward (2000). The groups considered in this study are: prostrate taxa that grow close to the surface and are attached tightly; motile taxa that can be low growing but are not attached to the surface and have the ability to move around the biofilm; erect taxa that can reach the canopy and sometimes form chains and stalked taxa that are attached to the substrate by a stalk with the diatom frustule at the surface of the biofilm (Wellnitz and Ward 2000). For this study erect and stalked taxa are together referred to as high-profile taxa and prostrate and motile taxa are considered to be low-profile. In general erect diatoms are more vulnerable to pollutants (as shown by the sensitivity values of the TDI) due to their larger surface area and their presence at the surface of the biofilm, and motile diatoms are least vulnerable, due to their ability to move around (Kelly and Whitton 1995). The TDI sensitivity values range from 1, being very sensitive, to 5 being tolerant,

meaning that a high score for the TDI represents poor water quality (Kelly and Whitton 1995). All trait groups show the whole range of TDI sensitivity values but the trend is for erect diatoms to be the most sensitive (Figure 2.1).

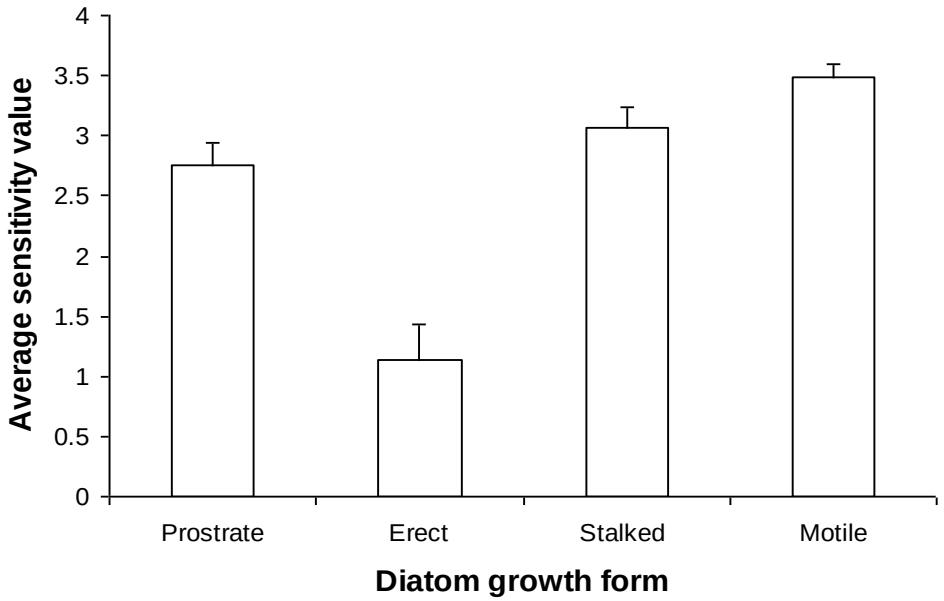

Figure 2.1: Average diatom sensitivity values (TDI) for species in different trait groups (Error bars represent 1 standard error). Values were taken from tables in Kelly *et al* (2007).

The relative abundance of high-profile diatoms is partly determined by abiotic factors, such as nutrients and flow (Passy 2007). However, grazing can also affect the relative abundance of different growth forms and hence has the potential to confound the interpretation of biomonitoring information and the assessment of water quality. Different growth forms of diatoms tend to differ in their susceptibility to grazing by benthic macroinvertebrates (Steinman *et al* 1987): large, high-profile diatom species tend to be more readily grazed than small low-profile species (Peterson 1987, Steinman *et al* 1987, Holomuzki and Biggs 2006). An algal assemblage progresses from a low-profile pioneer assemblage to an advanced assemblage made up of many high-profile algae (Yallop and Kelly 2006). In this study only the diatom assemblage is investigated, as they are often the most abundant algae in UK Rivers and are used in monitoring indices as a successful representative of all algae (Kelly *et al* 2007). Selective grazing by macroinvertebrates has the potential to alter the composition of the diatom assemblage

and as a consequence, highly grazed assemblages often resemble a pioneer assemblage made up of mainly prostrate, low-profile diatoms (Pan and Lowe 1994, Jordan and Lake 1996, Kelly *et al* 2008). As discussed, high-profile diatoms will often have a lower average score than low-profile diatoms due to many high-profile species being more vulnerable to pollutants because of their greater surface area, easier accessibility and slower growth. If the high-profile diatoms in an assemblage have a lower average score than the low-profile diatoms, grazing that decreases the relative abundance of high-profile diatoms has the potential to change the index.

The way grazers impact diatom assemblages is a function of their mouthpart morphology (Karouna and Fuller 1992), mobility (McKenny 2005), feeding rate (McKenny 2005) and body size (Holomuzki and Biggs 2006). For example, snails have rasping radula mouthparts that can lead to snail-grazed assemblages being distinct from those grazed by surface feeders with brushing mouthparts, such as mayflies (Holomuzki and Biggs 2006). Artificial stream studies have generally found that high-profile diatoms have lower absolute and/or relative abundance with mayfly grazing (Hill and Knight 1987, Colletti *et al* 1987, Holomuzki and Biggs 2006). In contrast, snails have been found to have variable effects due to being less mobile than mayflies thus grazing patches more intensively before moving on (McKenny 2005). However, even for the same species of grazer, there is considerable variation in effects across experiments. For instance Holomuzki and Biggs (2006) found the snail, *Potomopyrgus antipodarum*, had no influence on diatom assemblage structure whereas Holomuzki *et al* (2006) observed that it decreased the relative abundance of high-profile diatoms. A possible explanation for this variation might be related to experimental design (i.e. food limited conditions) or the use of different starting assemblages of diatoms.

Most previous studies on the effect of grazing on diatom structure have only investigated one type of mayfly grazing on one type of diatom assemblage (e.g. Colletti *et al* 1987, Holomuzki and Biggs 2006) meaning that conclusions are not necessarily widely applicable because there is much variation in diatom assemblages and conclusions are limited to that which was studied. As stated above mayfly grazing has generally been found to result in a decrease of high-profile diatoms (Hill and Knight 1987, Colletti *et al* 1987, Holomuzki and Biggs 2006), however, it is important to consider the impacts that

combinations of grazers have on different types of diatom assemblages. Different combinations of grazers may have different effects to those expected from single species trials, due to interactions such as feeding segregation, competition and predation (Holomuzki *et al* 2006, Tall *et al* 2006). There have been very few studies, if any, that have investigated the impact of grazing on diatom ecological indices, it is therefore unknown whether grazing can influence the results of monitoring (Kelly *et al* 2007). Past work allows us to hypothesise what is likely to occur with various types of grazing and types of diatom assemblage and enables us to predict a possible influence of grazing on diatoms indices (illustrated by the TDI) that has not yet been tested.

The aim of this study was to determine the selective grazing effects of macroinvertebrates with different functional traits (scraping snail grazers and surface feeding mayflies); both in isolation and in combination, on the structural composition of diatom assemblages and the potential consequences for diatom based ecological quality indices. The Trophic Diatom index (TDI) was used as an example of an ecological quality index as it is widely used in the UK and is based on similar principles to other diatom indices used across Europe (Kelly *et al* 2007). The consistency of grazing effects across families within the same grazing guild (surface feeding mayflies) and for two different diatom assemblages was investigated. Mayflies were selected because they are considered to be particularly selective grazers and are one of the most abundant grazers in many streams (Olsen *et al* 2001). The two different types of diatom assemblages used were indicative of different water qualities: good and moderate/poor water. The specific predictions tested were that grazing will decrease the relative abundance of high-profile diatoms in the assemblage and that the effect will be most pronounced for mayflies. It is also predicted that a change in diatom structure (i.e. decrease high-profile diatoms) will lead to an increase in TDI (indicative of poorer water quality). This study is the first to consider the potential impact of grazing on diatom based ecological quality indices.

2.2 Materials and Methods

2.2.1 Source of Organisms

Diatom assemblages were sourced from seven sites in South Yorkshire and North Derbyshire (Table 2.1).

Table 2.1: Sites used for diatom seeding solutions in artificial streams experiment. For each site TDI scores, status based on TDI, dominant diatom species with their respective sensitivity to nutrients, and growth forms are given. Sensitivity ranges from 1-5, with 1 being most sensitive, 5 being most tolerant. Samples were taken in June 2007.

Site name	TDI	Status	Dominant species	Sensitivity	Growth form
Peakshole Water	39.19	Good	*Achnanthidium minutissimum*	2	Prostrate
			Achnanthes oblongella	1	Prostrate
			Navicula lanceolata	3	Motile
Rivelin	27.70	Good	*A. minutissimum*	2	Prostrate
			Synedra ulna	2	Erect
			Nitzschia palea	3	Motile
Loxley	36.72	Good	*A. minutissimum*	2	Prostrate
			Fragilaria capucina	1	Erect
			Navicula gregaria	3	Motile
Ughill Brook	36.65	Good	*A. minutissimum*	2	Prostrate
			Cocconeis placentula	2	Prostrate
			Gomphonema olivaceum	3	Stalked
Little Don	52.95	Moderate	*Navicula minima*	3	Motile
			Fragilaria vaucheriae	2	Erect
			Achnanthes conspicua	5	Prostrate
Rother 2	49.05	Moderate	*Navicula gregaria*	3	Motile
			A. minutissimum	2	Prostrate
			Planothidium lanceolatum	4	Prostrate
Rother 3	59.31	Poor	*Reimeria sinata*	3	Stalked
			A. minutissimum	2	Prostrate
			Amphora pedicus	5	Stalked

Using the TDI as a measure of ecological quality, four sites were classified as ‘good’, two were classified as ‘moderate’ and one as ‘poor’. A ‘seeding solution’ was created by scraping approximately 20 stones from each ‘good’ quality river for the ‘good’ diatom assemblage and the ‘moderate/poor’ rivers for the ‘poor’ diatom assemblage (method as Lamberti *et al* 1987). The scrapings were collected into a tray containing filtered river water previously collected from the River Rivelin (SK 289871), a relatively unimpacted third order stream, which contains both snails and mayflies, and transported to the laboratory in plastic bottles, where the solution was made up to 30 Litres (with water

from Rivelin) and well mixed. At the start of each experiment 1 Litre of the diatom mixture was added to each artificial stream. Nutrient concentrations are classified as low according to the standards used by the Environment Agency of England and Wales (Table 2.2).

Table 2.2: Water chemistry for sites used for water and diatoms in preliminary experiment. Water quality classes for Phosphate based on standards on the Environment Agency given in parentheses.

River	Rivelin ("good" diatoms)	Little Don ("Poor" diatoms)	River Rother ("poor" diatoms)
Phosphate (mg/l P)	0.066 (Low)	0.15 (Moderate – High)	0.24 (High)
Nitrate (mg/l NO_3)	0.15 (class 1)	0.18 (class 1)	0.57 (class 1)
Nitrite (mg/l N)	0.030	0.034	0.051
Ammonia (mg/l N)	0.11	0.07	0.30
Alkalinity (mg/l $CaCO_3$)	60	30	95

Grazers were collected mainly from the River Rivelin (SK 289871) and three other regional streams with similar water quality (Porter Brook SK 318855, Peakshole Water SK 170834 and Brookside Beck SK 348706). The snail used was *Potamopyrgus jenkinsi* (Family Hydrobiidae), which is the most abundant snail species found in regional macroinvertebrate assemblages (Chapter 4). The mayfly families used were Baetidae, Ephemerellidae and Heptageniidae. The dominant species in each family were *Baetis rhodani*, *Ephmerella ignita* and *Heptagenia fuscogrisa*, respectively. Each taxon was kept in separate containers in river water, with biofilm-covered cobbles from the River Rivelin and acclimatised to experimental conditions for a minimum of 24 hours.

2.2.2 Experimental system

Experiments were conducted in artificial streams (150 cm length x 9.5 cm width x 9 cm depth) constructed from white plastic electrical ducting material (Figure 2.2). Each stream was divided into four compartments (20 cm in length, with space at the ends for pump and tube) using fine mesh (120 µm x 120 µm mesh size from Plaskok ®) attached

with waterproof non-toxic silicon sealant and filled to a depth of 8 cm with water from the River Rivelin, which had been filtered through a plankton net (mesh size; 56µm). Water levels were maintained at this level throughout by topping up with river water (from the River Rivelin) as needed.

Figure 2.2: Picture of the artificial stream systems used in these experiments. Arrows indicate the direction of flow.

The same river water was used for all experiments to enable the separation of compositional effects from water quality effects (i.e. increased diatom productivity, altered grazer behaviour/ fitness). A preliminary experiment confirmed that diatom assemblages indicative of poor water quality could be maintained for 2 weeks (the duration of the experiment) in good quality water (Appendix 1.1). Small pre-washed (with distilled water) cobbles (diameter: 3-6 cm) were added to each stream compartment (covering the channel bottom) as substrate and water was circulated through each channel using plastic tubing at a rate of approximately 83 ml/s using an Aquaclear power head 201 Hagen ® pump. The channels were illuminated using fluorescent strip light canopy (Model 200831, controlled environments Ltd, Winnipeg, Manitoba, Canada) giving a light intensity of approximately 3000 LUX, which is similar to a moderately shaded stream (Allen 1995). Steams were subjected to a 14 hour day: 10 hour night to replicate late summer/ early autumn conditions. Water temperature was maintained at 16-18 ºC by running the circulating pipes through buckets of iced water.

Each experiment consisted of 30 streams, each containing a snail grazing, mayfly grazing, combined snail and mayfly grazing and a control (ungrazed) treatment. The experiment was repeated three times for the different mayfly families (Baetidae, Ephemerellidae and Heptageniidae) using the same diatom assemblage (seeding solution) and once using Baetidae and the 'moderate/poor' diatom assemblage as the seeding solution. Baetidae was the most common family of grazer in the regional stream assemblages (Chapter 4) and is more tolerant of nutrient enrichment than the other mayfly families, thus likely to be found associated with the poorer assemblage. Each experiment lasted 14 days, water and diatom "seeding solution" being added on day 1 and animals being added on day 8. The streams ran for 1 week after the addition of grazers. There was 1 week between each experiment to allow the streams to be cleaned and fresh water and diatom "seeding solution" to be collected. There were small differences in the starting assemblages of the "good" diatom "seeding solution" due to being taken at slightly different times but none that were significant.

2.2.3 Stocking stream channels

To ensure any difference in grazing effect was due to differences in feeding strategy and not biomass, approximately the same biomass of each taxon was used in each experiment. The average grazer dry weight for 0.0625 m^2 surber samples taken from 24 relatively un-impacted streams (summer 2006) was 27.5 mg of grazer/0.0625 m^2. This was used to calculate that the dry mass of grazers needed in each experimental chamber to give the same density was 9.2 mg. To determine how many individuals of each taxon was needed to give approximately 9.2 mg, average dry mass was calculated from 10 individuals, sampled in May and June 2007 representing medium sized specimens. Dry weights were performed by drying at 60 ºC for 4 days then weighing on a micro-balance to 0.001mg. *Potamopyrgus jenkinsi* had an average dry weight per individual of 1.152 mg resulting in 8 being used for single treatments and 4 for combined treatments. Baetidae had an average dry weight of 0.383 mg, Ephemerellidae 0.521 mg and Heptageniidae 0.671 mg meaning that 24, 18 and 14 individuals were used in single species treatments respectively and 12, 9 and 7 were used for combined treatments.

2.2.4 Sampling and processing

Diatom samples were taken at the end of each experiment by scrubbing 5 cobbles from each stream with a toothbrush into a labelled vial and preserving in Lugol's iodine. Diatoms were identified from the lugol's iodine preserved sample to determine species composition. The samples were put in conical flasks and boiled for approximately 20 minutes, in 30 % hydrogen peroxide until the solution became clear. The resulting solution was centrifuged for 10 minutes at 3000 rpm (Centaur 2). The supernatant was drained away and the pellet re-suspended in water, and centrifuged again. This process was repeated 5 more times to give 6 washes in total. The samples were then diluted so only a slight turbidity was noticeable and 0.8 ml placed on a cover slip. The cover slip was left over night for the liquid to evaporate. A microscope slide was heated on a hot plate and a drop of Naphrax mounting medium (Brunel Microscopes Ltd, Chippenham, UK) placed on it; the cover slip was inverted onto the naphrax to fix the diatoms. Slides were labeled and left to harden before being observed under a x 1000 magnification oil imersion lens (Nikon type 120 microscope). Diatoms were identified using appropriate keys (Krammer and Lange-Bertalot 1986-1991, Round *et al* 1990 and Kelly 1998) for a total of 300 valves as described in Kelly *et al* (2007).

Grazers were collected at the end of each experiment from each stream compartment and their mass determined by drying at 60 ºC and weighing to 0.1µg using a Cahn 25 microbalance. Throughout the experiment, pH and temperature were measured using a Jenway 3100 pH meter; conductivity was measured using a Jenway 4071 conductivity meter; dissolved oxygen content was measured using a Hanna HI 9146 dissolved oxygen meter and flow rate was determined by measuring the amount of water expelled in 10 seconds from the pipe into a measuring cylinder. If necessary, flow rates were adjusted daily to ensure consistency across stream channels.

2.2.5 Data analysis

Diatoms were divided into growth forms (erect, stalked, prostrate and motile) using the guidelines of Wellnitz and Ward (2000). Erect and stalked were classified as 'high-profile' and prostrate and motile were classified 'low-profile' (See Chapter 4 Table 4.3). For each experiment the effects of grazers on the relative abundance of different growth forms and dominant diatom species were assessed using ANOVAs (as General linear models with stream as a random factor and type of grazer(s) as treatment). Percentage data were arcsine square root transformed before analysis. Similar analyses were performed for individual species that comprised more than 10 % of the diatom assemblage and for TDI value. Differences between diatom assemblage types were assessed using 2-way ANOVA to assess for interactions between diatom assemblage quality and grazer type. The TDI was calculated using the revised methods and scoring tables of Kelly *et al* (2008):

$$TDI = (WMS \times 25) + 25 \qquad \text{Equation 2.1}$$

Where WMS, the working mean score, has a value between 0-100) – based on the weighted average equation of Zelinka and Marvan 1961 (Kelly *et al* 2008):

$$\text{WMS} = \frac{\sum a_j s_j}{\sum a_j} \qquad \text{Equation 2.2}$$

Where a_j is abundance of species j in the sample, s_j is pollution sensitivity (1-5 obtained from Kelly *et al* 2007) of species j.

The TDI ranges from 0-100 with lower values indicating high water quality and higher values indicating higher levels of eutrophication and nutrient enrichment.

Throughout the analyses Tukey's multiple comparison tests were used to determine instances where significant differences occurred amongst treatments. Average sensitivity values (from the TDI tables in Kelly *et al* 2007) of the diatoms that make up the high and low-profile diatoms in the assemblages were analysed to determine if these differed

between the groups within the assemblage. All statistics were performed using Minitab for Windows version 14.0.

2.3 Results

In each experiment there was no significant difference in the mass of different grazers at the end added to each treatment ($F_{2,29} \leq 1.97$, $P > 0.05$). There were also no significant differences in pH, temperature, conductivity or dissolved oxygen content across the artificial streams and in stream sections ($F_{2,29} \leq 1.78$ $P < 0.05$).

2.3.1 Structural differences in the diatom assemblage between grazed treatments

There was a significant effect of grazing on the proportion of erect taxa in diatom assemblages from both 'good' and 'moderate/poor' quality sites ($F_{3,116} \leq 33.87$, $P < 0.001$). Grazing by the three mayfly families investigated resulted in a significant reduction in the proportion of erect diatoms both when in isolation and when in combination with the snail grazer (Figure 2.3, Tukey's tests:$P < 0.001$). Snail grazing only resulted in a significant reduction of erect diatoms in one of the three experiments using a 'good' diatom assemblage (Figure 2.3a, Tukey's test: $T = 4.68$, $P < 0.001$) and the experiment using the 'moderate/poor' diatom assemblage ($T = 5.31$, $P < 0.001$).

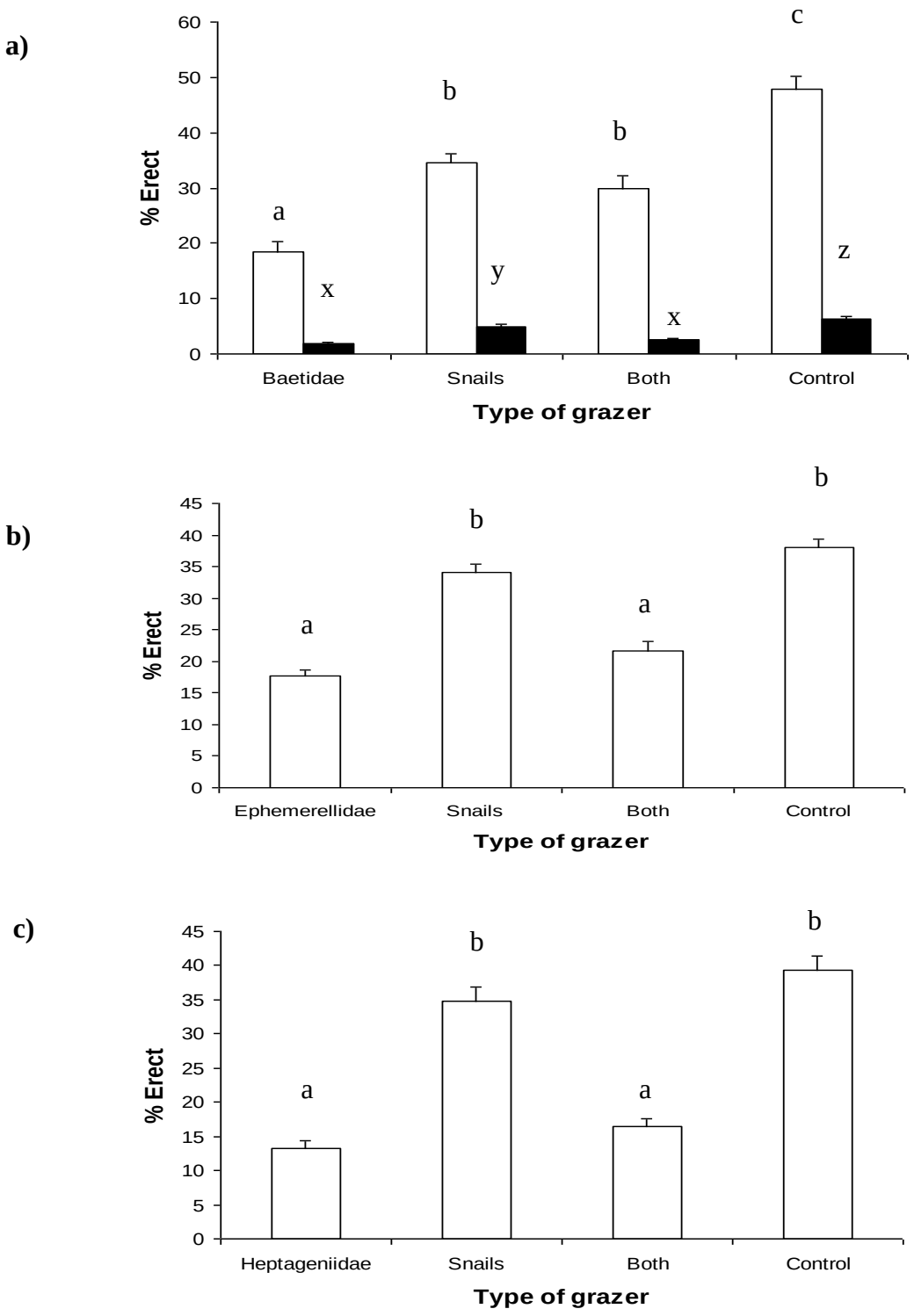

Figure 2.3 Mean (+SE) proportion of erect diatoms in different grazing treatments: a) Baetidae (open bars "good" diatom assemblage, closed bars "moderate/poor" diatom assemblage), b) Ephemerellidae and c) Heptageniidae. Different letters show significant difference between treatments in experiments (Tukey's test, $P < 0.05$).

2.3.2 Influence of grazing on the TDI

Grazing resulted in a significant change in the TDI of 'good' quality streams ($F_{3,116} \geq 13.96$, $P < 0.001$) but had no effect on the TDI of diatom assemblages from 'moderate/ poor' streams ($F_{3,116} = 1.15$, $P > 0.05$). Grazing-induced effects on TDI followed those reported for the abundance of high-profile (erect) diatoms (Figure 2.2). Mayfly grazing of diatom assemblages from 'good' quality sites, either in the presence or absence of snails, resulted in a significant increase in the TDI (Tukey's test: $T \geq 4.42$, $P < 0.001$) but snail grazing generally did not ($T \leq 2.62$, $P > 0.05$) the exception being the snail grazers in the Baetidae experiment ($T = 2.984$, $P < 0.05$). The increase in TDI as a result of grazing ranged from 4.74 – 5.6 on average for the three experiments (Figure 2.4).

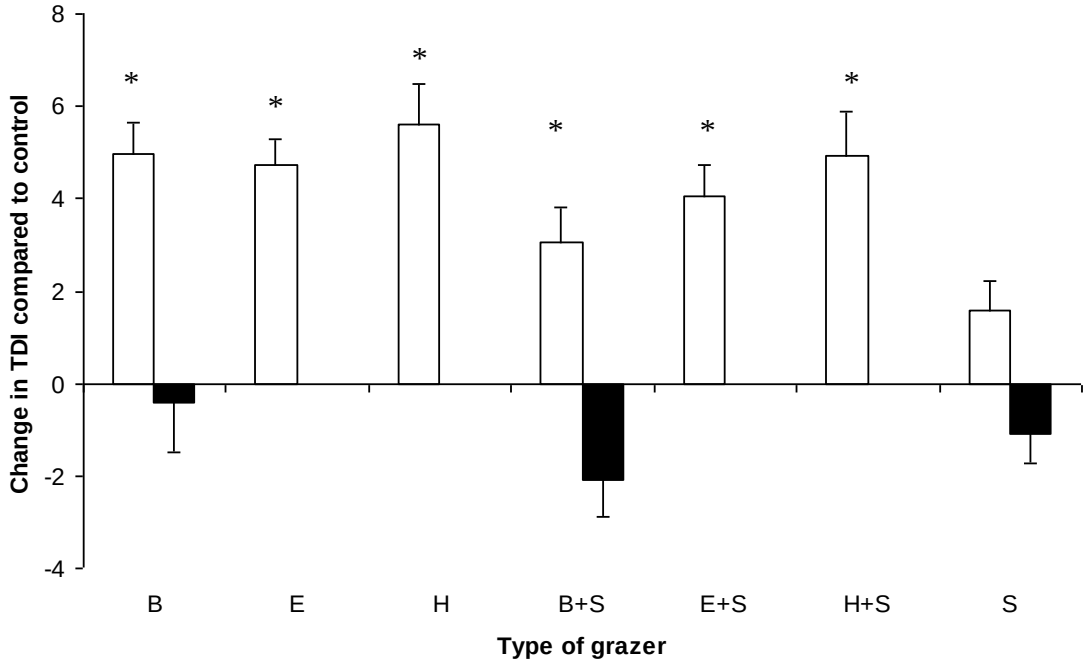

Figure 2.4: Influence of different types of grazing on the TDI. Bars represent change in TDI compared to the control treatment. Open bars represent the 'good' diatom assemblage; closed bars represent the 'moderate/poor' diatom assemblage. B = Baetidae, E = Ephemerellidae, H = Heptageniidae and S = average Hydrobiidae. * show significant difference to control (Tukey tests $P < 0.05$)

2.3 Influence of diatom sensitivity values on the impact of grazing

The average sensitivity score (out of 5) for all the low-profile diatom species in the "good" assemblage across treatments was 2.24, 2.34 and 2.44 for the Baetidae, Ephemerellidae and Heptageniidae experiments respectively. The corresponding average sensitivity score for high-profile diatom species in the "good" diatom assemblage across treatments was 1.65, 1.33 and 1.40. The average sensitivity value for high-profile diatoms in each experiment was significantly lower than that for low-profile diatoms ($F_{3, 116} \leq 233.86$, $P < 0.001$). The averages for the "moderate/poor" diatom assemblage are 2.75 and 3.13 for low-profile and high-profile diatom species respectively, which is the opposite of the 'good' diatom assemblage but there was no significant difference between the sensitivity values ($F_{3, 116} = 1.07$, $P > 0.05$). The average sensitivity values for erect diatoms was lower than for prostrate diatoms, in the 'good' diatom assemblage and stalked was lower than the motile diatoms, this was the other way around for the 'moderate/poor' diatom assemblage (Figures 2.5 a, b and c).

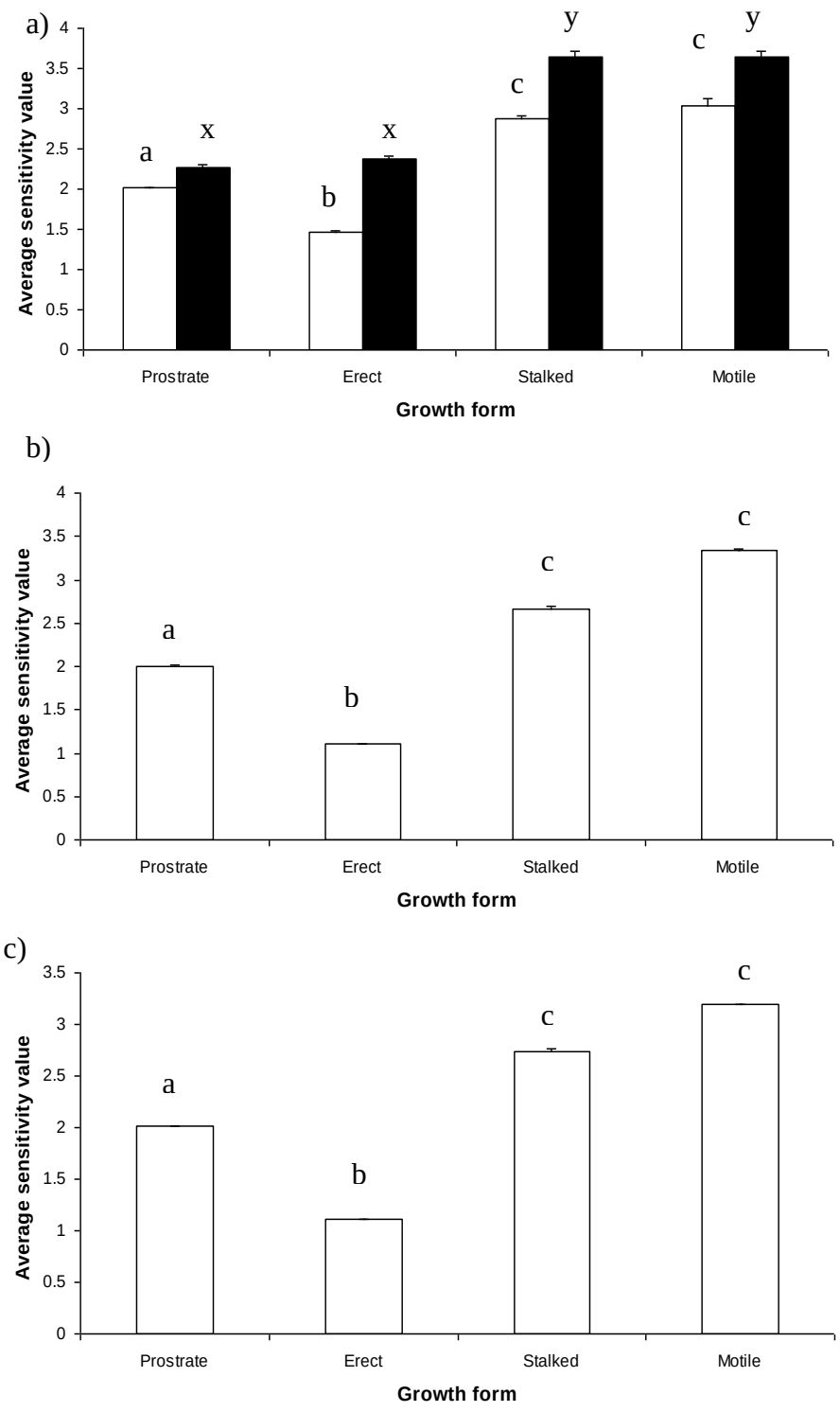

Figure 2.5: Average sensitivity value of different growth forms in different experiments: 2.5a open bars 'good' baetidae experiment, closed bars 'moderate/poor' baetidae experiment, 2.5b Ephemerellidae experiment and 2.4c Heptageniidae experiment. Error bars represent 1 standard error, different letters = significant differences by 1-way ANOVA ($P < 0.05$).

2.4 Influence of grazing on diatom species composition

The diatom assemblage from 'good' quality sites comprised 38 species, with two species (*Achnanthidium minutissimum* and *Fragilaria capucina*) accounting for more than 10 % of the diatoms in each experiment (for full species list and analysis of species present at more than 2% in at least one stream see Appendix 1: Tables A, B, C and D). Additionally, *Synedra ulna* accounted for more than 10 % of the diatoms in the Baetidae trial and *Nitzschia paleacea* accounted for more than 10 % of the diatoms in the Ephemerellidae and Heptageniidae trials. The 'poor/moderate' diatom assemblage comprised 27 different species and had a very different assemblage structure with fewer high-profile diatoms overall and a greater relative abundance of stalked taxa (5.42 % in the control) within the high profile diatoms and motile taxa (36.52 % in the control) within the low profile diatoms. Only *A. minutissimum* and *Navicula lanceolata* account for more than 10 % of the diatoms in the 'moderate/poor' diatom assemblage (Appendix 1 Table D).

Grazing resulted in a significantly greater relative abundance of the prostrate diatom *Achnanthidium minutissimum* in the 'good' assemblages ($F_{3,116} \geq 20.96$, $P \leq 0.005$) (Figure 2.5). No significant differences in *A. minutissimum* relative abundance were found for the 'moderate/poor' diatom assemblage ($F_{3,116} = 2.6$, $P = 0.055$). The mayfly grazed assemblages had significantly less relative abundance of the erect diatom *Fragilaria capucina* for the 'good' assemblage ($F_{3,116} \geq 18.68$, $P < 0.001$) (Figure 2.6). Therefore grazing resulted in increased *A. minutissimum* relative abundance with a corresponding decrease in *F. capucina* relative abundance. Additionally the Baetidae experiment ('good' diatom assemblage) had significantly less relative abundance of *S. ulna* in the grazed treatments ($F_{3,116} = 32.87$, $P < 0.001$).

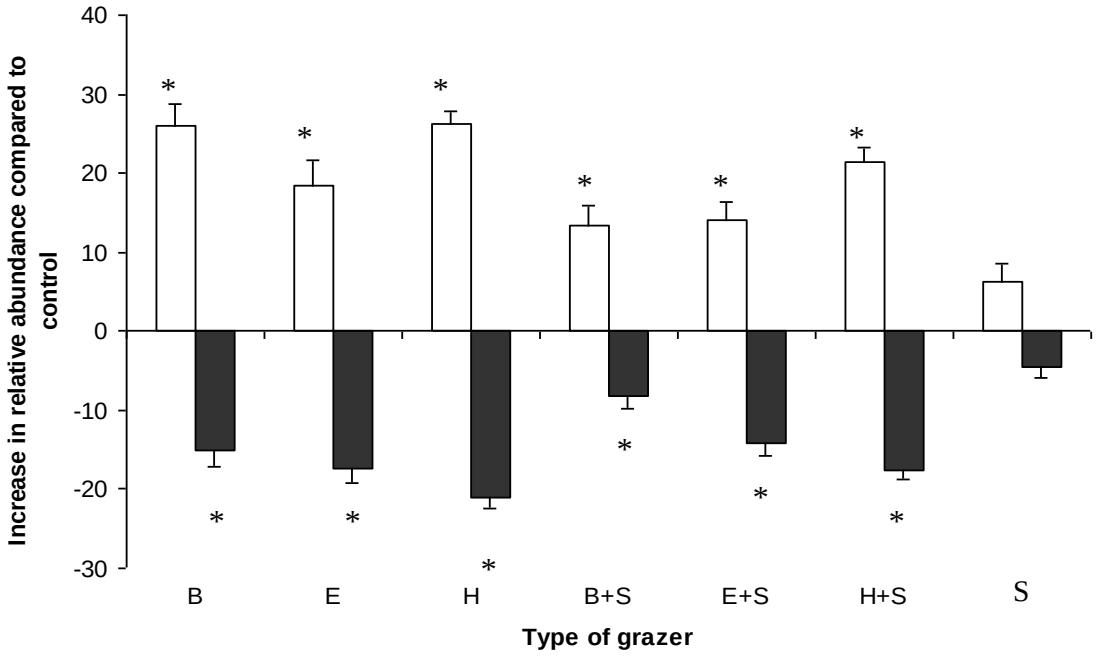

Figure 2.6: Effect of different types of grazing on the relative abundances of the most abundant diatom species in the 'good' diatom assemblage (open bars *Achnanthidium minutissimum*, closed bars *Fragilaria capucina*). Bars represent changes in relative abundance compared to control treatment B = Baetidae, E = Ephemerellidae, H = Heptageniidae and S = Average snails (Hydrobiidae). * indicates significant differences from the control (Tukey tests $P < 0.05$)

In the experiments using Baetidae, no significant differences were found between diatom assemblage types ('good' vs. 'moderate/poor') for the proportion of high-profile diatoms by percentage change between grazed and control treatments (2-way ANOVA found significant difference between grazers: $F_{2,174} = 92.01$, $P < 0.001$, but no difference between diatom assemblage or the interaction: $F_{1, 174} \leq 0.58$ $P > 0.05$) (Figure 2.7)). For the TDI there were significant differences between the 'good' assemblage and the 'moderate/poor' assemblage for percentage change for both the Baetidae alone and Baetidae and snail grazed treatments ($F_{1,174} = 85.06$, $P < 0.001$). There was also a significant difference between grazer types ($F_{2, 174} = 4.83$, $P = 0.009$) but the interaction was narrowly non-significant ($F_{2,174} = 3.02$, $P = 0.051$) (Figure 2.8). This means that for the 'moderate/poor' diatom assemblage the grazed treatments were about 100 % of the control TDI, whereas the 'good' diatom assemblage was only around 80 % of the control TDI.

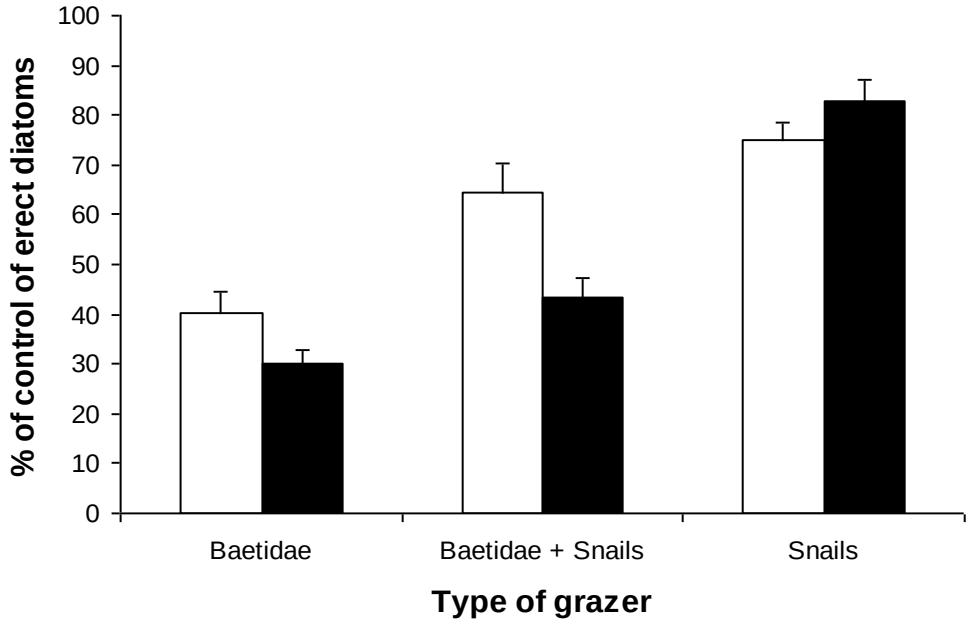

Figure 2.7: Percentage of erect diatoms at differently grazed treatments compared to the control (open bars 'good' diatom assemblage, closed bars 'moderate/poor' diatom assemblage). No significant differences were found between assemblage types (P > 0.05).

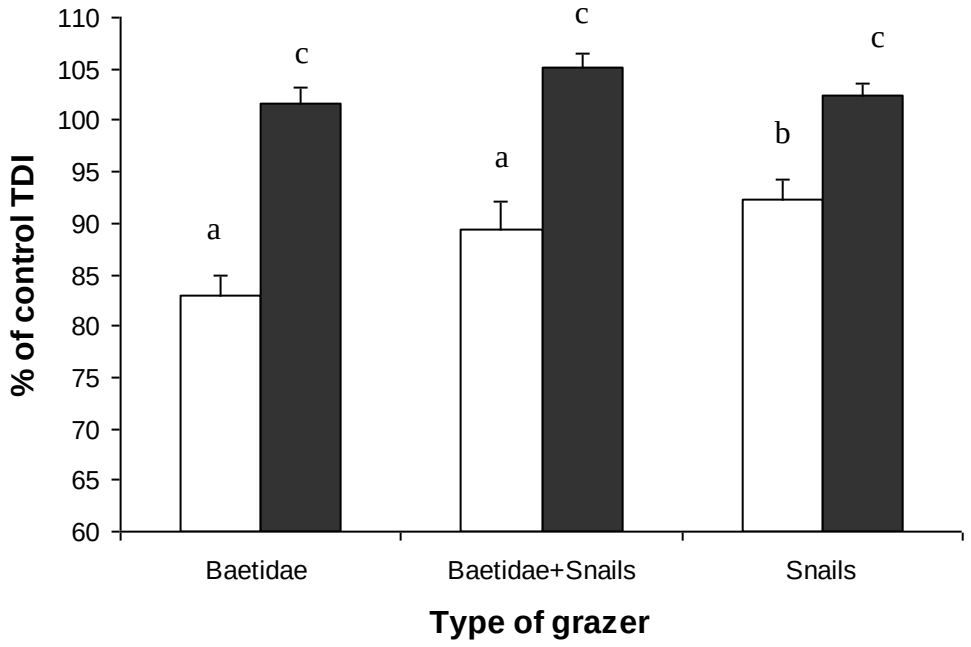

Figure 2.8: Percentage of control TDI at differently grazed treatments. Open bars represent the 'good' diatom assemblage; closed bars represent the 'moderate/poor' diatom assemblage). Significant differences between types of diatom assemblage are indicated by different letters (P > 0.05)

2.4 Discussion

Grazing by the three families of mayflies (Baetidae, Ephemerellidae and Heptageniidae) altered the structure of the diatom assemblages by reducing the relative abundance of erect diatoms. Grazing by a combination of mayflies and snails had the same effect, but snail grazing alone did not. These results confirm those of previous studies that have investigated mayfly grazing with isolated treatments, and establish that natural abundances of mayfly grazers belonging to different families can cause similar changes in the diatom assemblage when associated with other grazers (snails) (Table 2.2). Grazing mayflies decreased the relative abundance of erect diatoms whether the diatom assemblage was indicative of 'good' water quality or of 'moderate/poor' water quality, thus showing that it is not dependent on the identity of the diatoms but the traits that they have suggesting that grazers will consume what is accessible to them.

Previous studies have also found that mayflies are able to decrease the proportion of erect diatoms in the assemblage (Table 2.2). The literature cites multiple reasons why certain diatom species are grazed preferentially, all are due to being more available to grazers than other, whether this is size (Steinman *et al* 1992, DeNicola *et al* 1990), mode of attachment (Hill and Knight 1987), position in the diatom matrix (Sarnelle *et al* 1993, Tall *et al* 2006) or growth form (Villanueva *et al* 2004, Pan and Lowe 1994). In contrast to the majority of studies (Table 2.2) the current study found little effect of snail grazing on the structure of the diatom assemblage.

Table 2.2: Summary of papers examining the influence of mayfly and snail grazing on diatom assemblage structure in flowing waters. A total of 26 papers were found in the literature.

Type of grazer	Effect on diatom structure	Number of studies	*References*
Mayfly	Decrease relative abundance of erect/high profile, upright or large taxa	13	Hill and Knight (1987) and (1988), Colletti *et al* (1987), Lamberti *et al* (1987), Wellnitz and Ward (1998), Holomuzki and Biggs (2006), Pan and Lowe (1994), Holomuzki *et al* (2006), Peterson *et al* (2001), Villanueva and Modenutti (2004), DeNicola *et al* (1990) and Villanueva *et al* (2004), Dudley (1992), **Present study**
	No influence on diatom assemblage structure	3	Karouna and Fuller (1992), Lamberti et al (1995) and Steinman *et al* (1987)
Snails	Decrease relative abundance of erect/high profile, upright or large taxa	13	Lambert *et al* (1987), Lamberti *et al* (1995), Steinman *et al* (1987), Holomuzki *et al* (2006), McCormack and Stevenson (1991) and (1989), Steinman *et al* (1992), McCormack (1994) and DeNicola *et al* (1990), Rosemond *et al* (1993)
	No influence on diatom assemblage structure	2	Holomuzki and Biggs (2006), Villanueva *et al* (2004), **Present study**
	Increase over-storey	1	Sarnelle *et al* (1993)
General grazing/ whole assemblage	Decrease relative abundance of erect/high profile, upright or large taxa	1	Opsahl *et al* (2003)

The impact of different grazers on the diatom assemblage is likely to be due to the traits of the grazers present because the biomass of snails was not significantly different from mayflies meaning that a difference in biomass would not have been responsible for the difference in effect on the diatom assemblage. The current study found that grazing by the snail *Potamopyrgus jenkinsi* had less effect on the diatom assemblage structure than the mayflies, with only 2 out of the 4 trials with the snail having any significant change, and those that did had only a small change. This agreed with those studies that assessed snails to be non-selective feeders due to their mouthpart morphology (McCormack and Stevenson 1989, Dillon 1998). Snails are able to use a wide range of stroking, gauging

and biting motions to graze, unlike the mayflies that have less versatile mouthparts (Dillon 1998). It is also possible that a preference for a certain food type would only occur when the snail is fully sated with them clearing all food present unless a preferred food source was very abundant and readily available. (Dillon 1998). Due to the growth time in the current experiments, the diatom assemblage may not have provided enough food for the less active snails to be selective. The lesser mobility of snails could also result in less grazing over one week, relative to their biomass than the active mayflies (McKenny 2005). This could also explain why some trials had a small significant change as it may be that if the experiments had ran for more time the less active snails would also have decreased the relative abundance of high-profile diatoms significantly (McKenny 2005).

Other studies have found that snails can have a significant influence on the diatom assemblage and show some selective grazing (Table 2.2). Food choice has been demonstrated for snails, for example a pond trial using the snails' *Lymnea peregra* and *Planorbis vortex* found that they preferred filamentous green algae and diatoms respectively; demonstrating that feeding varies within the snail guild at least when in competition (Lodge 1986). A variety of different snail taxa have been used in previous experiments, so diversity in traits/feeding among the snail group may explain observed differences (Lamberti *et al* 1987, Steinman *et al* 1987, Lowe and Hunter 1988, McCormack and Stevenson 1989). Snails have even been found to have a positive effect on over-storey algae suggesting that snails can be selective in grazing under-story algae or otherwise cause conditions to favour the over-storey (Sarnelle *et al* 1993). High-profile diatoms often have a slower growth rate than low-profile diatoms so could decrease in relative abundance when grazed equally, thus explaining studies that have found snails decreasing the relative abundance of high-profile diatoms when in theory they should be non-selective (McCormack and Stevenson 1989). However, active avoidance of a diatom species has also been described for the snail, *Elmia clavea*, that was found to avoid gelatinous diatoms, possibly due to the diatom growth form being a successful defence against grazing by this type of snail, showing that even snail radula cannot eat all algal forms (Steinman *et al* 1992). Some studies have found snails to influence the diatom assemblage when mayflies do not. This could be due to different

species being used or the conditions that the experiment (natural densities rather than equal densities, initial diatom assemblage) was set up in (Holomuzki *et al* 2006). For snails we can say that interactions with diatoms are less predictable than for mayflies and often less significant, however some taxa do have the ability to influence the diatom assemblage structure as well as biomass.

The results for the snail and mayfly combined treatments demonstrate that snail presence does not interfere with mayfly induced changes of the diatom assemblage. Treatments that were grazed by both snails and mayflies were not significantly different to the mayfly-only grazed assemblages. This indicated that half the biomass of mayflies as used in the mayfly only treatments had a significant impact on the diatom assemblage. This represented a more realistic situation as it is unlikely that mayflies would be the sole grazer in natural conditions. Therefore this work indicates the potential that grazing has for influencing the relative abundances of different diatom species in the assemblage in rivers with multiple grazers present. Studies that have investigated snails and mayflies on the same diatom assemblage (but in different treatments) have often found mayflies have a greater impact. An example being Villanueva *et al* (1994) found that the snail (*Chilina dombeiana)* did not have any effect on the diatom assemblage when the mayfly (*Merialaris chiloeenis)* caused a decrease in erect diatoms, concurring with the current study. Holomuzki and Biggs (2006) also found that a mayfly and a caddisfly decreased erect diatoms in microcosms but that a snail did not. However, in the latter case results could have been influenced by the snails burying into the substrate (Holomuzki and Biggs 2006), something which could not have occurred in the present study as only cobbles were used not sand or gravel.

Grazing by mayflies on the diatom assemblage indicative of good water quality resulted in a significant increase in the TDI compared to the control, as well as a change in diatom assemblage structure. This was due to the sensitivity values of the diatoms that dominated the high-profile group being lower than the sensitivity value of the dominant low-profile diatoms present in the assemblage. Although there was a decrease of high-profile diatom relative abundance in the ‘moderate/poor’ diatom assemblage, due to grazing by mayflies, the TDI was unaffected. This was because of the average sensitivity

values of the high and low-profile diatoms not being significantly different, meaning that no change in TDI resulted from grazing. As expected by the lack of structural change, snail grazing alone resulted in no change in the TDI for both diatom assemblage types. This was the result of type of grazing rather than the sensitivity values of the diatoms present. These results demonstrate that in certain situations the presence of grazing mayflies can change the score of indices that are based on relative abundances of diatoms but the impact of grazing on the TDI is dependant on the identity of both the grazers present and identity of the dominant diatom species of different trait groups in the assemblage.

Grazing by mayflies has been found to alter the diatom assemblage composition, and due to most commonly used diatom indices being based on the relative abundance of different diatom species with different sensitivity values to impacts, there is the potential for indices to be altered (i.e. TDI). This study shows that a potential reason for the high variability in diatom assemblages and diatom based indices could be due to grazing or the types of grazing that it is exposed to. The current work has demonstrated that, as well as having different sensitivities to pollution (Kelly and Whitton 1995, Prygiel *et al* 1996), different diatom species also have different vulnerabilities to grazing. Thus grazing has the potential to influence ecological monitoring results, depending on the sensitivity and growth forms of the dominant species of diatom.

This chapter found that most of the change observed for the experiments using the good diatom assemblage was due to two dominant species; an erect/high profile species, *Fragilaria capucina* and a prostrate/low profile species, *Achnanthidium minutissimum.* This showed that just two very abundant species were driving the patterns observed in the trials. The dominance of these two species is fairly typical of assemblages found in good/excellent water quality conditions (Kelly *et al* 2007). *F. capucina* has a TDI sensitivity value of 1 whereas *A. minutissimum* has a TDI sensitivity value of 2 (Kelly *et al* 2008). This difference in sensitivity values indicates that an increase in the relative abundance of *A. minutissimum* coupled with an associated decrease of *F .capucina* can explain the result that mayfly-grazed assemblages have a higher TDI because of the proportions of these abundant species changing. As a consequence of the dominance of

two species (*A, minutissimum* and *F. capucina*) there may not have been high enough relative abundance of the other species to determine if grazing was influencing them. The structure of the 'moderate/poor' diatom assemblage was influenced in a similar way but the high-profile diatoms made up proportionally less of the diatom assemblage. The species present in the 'moderate/poor' and 'good' assemblages were very different, as typified by their different TDI scores. There were more motile diatoms present in the poor assemblage as well as fewer high-profile diatoms overall. The decrease of high-profile diatoms due to grazing in the 'moderate/poor' diatom assemblage was caused by decreases in the overall abundance of high-profile species rather than any specific dominant species. The species number was similar between the diatoms obtained from different water qualities, agreeing with studies that have shown that identity and assemblage structure changes when diatoms are impacted rather than species richness (Hirst *et al* 2002).

These experiments have demonstrated that mayfly grazing can result in changes in the diatom assemblage structure and that under suitable conditions can result in a small but significant change in the TDI. To predict if grazing will have an influence on the diatom index (i.e. TDI) we need to consider the traits that make up the diatom assemblage, and whether there differences exist between the sensitivity values of high and low-profile diatoms. Ecological quality monitoring could be benefited by determining what types of grazers are present, to assess if variability in the diatom assemblage is likely to be due to grazing. This study indicates that grazing may cause most change in "good" diatom assemblages because of both the type of diatoms present and the type of grazers present, as mayflies prefer good water quality (Olsen *et al* 2001). Our results suggests that monitoring the macroinvertebrate assemblage and diatom assemblage at the same time would be useful for understanding the ecological quality of the system because if the grazers and diatoms present are known the possibility of variability being due to grazing can be calculated.

3.0 THE INFLUENCE OF MAYFLY DOMINATED GRAZER COMMUNITIES ON DIATOM ASSEMBLAGE STRUCTURE AND BIOMASS IN THE FIELD

3.1 Introduction

Grazing macroinvertebrates play an important role in determining the structure and biomass of the benthic diatom assemblage (Peterson *et al* 2001, McCormack and Stevenson 1989). Chapter 2 demonstrated that grazing by mayflies belonging to three different families could significantly influence the structure of diatom assemblages in artificial streams both on their own and when associated with snails. The influence of grazers on diatom assemblage structure is not only important ecologically but also for the potential effect a structural change could have on ecological biomonitoring results (Kelly *et al* 2007). Previous studies on the effect of grazing in natural streams have mainly focused on the less mobile crawling grazers, such as snails and caddisflies due to the relative ease with which they can be manipulated and their high abundance at some study sites (Jacoby 1987, McCormack and Stevenson 1989, Peterson *et al* 2001). More mobile, swimming grazers such as mayflies are much more difficult to exclude from feeding on the periphyton and hence most grazing studies using mayflies have been laboratory-based (Colletti *et al* 1987, Hill and Knight 1987, Lamberti *et al* 1987). An exception to this is Opsahl *et al* (2003) who excluded mayflies and some other grazers with electrical exclosures. However their study stream was strongly dominated by blue-green algae with minimal diatom biomass available for grazers. Evidence from laboratory trials suggests that mayflies could be at least as important as snails and caddisflies in the structuring of the diatom assemblage (Lamberti *et al* 1987). Mayfly

grazing could be especially important in circumneutral upland streams where they are often the most abundant grazers (Hirst *et al* 2004, Death and Joy 2004, Morley *et al* 2008, also many streams in Chapter 4).

The question of how mayfly grazing influences the diatom assemblage in natural conditions and in the presence of other organisms has not yet been answered, due partly to methodological limitations. Previous studies have been limited in answering this by numerous factors such as very short study durations (i.e. less than 24 hours) (Murphy 1984, Peterson 1987) or lacking a proper ungrazed control (Murphy 1984, Peterson 1987, Peterson *et al* 2001, Hirst *et al* 2002). Published studies have investigated grazer effects on algal biomass but lack the diatom assemblage data that would give insight into how the grazers affected the structure of the diatom assemblage (Lamberti and Resh 1983, Murphy 1984, Gawne and Lake 1995, Jordan and Lake 1996, Barbee 2005). Other studies have investigated specific stream conditions such as glacial snowmelt that is important in certain areas but are not applicable in the UK or many other parts of the world (Murphy 1984, Peterson *et al* 2001). Some studies have also only excluded some grazers from the "ungrazed" control meaning that, although interesting, only part of the macroinvertebrate assemblage is studied as opposed to finding out the influence of the assemblage as a whole (McAuliffe 1984, Jacoby 1987, Gawne and Lake 1995, Jordan and Lake 1996, Opsahl *et al* 2003, Barbee 2005, Greathouse *et al* 2006).

Different types of grazers differ in their modes of feeding with crawling and swimming grazers tending to differ in the way that they feed. The crawling snail and caddisfly grazers generally have scoop-shaped mandibles whilst the mayflies are surface grazers with inner grinding mouthparts (Cummins and Klug 1979). As a consequence of these differences in mouthpart morphology, they are likely to have different effects on the diatom assemblage, meaning that results found in the field using crawling grazers cannot be assumed to be applicable to other grazers. In field trials scraping grazers have been found to decrease both the biomass (sometimes measured as chlorophyll *a* or cell numbers) and the relative abundance of high-profile diatoms (erect and stalked diatoms that are available at the surface of the biofilm) in the assemblage (Lambert *et al* 1989, Rosemond 1993). Laboratory studies, using artificial streams or microcosms, have found

that grazing by mayflies has resulted in a decrease in high-profile or over-storey diatoms (Hill and Knight 1987, Colletti *et al* 1987, Hill and Knight 1988, Wellnitz and Ward 1998, Villanueva and Modenutti 2004, Holomuzki and Biggs 2006 and Holomuzki *et al* 2006, Chapter 2 of this thesis). Results for other grazers such as snails have been more variable, but the majority of studies have found a decrease in the relative abundance of high-profile diatom taxa (Lamberti *et al* 1987, McCormack and Stevenson 1989, Lamberti *et al* 1995 and Holomuzki *et al* 2006). However, other studies, using snails have observed no change in relative abundances of diatoms (Villanueva *et al* 2004 and Holomuzki and Biggs 2006). It has even been found that snails can have a positive influence on high-profile diatoms, possibly by selective grazing or by otherwise causing favourable conditions for high-profile diatom growth (Sarnelle *et al* 1993). Grazing macroinvertebrates may increase nutrients to the diatom under-storey suggesting that grazing can have a positive influence on some diatoms (McCormack and Stevenson 1989). Grazing caddisflies have been both associated with a decrease in the relative abundance of high-profile diatoms (Lamberti *et al* 1987, Peterson 1987, DeNicola *et al* 1990, Lamberti *et al* 1995 and Holomuzki and Biggs 2006) and with no change in assemblage structure (Hill and Knight 1988). Therefore the importance of determining what occurs in mayfly-dominated natural macroinvertebrate assemblages is highlighted.

Artificial streams can be used to show whether or not grazers influence diatoms and what types of grazer can cause changes, but in the real world there will generally be many different types of grazer present at any stream site, even when one species is dominant (Wallace and Webster 1996). The presence of several different grazer species at any one site is likely to produce competition, which could influence the outcome of grazing on the diatom assemblage (Tall *et al* 2006). More pronounced resource partitioning is likely to be present in nature, due to the abundance of different species, possibly with scraping grazers preferring tightly attached diatoms and surface feeding grazers preferring over-storey diatoms (Tall *et al* 2006). This niche differentiation could allow more coexistence between grazers and also result in the whole diatom assemblage being under greater pressure than when under single species trials (Tall *et al* 2006). When there are a high number of different grazer species, competition for primary

production resources may be stronger, resulting in different impacts on the periphyton than may be expected in a single species treatment (Wellnitz and Ward 1998).

Laboratory trials have resulted in useful insights into what could potentially happen in field situations, but due to the controlled conditions, which are essential for determining an effect; they do not inform us of what happens when there is predation or competition. This means that now we have knowledge of potential interactions from the laboratory we need to examine whether they still occur under more complicated natural conditions. Many macroinvertebrate taxa are generalists and will utilise more than one resource and therefore have the potential to influence the diatom assemblage whilst not being specifically grazer taxa (Mihuc 1997). For example *Asellus aquaticus*, *Gammarus pulex* and many chironomid species are all known to consume algae, but not as their primary food source (Moore 1979, Botts 1993). Predators can also result in changes in behaviour, for example increasing emigration rates via drift or changes in feeding rates (Bronmark *et al* 1992). All of these behavioural changes can alter the effect that grazers have on the diatom assemblage (Bronmark *et al* 1992, Wallace and Webster 1996). Studying grazer effects in nature is also important because other organism groups, such as fish and riverine birds will also interact with macroinvertebrates, influencing their abundance and behaviour (Harvey and Marti 1993, Forrester 1994). Organisms that do not graze the diatom assemblage can still directly affect it by non-consumptive disruption to the diatom mat and by nutrient cycling (Wallace and Webster 1996, Ledger and Hildrew 2000).

Changes in diatom structure can have a direct influence on diatom-based ecological indicators because they are based on the relative abundance of different taxa. To determine if grazing effects need to be considered when monitoring we need to find out how important they are under natural conditions. Diatom indices are being used to study general degradation within the requirements of recent legislation (Kelly and Whitton 1995, Kelly *et al* 2007), making it essential to understand how grazing by macroinvertebrates can influence indices in a real world situation as opposed to a laboratory trial as in Chapter 2. In this study the Trophic Diatom Index (TDI; Kelly *et al* 2007) is used as an example index but findings are widely applicable due to most indices

using similar numerical models based on species optima to assess water quality (Potapova *et al* 2004). By determining the likely influence of grazing on different diatom assemblages it will go some way to allowing us to predict how much variation in a sample may be due to grazing and will ideally result in this knowledge being built into monitoring assessments.

Most of the existing research on grazing macroinvertebrate-diatom interactions has focused on the effects of grazing on overall algal biomass, while less is known about the effects on diatoms. Our recent understanding of the grazing effects on diatom structure has been mainly derived from short-term manipulations, or a subset of the grazer community. Thus there is a need to find out more about the effects of entire macroinvertebrate communities and over longer durations in order to better understand the combined effects of all grazing macroinvertebrates on diatom communities in streams. The main question asked in this study is: how does macroinvertebrate grazing, by mayfly-dominated grazer assemblages influence the diatom assemblage under field conditions and how does this influence the TDI? To address this question we used field exclosures and enclosures in 10 streams in South Yorkshire and North Derbyshire. The following variables were measured: i) The relative abundance of high-profile diatoms, ii) the identity of the diatoms in the assemblage and iii) the chlorophyll *a* concentration (as a proxy for biomass).

3.2 Methods

3.2.1 Site descriptions

Sites were situated in or close to Sheffield, UK (Table 3.1) and were first to third order streams (1 m-7.0 m wide) with riparian vegetation comprising mainly of native deciduous trees, with an under-storey of herbs and grasses. All sites had a diatom assemblage indicative of good ecological quality as assessed by the methods set out in Kelly *et al* 2007. They also had previously been found to have a fairly high relative abundance of high-profile diatoms and a high abundance of grazing macroinvertebrates, including mayflies (Table 3.1).

Table 3.1: Sites used for enclosure/exclosure trials including some macroinvertebrate and diatom data. Data are from field surveys performed in July 2006 or May 2007. The TDI (trophic diatom index) goes from 0-100 with a lower number equalling better water quality (Kelly *et al* 2007). The grazer abundance was numbers found in a 3 minute kick sample.

Site	**Grid ref.**	**TDI**	**Ecological quality rating**	**Grazer abundance (Mayflies)**	**Number of grazer families**	**% High profile diatoms**
Peakshole Water	SK170834	37	Good	663 (595)	9	17.9
River Noe	SK168844	34.4	Good	81 (68)	9	16.7
Porter Clough	SK291849	32.9	Good	35 (24)	6	19.8
Loxley	SK295898	33.68	Good	543 (532)	5	20.35
Porter Brook	SK318855	35.0	Good	319 (302)	6	18
Rivelin	SK289871	28.96	High	1116 (1102)	6	16.10
Sheaf	SK328822	36.76	Good	1931 (1601)	9	25.14
Ewden Beck	SK241968	29.02	High	853 (750)	9	28.35
Trib. of Loxley	SK298895	31.3	Good	237 (220)	10	33.75
Ughill brook	SK262901	48.5	Good	41 (39)	4	27.9
Average	-	*34.75*	-	*582 (523)*	*7*	*22.4*

3.2.2 Enclosures/ Exclosures

The study took place in August 2008 during a period of stable flow. At each site, exclosures (caged, macroinvertebrates absent), enclosures (caged, macroinvertebrates present), and open control plots (no cage, macroinvertebrates present) were established (N = 3 per treatment). Exclosure/enclosure cages were made of containers with fine mesh (500 µm diameter) firmly attached using waterproof silicon sealant, similar to those used in Hauer and Lamberti (2005). Each cage contained at least 5 cobbles for diatom accumulation and placed on the riverbed and was fastened to a brick and/or tree

to prevent loss in the current. Cobbles (5-7 cm in diameter) previously placed in the river for 2 weeks in order for algae to colonise, were also used in cages to ensure that the substrate mimicked natural substrates, but so that an exact area could be sampled. Cobbles were exposed first to ensure that the macroinvertebrates in the caged treatments had some food and to ensure all cobbles started with a similar level of periphyton. The enclosures contained a natural assemblage of macroinvertebrates sampled from the same study sites using a Surber sample the same size as the cages (0.25 x 0.25 m). Cages were deployed for two weeks then collected and sampled. At the end of the experiment, macroinvertebrates were collected from the enclosures and preserved in Industrial Methylated Spirits (IMS) for counting, identification and dry mass measurements.

At the time of deployment, each stream was characterised by taking water samples that were analysed in triplicate using a Palintest ® kit for phosphates, nitrates, nitrites, ammonia and alkalinity. Hand-held meters were used to measure pH (Jenway 3100), conductivity (Jenway 4071), dissolved oxygen content (Hanna HI 9146) and temperature (Jenway 3100). Stream flow rate was measured using a hand-held flow meter (Valeport 801) and depth measurements were taken using the probe of the flow meter and width was measured with a tape measure. After 2 weeks, the same measurements were taken again, as well as samples of the macroinvertebrates in each cage at each site to determine the assemblage present. Five cobbles from each cage were sampled for diatoms using the methods of Kelly *et al* (2007), i.e. scrubbing the cobbles with a toothbrush and placing the sample into a small, labelled bottle containing Lugol's iodine. Samples were also taken for chlorophyll *a* analysis by scrubbing a standard-sized area (9.1 cm^2 bottle cap) of the cobbles. The chlorophyll *a* samples were kept on ice until they could be frozen at -18 ºC prior to analyse. Macroinvertebrates from enclosures were preserved in IMS for later counting and identification. Macroinvertebrates were counted and identified to family level using appropriate keys and then divided into mayfly grazers, non-mayfly grazers and non-grazers (using the Eurolimpacs website – Buffagni *et al* 2007) and dried at 60 ºC for 4 days before being weighed to the nearest microgram on a Cahn 25 microbalance (Scientific and Medical Products Ltd, Manchester, UK).

For diatom preparation and identification methods see Chapter 2.

Defrosted periphyton samples were processed for chlorophyll *a* content (a proxy for biomass) using a spectrophotometer method similar to Gregor and Marsalek (2004) and kept out of the light where possible. Samples were filtered through Whatman Number 1 filter papers, and then the filtrate placed in a foil-covered labelled tube with 10.0 ml of 90 % ethanol. Samples were well shaken before being placed in a hot water bath (80 ºC) for 7 minutes and left to cool for approximately 30 minutes. The samples were then placed in a cuvette and read at 664 nm and 750 nm, using a Cecil 1000 series spectrophotometer against a blank of 90% ethanol. The chlorophyll a content in $\mu g/cm^2$ can be calculated as follows:

$$\text{Chlorophyll } a \ (\mu g/cm^2) = E \times \frac{A_{664nm} - A_{750nm}}{C} \times V_{ext} \times DF \times L \qquad \text{Equation 3.1}$$

Where: E is the Extinction coefficient for chlorophyll *a* in 90 % ethanol at 664 nm (12.8), $A_{664\,nm}$ and $A_{750\,nm}$ are the absorption readings at 664 nm and 750 nm respectively, V_{ext} is the volume of extract in ml (i.e. 10.0), DF is the dilution factor, C is π x radius^2 (cm^2), and L is the cuvette path length (1 cm).

3.2.3 Data analysis

The trophic diatom index (TDI) was calculated for each site using Kelly *et al* 2007 (see Chapter 2) and were used as an example of a diatom index used in Europe (UK). The average sensitivity values of high and low-profile diatoms was also calculated for each site. In all analyses 'stream' was used as the independent replicate and the proportional data were arsine square route transformed prior to analysis. ANOVA's (as General linear models (GLM)), with treatment as the main factor and stream as a random factor, were used to determine if there was a significant difference between enclosures, exclosures and controls. Regression analysis was used to examine the relationship between grazing mayflies and the relative abundance of high-profile diatoms. Principal components analysis (PCA) was performed using a covariance matrix for diatom assemblages at

different sites and treatments to compare different diatom assemblages. Paired t-tests were used to test for differences between grazed and ungrazed treatments for both the first and second principal components of the diatom assemblage and also used to establish differences between enclosures and exclosures for macroinvertebrate number, biomass, grazer numbers, mayfly numbers and mayfly biomass. All statistics were performed using Minitab 14.0 for Windows. Diatom growth traits were assessed using the guidelines of Wellnitz and Ward (2000) and allocation of macroinvertebrates to functional feeding groups was performed using the Eurolimpacs database (Buffagni *et al* 2007).

3.3 Results

A significantly greater proportion of high-profile diatoms was found in ungrazed treatments (exclosures) compared to grazed treatments (enclosures) ($F_{2,18} = 24.44$, $P < 0.001$) (Figure 3.1.a). The proportion of high-profile diatoms in the assemblage did not differ significantly between the enclosures (caged grazers) and the open control plots (naturally grazed), thus caging did not have a significant influence on interactions. A similar pattern was demonstrated by the chlorophyll *a* concentration of periphyton, which was significantly higher in the ungrazed treatments compared to the grazed treatments ($F = 23.89_{2, 18}$, $P < 0.001$) (Figure 3.1.b).

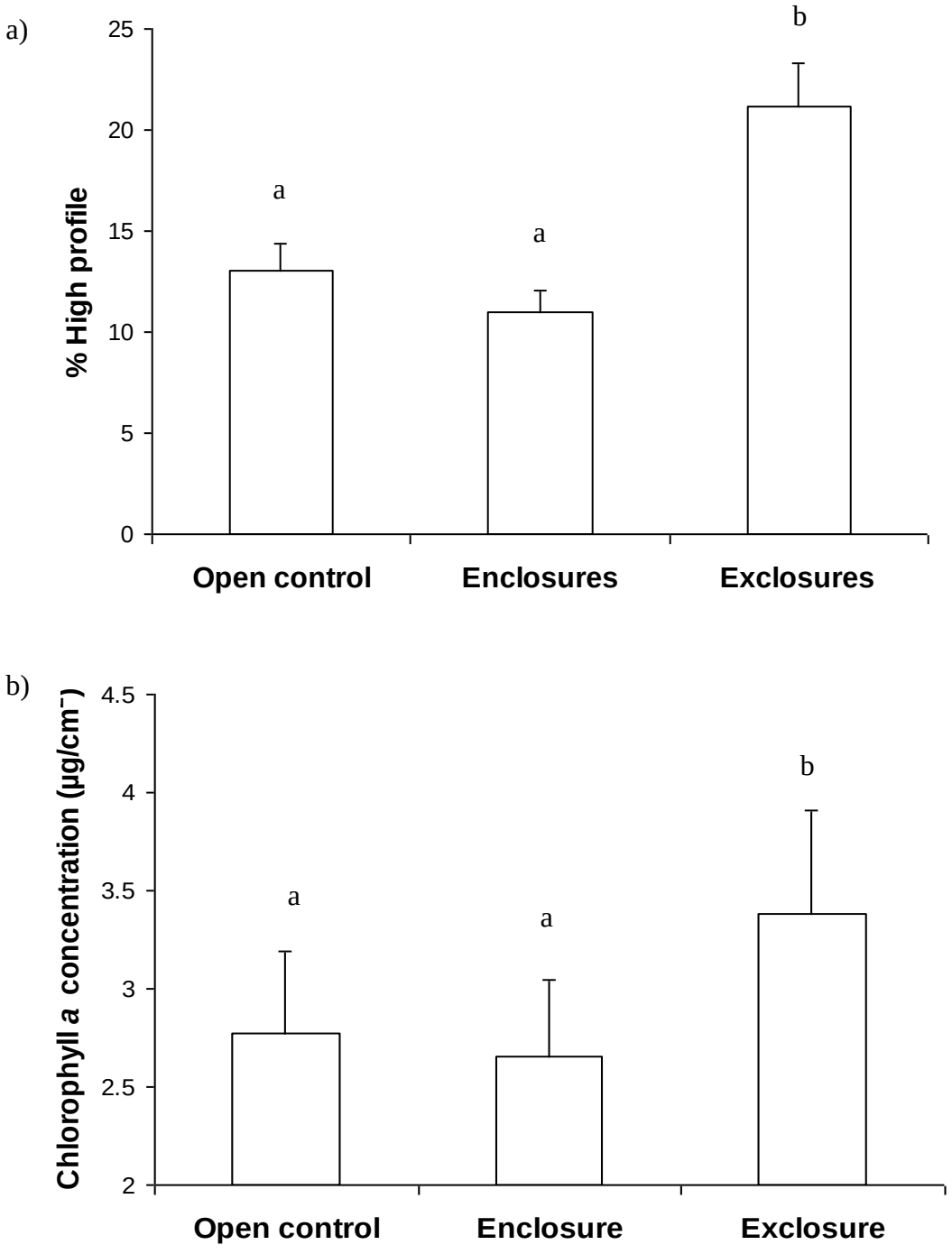

Figure 3.1: The influence of different grazer treatments on a) the proportion of high-profile diatoms and b) chlorophyll *a* concentration. Error bars represent 1 standard error; different letters represent significant differences by Tukey tests.

The relative abundance of erect diatoms was significantly lower in the grazed assemblages (enclosures and open plots) than in the ungrazed assemblages (exclosures) ($P < 0.001$, $F_{2,18} = 16.24$) whilst the converse was found for prostrate diatoms (Figure 3.2) ($F_{2,18} = 5.92$, $P < 0.05$). There were also significantly fewer stalked diatoms in the grazed treatment compared to the ungrazed treatment ($F_{2,18} = 5.96$, $P < 0.05$) but there was no significant difference in the relative abundance of motile diatoms. Prostrate diatoms were by far the most dominant group at all sites and treatments. The erect diatom, *Fragilaria capucina* was the only individual diatom species that had a significant difference in relative abundance between grazed and ungrazed trials ($F_{2,18} = 4.67$, $P < 0.001$).

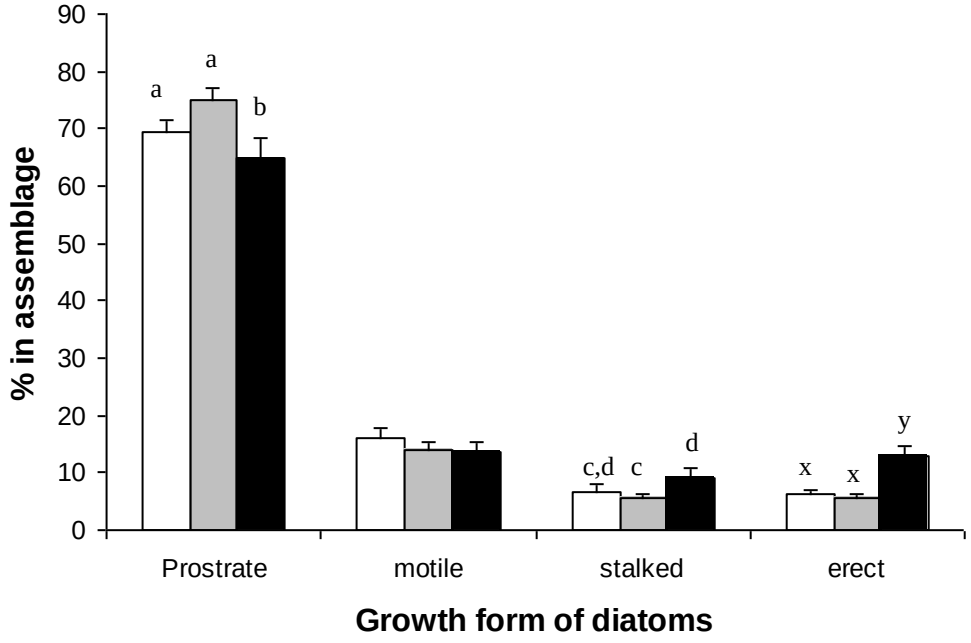

Figure 3.2: The average difference in relative abundance of different diatom growth forms at different treatments. Open bars are the natural treatment, grey bars are enclosures and black bars are exclosures. Different letters show significant differences between treatments (Tukey tests), error bars represent 1 standard error.

Mayflies were the numerically dominant macroinvertebrate at most of the 10 sites with most individuals belonging to either the Baetidae, Ephemerellidae or Heptagenidae. Study sites also contained small numbers of caddisflies, snails and other grazers such as Elminthidae beetles (Figure 3.3).

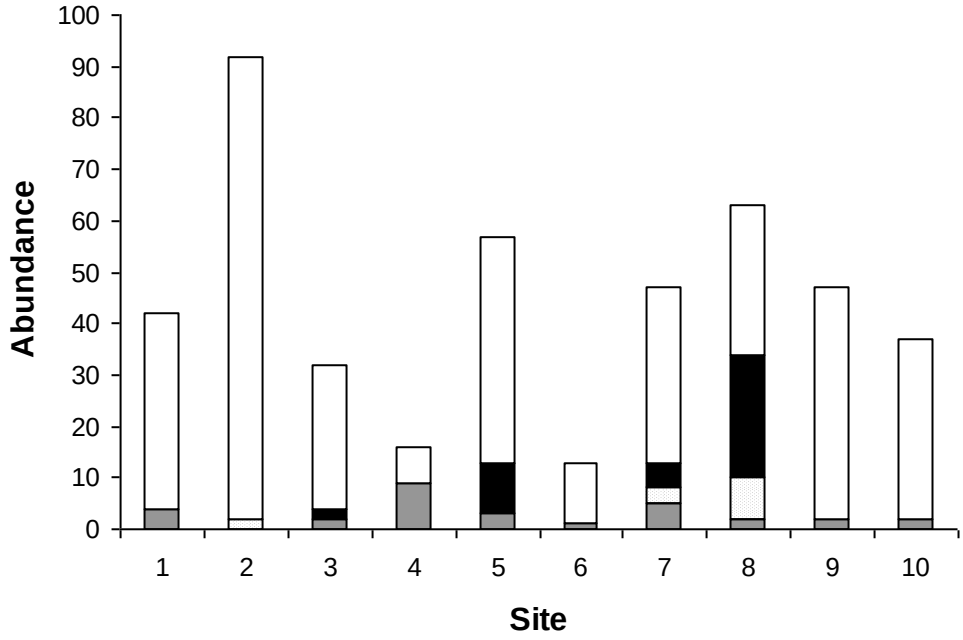

Figure 3.3: Average numbers of different grazing macroinvertebrates at sites. White bars denote mayfly abundance, black bars snail abundance, stippled bars caddisfly abundance and grey bars the abundance of other grazers.

There was no correlation between mayfly grazer biomass and non-mayfly grazer biomass indicating no co-variation ($P > 0.05$, $R^2 \leq 1.0$). There was no correlation between the average abundance of total macroinvertebrates, abundance of grazers or abundance of mayfly grazers and the difference between chlorophyll *a* at ungrazed and grazed sites (Regression analysis $P > 0.05$, $R^2 \leq 1.0$). Thus more grazers did not result in a greater decrease in biomass.

The abundance and biomass of mayfly grazers was found to be significantly positively correlated with the difference between the relative abundance of high-profile diatoms found in the ungrazed diatom assemblage and the grazed diatom assemblage ($R^2 = 0.33$, $P < 0.05$, $R^2 = 81.6\%$, $F_{1, 8} = 35.50$, $P < 0.001$ respectively) (Figure 3.4) (i.e. the more mayflies, the fewer high-profile diatoms found in the grazed treatment compared to its corresponding ungrazed treatment). There was no significant correlation between the total abundance (numbers) of grazers present or the total number of macroinvertebrates present, and the difference between the relative abundance of high-profile diatoms in grazed and ungrazed treatments ($P > 0.05$), but there was for total grazer biomass ($R^2 =$

87.3%, $F_{1, 8} = 54.91$, $P < 0.001$) (i.e. a greater biomass of grazers results in fewer high-profile diatoms)

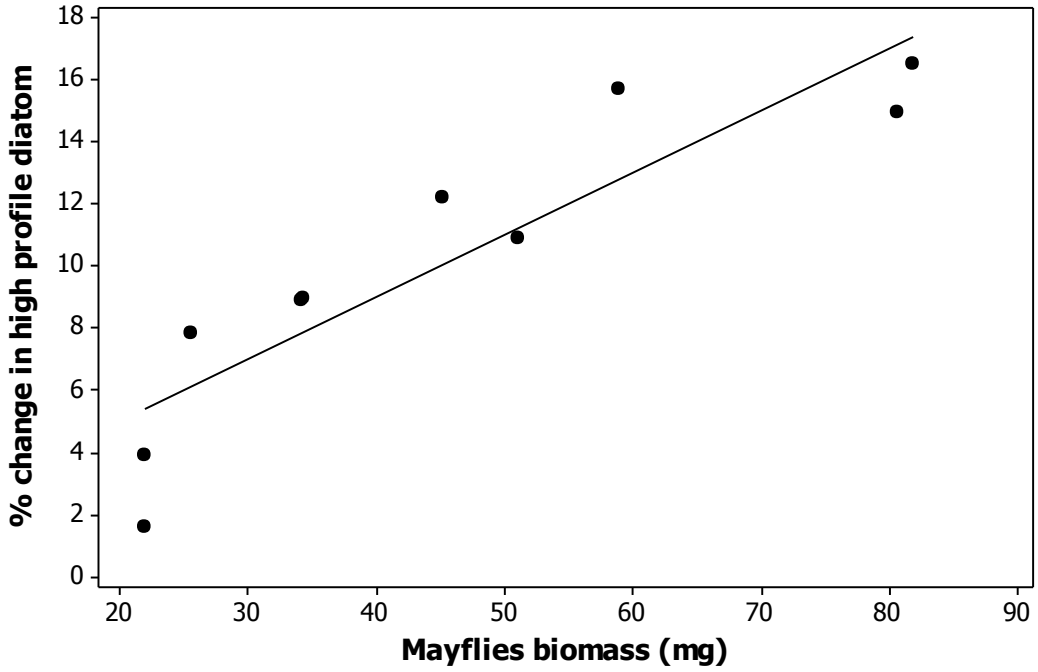

Figure 3.4: Differences between the % high profile diatoms in the grazed and ungrazed treatments diatom assemblage (i.e. ungrazed % - grazed %) compared to the average biomass of mayflies in grazed treatment.

Eighty-eight percent of variation in the grazed and ungrazed diatom assemblages across the 10 study sites was explained by the first two principal components of PCA (Figure 3.5). The grazed and ungrazed treatments separated along principal component 2, which although only explaining an additional 7 % of the variation in the assemblage, did result in a significant treatment effect (Paired t-test: $t = 5.20$, $P < 0.001$).

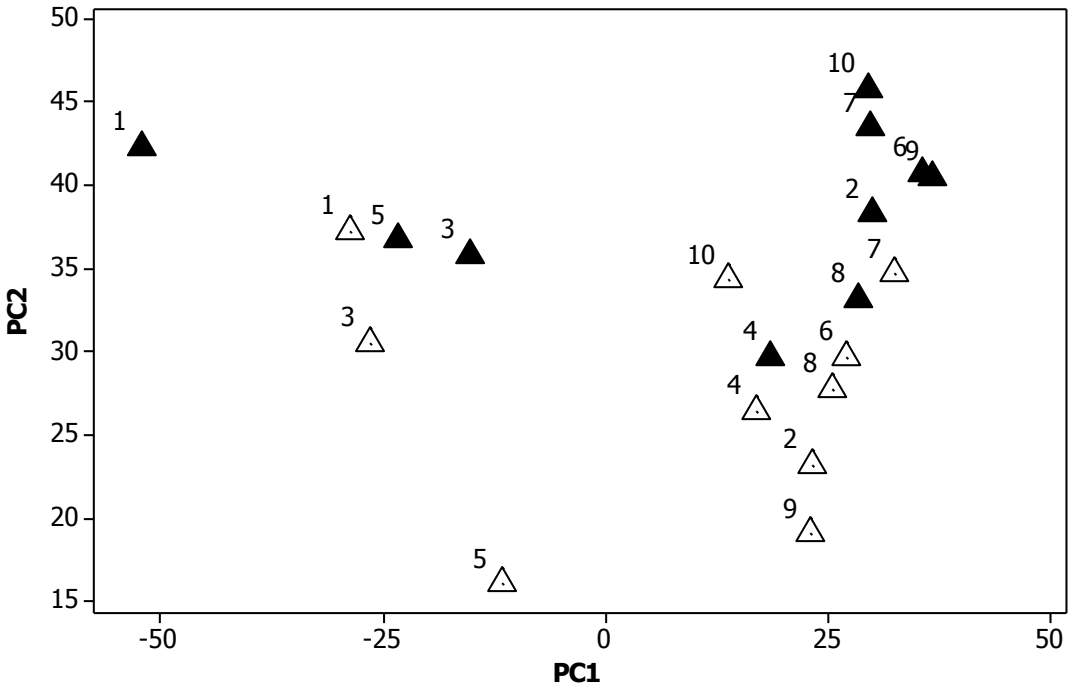

Figure 3.5: PCA plot of diatom assemblages at different sites, showing grazed sites as closed triangles and ungrazed sites as open triangles, sites are represented by numbers 1-10. PC1 represents 80% of variation; PC2 represents 7 % of variation.

3.3.2 Grazing influence on TDI

No significant difference was found between the TDI values of differently grazed treatments (Figure 3.6).

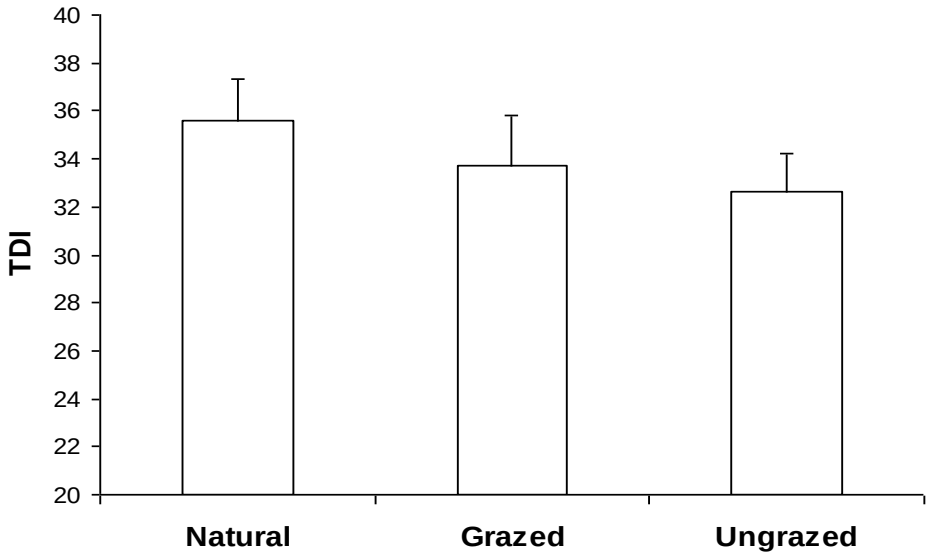

Figure 3.6: The TDI of differently grazed diatom assemblages (N = 10). Error bars equal 1 standard error (ANOVA non-significant).

No significant difference in TDI between grazed and ungrazed treatments was found because some sites had an increase in TDI with grazing (i.e. River Loxley; average grazed TDI = 30.65, average ungrazed TDI = 23.2) and at others a decrease (i.e. Porter brook; average grazed TDI = 41.22, average ungrazed TDI = 49.96), meaning that despite differences there was no consistent pattern (Table 3.2). However, the difference between the average sensitivity values (TDI score) of high and low-profile diatoms (Table 3.3) is positively correlated (R^2 = 39.6, P = 0.030) with the difference in TDI between grazed and ungrazed treatments (i.e. the larger the difference in average sensitivity value between high and low-profile diatoms the larger the difference in TDI) (Figure 3.7). For example the average sensitivity value of high-profile diatoms at the river Loxley is 1.95, whereas the value for low-profile diatoms is 2.08. An increase in the relative abundance of low-profile diatoms due to grazing would in this instance result in a higher TDI value. Conversely the average sensitivity value for high-profile diatoms at the Porter brook is 3.30 whereas the value for low-profile is 2.73. Therefore an increase in the relative abundance of low-profile diatoms due to grazing results in a lower TDI value. This indicates that differences in TDI can be due to structural (growth form relative abundance) changes in the diatom assemblage, which can reasonably be attributed to grazing. This shows that although there is not a pattern across sites there are differences between the grazed and ungrazed treatments in terms of TDI.

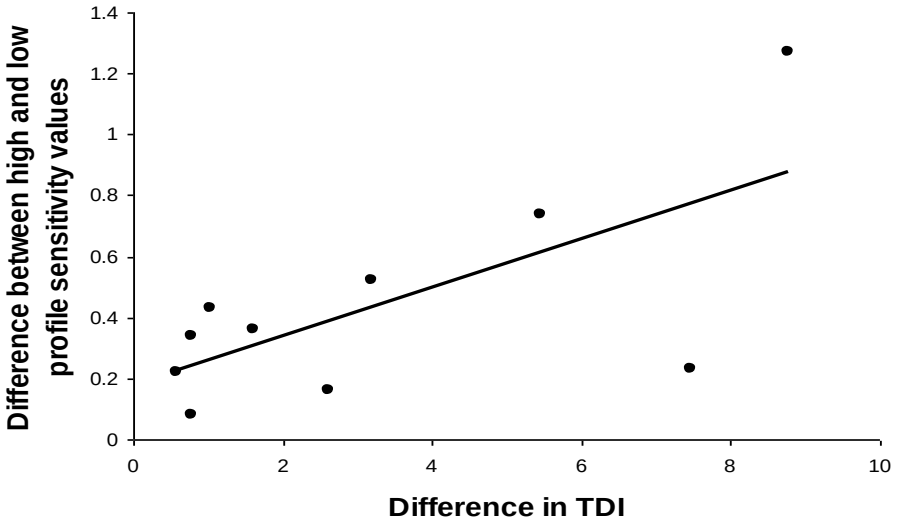

Figure 3.7: The difference in diatom sensitivity values (Kelly *et al* 2007) compared to the difference in TDI of differently grazed diatom assemblages (N = 10). Regression analysis was significant ($R^2 = 39.6$, $P < 0.05$).

Table 3.2: Trophic Diatom Index values and proportion of high profile diatoms at different sites and different treatments. There were no significant differences between treatments.

Site	**Natural**		**Caged-grazed**		**Ungrazed-caged**	
	Proportion of high-profile diatoms	**TDI**	**Proportion of high-profile diatoms**	**TDI**	**Proportion of high-profile diatoms**	**TDI**
Peakshole water	16.34	40.9	12.08	41.25	27.02	40.46
River Noe	12.27	33.54	13.4	30.08	25.6	26.88
Porter Clough	8.95	37.6	13.47	32.81	31.54	33.84
Loxley	14.91	39.03	12.7	30.65	14.48	23.2
Porter Brook	7.64	46.48	8.2	41.22	12.65	49.96
Rivelin	20.78	27.62	14.88	31.38	31.58	28.76
Sheaf	12.36	32.3	4.48	32.11	16.82	26.67
Ewden Beck	8.93	28.40	8.09	28.25	15.96	29.03
Trib. of Loxley	14.91	35.77	10.58	35.55	20.78	34.96
Ughill Brook	11.91	34.49	13.04	34.24	22.71	32.61

Table 3.3: Sensitivity values of low and high profile diatoms at different sites with different treatments. There were no significant differences between treatments or growth forms.

Site	Natural		Caged-grazed		Ungrazed-caged	
	Average sensitivity value Low profile diatoms	**Average sensitivity value high profile diatoms**	**Average sensitivity value Low profile diatoms**	**Average sensitivity value high profile diatoms**	**Average sensitivity value Low profile diatoms**	**Average sensitivity value high profile diatoms**
Peakshole water	2.65	2.58	2.70	2.30	2.77	2.23
River Noe	2.27	2.69	2.10	2.90	1.96	2.31
Porter Clough	2.40	3.08	2.20	3.16	2.28	1.93
Loxley	2.46	2.11	2.23	2.03	2.08	1.95
Porter Brook	2.54	4.09	2.40	4.10	2.73	3.30
Rivelin	2.09	2.20	2.28	2.15	2.25	1.81
Sheaf	2.23	3.13	2.21	3.20	2.13	2.47
Ewden Beck	2.14	2.12	2.11	2.33	2.16	2.17
Trib. of Loxley	2.40	2.67	2.40	2.03	2.48	1.93
Ughill brook	2.40	2.24	2.40	2.18	2.46	1.75

3.4 Discussion

The present study demonstrated that mayfly-dominated grazer assemblages can have a significant impact on the benthic algal assemblage in natural conditions. It also shows that mayfly grazing is associated with the structuring of the diatom assemblage, additional to studies that indicate the influence of caddisfly larvae (Peterson *et al* 2001) and snails (McCormack and Stevenson 1989). This is particularly important in streams where mayflies are the dominant grazers, as with most of the study sites used in the current investigation. Although other grazing macroinvertebrates were present, mayflies are likely to have had the greatest influence on the diatom assemblage in this study, because they were the most abundant grazer. Also sites with the highest numbers and biomass of mayflies showed the most dramatic reduction in high-profile diatoms in the grazed treatments compared with the ungrazed treatments. There was also no co-variation between numbers and biomass of other macroinvertebrates and the numbers and biomass of mayflies, meaning that the effect was probably predominantly due to the mayflies rather than any co-variation with other grazers.

The decreased proportion of high-profile diatoms in the assemblage and decrease in overall biomass found in grazed treatments is concordant with most studies using non-mayfly grazers and studies that have used mayflies in laboratory trials (Hill and Knight 1987 and 1988, Colletti *et al* 1987, Karouna and Fuller 1992, Pan and Lowe 1994, Holomuzki *et al* 2006). Mayflies are expected to preferentially consume high-profile diatoms due to their mouthpart morphology, which is thought to be more selective than the scraping mouthparts of caddisflies and snails (McKenny 2005). Like Wellnitz and Ward (1998), the current study found that motile diatoms were unaffected by grazing suggesting that they are structured by other (abiotic) factors. Motile diatoms are able to move by a raphe system allowing them to move around the biofilm (Yallop and Kelly 2006), which could explain why they are not influenced by grazing. The only diatom species that was found to be affected (decreased or increased) in relative abundance by grazing was the erect diatom *Fragilaria capucina*. This species has previously been found to be strongly influenced by mayfly grazing in artificial streams (Chapter 2). The lack of any other individual species changes due to grazing suggested that, particularly

for the prostrate group of diatoms, it is the trait of the diatom that determines whether it is preferentially grazed and not anything to do with its identity.

This study found that not only did the presence of mayflies result in fewer erect diatoms in the assemblage, but that the greater the biomass of mayflies present, the greater the difference in the proportion of erect diatoms between the grazed and ungrazed treatments. This provides further evidence that grazing by mayflies caused the decrease in high-profile diatoms and that the effect is controlled by the mayfly densities. This was similar to a laboratory trial that used different abundances of Heptagenidae mayflies that determined that the higher the number grazing, the greater the decrease in erect diatoms (Colletti *et al* 1987).

Some studies have found that grazing snails or caddisflies have greater effects on the diatom assemblage than mayflies, especially if periphyton biomass is the parameter being investigated (Lamberti *et al* 1987, DeNicola *et al* 1990). Alternatively, other studies have found that mayflies have a greater affect on diatom assemblage structure compared to other grazers (Villanueva *et al* 2004, Holomuzki and Biggs 2006). In this study biomass of mayflies was found to be important in structuring the diatom assemblage and biomass, as was total number/biomass of grazers, suggesting that grazing mayflies can be as important as less mobile grazers, or even more so in certain stream types. Caddisflies and snails may have greater effects individually due to their size and ability to clear whole areas of periphyton, but for sites which lack large numbers of caddisflies or snails this study shows that mayflies can have significant influence on the assemblage structure and biomass. Even if it is the case that mayflies have smaller affects than other individual grazers, they are often present in very large numbers at certain times of year and in certain stream types (Giller and Malmqvist 1998), and therefore can have an enormous influence on the algal community.

The present study agreed with previous findings that the presence of grazers' results in a decrease in algal biomass compared to ungrazed treatments (Lamberti and Resh 1983, Murphy 1984, Jordan and Lake 1996 and Greathouse *et al* 2006). This means that grazing decreases algal biomass and indicates 'top-down' control is operating in the

system and that primary producers are not able to keep up with the levels of grazing. However, it is likely that some species of diatom benefit from grazing: the more edible species, often better competitors for light, are decreased making the grazer-resistant species more dominant (Sarnelle *et al* 1993). There is also evidence of food preferences from gut content analyses of Hydroptilidae caddis flies. The caddis flies were found to prefer adnate diatoms and avoid the over-storey (Tall *et al* 2006). This showed that in a diverse grazer assemblage all of the resource can be utilised, potentially reducing the differences in relative abundances between grazed and ungrazed treatments. In the current study of mayfly-dominated streams it was found that the grazed assemblage is distinct from the ungrazed, demonstrating that grazing plays an important role in structuring the diatom assemblage in mayfly-dominated streams. However this may not be so pronounced in more diverse and/or even grazer assemblages if resource partitioning was operating (Tall *et al* 2006).

There was no significant effect of grazing on the TDI in this study when the 10 sites were considered as a whole. However, there was some evidence to suggest that mayfly-dominated grazer assemblages could have an influence on diatom structure that had the potential to change indices based on diatom relative abundance. Some of the diatom assemblages had high-profile diatoms with high average sensitivity values and/or low-profile diatoms with low sensitivity values and *vice versa*, meaning that there was no consistent pattern in whether grazed or ungrazed assemblages were likely to have higher TDI values. This does not mean that there is no potential for the TDI to be influenced by grazing because if there is a sufficient difference in the sensitivity values of the high and low-profile diatoms then there can be a change in TDI due to grazing but the direction of this change depends on the individual diatoms present and their sensitivity values. For example, if the average sensitivity value of high-profile diatoms in an assemblage was 1.5 and for low-profile diatoms it was 3, an increase in the relative abundance of low-profile diatoms could result in an increase in TDI and *vice versa*. At individual sites there were changes in TDI, but not as an overall consensus across sites for the direction of the change. This is due to the TDI depending on the sensitivity of the species in the assemblage, their relative abundances and this varying between sites, even of the same water quality (i.e. different diatom assemblages can have the same TDI).

The unpredictable effects of grazing on the TDI leaves us with a problem, because the TDI can be affected by grazing but to what extent depends on both the identity of the grazers, the diatoms that are present and the interactions between the two. The results of interactions have been found to be site-specific meaning that grazing can cause variation in the TDI. Thus grazing should be considered a possible cause of unexplained deviations from what may be expected for a site based on chemical or other biotic assessments (i.e. macroinvertebrates). However the outcome of the interaction on the index can only be predicted on an individual basis. If current recommendations to monitor multiple taxonomic groups are followed, dilemmas like this can be evaluated by assessing the grazer assemblage alongside the diatom assemblage, and predicting possible effects. For example, if it is known that there are many mayfly grazers at a site and combined with the high-profile diatoms having lower sensitivity values than low-profile diatoms (from TDI), then grazing is likely to result in an increase of TDI. This is because high-profile diatoms will be decreased in relative abundance resulting in a corresponding increase in the relative abundance of low-profile diatoms, which have a higher average sensitivity. The consequence of this is an increase in the TDI, indicating a worse ecological status than if the diatom assemblage was ungrazed.

CHAPTER 4: RELATIONSHIPS BETWEEN DIATOM AND MACROINVERTEBRATE STRUCTURE, BIOMASS, COMPOSITION AND INDICES.

4.1 Introduction

The previous two chapters have found that grazing by macroinvertebrates, in particular mayflies, can influence diatom assemblage structure and has the potential to influence indices based on it in some circumstances. This shows that two different taxonomic groups from different trophic levels can influence each other and demonstrates the importance of biotic interactions in structuring assemblages additional to abiotic factors. It is therefore important to determine whether trophic links can be observed in the field as well as in controlled experiments, as this is where monitoring is carried out. Assessing whether there are correlations between the diversity, biomass and structure of multiple taxonomic groups is also important for understanding the biotic processes involved in structuring freshwater ecosystems (Petts *et al* 2006). Understanding what structures freshwater ecosystems is important for understanding and interpreting the results of structural based indices (Petts *et al* 2006). Assessing multiple groups could be useful as it may decrease the chance of assessment error as well as providing extra

information to determine how the status of a river can be improved or maintained (Johnson *et al* 2006).

It has been increasingly recommended that more than one taxonomic group is used for monitoring; there are many advantages to this approach (Hering *et al* 2006). Rivers are under multiple pressures that operate over different temporal and spatial scales, thus the use of more than one biotic group for monitoring should allow for a more complete assessment of the state of a system than just using a single group (Soininin and Kononen 2004). Different organism groups have been found to provide distinct information about a system and react most strongly to different environmental variables depending on their ecology; therefore using multiple groups is unlikely to yield redundant information (or data) (Flinders *et al* 2008, Virtanen *et al* 2009). However, there are disadvantages to using multiple groups to assess water quality because it will take more time, money and sampling effort, which could potentially take resources away from other important areas (Johnson *et al* 2006). Thus, if it could be established with confidence that a single biotic group could predict the response of one or more other groups to environmental pressures it could be used as a proxy for a broader assessment of the ecology (Johnson *et al* 2006).

Four taxonomic groups have been highlighted as the elements to be used for monitoring the ecological quality of surface waters for the implementation of the European Water Framework Directive (WFD) (Hering *et al* 2006). These groups are macroinvertebrates, fish, macrophytes and algae (Hering *et al* 2006). These groups represent different trophic levels; therefore monitoring them at the same time should provide a comprehensive assessment of the system at different temporal scales and to various impacts (Herring *et al* 2006). Traditionally macroinvertebrates have been used for monitoring (and often assumed to be a proxy for other taxonomic groups) because they are easy to sample, are widespread, have a well-defined taxonomy, are relatively small and are abundant at most sites (Bonada *et al* 2006, Resh 2008). Disadvantages to using macroinvertebrates include the need for expertise in their identification, the fact that they are influenced by biotic interactions and have seasonal fluctuations unrelated to degradation (Resh 2008). Fish are a useful group for monitoring because of their importance within an ecosystem: long generation times, which are useful for assessing

changes over a long temporal scale: their ease of identification and the interest to the public as an enigmatic group involved in popular recreation (Resh 2008). Using fish alone for biomonitoring is problematic because they are highly mobile and may exhibit pollution effects far from where they have been sampled; in addition, due to their size they are not present in very small streams – or at least not in any great diversity or numbers (Resh 2008). In contrast to fish or macroinvertebrates macrophytes cannot move away from a stressors and therefore can exhibit responses to environmental variables present at the time and place when sampling occurs, as well as indicating change over a period of years, they are also relatively easy to identify (Szoszkiewicz *et al* 2006). However, there are also problems with using macrophytes as they can often grow outside of their optima, slow to establish and are slow to respond to change and they are not found at great diversity in many river types (Szoszkiewicz *et al* 2006). Algae, of which diatoms are usually used as a successful proxy (Kelly *et al* 1998) are useful for biomonitoring because they are ubiquitous, diverse, important for ecosystems, have a short generation time - meaning they respond rapidly to disturbance, are easy to sample in the field and they respond to plant specific stresses (Resh 2008). Limitations in the use of diatoms: include that they are influenced strongly by non-anthropogenic changes, they are not suitable for assessing long-term changes due to their short generation times and they require expertise in identification (Resh 2008). The advantages and disadvantages of the potential taxa for monitoring suggests that different groups will be more affected by some conditions than others and show which would be most useful for monitoring in different circumstances. This means that for the general assessment of ecological quality of a watercourse, the monitoring of all four groups may give the most complete assessment.

To date, there have been few studies that have investigated the links between the diversity of different biotic groups in freshwaters. However, there has recently been a surge in interest due to the WFD, but results so far have been unclear and inconclusive (Johnson and Herring 2009). Some studies found no correlations, for example, a study of the taxonomic richness across several taxonomic groups, including macroinvertebrates, zooplankton and birds, performed in the great lakes of the United States (Allen *et al* 1999). In this study different taxa that were potentially trophically linked did not have

observable correlations between them (Allen *et al* 1999). Another study undertaken in Finnish springs found that bryophytes and macroinvertebrates showed no significant concordance in their diversity (Virtanen *et al* 2009). Macroinvertebrates and fish have also been found to lack congruence in measures of their diversity, despite being linked through the food chain (Kilgour and Barton 1999, Infante *et al* 2009). Studies that have investigated macroinvertebrates and diatoms together have generally found no congruence in diversity, despite the fact that diatoms are an important food source for many macroinvertebrate grazers (Paavola *et al* 2006, Johnson and Herring 2009). Some evidence of congruence between different taxonomic groups has emerged for example a French study found a weak correlation between fish and diatom species richness which was most likely due to a similar response to a longitudinal gradient (Grenouillet *et al* 2008). The same study also found that fish and macroinvertebrates were correlated; due to biotic interactions, however no relationship was observed between diatoms and macroinvertebrates (Grenouillet *et al* 2008).

Studies that have focused on lower taxonomic levels, such as those comparing species richness of different orders of macroinvertebrate, have found concordance, possibly because similar organisms respond in similar ways to environmental variables (Bilton *et al* 2004, Sanchez-Fernandez *et al* 2006). Some studies have observed a positive correlation between the biomass of periphyton and macroinvertebrates, but not between their diversity, meaning that some aspects of their ecology can be correlated when others are not (Sponseller *et al* 2001, Tolonen *et al* 2005). Ecological indices based on fish, macrophytes, macroinvertebrates and diatoms have generally been found not to correlate, possibly reflecting their response to different environmental factors (Springe *et al* 2006). A study that assessed macroinvertebrates, fish and diatoms together found that the three groups never gave the same response to impacted systems (Carlisle *et al* 2006). This study shows that there is a risk of making incorrect assumptions when the responses of one group are used as a proxy for the ecological quality of the whole system (Carlisle *et al* 2008). Despite a number of recent studies the variation in rivers and sampling methods between studies is such that there is still a need for more data in order to determine whether correlations exist between multiple organism groups and in what situations these become apparent (Heino 2009).

Rivers and their biota are extremely variable because of the multiple factors influencing a river at any one time mean that any correlations found tend to be weak and variable and thus, may not always be observable (Lewis *et al* 2007). A large-scale study found that different groups were sensitive to different pressures and concluded that it was unlikely that any one group could best assess general degradation alone (Herring *et al* 2006). However, the response of different groups to different environmental variables could mean that certain taxa are best suited to assessing particular impacts that they respond most strongly to (Johnson and Hering 2009). If the stressor of interest is known, then there may be potential for the most sensitive group to that impact to be used as a surrogate for the ecology of the system (for example diatoms for nutrients) (Johnson *et al* 2006a, Johnson and Herring 2009). Different groups are also more suitable in different types of river, for example: in small streams macroinvertebrates and diatoms are most useful for monitoring due to the lack of species richness and abundance of fish and macrophytes (Herring *et al* 2006b). Where studies have found concordance between different groups, it has generally been in response to a strong environmental gradient (Heino 2009). Nonetheless, it is especially important to determine what, if any, links exist between different organism groups in relatively unimpacted streams in order to for relationships between groups to be assessed without being obscured by the presence of strong abiotic factors (Heino 2009). The relationship between responses in different groups must be determined at sites with the lowest human impacts. These sites act as reference sites, showing what relationships are present in an ideal environment and therefore provide baseline data with which to assess water courses (EU Water Framework Directive 2000/60/EC).

The structure of the macroinvertebrate and diatom assemblages is dependent both on the abiotic (physical, chemical and structural) and the biotic (interactions with other taxa) characteristics of that stream with the relative importance of each depending on the scale of the assessment (Heino *et al* 2004). Congruence between different taxonomic groups could be due to five different mechanisms, which are: a random draw of species from the species pool, a similar response to the same environmental gradient, a response to different, but correlated environmental gradients, biotic interactions or inconsistent

sampling effort (Heino 2009). This means that diatoms and macroinvertebrates may show concordance by responding to the same environmental variable(s) or by directly influencing each other through feeding interactions (Paavola *et al* 2003), assuming that sampling errors are not an issue. Macroinvertebrates and diatoms are known to be influenced by some of the same environmental factors (i.e. nutrients) but react differently to others such as land use parameters and channel morphology (Hirst *et al* 2002, Johnson *et al* 2006 and Lewis *et al* 2007). In some situations, diatoms and macroinvertebrates are responding to the same environmental variable, but the nature of that response is different (Sonneman *et al* 2001, Hirst *et al* 2002). Generally, macroinvertebrates decrease in species richness and abundance in response to a negative impact, whereas diatoms respond by a change in the identity of the species present (Sponseller *et al* 2001, Hirst *et al* 2002).

Diatoms, as one of the main primary producers in the aquatic system, are an important food source for grazing macroinvertebrates, and could therefore influence the macroinvertebrate assemblage (Juttner *et al* 2003). Feeding links between macroinvertebrates and diatoms mean that they could influence each other's diversity, structure and abundance as was shown in chapters 2 and 3. High diatom species richness may provide a greater number of feeding niches for macroinvertebrate grazers resulting in greater family richness and/or abundance of grazers (Tilman 1999). A high species richness of macroinvertebrates may reduce competitive diatom species to levels that allow less competitive species to survive, leading to greater diatom species richness (Tilman 1999). However, a study that excluded macroinvertebrates from areas to evaluate grazing effects found that ungrazed treatments had more diatom species than grazed treatments indicating that grazing macroinvertebrates have the potential to decrease the number of diatom taxa (richness) found in a system, by consuming some species to extinction (Opsahl *et al* 2003). Results presented in chapter 3 showed that the structure of grazed diatom assemblages was significantly different to the structure of ungrazed ones, but it is unclear whether any associations would be observable as correlations in a field survey. A study on the effect of scour on the periphyton demonstrated a two-way interaction where the initial diatom assemblage determined how it was affected by grazing (Wellnitz and Radar 2003). This suggests that the

identity of the diatoms initially present can influence the effect that grazers can have on them (Wellnitz and Radar 2003).

The abundance of macroinvertebrates is likely to influence the abundance of diatoms and *vice versa*; therefore determining if there are trophic interactions occurring is particularly important for aiding the understanding of structural based indices as it indicates whether biotic interactions have the potential to influence the indices (Petts *et al* 2006). A bottom-up controlled system would be expected to show that a greater biomass of diatoms (periphyton) would be associated with a greater biomass of macroinvertebrates, particularly grazers, due to more food being available (Alstad 1987). Studies have found that any factor that increases algal abundance or chlorophyll *a* content, such as increased nutrients or greater light availability, results in greater numbers and/or biomass of macroinvertebrates (Alstad 1987, Scimegeour and Winterbourn 1989, Thompson and Townsend 1999 and Sponseller *et al* 2001). Alternatively, some studies found no association between algal and grazing macroinvertebrate abundance showing that associations vary and may depend on the specific environment (Robson and Barmuta 1998, Hirst *et al* 2003). If the system is top-down controlled it would be expected that a greater biomass of macroinvertebrate grazers would result in reduced periphyton biomass, as they would be consumed at a greater rate than they can grow back. Several different studies have found evidence that exclusion or decrease of grazers results in a greater diatom density and/or algal abundance or similarly an increase in grazing results in a lower diatom density and algal abundance (Jordan and Lake 1996, Opsahl *et al* 2003, Wellnitz and Radar 2003, Hillebrand 2005 and Holomuzki and Biggs 2006). The influence of periphyton abundance on macroinvertebrate assemblage structure could also be important but Braccia and Voshell (2006) found that chlorophyll *a* concentration had no relationship with macroinvertebrate assemblage structure suggesting that in this case biomass of periphyton did not influence the type or identity of the macroinvertebrates present.

Abiotic factors, such as current velocity, can influence the interactions between macroinvertebrates and diatoms (Poff *et al* 2003). Macroinvertebrates are not able to graze as effectively on periphyton in strong current, therefore grazing intensity is not

only influenced by the number and type of grazers (Poff *et al* 2003). Abiotic factors can change over the course of a year leading to different types and severity of pressures over time. Seasonal factors that can influence periphyton can indirectly affect grazing macroinvertebrates and *vice versa*; such as increased shading leading to a decrease in grazers due to the decrease of food source (Dudgeon and Chan 1992). Some studies have found that algal abundance only influences some macroinvertebrate functional feeding groups, such as a positive association only being found between grazing macroinvertebrates and algal abundance in New Guinea streams (Dudgeon 1994).

Seasonality is important in rivers with most monitoring protocols requiring assessments to be made in the same season that the model was formulated (Feio *et al* 2006). Macroinvertebrates and diatoms will change naturally over a season both due to changing environmental conditions (abiotic and biotic) and by their different life histories and emergence times (for macroinvertebrates) (Lancaster *et al* 1996). For example biomass and density of macroinvertebrates are expected to peak in the spring and autumn in headwater streams (Giller and Malmqvist 1998). Thus seasonality needs to be considered when determining the influence different groups have on each other; changes in one group could be a result of a change in the other group mediated by season. Concordance between macroinvertebrates and diatoms has rarely been investigated at the same sites over several months, so it is unknown whether the two groups show any covariation over time. Patterns of change in macroinvertebrate assemblages are often inconsistent, but these changes need to be understood in order to investigate their impact on their food source (i.e. diatoms) and assessments based on this (Kay *et al* 2001, Beche *et al* 2006). Variability of macroinvertebrate and diatom assemblages in acid streams has been found to be greater over seasons than between years demonstrating the importance of seasonality (Lancaster *et al* 1996). Seasonal variations in food supply (i.e. primary production) have been found to structure the macroinvertebrate community by determining the proportion and biomass of grazing species (Thompson and Townsend 1999). Some temporal variation is predictable, for example chironomids have been found to dominate a well-studied UK stream in the summer months whereas stoneflies are dominant for rest of the year (Woodward *et al* 2002).

In order to monitor ecological quality, indices have been developed based on the structure of the different taxonomic groups found in rivers. Any processes or impacts that influence the structure of the groups can therefore influence indices based on them (Petts *et al* 2006). Indices used in biomonitoring are based on the characteristics of groups so a full understanding of how the underlying properties of the groups (biomass, species/ family richness, abundance, identity) relate to each other is needed to understand how indices may correspond to each other and why this may be. The most frequently used metrics for macroinvertebrates and diatoms in the UK are the Average Score Per Taxon (ASPT) and the Trophic Diatom Index (TDI) respectively (Wright *et al* 1998, Kelly and Whitton 1995). The ASPT score and TDI can be compared by dividing the observed score by the expected (that which would be found in pristine conditions) to get an Ecological Quality Index or ratio (EQI) for the site. Due to the factors mentioned above, these indices may agree or they may be complementary and tell us different things about the site in question.

Sites that are relatively unimpacted are used in this study in order to investigate fundamental correlations and not those influenced by anthropogenic impacts. Identifying relationships found between organisms at unimpacted sites allow us to separate naturally occurring interactions from interactions from those that have been affected by human impacts. Macrophytes and fish are not considered in this study due to the low numbers that are present in small streams, making diatoms and macroinvertebrates the more robust indicators in this case (Herring *et al* 2006). The questions addressed by this study were: i). Are there correlations between macroinvertebrate and diatom assemblages in relatively unimpacted North of England streams?
ii). Do the indices based on these groups give the same assessment of the streams ecological quality?
iii). Are there trends for individual sites over time?

This study aims to determine if there is any correlation/ concordance between diatoms and macroinvertebrates (species/family richness, abundance, biomass, species composition, traits) in minimally impacted streams. It also aims to find out if indices

based on these two groups show concordance and therefore determine if one can be predicted from the other. This chapter aims to assess what aspects of macroinvertebrate and diatom assemblages are linked in order to explain what may be happening to structure the indices and find out if correlations occur over time.

4.2 Methods

4.2.1 Site Descriptions

Twenty-four study sites (Table 4.1), classified as either excellent or good ecological quality based on macroinvertebrate data (Environment Agency A or B classified sites from 2002-2005) were chosen to reflect a range of macroinvertebrate taxon richness. A sub-set of 10 of these sites were chosen, to represent a variety of different sized rivers, to be assessed May 2007 – October 2007 and May, July and August 2008. One of these sites (Upstream of Damflask) was not sampled in August 2008 due to a road closure, leaving a total of 89 samples.

A 50 m long study reach was identified at each site and characterised in terms of channel-width, water-depth, substrate composition, flow rate and canopy cover. The dominant riparian vegetation was identified. Width was measured in three places (start, middle and end of study-reach) with a tape measure, substrate composition was approximated using RIVPACS methodology (Murray-Bligh *et al* 1997), flow rate was measured in ten random places within the study reach using an electromagnet flow meter

(Valeport 801) and depth was measured at the same time as the flow. Riparian vegetation and canopy cover was assessed by eye.

Table 4.1: Study sites for field study undertaken in July 2006. Those in bold were also sampled in May 2007 – October 2007 and May, July and August 2008.

Site name	Grid Reference	Water course name
East of Alton	SK370643	Press Brook
Hipper	**SK357703**	**River Hipper**
River Sheaf	**SK324813**	**River Sheaf**
Lathkill	SK221646	River Lathkill
Peakshole Water	**SK170834**	**Peakshole Water**
Redleadmill Brook	SK391666	Redleadmill Brook
River Noe	**SK168844**	**River Noe**
Oughtibridge	SK305933	Cournes Brook
South of Fulwood Hall	**SK291849**	**Carr Brook**
River Wye	SK104725	River Wye
Stubbing Court	SK344766	Tributary of Barlow Brook
Brindwoodgate	SK334759	Black Carr Lumb
Berrymoor	SE296071	Silkstone Dike
Rivelin	**SK289871**	**River Rivelin**
Milthorpe	SK325761	Dunston Brook
Brookside Beck	**SK348706**	**Brookside Beck**
Holymoorside	SK337688	Trickett Brook
Loxley	**SK298895**	**River Loxley**
Redmires	SK273865	Wyoming Brook
Upstream of Damflask	**SK262091**	**Ughill Brook**
Thurgoland	SK296998	River Don
Meersbrook	SK359844	Meers Brook

4.2.2 Samples and processing

At each site, measurements of water pH (Jenway 3100 pH meter), temperature (Jenway 3100 pH meter), dissolved oxygen (Hanna HI 9146 dissolved oxygen meter) and conductivity (Jenway 4071 conductivity meter) were taken from the start, middle and end of the study reach. Water samples were taken, in triplicate, from below the surface of the water using 150 ml plastic bottles (previously cleaned with distilled water) for the analysis of alkalinity, total phosphorus content, nitrate and nitrites. Samples were transported to the laboratory on ice and then either processed immediately on return or frozen at -18 ºC for later analysis. The water samples were analysed in the laboratory in triplicate using a Palintest ® kit.

Macroinvertebrates were sampled from each site using the Environment Agency's standard 3-minute kick sample: 1 minute search of the surface, 3 minute kick sample, using a 25 cm by 20 cm net with a 500 µm mesh size, and 1 minute turning of rocks (Murray-Bligh *et al* 1997). Macroinvertebrates and debris were put in 500 ml plastic tubs and stored in 70 % IMS. Macroinvertebrate biomass was sampled by taking ten random 0.0625 m^2 surber samples, using a surber sampler with a 500 µm mesh size, at each site and stored in the same way as the kick samples. Diatom samples for species identification were sampled using the method of Kelly *et al* (2007): 5 cobbles (5 cm – 15 cm in diameter) were scraped using a stiff nylon toothbrush and combined to form one sample that was stored in Lugols Iodine. Periphyton biomass (as chlorophyll *a)* was sampled (in July 2006 only), by taking samples from the upper surface of 10 cobbles, randomly chosen from a riffle section of the study reach. A specific area (9.1 cm^2) was sampled from each cobble by holding a bottle cap closely to the stone then brushing the periphyton vigorously with a toothbrush to remove all periphyton on the stone except for that under the bottle cap. The bottle cap was then removed and the remaining periphyton brushed carefully into a plastic container. The periphyton was transferred carefully into a labeled tube, kept on ice and in the dark for transport back to the laboratory. Samples were stored, frozen at -18 ºC until they could be processed.

Macroinvertebrates were identified to family level using the appropriate keys (Macan 1959, Elliot and Mann 1979, Hynes 1984, Wallace *et al* 1990, Nilsson 1997 and Elliot *et al* 1998) and the numbers of individuals in each family recorded. Chlorophyll *a* samples were processed by the method described in Chapter 3 (3.3.2). Additional to the 10 replicate chlorophyll *a* samples, 2 of the samples had each individual sample (cobble sample) split into 4, and each of these 4 analysed separately, in order to assess the variability of the method. The 2 split samples had standard deviations of 0.03 and 0.015 compared to a standard deviation of 0.78 for the 10 samples from different stones. This shows that the high variation in samples is due to natural variability of the periphyton on the cobbles not variability within the assay.

For the method for diatom processing and identification see Chapter 2 (Section 2.2.4).

Functional feeding groups for macroinvertebrates were assessed using the Eurolimpacs database (Buffagni *et al* 2007) with all species being allocated to non-grazer or grazer categories on the basis of their predominant food source (periphyton or other). Taxa that had scored 5 or more (out of a total of 10 points) in the grazer category were considered to be grazers for the purpose of this project. For families with different species that where not all considered grazers (i.e. scoring 5 or more in the grazer category) the family is classed as a grazer family if the score for grazers divided by the number of species in the family is greater or equal to 5 (i.e. the overall average score is that of a grazer as defined above). The grazer functional feeding group was split into surface feeding grazers, those that predominantly graze the top surface of the assemblage and "scraper" grazers, those that consume the whole of the assemblage due to their scraping mouth parts, assessed from the literature (Karouna and Fuller 1992, Wellnitz and Ward 2002, Holomuzki and Biggs 2006 and Tall *et al* 2006) (Table 4.2).

Table 4.2: Classification of grazers found at study sites used in this study.

Surface feeder	Scraper
Baetidae	Hydrobiidae
Ephemerellidae	Elmidae (Adults)
Heptageniidae	Elmidae (larvae)

Glossosomatidae
Ancyclidae
Planorbidae

Diatoms were assigned trait groups (erect and stalked are considered high-profile, prostrate and motile are considered low-profile) Wellnitz and Ward (2000) as a guideline (Table 4.3).

Table 4.3: Classification of diatom taxa (found at > 1% relative abundance at one or more site) by growth form at sites used in this study.

Low Profile	High Profile
Achnanthes spp	*Encyonema spp*
Achnanthidium spp.	*Fragilaria spp*
Planothidium spp.	*Cymbella spp*
Cocconeis spp.	*Gomphonema spp*
Eunotia spp.	*Amphora spp.*
Navicula spp.	*Diatoma spp*
Nitszchia spp	*Meridion circulaire*
Surrirella spp.	*Reimeria sinuata*
Cocconeis spp.	*Rhoicosphenia abbreviata*

4.2.3 Data analysis

Macroinvertebrate Average score per taxon (ASPT) was calculated using the methods described in Wright *et al* (2000). Ecological quality index/ratio (EQI) was calculated using the observed ASPT score divided by that expected at the site in pristine conditions as described by Wright *et al* (2000). Ecological quality class boundaries are those described by Kelly *et al* (2008) for diatoms and Clarke *et al* (2004) for macroinvertebrates and are those currently recommended for use in the implementation of the WFD.

The trophic diatom index (TDI) was calculated using the revised scoring table found in Kelly *et al* (2007) as described in Chapter 2 (Section 2.2.5).

Correlation analyses were performed to determine if there were significant correlations between parameters investigated. Chi-squared analysis was used to determine if there was significant difference between the numbers of study reaches falling in the same ecological quality class for both macroinvertebrates and diatoms. T-tests were used to determine if there were significant differences in abiotic characteristics between sites that have the same EQI rating (i.e. both good or both poor) for diatom and macroinvertebrates and diatom and those that differ. All statistics were performed using Minitab version 14.0 for Windows.

2.3 Results

Study sites were sampled in July 2006 during dry, warm, sunny weather. The 10 sites assessed between July 2006 and August 2008 were sampled during a variety of weather and conditions including after a large flood event (see chapter 5). Sites ranged from 0.5 m and 9 m in width and 5 cm and 40 cm in depth. Most sites had riparian vegetation of deciduous trees, holly and various herbs. The substrate of the riverbed was usually cobbles with some boulders, sand and silt. Water birds such as grey wagtail and dipper were often sighted. At several sites fish were observed, mainly bullheads and young trout.

4.3.1. Correlation between macroinvertebrate and diatom taxon richness

There was no relationship between diatom diversity and macroinvertebrate diversity (as species number and family number respectively) (Figure 4.1). Correlation analysis found no correlation between numbers of taxa in the two groups or number of taxa and biomass of the two groups ($F_{1,22} \leq 0.03$, $P > 0.05$). Taxon richness was not related to ecological quality class for TDI or ASPT score. There was slight positive correlation between diatom and macroinvertebrate richness over the 9 months that were sampled over 3 years ($R^2 = 4.9$, $F_{1, 86} = 5.51$, $P = 0.021$). But there was no consistent trend for

diversity at individual sites over time, with no pattern indicating that the sampling when the highest number of macroinvertebrate families was recorded was associated with the highest number of diatoms recorded (Figure 4.2).

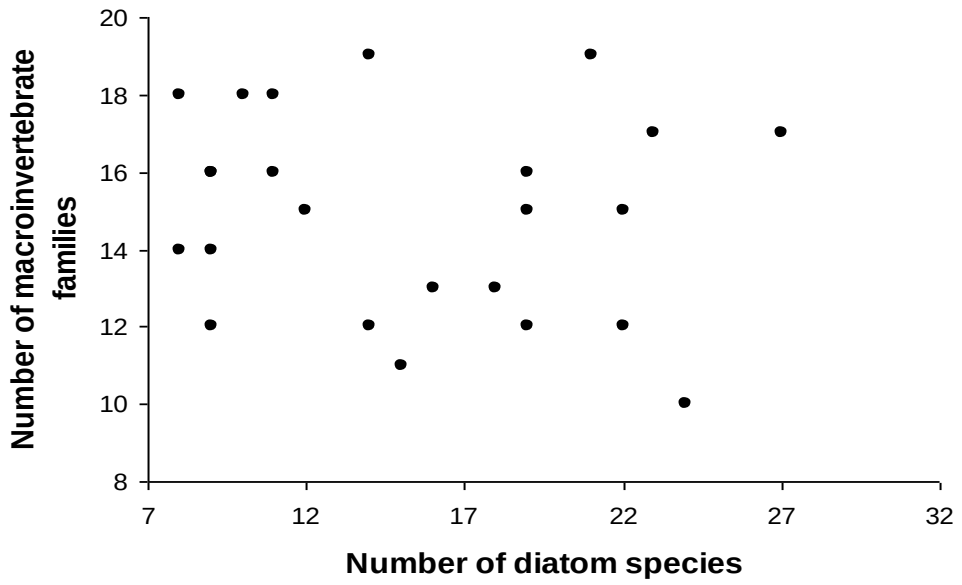

Figure 4.1: The number of macroinvertebrate families compared to the number of diatom species present in samples. There is no correlation between the 2 groups ($P > 0.05$).

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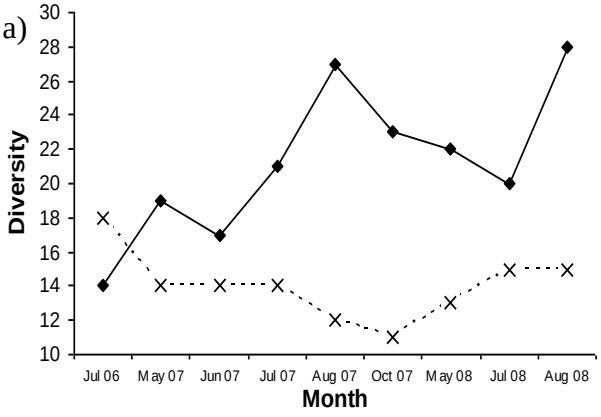
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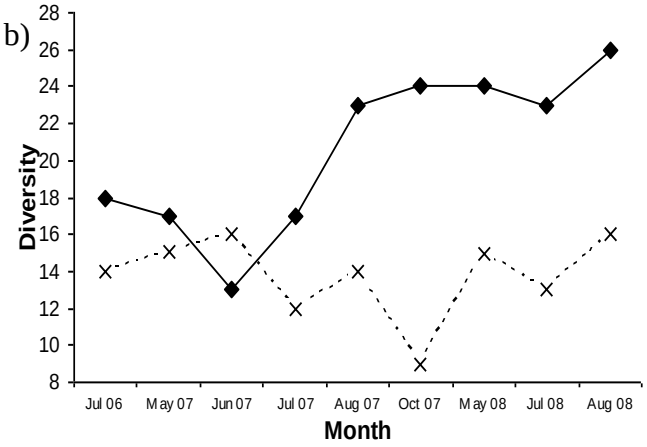
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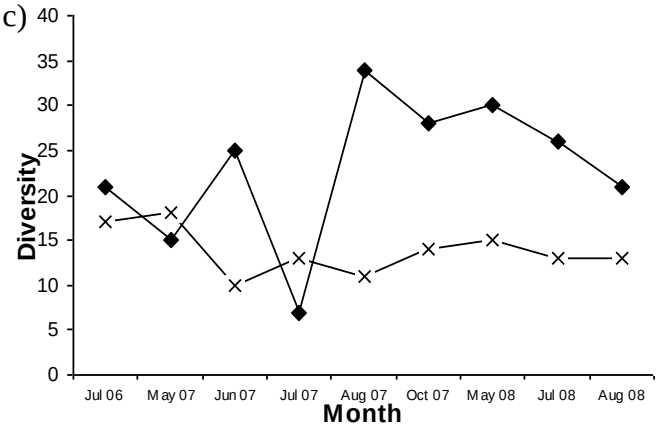
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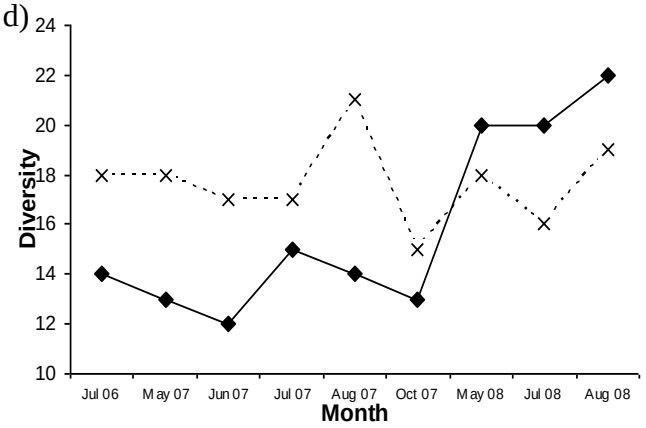
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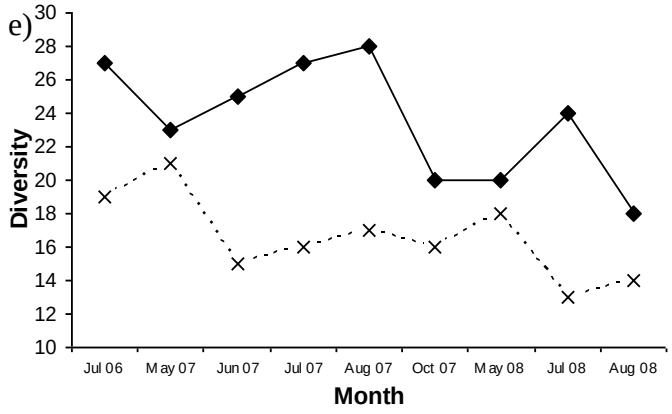
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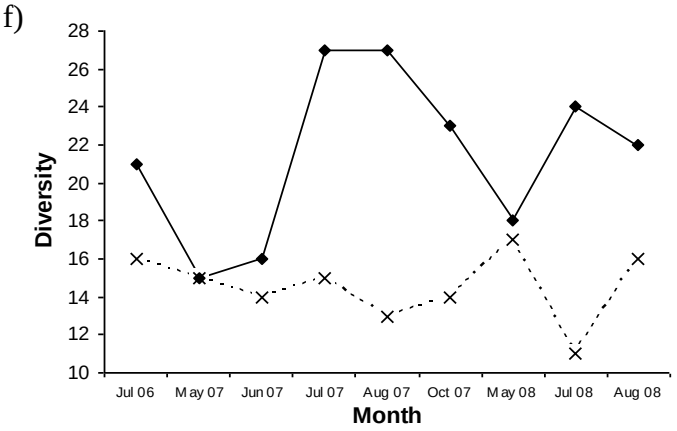
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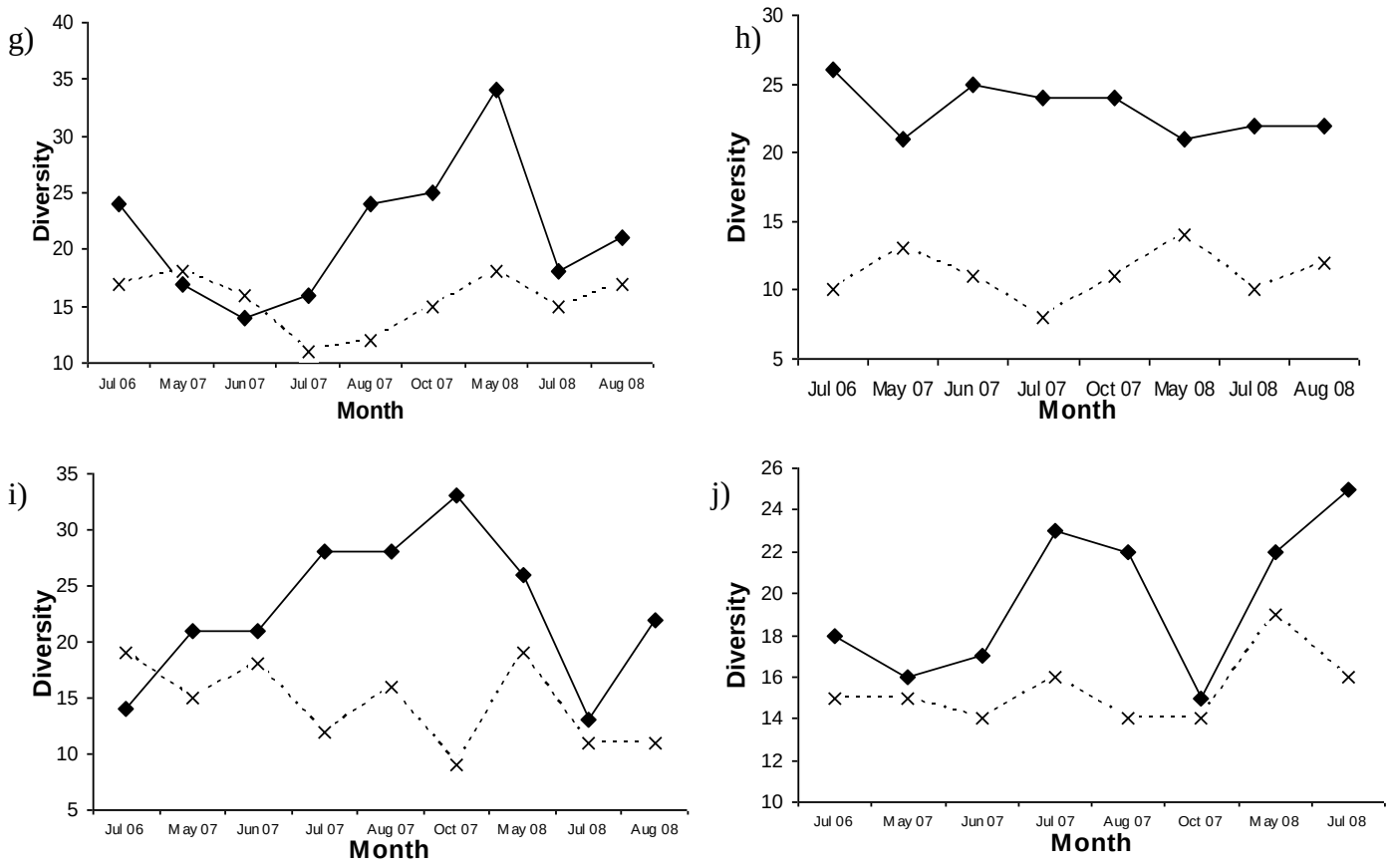


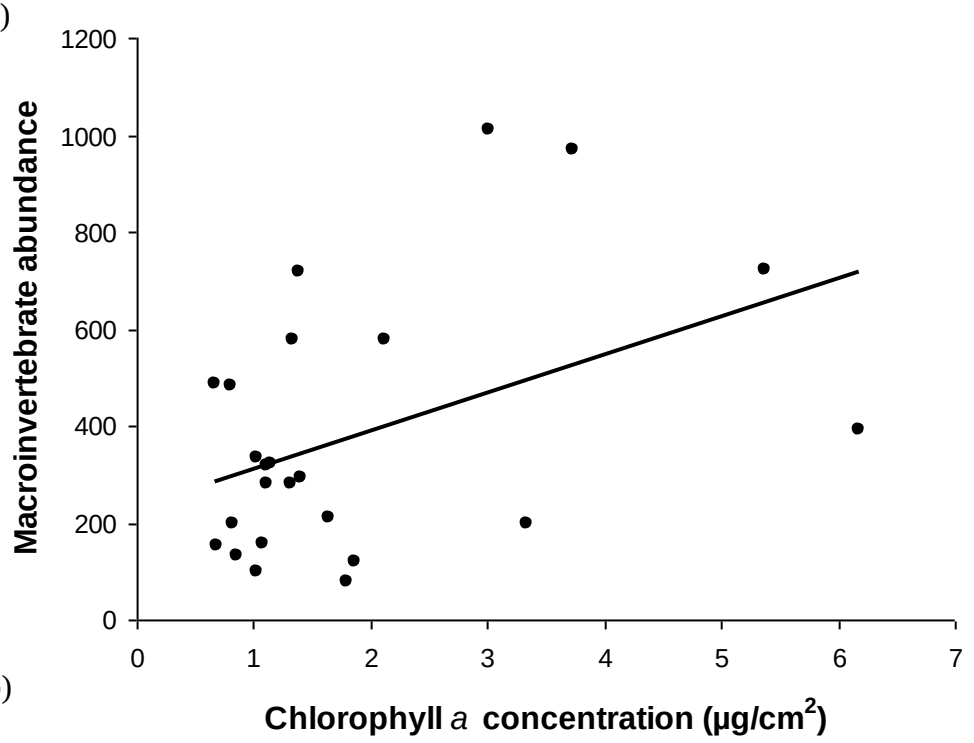
Figure 4.2: Richness (as species number for diatoms – solid line, as family number for macroinvertebrates – dashed line) for a) Porter Brook, b) River Hipper, c) River Loxley, d) Brookside Beck, e) Peakshole Water, f) River Sheath, g) River Noe, h) Upstream of Damflask, i) River Rivelin and j) South of Fulwood hall over 9 months across 3 years.

4.3.2. Correlation between biomass of Macroinvertebrates and Periphyton

Chlorophyll *a* (a proxy for periphyton biomass) was positively correlated with both macroinvertebrate abundance (numbers) and biomass (Figures 4.3 and 4.4.). For numerical abundance of macroinvertebrates correlation was found to be stronger when just grazers (Figure 4.3b) are assessed (R^2 = 36.9, $F_{1, 22}$ = 10.33, P < 0.05) compared to the numerical abundance of total macroinvertebrates (Figure 4.3a) (R^2 = 18.7, $F_{1, 22}$ = 5.67, P = 0.05). There was significant positive correlation between chlorophyll *a* concentration and macroinvertebrate biomass (Figure 4.4a) (R^2 = 33.3, $F_{1, 22}$ = 11.0 P < 0.001) and for just grazer biomass (Figure 4.4b) but values were less for grazers alone, and narrowly insignificant (i.e. a lower R^2 value (R^2 =14.9, $F_{1, 22}$ = 3.87, P = 0.062). The results clustered at low levels of chlorophyll *a* indicating that many of the streams in this

study were quite similar in terms of primary and secondary production (Figure 4.3 and 4.4).

a)



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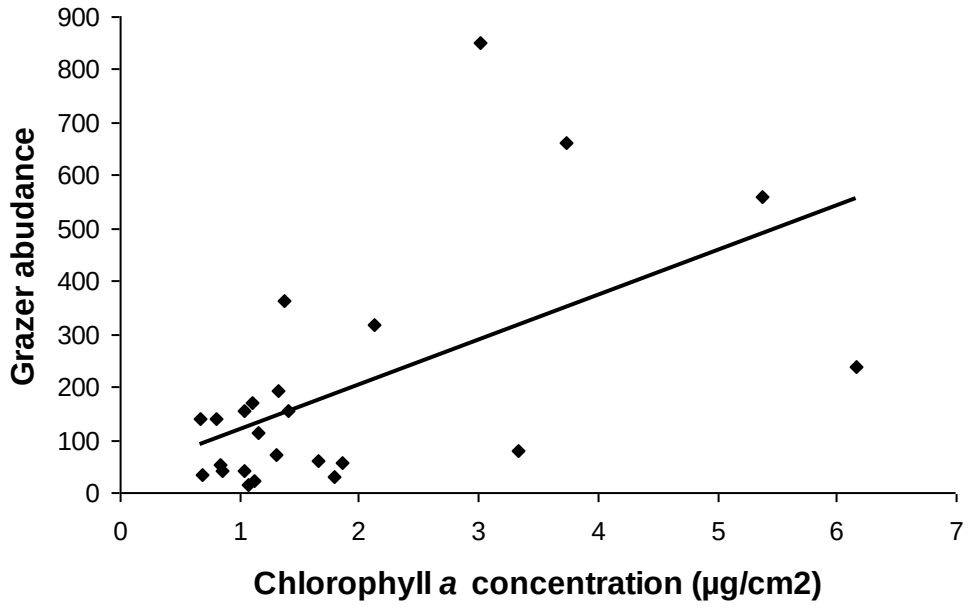


Figure 4.3: Chlorophyll *a* concentration at 24 Northern England stream sites compared to a) number of macroinvertebrates and b) number of grazing macroinvertebrates.

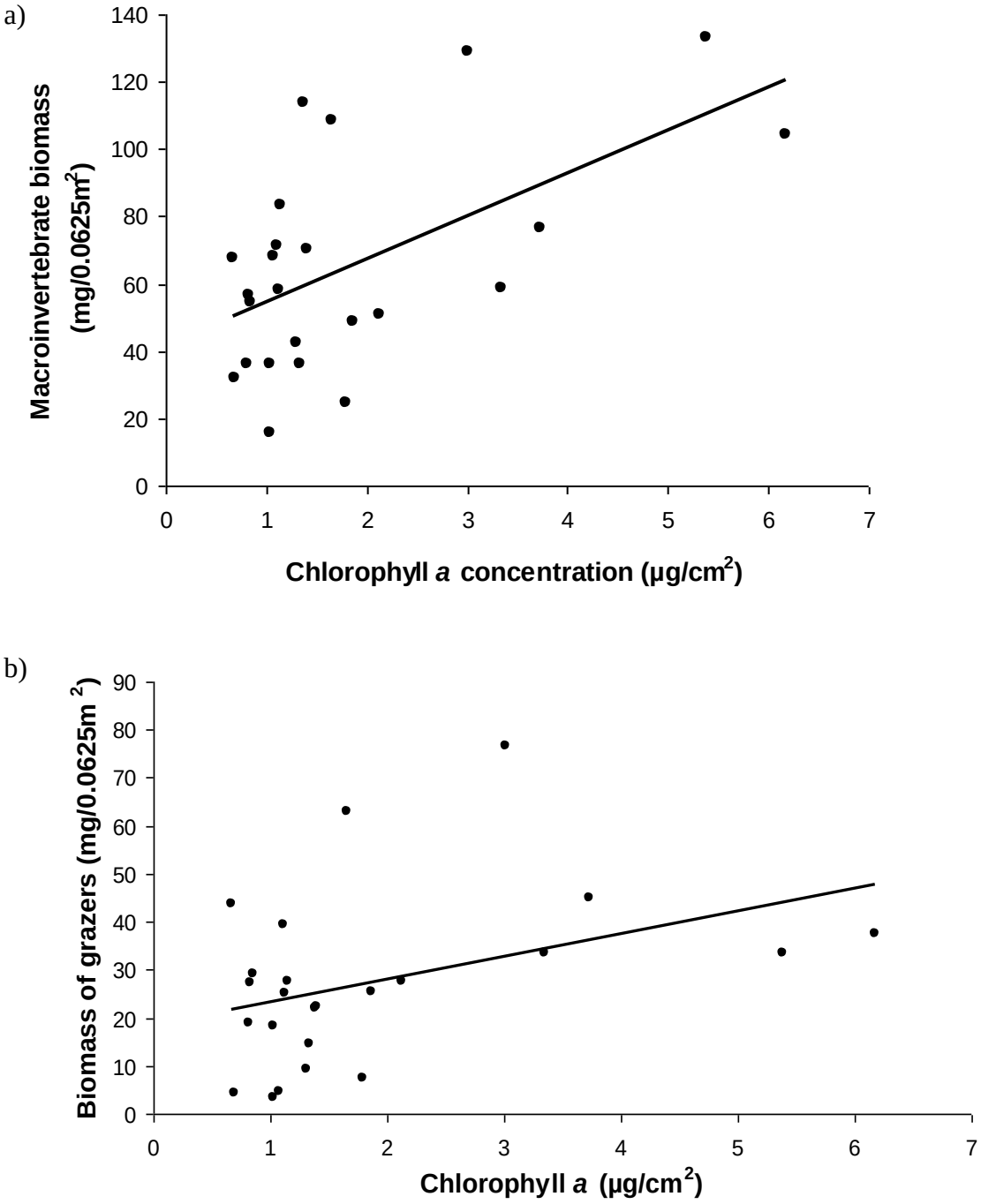


Figure 4.4: Chlorophyll *a* concentration at sites compared to a) biomass of macroinvertebrates and b) biomass of grazing macroinvertebrates.

Chlorophyll *a* concentration was significantly correlated to the number of grazing mayflies in the macroinvertebrate assemblage ($R^2 = 43.37$, $F_{1, 22} = 13.87$, $P < 0.001$) by a value higher than for total macroinvertebrates or for all grazers (Figure 4.5). The

strongest correlation for periphyton biomass was therefore found with grazing mayfly numbers. No significant correlation for other macroinvertebrate groups alone was found ($F_{1,22} \leq 0.03$, $P > 0.05$).

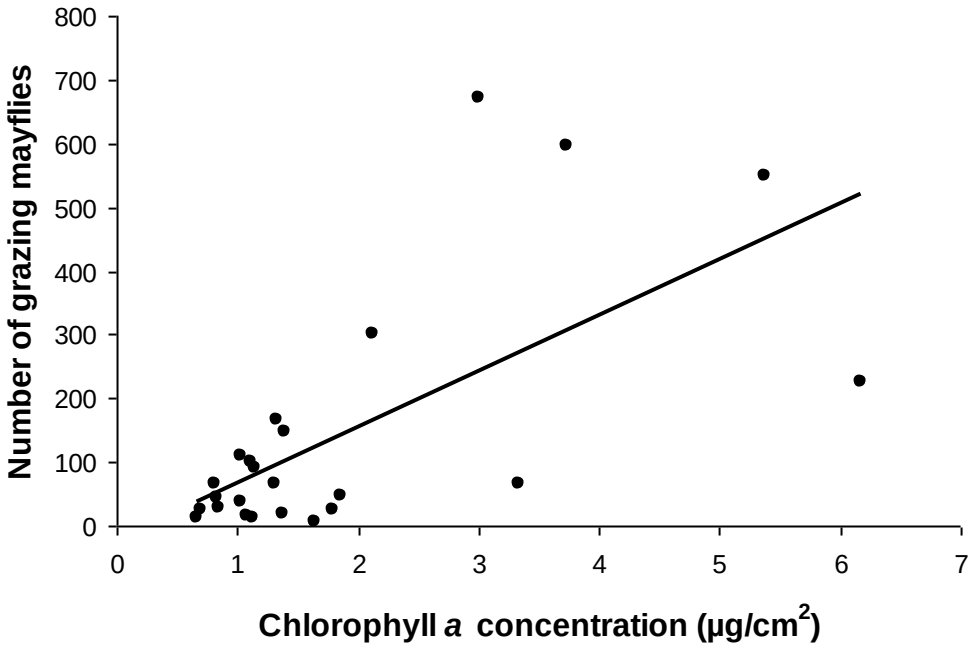


Figure 4.5: Chlorophyll *a* concentration (biomass) of the algal assemblage compared to numbers of mayfly grazers.

4.3.3. Correlation between traits of macroinvertebrates and diatoms

The percentage of high-profile diatoms in the assemblage was significantly positively correlated with mayfly grazers both over the 24 study reaches sampled in 2006 (R^2 =17.65, $P < 0.05$) and the 89 samples over the 3 years ($R^2 = 9.3$, $F_{1, 86} = 7.93$, $P < 0.01$) (Figure 4.6 and 4.7). However, there was greater correlation in July 2006 than over the 3 years.

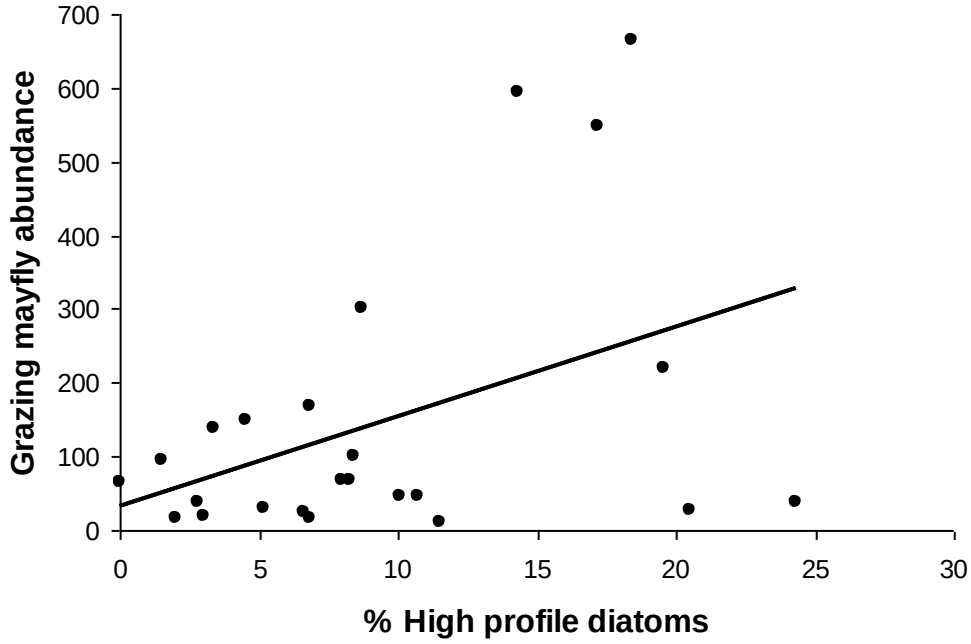


Figure 4.6: Proportion of high profile diatoms found in the assemblage compared to the numbers of mayfly grazers in the assemblage.

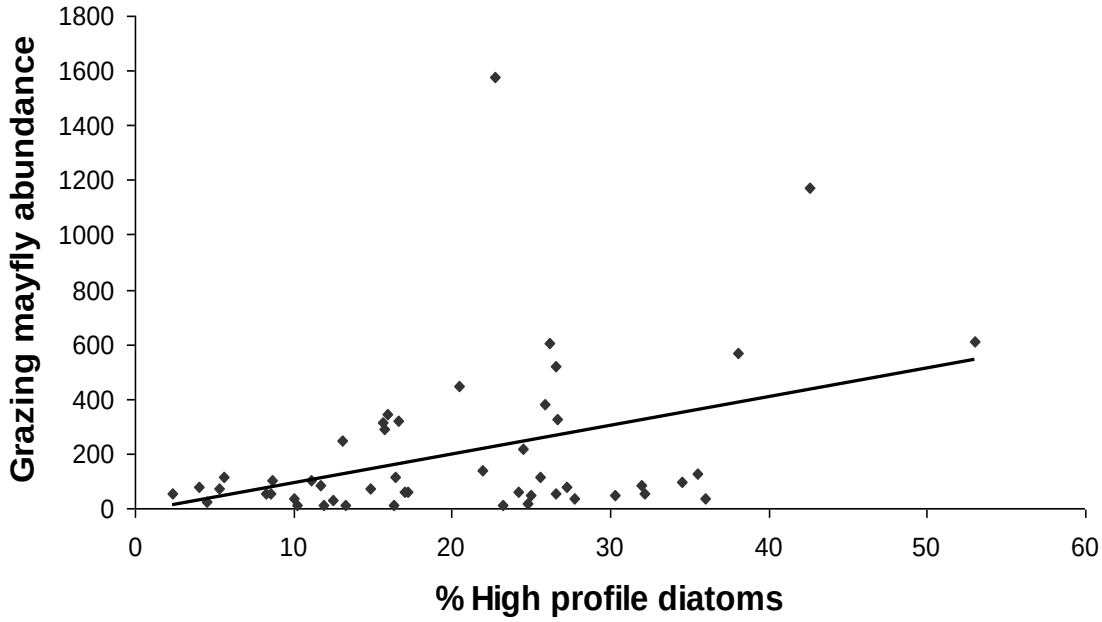


Figure 4.7: Proportion of high profile diatoms found in the assemblage compared to the numbers number of Baetidae grazers in the assemblage in the extensive survey.

4.3.4. Ecological Quality indices

No concordance was found for the Ecological Quality Index (EQI) between macroinvertebrates and diatoms for July 2006 (Figure 4.8). Three sites were classified as moderate for macroinvertebrates. Of these study reaches one was also classified moderate for diatoms, the other two study reaches were classified as good for diatoms. Thirteen study reaches were good or above for both diatoms and macroinvertebrates meaning that they achieved the target of good ecological quality. Therefore 34 % of study reaches in July were ranked as good or above for both groups. Of these study reaches five achieved the same EQI class for both groups (i.e. high and high or good and good). Six study reaches were good for macroinvertebrates but high for diatoms and three study reaches were high for macroinvertebrates and good for diatoms. Of the 21 study reaches that ranked good or above for macroinvertebrates seven of these study reaches had diatom rankings of moderate or below. Two study reaches were on the borderline between good and moderate for diatoms. Chi-squared analysis found that when study reaches that were good or above for diatoms and macroinvertebrates were compared to sites which were not good or above for both there was a significant difference ($\chi^2 = 6.75$, P = 0.05, d.f. = 1), thus there was significant difference in quality class assessed by diatoms compared to macroinvertebrates. There was a greater difference found when study reaches in the exact same class (i.e. high and high, good and good etc) were compared to sites in a different class ($\chi^2 = 27.0$, P = 0.001, d.f. = 1).

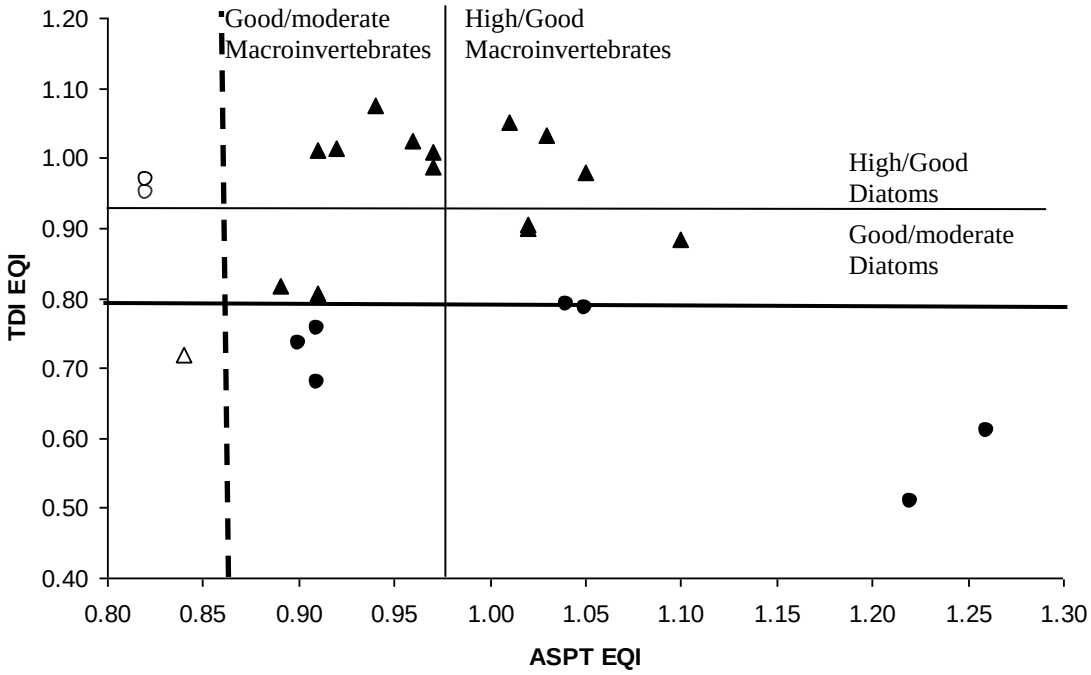


Figure 4.8: Ecological quality indices (EQI) of macroinvertebrate and diatom assemblages for 24 study reaches at Northern England Rivers taken in July 2006. Boundaries between ecological quality classes are marked and labeled. Triangles indicate sites that are classified as the same status, with closed indicating good or above. Closed circles indicate sites that are good or above for macroinvertebrates and moderate or below for diatoms, sites marked with open circles indicate sites that are classed below good for macroinvertebrates and above for diatoms.
* 2 sites (Good for diatoms and macroinvertebrates) had identical EQI's so only 23 sites observable on the figure.

There were no correlations found in EQI values for individual sites over time (Figure 4.9) ($R^2 \leq 1.0$, $P > 0.05$). Out of the 89 samples from 10 sites only 1 sample at one study reach is good or better for diatoms and moderate for macroinvertebrates, 16 samples (18.82 %) are below good for diatoms and good or above for macroinvertebrates. 72 sites (80.89 %) sites ranked good or above for both groups (higher than found in July 2006 only), 7 samples were good for both groups, 30 sites are high for both groups. 37 samples had the same specific EQI class (41.57 %), 19 samples ranked high for macroinvertebrates and good for diatoms (21.34 %) and 16 sites ranked as good for macroinvertebrates and high for diatoms (17.98 %).

Assessment by diatoms was therefore significantly different to assessment by macroinvertebrates when study reaches that were good or above for diatoms and

macroinvertebrate indices were compared to study reaches that were not good or above for both groups by chi-squared test ($\chi^2 = 8.77$, $P < 0.05$, d.f. = 1) and those in the exact same EQI class ($\chi^2 = 199.45$, $P < 0.001$, d.f. = 2). This means that diatom values are significantly different from those that would be predicted by macroinvertebrate values. Out of the 16 samples that were good or above for macroinvertebrates but below good for diatoms 6 were from the Porter brook (July 2007, August 2007, October 2007, May 2007, July 2007 and August 2007), 5 were from Brookside Beck (August 2007, October 2007, May 2008, July 2008 and August 2008), 2 were from the river Hipper (July 2007 and August 2007) 2 were from the River Sheath (June 2007, July 2007) and 1 was from the River Loxley (August 2007). This shows that 5 of the sites were always ranked good or above for both groups.

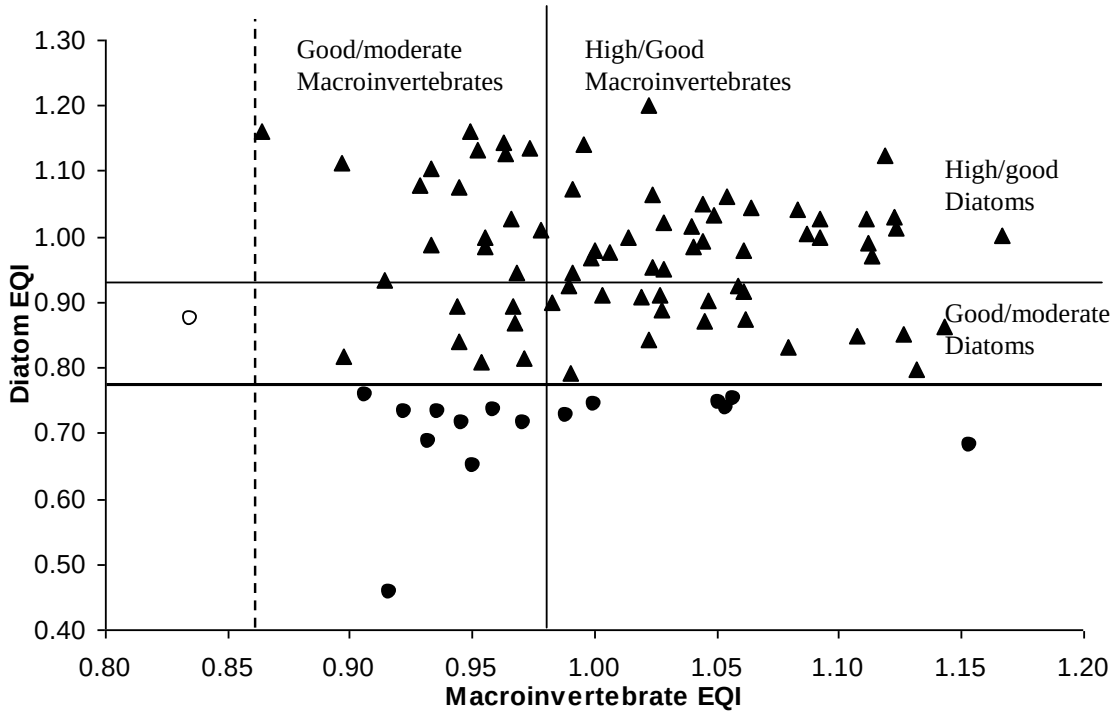


Figure 4.9: Ecological quality indices (EQI) of macroinvertebrates and diatoms for 10 sites with results taken July 06, May 07 – Oct 07 and May 08, July 08 and Aug 08, from Northern England Rivers. Boundaries between classes are marked and labeled. Triangles indicate sites that are classified in the same status. Closed circles indicate sites that were good or above for macroinvertebrates and moderate or below for diatoms, sites marked with open circles indicate sites that were classed below good for macroinvertebrates and good or above for diatoms.

There was no concordance between the diatom TDI and either the macroinvertebrate ASPT score or BMWP score before they were converted into EQI s for both the July 2006 survey and the 10 site in-depth survey (Correlation analysis $P > 0.05$, $R^2 \leq 1.0$).

4.2.5 Physical/ chemical measurements

Abiotic parameters for sites with the same EQI for diatoms and macroinvertebrates were compared to sites with different EQIs by t-tests. It was found that all chemical and physical variables measured in this study were not significantly different except for nitrates ($t = 2.27$, d.f. = 14, $P = 0.039$) that were found to be slightly significantly higher at sites that had lower EQI values for diatoms. Thus diatoms appear to respond more strongly to Nitrates than macroinvertebrates. For full recordings of abiotic measurements at all sites see Appendix 3 table A9.

4.4 Discussion

Significant associations between groups were indicated by the positive correlation observed between macroinvertebrate and periphyton abundance and biomass. Positive correlation was also found between numbers of mayflies and the proportion of high-profile diatoms both in July 2006 and across the 9 months sampled. There was no correlation found between any aspects of diversity for the two groups. The lack of correlation in diversity was replicated by the lack of correlation between indices based on the two groups. This indicates that macroinvertebrates cannot reliably predict the ecological status class for diatoms and *vice versa*. The relatively low R^2 values found for the abundance and biomass suggest that multiple factors determine abundance and biomass of these organisms and that interaction with each other, although important, are just one factor of many.

Previous studies on concordance between different groups have rarely been performed in freshwater but those that have (groups studied include macroinvertebrates, diatoms, zooplankton, bryophytes, water birds and fish), like this study have tended not to find correlation between the richness of different groups (Allen *et al* 1999, Heino *et al* 2002,

Soininin and Kononen 2004 and Heino *et al* 2005). One reason given for the lack of concordance is that different groups often respond most strongly to different environmental parameters (Heino *et al* 2005). Another reason is that when different groups respond to the same abiotic variable they react in different ways. An example of this is that macroinvertebrates have been found to respond to metals by a decrease in diversity and abundance but diatoms respond by a change in the identity of taxa present (Hirst *et al* 2002). Different taxonomic groups were also found to respond differently to urbanisation in Australian stream systems (Sonneman *et al* 2001). The lack of any correlation in the richness of macroinvertebrates and diatoms could partly explain the finding of no concordance between indices because indices are based on the structural composition of biotic assemblages. Previous work has also found that indices often do not give the same ecological quality class for different groups. For example, Paavola *et al* (2003) found that classification of headwater streams was not concordant across different taxonomic groups in Finland. They suggested that from their findings great care should be taken in assuming a typology for a river, based on just one group indicating the quality of the whole system (Paavola *et al* 2003).

Studies that have considered congruence in community structure, rather than species richness, using macroinvertebrates, fish and birds have sometimes found significant correlations (Kilgour and Barton 1999, Paskowski and Tonn 2000). This indicates that although diversity itself may not correlate, other aspects of community structure may be linked, at least for some organism groups. The current study illustrated this by the positive correlation found between high-profile diatoms and the number of mayfly grazers. Macroinvertebrates and diatom are thus not independent of each other but many aspects do not correlate in a way that can be measured by a one-off survey. This disagrees with those studies that have yielded concordance in such a way that suggestions have been made that one group could predict quality for others. An example of this was Kilgour and Barton (1999) postulating that, due to significant correlations observed between fish and benthic macroinvertebrates, surveys of macroinvertebrates could be used to make assumptions about the fish population. Biotic groups have also been found to correlate when they have a shared food source (Paszkowsi and Tonn 2000). The reason for this being, that they respond to environmental variables in the

same way because this is mediated by the shared food source (Paszkowski and Tonn 2000). These observations suggest that concordance is more likely between groups if they have similar feeding habits (same tropic level) and are of a similar size. This is not the case for macroinvertebrates and diatoms further suggesting that predicting one from the other is unlikely. However, Hering *et al* (2006) advised that it might be possible to decrease monitoring effort if the type of degradation and the most sensitive group to it is known. The different stressors present in the system to be monitored would have to be known with confidence but this approach could work in some cases. In order for this approach to work a full understanding of what organism group is most sensitive to what stressor would have also need be established categorically.

The current study found that biomass of macroinvertebrates and diatoms did not relate to the species/family of the other group. This suggested that more food source did not result in greater numbers of consumer taxa (grazing macroinvertebrates), and that more predation (grazing) was not associated with less (or more) diversity of diatoms. Alstad (1986) found that greater density of diatoms lead to more species of net-spinning caddisfly being present, suggesting that richness – biomass relationships are possible but may be very specific. Also, high numbers of grazers have been found associated with low algal diversity in a marine system, but this is influenced by different parameters than freshwater (Breitburg 1985). Some freshwater studies that have included ungrazed trials have found that grazed trials have less algal species than ungrazed, indicting that in some cases grazing can decrease richness (Opsahl *et al* 2003). However there were no ungrazed controls in the streams in this study so what the diatom assemblage would be like without their presence could not be assessed. As well as the current study many others have found no affect of grazers on the diatom assemblage (i.e. Hirst *et al* 2003). The lack of correlation between macroinvertebrate family number and algal biomass in this study could have partly been due to macroinvertebrates only being identified to family level. As closely related species may increase with more food source because there would be less competition. The lack of concordance could also be because the study was performed in summer when primary production is high, meaning that competition for food may not have been important in structuring what grazers were present due it being abundant (i.e. bottom-up control). Another consideration is that

other organism groups (i.e. fish, birds) were not measured in this study and would also control and interact with macroinvertebrates. In addition the biomass of periphyton included that of other algae, not only diatoms, so total algal diversity may have been different to that measured by diatom species number and could have influenced (or be influenced by) macroinvertebrates.

Higher biomass of periphyton (as measured by chlorophyll *a)* was correlated with a greater abundance (number) and biomass of macroinvertebrates. Periphyton biomass was also correlated, more strongly, to grazing macroinvertebrate numbers. The positive association agrees with several other studies that also indicate bottom-up (i.e. more food = more consumers) control (Scrimegeour and Winterbourn 1989 and Sponseller *et al* 2001). However, this contrasts to studies that find increased grazers result in decreased algae, suggesting top-down control (i.e. more consumers = less food source) (Jordan and Lake 1996, Opsahl *et al* 2003 and Hillebrand 2005). It is also in contrast to those studies that observe no relationship between algal biomass and macroinvertebrate biomass (Robson and Barmuta 1998 and Hirst *et al* 2003). The observations in this study suggest that rivers in this survey were structured partly due to bottom-up control, with a high food source associated with high numbers of consumers. It is particularly likely due to grazer numbers, which directly utilise the resource, being strongly positively correlated with algal biomass. The current study was carried out in summer, meaning that this could be expected, as primary production would be high, due to long daylight hours and high temperatures.

The observation that the grazer functional feeding group was more closely correlated to chlorophyll *a* concentration than total macroinvertebrates suggested that links in abundance were due to food chain interactions not just good conditions for the biomass of one group being good conditions for another group. This is similar to what Dudgeon and Chan (1992) and Dudgeon (1994) found when studying shading: out of the macroinvertebrates only grazers decreased with increased shading (because only grazers responding to algae). However, when biomass was considered, this study found that periphyton only correlated significantly with total macroinvertebrate biomass and not grazer biomass (although only narrowly insignificant). This could be because of many

of the grazers are insect larvae (i.e. mayflies), and many of them emerging in the summer (thus not measured), although having used the food source. Also, although large numbers of these grazing insect larvae are found, the ones present are small (due again to emergence of larger individuals). Additional to this the anomaly could also be explained by predators (i.e. some stoneflies, diptera and caddisflies) benefiting from a high biomass of periphyton via the grazers they prey on. Predators tend to be large, so although numbers of predators were fairly low each individual had a large biomass. As well as predators benefiting indirectly from instream production, most macroinvertebrate are generalists, to a certain extent, and will utilise a plentiful food source (Mihuc 1997). This is especially true in summer months when some external inputs are limited (i.e. leaf fall) (Mihuc 1997).

Positive correlation observed between the number of mayfly grazers and the percentage of high-profile diatoms in the assemblage also indicates bottom-up control. Demonstrate by the more preferred food source (high-profile diatoms as established by chapters 2 and 3) being associated with more consumers. A more pronounced correlation was found between mayfly numbers and percentage high-profile diatoms in July 2006 than over time all the months studied. This suggested that in high summer, when primary production is high, a greater control from the food source is present than at other times of the year when abiotic factors may be more important in structuring the diatom assemblage. Villanueva and Modenuti (2004) found that whether grazing decreases periphyton abundance is dependent on the starting assemblage of the periphyton. Possibly explaining why different studies and systems find different results for periphyton – grazer interactions.

Several studies have found that in controlled trials more surface feeding grazers (top-down control), such as mayflies, results in less erect diatoms than controls, because of feeding interactions (Wellnitz and Ward 1998, Holomuzki and Biggs 2006). This demonstrates that an increase in preferred food source encourages large numbers of consumers indicating bottom-up control. It also shows that traits of the two groups can be related, even when taxonomic diversity is not, suggesting that perhaps indices based on traits of organisms could predict other groups better than those based on identity

(Statzner *et al* 2005). The idea could be something to investigate in the future. Other studies have found that some grazers preferentially feed on certain diatom growth forms suggesting that macroinvertebrates can influence the structure of the diatom assemblage depending on the feeding habits of the grazers present (Hill and Knight 1988, Lawrence *et al* 2002, Chapter 2). These links suggest that although the macroinvertebrates and diatoms do not correlate to each other on a one off sample, they can have an influence on the structure of the other group. The influence that each group has on the other does not occur in a manner that is observable by indices based on assemblage composition. However this does not mean that interactions between them are unimportant in the results of indices. The variability found in index results shows that any factor(s) that can influence them need to be understood. Biological interactions could contribute to the variation found in indices as demonstrated in Chapter 2.

In the current study Nitrates were marginally, but significantly, higher at sites that had a worse ecological quality rating for diatoms than for macroinvertebrates. This suggested that Nitrates was an important factor for the TDI, and therefore the species composition of the diatom assemblage. The TDI is designed to assess nutrient impacts (Kelly and Whitton 1995) so the above result that diatoms are more strongly influenced by Nitrates than macroinvertebrates, is what may be expected. However, it is surprising that Phosphates did not show the same trend, as the TDI was designed partly with this is mind (Kelly and Whitton 1995). Although the idea of biotic indices is to assess longer term changes so the spot measurement of chemicals may not represent what the organisms had been responding to. The one off measurements of chemical parameters may, therefore, not be accurate in describing the long-term pressures affecting the system and some associations between biota and chemical aspects may have been missed.

This study demonstrates that correlations can be found between diatom and macroinvertebrate assemblages, specifically each other's structure and abundance. However, these links were not found to translate to concordance that could be used reliably for predicting ecological quality of one group from another. Thus this work agrees with the argument put forward by other groups, that multiple taxonomic groups

should be used when assessing ecological quality of freshwaters (Soininin and Kononen 2004, Cao *et al* 2007) rather than one as an indicator for others (Kilgour and Barton 1999). Therefore, it is likely that diatoms and macroinvertebrates act as complementary, rather than surrogate indicators. On the evidence gathered so far it is important to monitor using multiple groups of organisms to ensure good ecological quality is reached, and maintained, for rivers and streams.

5.0 THE RESPONSE OF MACROINVERTEBRATE AND DIATOM ASSEMBLAGES TO A SIGNIFICANT DISTURBANCE EVENT.

5.1 Introduction

Flow is one of the most important factors in structuring lotic ecosystems making understanding what occurs in extreme flow (flood) events very important (Uehlinger *et al* 2003). A flood is defined as "a flow that overtops stream banks" or ecologically as "a discharge which scoured substrates and disrupted biota" (Gordon *et al* 2004). A flood therefore fits the definition of a disturbance, defined as "discrete events that disrupt population, community, or ecosystem structure either directly by killing or displacing organisms or indirectly by changing resource abundance or the physical environment" (Snyder and Johnson 2006). Some authors argue that a flood event only represents a disturbance to the system if the timing is atypical and unpredictable (Brewin *et al* 2000). However, it has been found that repetition of a stressor can have a greater effect than a one off rare event, meaning that frequent and predictable flood events can also be a disturbance, thus any flood event could have an influence on the biota (Riddle *et al* 2009). The flood of summer 2007 was likely to represent a disturbance event for the biota that was present in the rivers at that time on all of the above criteria.

The main consequence of flooding is changes in hydrology, which is known to be one of the main factors that cause temporal change in macroinvertebrate assemblages for stream systems (Brewin *et al* 2001). Water temperature is also changed by flooding, due to an influx of water from elsewhere, and can influence the number of macroinvertebrate taxa present (Milner *et al* 2001). The extent of the impact that a flood has may depend

on the type of substrate in the system combined with the amount of bed movement that occurred, as it is thought to be bed movement and not flow *per se* that results in the greatest impact on the biota, thus indicating that floods will have different affects in different systems (O'Connor and Lake 1994, Snyder and Johnson 2006). For example a stream that was impacted by sand had a large decrease in macroinvertebrate abundance and species richness when flooding occurred but patches that were not impacted by sand had stable macroinvertebrate assemblages despite the occurrence of flooding (O'Connor and Lake 1994).

Floods can affect the ecology (including diatoms and macroinvertebrates) by rearranging stream habitats, scouring away aquatic and/or riparian plants and increasing macroinvertebrate drift (Gordon *et al* 2004). Frequent hydrological change has been found to result in macroinvertebrate assemblages with a lot of temporal variation (Breuin *et al* 2001). Changes in hydrological conditions are also a key factor in determining pattern and process (i.e. biomass, species composition, physiology and growth form) in algal communities (Peterson and Boulton 1999). Diatoms and macroinvertebrates are likely to respond differently to flooding because of their different generation times and body sizes (Soininen and Eloranta 2004). Flood events can result in low macroinvertebrate abundance, for example a flood prone system was found to have its lowest abundance immediately after flooding and highest abundance after a long period of stable, low flow (Scrimgeour and Winterbourn 1989). It has also been found that there is more stability in the taxonomic composition of macroinvertebrates in dry years than wet years indicating that floods increase the variability of the assemblage (Beche *et al* 2006). The relative abundance of different macroinvertebrates can be changed by disturbance, and may be of greater significance than compositional changes (Scarsbrook 2002). This is because the dominance of species may change, even though the same species are present after as before a flood thus, biological interactions could be influenced (Scarsbrook 2002). Changes in macroinvertebrate assemblage, in response to a flood event, may be less than expected due to adaptations that enable them to exist in a harsh riverine environment. Adaptations may allow them to cope with even extreme flood events without significant lasting impact (Snyder and Johnson 2006).

Flooding generally decreases algal biomass, due to scour caused by abrupt changes in current velocity and turbulence (Peterson *et al* 2001). Light intensity and temperature can also be changed by flooding and can influence algal biomass (Oemke and Burton 1986). It is less well established what occurs to the diversity and identity of diatoms after a flood but generally diatoms are found to respond to stress by change in species composition rather than a change in diversity (Hirst *et al* 2002, De Jonge *et al* 2008). The diatom assemblage is structured by many physical, chemical and biological factors and a large physical disturbance like a flood can alter the outcomes of these interactions (Peterson *et al* 2001). If a flood changes the temperature of a system algal diversity may be influenced, as it has been found that diatom diversity is higher when the water is colder, possibly due to a decrease in dominance by any one species (Oemke and Burton 1986). Flooding may result in the diatom assemblage being more controlled by abiotic factors, as predicted by the harsh-benign hypothesis, whereas in stable conditions the assemblage is controlled more by biotic factors such as macroinvertebrate grazing (Garnier *et al* 1995). The algal response to scour depends on the magnitude, timing and duration of the flood and what the structural properties of the diatom assemblage are (Peterson and Stevenson 1992, Peterson *et al* 2001). Most diatom assemblages are likely to vary a lot through a season due to high turnover rates and opportunistic life history strategies (Biggs *et al* 1999). The implication being that the effect of a flood on diatoms could be hard to measure because of their highly variable natural state (Biggs *et al* 1999). Disturbance regime, along with grazer activity and light availability are thought to contribute to the seasonality of the structure of the diatom assemblage, meaning that an atypical flood event could change this trajectory, especially if grazing assemblages are changed significantly (Biggs 1996). Species richness and identity may vary across seasons to a greater extent than community structure, suggesting that identity is less important than the traits of the organisms in determining what is present (Thompson and Townsend 1999).

The stability of any community depends on how resistant and resilient it is to change (Soininen and Eloranta 2004). Periphyton communities established in fast current conditions are likely to have greater resistance to flooding than those that are established in slow current conditions due to the assemblage being adapted to some of the pressures

created by a flood in the former scenario (Peterson and Stevenson 1992). However, resilience has been found to be higher in diatom communities in slow currents with them returning to previous biomass and structure faster than communities grown in fast currents (Peterson and Stevenson 1992). Diatoms have high turnover rates and opportunistic life cycles that are well adapted to the harsh conditions that rivers and streams provide, these traits are also suited to withstanding flooding (Stevenson 1997). The short algal succession rate means that recovery from a flood event can be rapid (Biggs and Smith 2002). Flooding can release organisms from competitive pressures allowing these communities to reproduce quickly and return to their former community (Peterson and Stevenson 1992). The timing of the flood, successional stage of the algae and susceptibility of the assemblage all determine how affected by a flood disturbance the algal assemblage is (Peterson and Stevenson 1992). There is more likely to be a flood affect on diatoms if there is bed movement as algae on armoured sediments have been found to be unaffected by flow perturbations (Biggs and Smith 2002).

The degree of change caused by a flood to the algal assemblage depends on the age of the community and the initial current of the stream (Peterson and Stevenson 1992). After a scour event a succession of diatoms going from a pioneer assemblage of low attached diatoms to filamentous ones takes place (Stevenson *et al* 2000). Directly after a flood the diatom community is likely to be made up of small diatoms such as *Achnanthus minutissima* which, will be superseded by a dominance of filamentous species such as *Synedra spp*. (Peterson and Stevenson 1992). Diatoms that are able to persist after a scour event are generally low-profile and well attached to the substrate (i.e. *A. minutissima, Cocconeis pedicus*) (Johnson *et al* 1997). Flooding can also determine the patchiness of algal distribution with sheltered areas representing refuges for the rapid replacement of algae (Matthaei *et al* 2003). This suggests that flood events may not affect the overall algal assemblage at a site but may change how it is distributed amongst patches (Matthaei *et al* 2003). Sites exposed to frequent flooding may have greater algal species diversity, than more stable systems, due to less dominance by any one species (Biggs and Smith 2002).

Interactions between macroinvertebrates and diatoms can result in indirect effects of flooding but this depends on the timing of the flood (Peterson 1999). The macroinvertebrate assemblage can be influenced by indirect affects such as scour causing a decrease in primary production and decreasing grazers' food source (Scrimgeour and Winterbourn 1989, Thompson and Townsend 1999). A decrease in preferred food source can also occur because some traits of diatoms that confer flood resistance (low-profile) also confer resistance to grazing (Scrimgeour and Winterbourn 1989). Chapters 2 and 3 demonstrate that macroinvertebrate grazing can significantly influence the diatom assemblage meaning that if this is changed it could have an affect on the diatoms mediated by food-chain interactions. It may be that due to the small size and short life cycles of diatoms they are less affected by the flood than macroinvertebrates but the change in macroinvertebrates may lead to an observable difference in diatom assemblage structure.

Variation in both biotic and abiotic factors is natural in a dynamic stream system meaning that there is a lot of change within a system even without a major event to drive this (Lancaster *et al* 1996). Distribution patterns and community composition are often variable and inconsistent suggesting that an extreme event may cause a directional change that could be observed above the natural variation (Kay *et al* 2001). Some temporal variation can be predictable, for example chironomids have been found to dominate a well-studied UK stream in the summer with stoneflies dominant for rest of the year (Woodward *et al* 2002).

Seasonality has been found to be important in rivers, with most monitoring protocols requiring assessments to be made in the same season that the model was formulated meaning that different assemblages will be found at different times of the year regardless of any impact (Feio *et al* 2006). Seasonality of the macroinvertebrate species present is expected due to their different life histories and emergent times, meaning that sampling at different times of the year will result in a different assemblage being assessed regardless as to whether a disturbance has occurred (Wallace 1990). Seasonal changes within the stream can be related to fine particulate organic matter, which is also influenced by floods, suggesting that the flood event could change the macroinvertebrate

assemblage using the same mechanism as seasonal changes (Giller and Malmqvist 1998). Diatom assemblages have been found to generally have less seasonal variation than macroinvertebrates (De Jonge *et al* 2008).

Floods are important for many natural river systems, for example regulated rivers without floods have an uncharacteristic macroinvertebrate assemblage that can be restored by the re-introduction of flooding (Robinson and Uehlinger 2008). If a flood is a predictable, regular occurrence in a system it is less likely to have significant effects (Brewin *et al* 2000). In systems that are prone to frequent, predictable floods it has been established that the organisms present adapt their life histories to cope with these disturbances (Brewin *et al* 2000). An example of this is the decrease of macroinvertebrate abundance observed *before* monsoon floods occur in Nepal (Brewin *et al* 2000). This means that systems can adapt to regular, predictable spates with changes in the macroinvertebrate assemblage being consistent with seasonal change rather than flooding (Boulton *et al* 1992, Brewin *et al* 2000). However, an atypical extreme flood event is more likely to have large effects due to organisms being less adapted to cope with it due to it either being an unusual event for the system or an unusual timing. Few studies have addressed atypical floods due to their unpredictability and the lack of pre-flood data. For example a study that investigated the influence of a massive flood event was performed 3 years after its occurrence but did find significant lasting changes but it is hard to say whether this was due to the flood as there was no before flood data from that specific river (Snyder and Johnson 2006).

The June 2007 flood that occurred in South Yorkshire gave us an almost unique opportunity to study the immediate effect of a flood compared to samples taken before it happened due to having sampled in May 2007. In this study the flood was atypical and extreme, of the magnitude that has not been witnessed in the UK since 1947 (Marsh and Hannaford 2007). It was a very rare flood event due to its timing and unprecedented severity but floods of this nature are likely to become more frequent with predicted climate change (Marsh and Hannaford 2007). It is therefore important to determine if the organisms present were able to cope with it by their existing adaptations or if they suffered significant changes. Assessment using both macroinvertebrates and diatoms is

useful to assess flood affects because more information can be gained due to the representation of two trophic levels (Lancaster *et al* 1996).

This study aims to assess the effect of a large, unusual summer flood event on both macroinvertebrate and diatom diversity and community structure, whilst unravelling the flood affects from seasonal variation. This will allow us to assess how the biota will be affected by atypical flood events that may become more frequent with expected changes in climate. The importance of flooding as a disturbance in river systems will be evaluated.

5.2 METHODS

5.2.1 Sites

Seven sites were chosen in the South Yorkshire and North Derbyshire area, initially to determine seasonal variations in the diatom and macroinvertebrate assemblage (Table 5.1). These sites were a subset from those sampled in July 2006 and chosen to represent a variety of trophic diatom index values (Chapter 4). Sites were sampled in May 2007 (Pre-flood), July 2007 (post-flood) and May 2008 and July 2008 for comparison to a non-flood year. A large-scale flood event occurred in late June 2007, resulting in an opportunity to study the effect of a significant flood event on the biota of these streams. Sites were classed as being significantly affected by the flood if they had an increase in the ratio of cobbles/boulders to sand/gravel and if they had burst their banks, resulting in 7 sites from an initial 10 (Table 5.1).

Table 5.1: Stream sites used to investigate the effect of the June 2007 flood on diatoms and macroinvertebrates.

Site	Grid ref.	Width (M)	% Increase in ratio of cobbles/boulders to gravel/sand, silt between May07 and July07	Burst banks due to flood
Peakshole water	SK170834	4.2	20	Yes
River Noe	SK168844	6.9	21	Yes

Loxley	SK295898	9.1	28.6	Yes
Porter Brook	SK318855	5.2	20	Yes
Rivelin	SK289871	3.0	50	Yes
Sheaf	SK328822	5.6	50	Yes
Brookside beck	SK248706	2.3	28.6	Yes

5.2.2 Sampling strategy

For the purpose of this investigation each site was sampled once for macroinvertebrates and diatoms in May 2007, July 2007, May 2008 and July 2008. Samples were taken on the same day for all sites each month to minimize variation due to sampling time. Site characterisation, for physical and chemical information was performed in the same way as Chapter 4.

Macroinvertebrates were sampled from each site using the Environment Agency's standard 3-minute kick sample. This involves 1-minute search of the surface, 3-minute kick sample and 1 minute of turning rocks (Murray-Bligh *et al* 1998). Macroinvertebrates and debris were put in 500 ml plastic tubs and stored in 70 % IMS. Algal samples were taken using the standard method used by the Environment Agency; 5 cobbles were scraped and combined to form one sample that was stored in Lugol's iodine (Kelly *et al* 2007). This sample was used for the identification of diatoms.

5.2.3 Laboratory processing

Macroinvertebrates and diatom samples were processed and identified in the same way as Chapter 4.

5.2.4 Data analysis

Abundance data were log-transformed and proportion data were arcsine square root transformed before data analysis. The difference between the months of May and July

were used to determine if there were any difference between years that could be attributed to the flood rather than seasonal differences that occur between months in non-flood affected years. Paired T-tests were used to compare different attributes of sites in different years (Macroinvertebrate family number, diatom species number, macroinvertebrate total abundance, abundance of dominant macroinvertebrate families, relative abundances of diatom trait groups and diversity and similarity indices for both macroinvertebrate and diatoms). General linear models, along with Tukey comparison tests, with site and month as the model and site as a random factor were used to determine differences between individual months for the aspects of the assemblages stated above. Bray-Curtis similarity was calculated by:

$$BCij = \sum \frac{|nik - njk|}{(nik + njk)} \qquad \text{Equation 5.1}$$

Where n is number of individuals, i is the first site, j is the second site and k is species/family.

Shannon-Weiner diversity was calculated by:

$$H' = -\sum_{i=1}^{s} PiInPi \qquad \text{Equation 5.2}$$

Where i is the number of individuals of each species (family), s is Number of species (families), Pi is the relative abundance of each species (family) as ($\frac{ni}{N}$)

Shannon-Weiner evenness was calculated by:

$$E = \frac{H'}{H'\max} \qquad \text{Equation 5.3}$$

Where H^1_{max}:

$$-\sum_{i=1}^{s}\frac{1}{s}In\frac{1}{s}=InS \qquad \text{Equation 5.4}$$

Berger-Parker dominance (Berger and Parker 1970) was calculated by:

$$DBP=\frac{N\max}{N}\ \text{Expressed as}\ \frac{1}{DBP} \qquad \text{Equation 5.5}$$

Where N_{max} is total number of individuals of most common group and N = total number of individuals in the community.

Averages and standard errors were calculated for all parameters investigated. All analyses were carried out using Minitab 14.0 for Windows.

5.3 RESULTS

5.3.1 Macroinvertebrate family richness

There was a significantly greater number of macroinvertebrates in May 2007 than July 2007 ($F_{1, 1, 24} = 17.46$, $P < 0.01$) but not between May 2008 and July 2008 and no significant difference between years or the interaction between the month and the year ($F_{1, 1, 24} \geq 0.55$, $P > 0.05$) (Figure 5.1). This shows that there was a significant difference in 2007 between months but not in 2008.

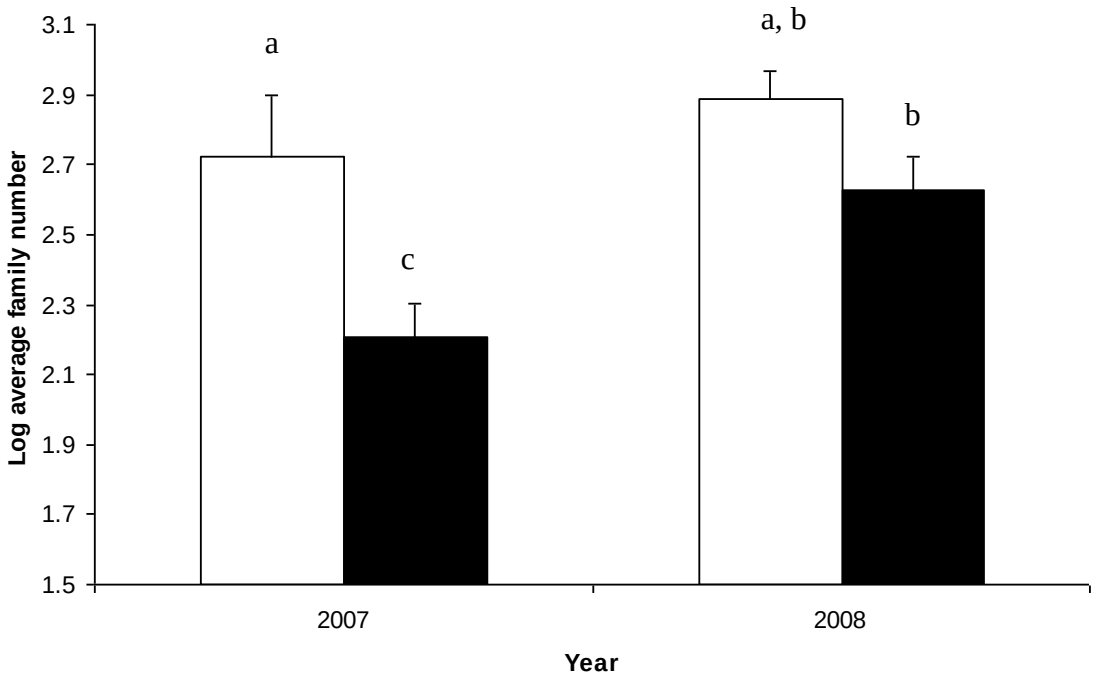


Figure 5.1: Average macroinvertebrate family number in May and July for 2007 and 2008 for 7 sites. Open bars are May and closed bars are July. Error bars show 1 standard error, different letters show significant differences ($P < 0.05$).

5.3.2 Diatom species richness

The difference between 2007 and 2008 for diatom species diversity for month, flood and the interaction was non-significant ($F_{1, 1, 24} \leq 3.90$ $P > 0.05$) although the average for July 2007 was lower than May and the opposite occurred in 2008 (more species present in post flood than pre-flood), the variability was too large for this to be significant (Figure 5.2).

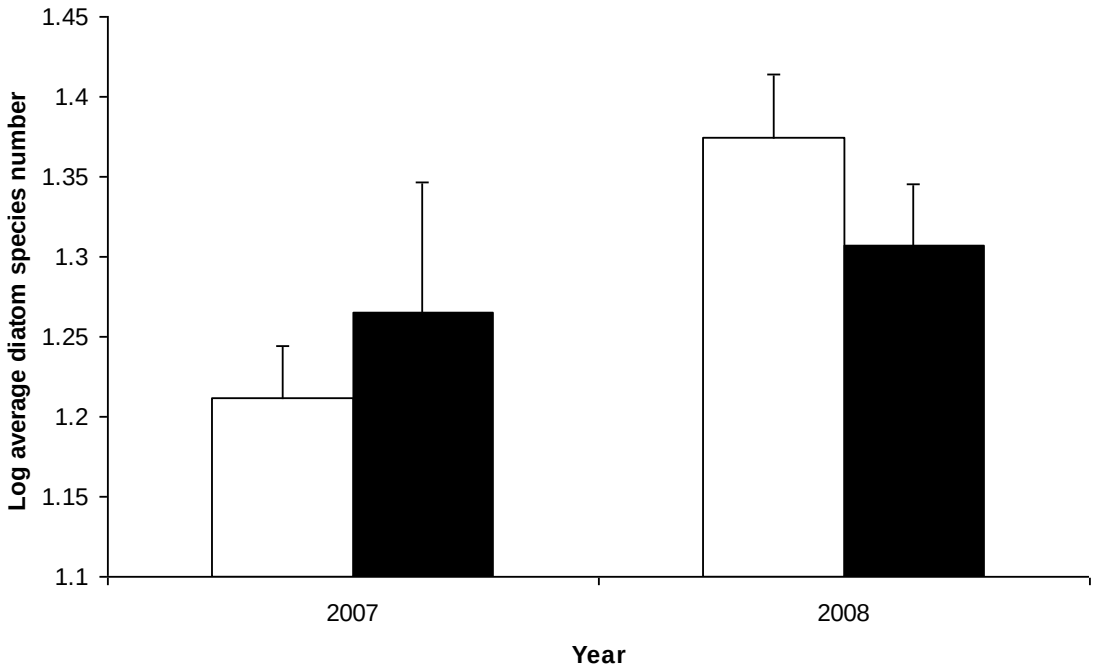


Figure 5.2: The difference between diatom species diversity at 7 flood affected sites between May and July. Open bars are May and closed bars are July. Error bars equal 1 standard error.

5.3.3 Macroinvertebrate abundance

There were significantly fewer macroinvertebrates present in July 2007 than any other month ($F_{3, 24} = 7.69$, $P = 0.001$). There was a significant difference between months and between the flood year and non-flood-year ($F_{1, 1, 24} \geq 7.77$, $P < 0.001$) but no significant interaction ($F_{1, 1, 24} = 1.49$, $P = 0.235$).

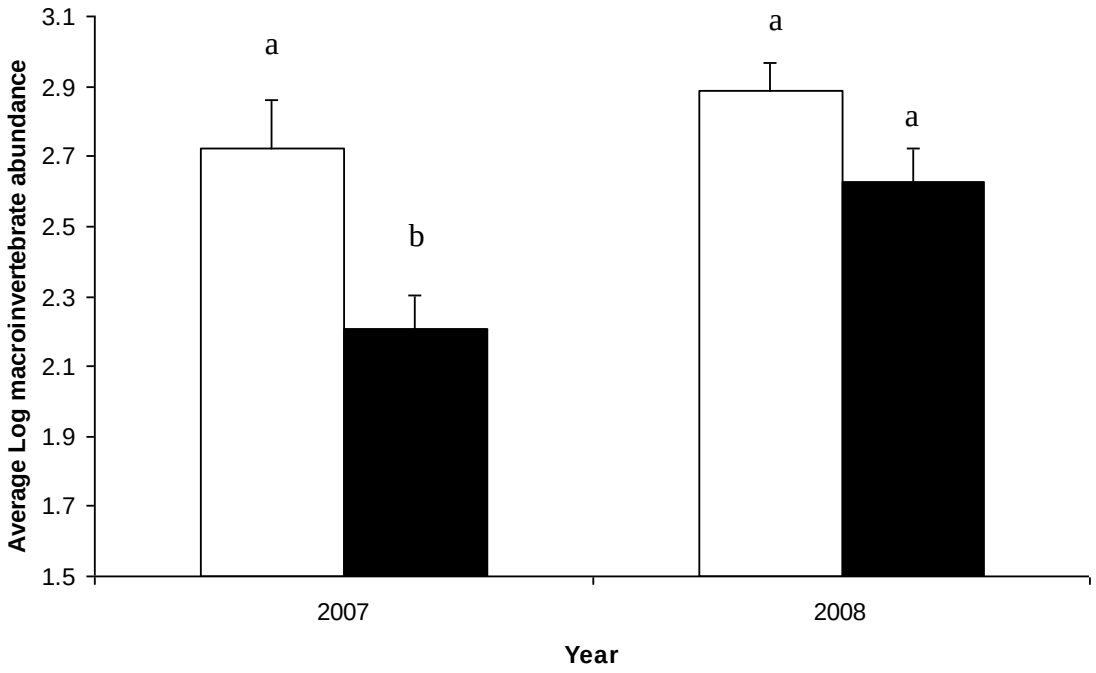


Figure 5.3: Average Log abundance of macroinvertebrates at 7 sites. Open bars represent May and closed bars represent July. Error bars show 1 standard error and difference letters show significant differences ($P < 0.05$).

5.4 Diversity indices for macroinvertebrates and diatoms

Differences between months were observed for Shannon-Weiner diversity and evenness for macroinvertebrates but not diatoms (GLM: $F_{3, 18} = 25.53$, $P < 0.001$ and $F_{3, 18} = 27.06$, $P < 0.001$ for macroinvertebrates and diatoms respectively) indicating that there were significant seasonal differences in diversity for macroinvertebrates but that this was unlikely to have been caused by the flood (Figures 5.4 and 5.5). It can also be observed that values for Shannon-Weiner diversity were on average higher for diatoms but this is probably due to species being used for diatoms and family level for macroinvertebrates. Values for evenness were similar for diatoms and macroinvertebrates except for May 2008, possibly due to large numbers of mayflies being present in May 2008 resulting in a decrease in evenness.

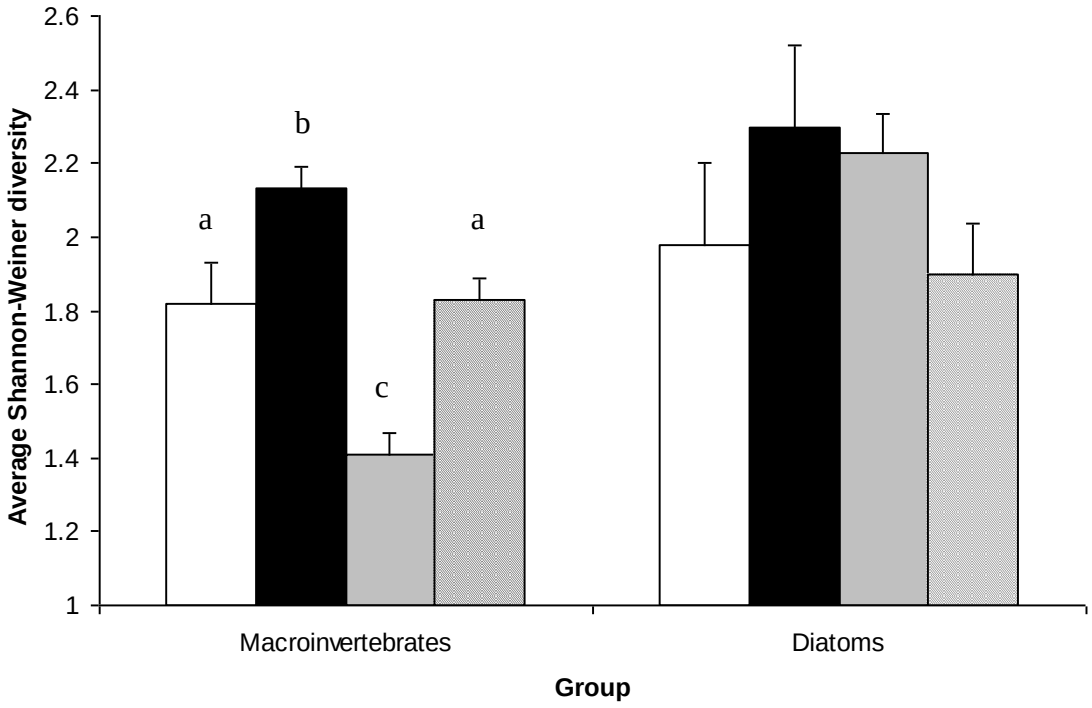


Figure 5.4: Average difference in Shannon-Weiner diversity between May and July of assemblages at 7 sites, a significant flood event occurred in June 2007. Different letter represent significant differences ($P < 0.05$). Error bars represent 1 standard error, open bars represent May 2007, and closed bars represent July 2007, grey bars May 2008 and striped bars July 2008.

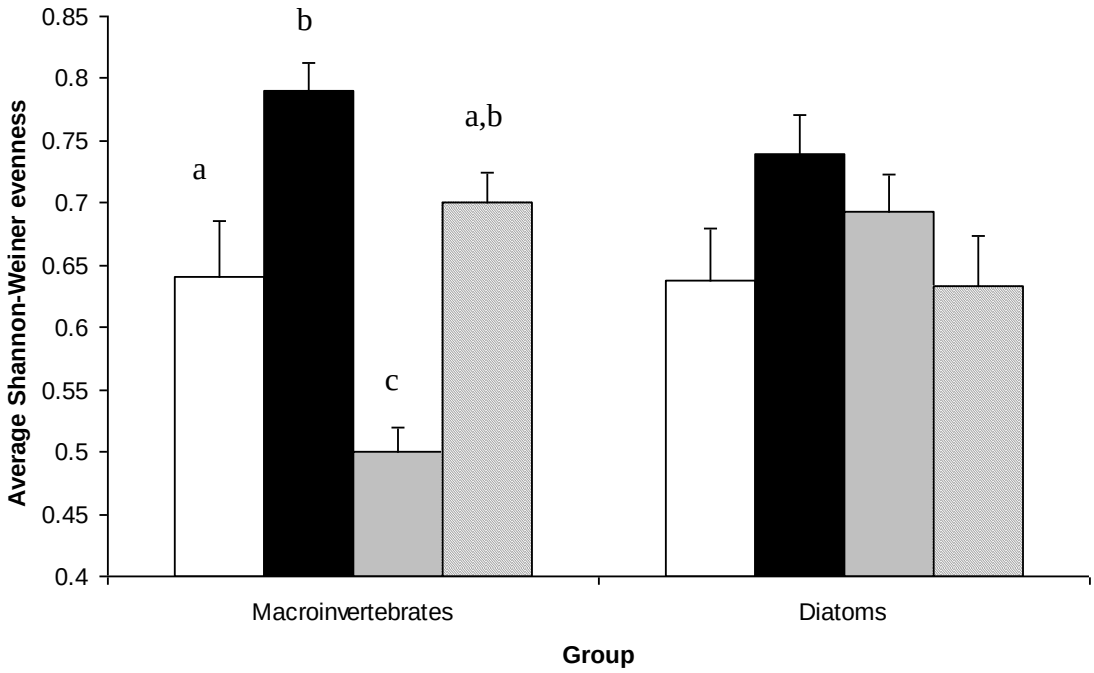


Figure 5.5: Average difference in Shannon-Weiner evenness between May and July of assemblages at 7 sites, a significant flood event occurred in June 2007. Different letter represent significant differences ($P < 0.05$). Error bars represent 1 standard error, opens bars represent May 2007, and closed bars represent diatoms July 2007, grey bars May 2008 and striped bars July 2008.

There was a significant difference in the difference between May and July for 2007 and 2008 for macroinvertebrate Berger-Parker dominance index (Paired T-test: $T = 2.94$, $P < 0.05$). That is to say there was a positive difference between May and July in 2007 (i.e. there was higher dominance in July than May in 2007) and a negative difference between May and July in 2008 (i.e. there was lower dominance in July than May in 2008). The trend was the other way round for diatoms but was non-significant. There were also significant differences between months for Berger-Parker dominance for the macroinvertebrate assemblage (GLM: $F_{3,18} = 7.86$, $P < 0.001$) with July 2008 having less dominance and therefore being more diverse than May 2008 for macroinvertebrates.

5.3.5 Similarity of assemblage (Bray-Curtis)

The average Bray-Curtis similarity between May and July was significantly different between 2007 and 2008 for macroinvertebrates (Paired T-test: $T = 4.55$, $P < 0.005$), with the assemblage in May and July being more similar in 2008 than they were in 2007. For diatoms there was no significant difference in Bray-Curtis similarity index (Paired T-test, $T = 0.06$, $P > 0.05$) (Figure 5.6). In 2007 the macroinvertebrate assemblage was more dissimilar between May and July than the diatom assemblage whereas in 2008 the diatom assemblage is more dissimilar. The difference between months was greater for macroinvertebrates in the flood year, whereas for diatoms it stays at a similar level.

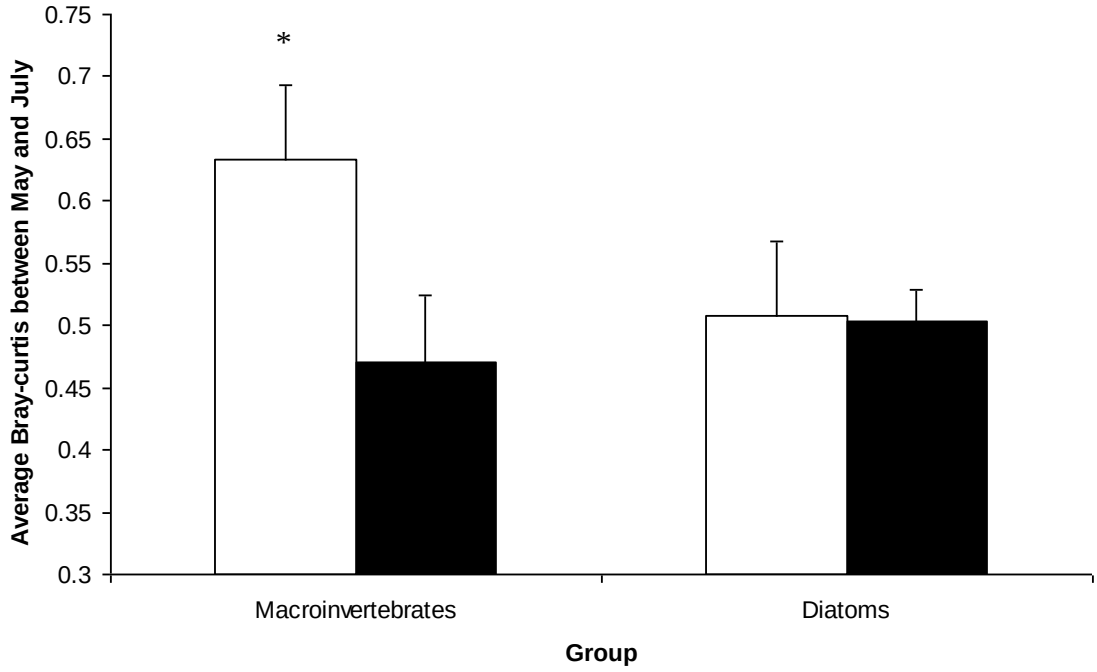
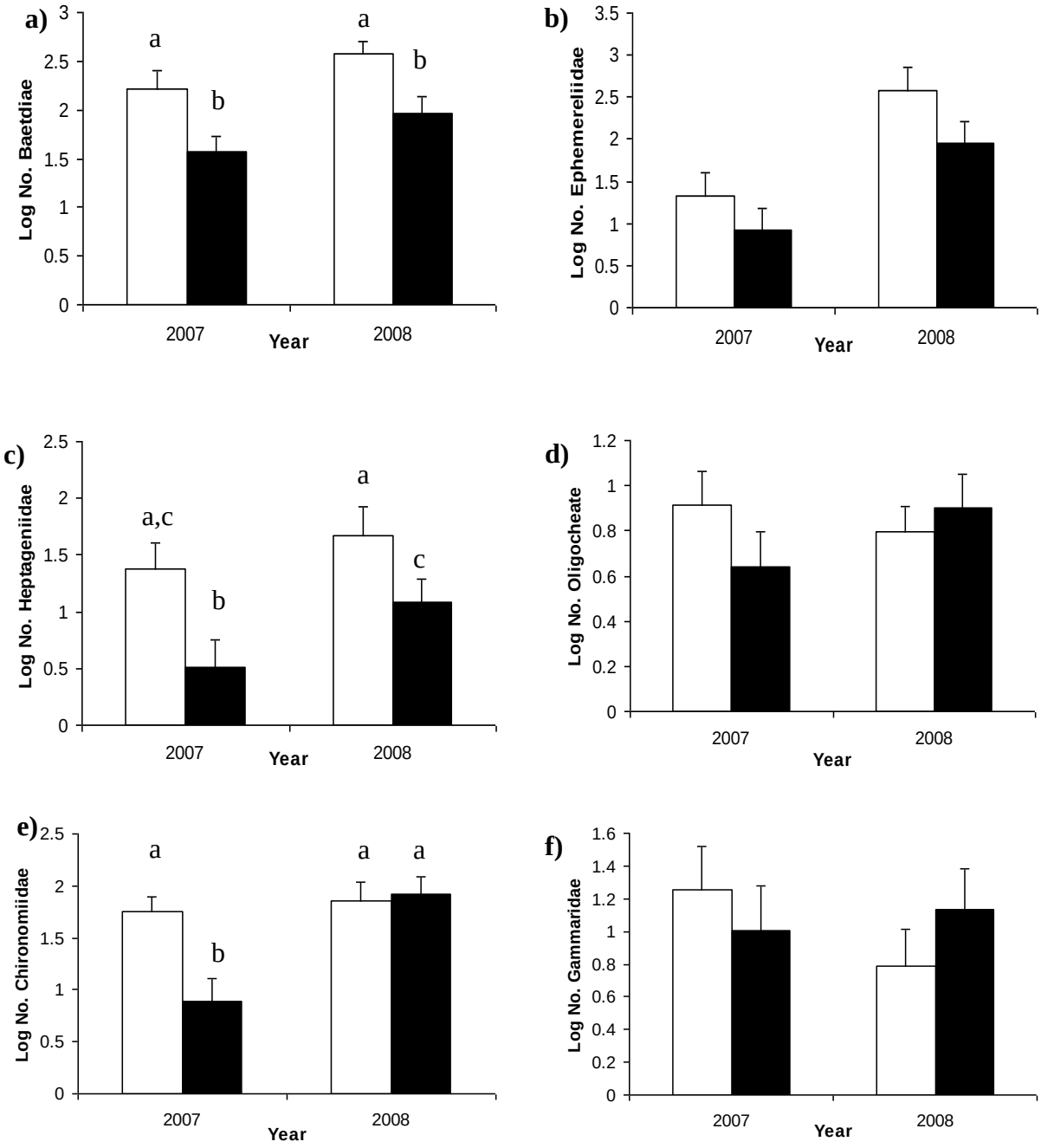


Figure 5.6: Average dissimilarity of assemblage between May and July at 7 sites, a significant flood event occurred in June 2007. Error bars represent 1 standard error, open bars represent 2007, and closed bars represent 2008. Significant difference is indicated by *.

5.6 Individual taxa of macroinvertebrates and diatoms

Significant differences were found in Log-transformed change in abundance between May and July between 2007 and 2008 for Oligocheata ($T = 3.12$, $P < 0.05$, d.f. = 1), Chironomidae ($T = 2.66$, $P < 0.05$, d.f. = 1), Ephemerellidae ($T = 2.85$, $P < 0.05$, d.f. = 1) and Gammaridae ($T = 5.57$, $P < 0.001$, d.f. = 1) for these taxa there was a greater difference in 2007 than 2008. No significant difference between years by paired t-tests was found for the following abundant macroinvertebrate families (Baetidae, Heptagenidae, Hydropsychidae, Capnidae, Simulidae, Ryacophilidae or Elmidae). No significant difference between years using the order of mayflies as a group was found either. There were some significant differences between months: Baetidae and Heptageniidae were both more abundant in May than July for both years (Figures 5.7 a) and c). For Chironomidae there were significantly fewer individuals in July 2007 compared to any other month (Figure 5.7 e). It can also be seen that mayflies, especially Baetidae were the most abundant group of macroinvertebrates present at these sites on average.

There were no significant differences between any of the log transformed dominant diatom species and the difference in relative abundance between May and July between years ($T \leq 1.23$, $P > 0.05$, d.f. = 1). No significant differences between years by paired t-tests for any of the diatom trait groups by relative abundance were found (i.e. % prostrate, motile, erect, and stalked and % high profile). There were few seasonal differences by GLM, except for July 07 having significantly less % erect diatoms than May 08 (Figure 5.8)


a)
Log No. Baetdiae
3
2.5
2
1.5
1
0.5
0
a
b
a
b
2007
Year
2008
b)
Log No. Ephemereliidae
3.5
3
2.5
2
1.5
1
0.5
0
2007
Year
2008
c)
Log No. Heptageniidae
2.5
2
1.5
1
0.5
0
a,c
b
a
c
2007
Year
2008
d)
Log No. Oligocheate
1.2
1
0.8
0.6
0.4
0.2
0
2007
Year
2008
e)
Log No. Chironomiidae
2.5
2
1.5
1
0.5
0
a
b
a
a
2007
Year
2008
f)
Log No. Gammaridae
1.6
1.4
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1
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0.6
0.4
0.2
0
2007
Year
2008

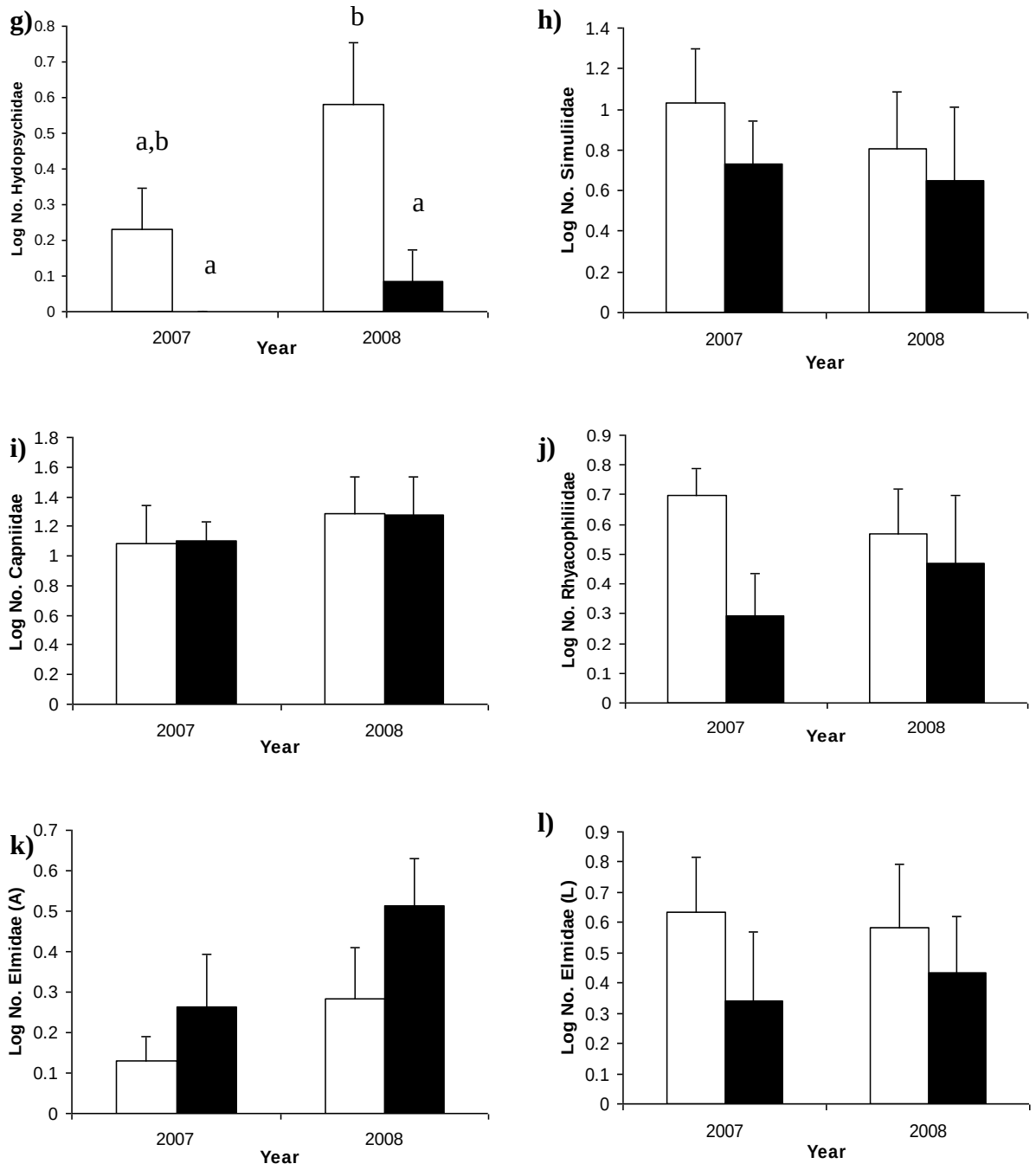

Figure 5.7: Average difference (Log transformed) in a) Baetidae, b) Ephemerellidae, c) Heptagenidae, d) Oligocheate, e) Chironomidae, f) Gammaridae, g) Hydropsyche, h) Simulidae, i) Capnidae, j) Rhyacophilidae, k) Adult Elmidae and l) Laval Elmidae log abundance between May and July of assemblage at 7 sites, a significant flood event occurred in June 2007. Different letter represent a significant difference ($P < 0.05$), Error bars represent 1 standard error, opens bars represent May, and closed bars represent July.

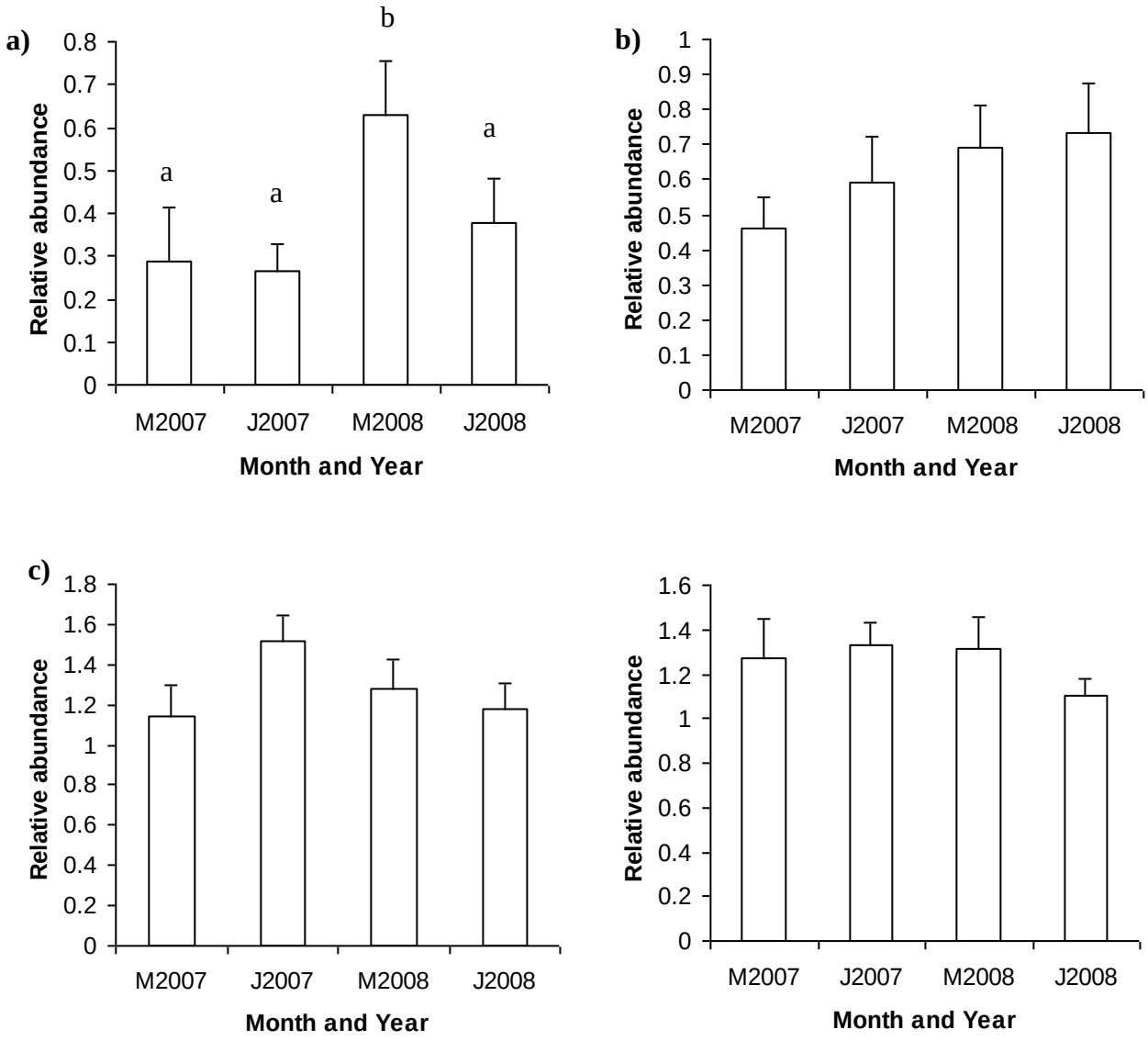

Figure 5.8: Proportion of diatom trait groups (Arcsine square root transformed).a) Proportion erect diatoms, b) proportion stalked diatoms, c) proportion prostrate diatoms and d) proportion motile diatoms. Different letters represent significant differences; error bars represent 1 standard error.

5.4 Discussion

Findings from this study indicate that the June 2007 flood influenced macroinvertebrate assemblage structure but that the diatom assemblage was not observably affected but was generally extremely variable. Bray-Curtis similarity demonstrated that May 2008 and July 2008 were significantly more similar than May 2007 and July 2007 for macroinvertebrates meaning that the flood may have been responsible for a change in structure that made the assemblages more different than would be expected just by seasonal change. This is despite other parameters being predominantly influenced by seasonality as opposed to the flood. Most values for macroinvertebrates were lower in July than May for both 2007 and 2008 indicating that seasonal change was significant. The diatom assemblage was very variable but the parameters we measured had no changes due to the flood or any clear seasonal patterns but were possibly not observed due to the small number of sites used. Macroinvertebrate assemblages appeared to be more affected by the flood than the diatom assemblages, possibly due to their larger size.

The results for individual taxa suggest that the flood occurred at a time when many macroinvertebrates (insect larvae) were low in abundance anyway (due to emergence etc) meaning that it had minimal effect on abundance. There were significantly fewer macroinvertebrates in July in 2007 than in May 2007 but not for 2008, but no significant difference was found for the amount of change meaning that there could have been some flood affect on abundance but it was not large. It is possible that if the flood had occurred in May the impact on the biota would have been more dramatic because more would have been present. Diatom assemblage composition was not influenced by the flood and also did not appear to be seasonally controlled. The lack of directional patterns in the diatom assemblage found could be due to the amount of variability in the diatom assemblage and at different sites meaning that they were influenced differently at specific sites.

The macroinvertebrate families that were found to have significantly greater value for the difference between May and July in 2007 than 2008 could have been due to their life

history strategies making them more vulnerable to the flood than the less affected families (Table 5.4).

Table 5.4: Life history traits of different families of macroinvertebrates that were most abundant at sampled sites. Information adapted from the Eurolimpacs website (Buffagni *et al* (2007)) unless otherwise stated.

Significant difference between 2007 and 2008	**Life history traits**	**No significant difference between 2007 and 2008**	**Life history traits**
Oligocheate	Entirely aquatic	Baetidae	Bivoltine, emerge spring and summer, long emergence period
Chironomidae	Some species univoltine, many bivoltine, some multivoltine	Heptagenidae	Mainly Univoltine, emerge spring, summer and autumn, long emergence
Ephemerellidae	Univoltine, emerge mainly in summer	Hydropsychidae	Univoltine, emerge mainly in summer
Gammaridae	Entirely aquatic. Mainly breed in the summer, most dense in the summer (Iversen and Jesson 1977)	Glossomatidae	Univoltine/flexible, emerge mainly in summer
		Simulidae	Variable/ little known life history
		Elmidae (Adult)	Entirely aquatic
		Elmidae (Larvae)	Entirely aquatic
		Capnidae	Univoltine, emerge spring
		Ryacophilidae	Univoltine, emerge mainly in summer
		Ceratopoginae	Variable/ little known life history

Oligocheata and Gammaridae have entirely aquatic life cycles so were more likely to be abundant at the time of the flood due to being present throughout the year. Ephemerellidae are univoltine and emerge mainly in the summer, suggesting that the flood could have occurred before their main emergence (Buffagni *et al* 2007). Chironomidae are multivoltine (although many species are poorly understood) and thought to often have overlapping cohorts (Huryn and Wallace 2000) so are likely to emerge at many times meaning that the flood probably occurred during a time when they were vulnerable,

evidenced by their high abundance in July 2008. For some taxa in the current study it was impossible to tell if the flood influenced them as they were found in insufficient numbers. Previous studies have found that different taxa respond differently to floods with some showing decreases, some increases and some remaining unchanged, indicating that some taxa are more able to cope with floods than others (Robinson and Uehlinger 2008).

The families that appeared unaffected by the flood are mainly those that are seasonally more abundant in May than July, indicating that the flood occurred when they were less vulnerable. For many of these families there were less in July 2007 than July 2008 but because May 2008 was also higher than May 2007 these results did not show as anything other than a seasonal pattern. This indicates that the flood may have had an affect on the individuals present but not at the population level. Elmidae beetles did not appear to be affected by the flood and are entirely aquatic meaning they could have other methods of coping with the flood, such as avoidance or clinging on. They showed a pattern of having more larvae in May and more adults in July, which was consistent across years (Figures 5.9 k and 5.9 i). Most macroinvertebrate families are able to cope with flood events successfully due to refuge seeking behaviour, re-colonisation strategies and flexible life histories (Scimgeour and Winterbourn 1989). Different families are likely to use different strategies to cope with the flood, i.e Elmidae swim to refuges, such as root systems and backwaters, to avoid the affects, other families having long flight periods ensuring colonists are always available and others having a high reproduction rate allowing them to repopulate rapidly (Scrimgeour and Winterbourn 1989). Infrequent, unpredictable flood events are a characteristic of many lotic systems so organisms may have adapted to this unpredictability by having un-seasonal life histories and flexible strategies to decrease the chance of losing a whole cohort, meaning that stream environments favour opportunism (Scrimgeour and Winterbourn 1989).

Even a once in 2000-year extreme flood event has been found to result in no lasting effects on taxon richness or assemblage stability of macroinvertebrates meaning that they are remarkable well adapted to cope with floods (Snyder and Johnson 2006). This shows that stream biota is even able to cope with highly unusual flood events as illustrated by the

current study finding no lasting effects. Macroinvertebrates and diatoms will change naturally over a season both due to changing environmental conditions (abiotic and biotic) and by natural variations (i.e seasonality) regardless to whether there is a flood event (Lancaster *et al* 1996), and this aspect needs to be considered when determining what impact a disturbance such as a flood has. Macroinvertebrate assemblages show much temporal variation in taxon richness and community composition but patterns are often inconsistent suggesting that flood influences could be difficult to detect above other variation, which could be the case for taxa that appear unaffected by flooding (Kay *et al* 2001).

Studies that have considered seasonal change have usually found that within year variation is higher than between year variability for both macroinvertebrates and diatoms, suggesting that the high seasonal variation observed in this study was not unusual and that it accounts for the significant differences found between months, but not years for many aspects of the assemblages (Lancaster *et al* 1996). Seasonality, in a regularly flooding river, has been found to be due to factors such as primary production and nitrogen flux and rather than flood events (Boulton *et al* 1992). A high level of natural variability in the diatom assemblage could be the reason for not finding a flood effect (Peterson and Stevenson 1992). In this study the post flood diatom samples varied from the pre- flood samples, but not consistently with some showing increases in diversity and others showing decreases. This is consistent with other studies that have found changes caused by flood events on the diatom assemblage depend on how susceptible the species present are, for example *Achnanthidium minutissimum* is likely to be flood resistant due to its fixed, low-profile growth form whereas large, less fixed species such as *Synedra radians* are decreased in relative abundance after flood events (Peterson and Stevenson 1992). It also agrees with results from frequently flooded New Zealand streams that found that often diatom richness decreased after floods but not in every stream and not after every flood (Biggs and Smith 2002). This means that some assemblages are likely to be unaffected by flooding but others are, thus flood induced change is dependent on what is present initially in the system for diatoms. Diatoms have very high resilience and can return to control levels in 3-6 days post flood in some circumstances (Peterson and Stevenson 1992). Diatom assemblages can

recover fast because of a high proportion of the diatom assemblage being made up of R-selected cells i.e. small pioneer species with fast growth (Yallop and Kelly 2006). It is also possible that adaptation of an algal assemblage to other aspects of the environment, such as grazing, also infer resistance to scour, meaning that although they were not adapted to this unusual flood event they could have been well adapted to cope with it through adaptation to other stresses (Stevenson 1997).

It is probable that if a flood occurred when there was less grazing pressure and the diatom assemblage was dominated by C-selected cells (those larger more competitive cells but with slower growth rates) (Yallop and Kelly 2006) there would have been an observable effect of the flood on the diatom assemblage structure. However, the flood occurred in summer when there are fairly high numbers of grazers and the diatom assemblage is likely to be maintained as a pioneer assemblage by this. The effect of flooding on the number of diatom taxa present has been found to be highly variable (Uehlinger *et al* 2002) with diatom richness varying erratically meaning that patterns are hard to determine and responses to floods being hard to predict (Biggs and Smith 2002). A flood can result in greater specie diversity if it results in a decrease of the dominance of any one species and increases the chance of co-existence (Oemke and Burton 1986, Yallop and Kelly 2006). Often the identity of the diatoms present rather than species richness is the element most likely to respond to environmental variation (Biggs and Smith 2002). High variation has been found in diatom samples with samples taken across seasons being found to explain more of the variance in chemical data than either a one off sample or a 2-month average for diatom index result (Lavoie *et al* 2009).

Temporal variation can influence the assessment of whether a site is degraded, and also from this study possibly the influence a flood can have meaning that taking seasonality into account in the assessment of a water body is essential (Linke *et al* 2001). This study indicates that seasonality can be more important than flood disturbance in structuring the assemblage but that timing of flooding could also be significant. Macroinvertebrates with entirely aquatic life histories are more likely to be influenced by flooding than those with an aerial phase. Most aquatic organisms present in lotic systems are well adapted to cope

with flood events even when they are large and atypical, possibly due to other adaptations that confer resistance and/or resilience to flood events as well. The evidence suggests that aquatic biota should cope well with flood events that may occur more frequently in the future but further studies are needed to assess the extent they can cope with frequent large flood events and if this can alter trophic interactions with higher organisms such as fish. Information is need on other flood events: those that occur at different times of the year and those in different stream systems. A greater replication of sites, than used in this study, would also be advantageous and allow a greater understanding of the impact flooding will have on aquatic systems and energy flow. Studying the influence floods have on ecosystems will allow us to find out if/how they may impact important ecosystem services.

6.0 DISCUSSION

The research reported in this thesis aimed to determine how associations between macroinvertebrates and diatoms can influence diatom assemblage structure and associated water quality indices. This was achieved by investigating the following: influence of grazers with different feeding modes (Mayflies and snails), on diatom assemblages made up of different species (Chapter 2); the influence of mayfly dominated grazer assemblages on the diatom assemblage was investigated in a field trial (Chapter 3); the relationships between the two groups were examined in a field survey of minimally impacted sites, to find out if there were observable patterns between them (Chapter 4); lastly to determine if macroinvertebrates and diatoms are linked by being influenced by the same impact the effect of a severe flood on their diversity and assemblage composition was studied (Chapter 5). Throughout the work the implication of the macroinvertebrate-diatom interactions on a diatom based index (the TDI was used as an example) was considered.

Mayflies had significant effects on the diatom assemblage by decreasing the percentage high-profile diatoms in the assemblage, both in artificial streams and in the field. Snail grazers were not found to have any significant effects on diatom assemblage structure. The amount that grazing macroinvertebrates decreased high-profile diatoms was dependent on the abundance of grazing mayflies, with more mayflies associated with greater decreases in high-profile diatom relative abundance. However correlations between diversity and ecological indices of macroinvertebrates and diatoms were not observed in the field survey or in their response to the flood event. This meant that despite evidence of food-chain links between them aspects relating to indices could not be observed in a survey. The field survey found that correlations between different groups were observed between abundance based variables, again indicating food-chain links. The lack of correlation observed for

indices and richness means that it is unlikely that the assemblage of one group can be used to predict the assemblage of another (e.g. Heino *et al* 2005, Chapter 4 and 5).

The first objective of this thesis was to investigate how different grazing macroinvertebrates influence diverse diatom assemblages, and what impact this has on the indices based on them. The effect of grazing on diatom community structure was investigated both in the laboratory (artificial stream studies) and in the field (experimental manipulation). The grazing effects of three mayfly families (Baetidae, Ephemerellidae and Heptageniidae) and one snail family (Hydrobiidae) on two different diatom assemblages were investigated in the artificial stream studies, and the effect of a mayfly-dominated assemblage investigated in the field. Mayfly grazing resulted in a decrease in the relative abundance of erect/high-profile diatoms in both diatom assemblages, whereas snail grazing had no effect on the structure of diatom assemblages. This meant that diatoms were not equal in their consumability: erect/high-profile diatoms (e.g. *Fragilaria spp.*) were more susceptible to grazing than prostrate taxa (e.g. *Achnanthes spp.*) (Peterson and Jones 2003). These results are consistent with those of previous studies (Villanueva *et al* 2004, Holomuzki and Biggs 2006) and are explained by differences in the mobility (McKenny 2005), feeding behaviour and mouthpart morphology (Karouna and Fuller 1992) of the two types of grazers. Mayflies have brushing mouthparts that cannot consume the low-profile diatoms (Holomuzki and Biggs 2006) as readily as snails with rasping radula mouthpart (Dillon 1998). Additionally mayflies are more mobile and will move to abundant patches, thus resulting in more selective grazing compared to sedentary grazers (McKenny 2005). This demonstrated the importance of a biotic interaction in influencing the structure of the diatom assemblage.

The field manipulation (Chapter 3) showed that grazed assemblages had a lower relative abundance of high-profile diatoms than ungrazed assemblages. Evidence to support the contention that the change in diatom community structure was primarily due to mayfly grazing was: (i) mayflies were the most abundant grazer group in the macroinvertebrate community; (ii) a greater difference in relative abundance of high-profile diatoms between the grazed and ungrazed treatments associated with greater mayfly numbers; (iii) diatom

community composition did not co-vary with the abundance of other grazers. This is the first study to establish the effect of mayfly grazing in the field on diatom assemblage structure and indices. This is inline with studies on scraping grazers, such as snails and caddisflies, which have reported decreases of high-profile diatoms in the field (McAuliffe 1984, Ferminella *et al* 1987). The observation that snails decrease the relative abundance of high-profile diatoms in the field contradicts the findings from the artificial stream study reported in this thesis. This apparent disagreement between studies could possibly be due to different relative growth rates under field and laboratory conditions. High-profile diatoms are slower growing than low-profile diatoms (Morin *et al* 2008), so even if eaten in the same ratio as low-profile diatoms they will be decreased in relative abundance. This effect may be more pronounced in natural stream environments than those found in artificial streams because conditions for growth are likely to be further from optimal and additional pressures will be present (Lamberti and Steinman 1993). The exact mechanism for why grazing tends to decrease high-profile diatoms in the field, even when grazers are non-specific, could be further investigated in the future. The mechanism could involve the grazer, the diatoms, the environment or a combination of these factors.

Diatom community structure is used to calculate water quality indices, such as the Trophic Diatom Index (TDI) (Kelly and Whitton 1995). Grazer-induced shifts in the relative abundance of high-profile diatoms may have consequences for the value of these indices and hence the assessment of water quality. The diatom assemblages used in the artificial stream study were representative of two different water qualities: 'good' or 'moderate/poor' ecological quality, as defined in the Water Framework Directive (WFD) and assessed using the TDI. Mayfly grazing of the 'good' ecological quality assemblage resulted in a significant increase in the TDI (that is to indicate poorer water quality). But there was no effect on the TDI for the 'moderate/poor' diatom assemblage. This study is the first demonstration that grazing, by surface feeding grazers, has the potential to change a biotic index. The change in index was small for the laboratory experiment (approximately 4-5 points), but any change could be significant for management goals if the assemblage is already close to an ecological class boundary, set for ecological assessment (Kelly *et al* 2008).

Changing the index score by a few points could move the assessment into a lower class meaning that it could appear to fail the standard set by legislation (i.e. drop from representing "good" to "moderate" ecological quality). Due to the nature of biotic indices and their scoring systems the same ecological assessment value can be made up of different identities and abundances of organisms. This means that two sites with very similar diatom assemblages with no grazing could have a different assemblage (and TDI value) with grazers present (i.e. if one site had many mayflies and the other with very few). This idea could go some way to explaining the lack of concordance in indicator values observed in Chapter 4.

The lack of influence of mayfly grazing on the TDI of the 'moderate/poor' ecological quality assemblage was due to the relative sensitivity of the diatoms present. There was no significant difference between the sensitivity values of the high and low-profile diatoms present in the 'moderate/poor' assemblage. Whereas, for the 'good' quality assemblage high-profile diatoms had a significantly lower average sensitivity value than the low-profile diatoms. The effects of grazing on the TDI in the field experiment were inconsistent, as there was no pattern for whether high or low-profile diatoms had the lower TDI score. However, there was significant positive correlation between the difference in TDI score between grazed and ungrazed treatments and the difference between high and low-profile diatom sensitivity values (i.e. a larger difference in average sensitivity value between high and low-profile diatoms resulted in a greater difference in TDI score for grazed and ungrazed diatom assemblages). Some grazed assemblages had significantly lower TDI values than the ungrazed but some had significantly higher values meaning that the affect was variable and dependent on the identity of the diatom species present. Thus, grazers have the potential to influence diatom indices but whether this occurs depends on the sensitivity values of the diatoms that make up the different trait groups. This is because diatom morphology determines whether a species is susceptible to grazing or not. Thus, the outcome of the interaction is dependent on both the identity of the grazer (whether they selectively graze) and the identities of the diatoms present (what sensitivity they have

according to index values). This illustrates how complicated predicting the outcome of grazing on the TDI is.

Mayfly grazing has been found to consistently result in less relative abundance of high-profile diatoms compared to ungrazed and can cause variation in the diatom assemblage, which has potential to influence indicators based on diatom relative abundance. If a diatom assemblage is found to have high-profile diatoms with lower average TDI sensitivity values than the low-profile diatoms it is likely that selective grazing will cause an increase in TDI. Therefore grazers are likely to cause some of the non-environmental variation in the diatom index (Figure 6.1).

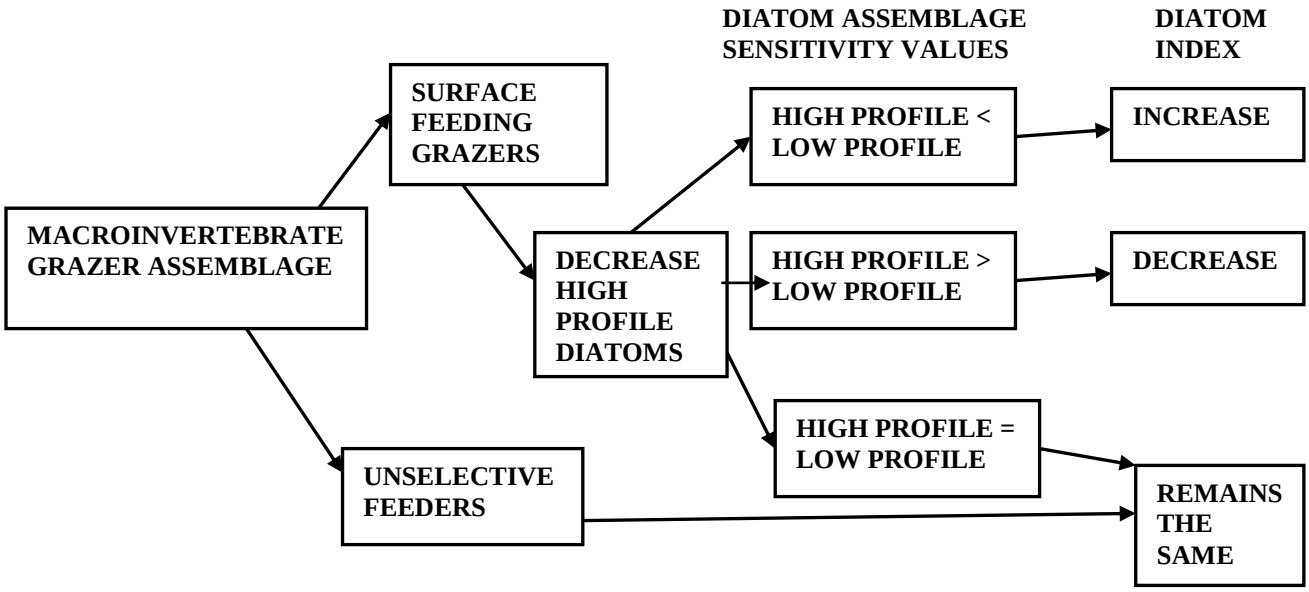

Figure 6.1: Schematic of possible outcomes with macroinvertebrate and diatom assemblages with different traits. An increase of grazers should result in increased influence from left to right of the diagram. The interactions are also influenced by environmental/abiotic variables but these are not shown on this figure.

At a large scale abiotic factors like climate and geomorphology will determine the species pool that *can* be present, but biotic interactions have been shown to be important for structuring at small scales, especially the ratio of organisms as opposed to their presence or absence (Jackson *et al* 2001). This study addressed the objective of how grazing macroinvertebrates influence diatom assemblage structure and shows that at small scales biotic interactions are important in determining what, from the possible abiotic-determined

species pool, is present and specifically for this study in what relative abundances. The results of this study may not be applicable to all situations and stream types, as the laboratory streams and the field study were performed in stable conditions so disturbance was not considered.

The first section of this thesis determined that macroinvertebrates *could* influence the structure of the diatom assemblage, indicating that links occur between the two groups. Therefore, the question of what the relationship between biomass, diversity and community structure of the diatom and macroinvertebrate communities in natural conditions was asked (Chapter 4). A field survey of relatively unimpacted sites was conducted to determine if correlation exists between the above aspects of diatom and macroinvertebrate assemblages and indices, to find out if the food chain links observed in experiments translated to correlations that were observable in nature (Chapter 4). There was no correlation between the assessment of water quality based on macroinvertebrate community structure (i.e. ASPT score) and the assessment based on diatom community structure (i.e. TDI). This agrees with other studies that have investigated diverse groups, such as: macroinvertebrates, fish, diatoms, macrophytes and bryophytes, and found that their indices do not correlate (Heino *et al* 2005, Hering *et al* 2006). The sites used in the current study were not under strong anthropogenic gradients, thus it may be that studies that have found concordance have been when organisms are responding to strong gradients that both are affected by (Soininin and Eloranta 2004). For example macroinvertebrates and diatoms have both been found to respond to nutrient gradients (Johnson *et al* 2006). However, different groups tend to have different response trajectories to different stressors, meaning that they give different information (Johnson and Hering 2009). It appears that in freshwaters, different biotic groups are often influenced by different environmental characteristics and that indicators will complement each other and strengthens inferences that are made about environmental condition (Heino 2009, Johnson and Hering 2009). Therefore, determining the ecological quality of a whole system based on one element is unrealistic and risky (see chapter 4) meaning that the use of multiple groups and indices for monitoring should allow the identification of environmental problem(s) and enable the selection of suitable measures to correct them (Heino 2009).

No correlation was observed between diatom species richness and macroinvertebrate family richness; both the biomass and abundance of macroinvertebrates were positively correlated with chlorophyll *a* content (used as a measure of algal (mainly diatom) biomass). The relationship was more pronounced when grazer biomass was compared, indicating the potential for bottom-up control. The association of increased algal biomass with more grazers has also been found in other studies (Scrimegeour and Winterbourn 1989, Sponseller *et al* 2001). Other investigations and the field manipulation experiment (Chapter 3) have found evidence for top-down control with increased grazers associated with decreased algae (Jordon and Lake 1996, Opsahl *et al* 2003, Holomuzki and Biggs 2006). Therefore, both top-down and bottom-up control is acting on these stream systems. The field study took place (July) when there was lots of light (i.e. potential for high primary production), meaning that bottom-up control was probably more likely than at other times of year. There was also a weak positive correlation found between the relative abundance of high-profile diatoms in the assemblage and the numbers of surface feeding grazers (mayflies). This meant that more grazers were associated with more high-profile diatoms. Findings from the survey contrasts results from artificial stream studies and field manipulation where more high-profile diatoms were present in ungrazed treatments than grazed treatments (Chapter 2 and 3). This can be explained by methodological differences revealing either top-down or bottom-up control, in a system that is likely to be influenced by both. Studies that have excluded grazers have predominantly found that there are less algae/ high-profile diatoms in the grazed treatment (i.e. Jordon and Lake 1996, Opsahl *et al* 2003, Holomuzki and Biggs 2006, Chapters 2 and 3) whereas, studies surveying the two groups find sites with more algal biomass/ high-profile diatoms associated with more grazers (Scrimegeour and Winterbourn 1989, Sponseller *et al* 2001, Chapter 4). This means that the two findings are not mutually exclusive, but that sites that have a high diatom (algal) biomass and/or high-profile diatoms are likely to have even higher values if grazers were not present.

Evidence of macroinvertebrates and diatoms being influenced by different environmental factors was provided by the two groups' structural responses to the summer 2007 flood

event (Chapter 5). The flood influenced the macroinvertebrate assemblage whereas the diatom assemblage was not observably influenced. No concordance was found for the response of macroinvertebrate and diatom diversity or structure to the flood. Only short-term affects of the flood were detected for macroinvertebrates, with some taxa being significantly decreased in the month after the flood occurred. The taxa that were most affected were those that spend all of their lives in the water (Gammaridae, Oligocheata), have multi-voltine emergence (Chironomidae), or are normally present in large numbers in early summer (Ephemerellidae). Many taxa (some mayflies, caddisflies, and stoneflies) are mainly aerial (Buffagni *et al* 2007) at the time the flood occurred, meaning that they may have avoided the flood. This suggests that results could have been different if the flood occurred at a different time of year. This study suggested that season had a greater influence on the presence and abundance of macroinvertebrates than flooding. This was demonstrated by significant differences between May and July for both 2007 and 2008 for several aspects of their ecology (Chapter 5).

The flood did not influence diatoms, possibly because they are able to re-colonise quickly and that they were only investigated for relative abundance. It is well known that diatom biomass would have initially decreased, as many studies have shown this (Rutherford *et al* 2000). Also diatoms tend to bloom after a flood due to recovering faster than grazers possibly because they are removed from top-down control (Rutherford *et al* 2000). It may therefore, be possible to use diatoms to assess rivers fairly soon after a flood because their indices are based on relative abundance. However results are likely to be variable because of the likelihood of assessing a pioneer community, which can have higher tolerance than a climax community (Yallop and Kelly 2006). But it could be possible to develop a range of assessments that include pioneer assemblages through to climax communities and could all represent the same water quality (Yallop and Kelly 2006). This would remove the chance of missing a species expected to be present at a site simply because the assemblage was at the wrong point in its trajectory (Yallop and Kelly 2006). Although not detected in this study, the flood could affect diatoms indirectly by changes in macroinvertebrate assemblage. For example if macroinvertebrate grazers (Consumers) are reduced the

diatoms (prey) have less grazing pressure meaning they could sustain or increase their assemblage as in a "consumer stress model" (Thompson *et al* 2002).

There are many interrelated factors; both biotic (i.e. grazing) and abiotic (i.e. hydrology) that can influence diatom assemblage structure and therefore indices based on this (TDI) (Figure 6.2). The current work helps to solve the problem of predicting factors that determine how an algal assemblage is structured, which is considered one of the greatest "challenges for benthic algal ecologists" (Stevenson 1997).

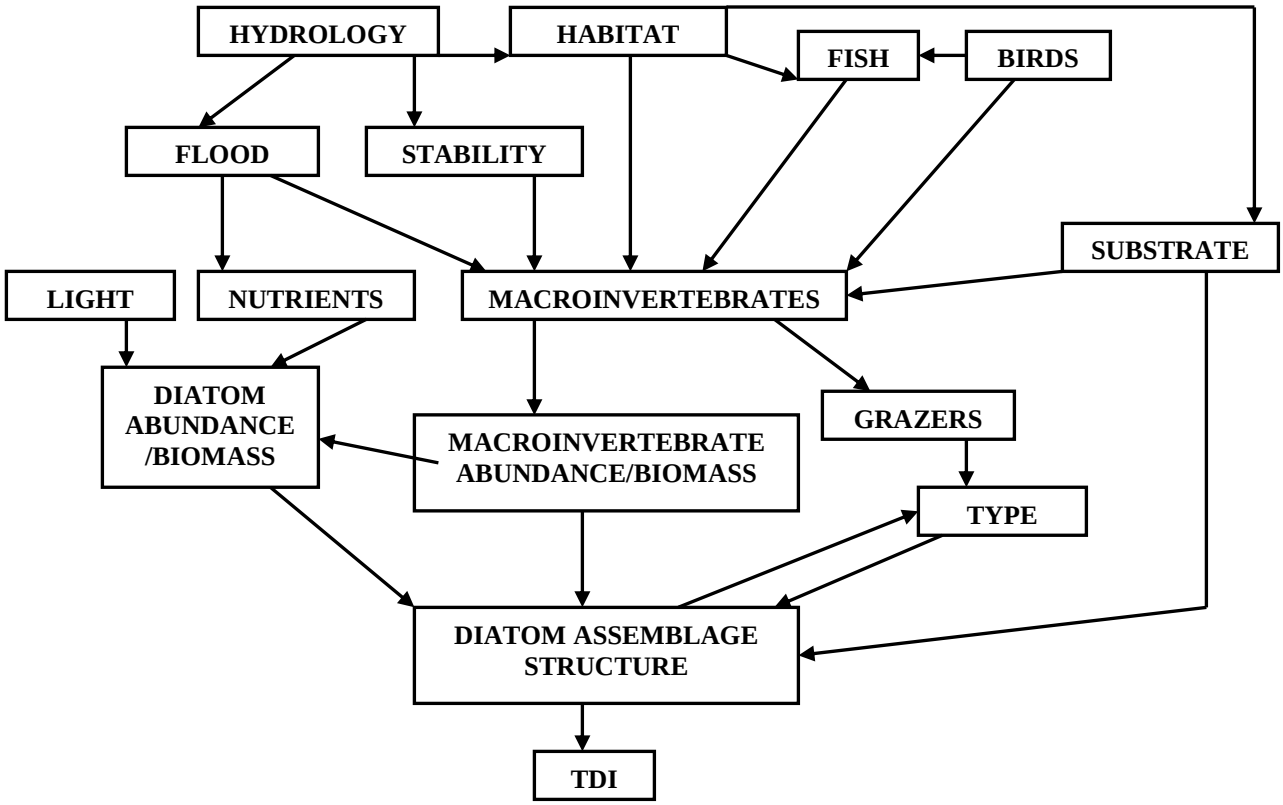

Figure 6.2: Factors that influence the stream diatom assemblage structure and thus the indices based on this (e.g. TDI). Partly developed from Stevenson 1997.

Elucidating how the algal assemblage is structured should also help in the understanding of indices that use relative abundances of species. All biotic assemblages are subject to both spatial and temporal variation meaning that all biotic indices will have uncertainty associated with them, therefore the more we understand this variation the more robust these indices can be made (Kelly *et al* 2009). A recent study found that the greatest amount of

temporal variation in the TDI was found at the moderate/good status (the target of the WFD) boundary meaning that any knowledge gained on diatom variability is vital for assessing whether measures need to be taken for a narrowly failing/passing water body (Kelly *et al* 2009). The amount of variation found by Kelly *et al* (2009) could be up to 0.2 of an EQI point (15-20 TDI points) which is higher than the differences found with grazed or ungrazed treatments in this study (~5 TDI points) but this indicates that differences in grazing pressure could explain some of the observed changes. Kelly *et al* (2009) suggest that due to the variability of diatoms assessments should be made over several years and several samples. However, this is time consuming and may not always be practical so combining knowledge gained from other groups (Macroinvertebrates, fish, macrophytes) sampled at the same time may give an explanation for variability. It also means that the more we elucidate what causes variation in diatom assemblages the more this can be taken into account with monitoring.

Diatom indices are based on assemblage structure and are therefore a product of many biotic and abiotic factors, thus integrating the whole environment they live in. Interactions between diatoms and grazing macroinvertebrates are an important local-scale factor for structuring assemblages, as shown by this thesis. Local scale factors are influenced by higher-level factors like hydrology and climate (Stevenson 1997). Thus, the ultimate diatom assemblage is not exclusively structured by any one factor but is the result of a hierarchy of factors that still need to be fully untangled and understood (Stevenson 1997).

The demonstration by this study that biotic factors, namely macroinvertebrate grazing on benthic diatoms, can influence the structure of part of a system highlights the importance of these local interactions. The finding that biotic interactions influence structure means that monitoring results not only reflect abiotic factors, which are present in the system, but biotic interactions as well. Large-scale factors, such as climate and geology, determine what can be present (constrain the extent of local scale effects), but what is actually present, and in what proportion, is structured by local factors such as biotic interactions between groups (Stevenson 1997).

Most of the work in this study was carried out in fairly benign conditions (the artificial streams, exclosure experiment, July 2006 field survey) meaning that the harsh-benign hypothesis (Menge and Sutherland 1976) would predict biotic factors would have a greater influence than in a harsher environment. This means that results from this study could have been different in other conditions. A benign environment is more likely to be influenced by biotic interactions because organisms do not have to cope with harsh, abiotic factors, leading to competition and predation being more common (McAuliffe 1984, Poff and Ward 1995). Rivers and streams often have harsh environments (Lancaster 1996), but it may be possible that adaptations of stream organisms to the stream environment make what is considered a harsh environment relatively benign. The finding of no evidence for links between the two groups after the flood could also be explained by the conditions being harsh, thus abiotic factors could have been dominating the assemblage structuring. Evidence suggests that a stable flow is needed for the algal assemblage to be structured by biotic affects, meaning that a flood event could have disrupted this (Stevenson 1997 and references within). The result of macroinvertebrates being more affected by the flood than diatoms could be due to a "consumer stress model" response, in that diatoms were released from grazing pressure by macroinvertebrates being more affected by a stressor (flood) (Thompson *et al* 2002). The structuring of any system is influenced by many factors that are interrelated, meaning that better understanding of these elements should lead to management that can better maintain ecosystems and their functions. This study goes some way to achieving an understanding of how changes in one element of the environment (macroinvertebrates) can influence another (diatoms), and will aid in predicting what may occur in these systems (Stevenson 1997).

The WFD requires that multiple groups be used for assessing ecological quality; usually accepted to mean macroinvertebrates, macrophytes, fish and periphyton (of which diatoms are an accepted proxy) (e.g. Hering *et al* 2006). If it had been found that these groups were concordant in their assessments, time and money could have been saved by using one group as a surrogate for the other taxa. However, this study adds to other recent evidence that found this is unlikely to be a successful strategy, meaning that it is best to assess freshwater ecological quality using more than one group (Heino *et al* 2005, Heino 2009). The findings

of this study suggest that measuring diatoms and macroinvertebrates at the same time when monitoring should be beneficial because: i) it gives a more accurate idea of the quality of the system, ii) there is additional ecological information to be gained by knowing what is present for both groups and iii) discrepancies in results may be untangled with additional taxonomic data. For example, if it is known that a site has lots of grazing mayflies and the diatom index is found to be on the good/moderate borderline it may be that, the site could be considered good for diatoms, as the slightly lower value could be due to the erect diatoms being preferentially grazed (Figure 6.1). Therefore, this is something to consider when a site is narrowly failing for one group, as it could be due to biotic interactions rather than some kind of impact, especially if chemical and hydrological assessments are also good.

If information about more than one taxonomic group is known, not only will it give a better idea of the status of the system, it will also tell us more about the ecology and allow us some insight into any discrepancies that may be caused by biotic interactions. The current evidence suggests that development of successful indices for groups other than macroinvertebrates should be continued and ensured to be applicable for surveillance, operational and investigative monitoring as required by the WFD, as just using macroinvertebrates is unlikely to meet current and future monitoring and management goals (Allen *et al* 2006).

This study only investigated the interactions between two of the four ecological quality elements required by the WFD. The next step is to be able to add fish and macrophytes into the investigation and determine how they may influence each other's structure. Determining the effect of fish on macroinvertebrate assemblage structure and consequently the diatom assemblage, and thus indices based on this, would be a useful and ecologically interesting topic to investigate. It would also be interesting to see how the corresponding indices would be affected by any trophic interactions. Additionally some of the investigations in this project were limited by lack of replication, especially the floods work, as more replicates may have resulted in some more significant results. Another aspect to consider is that some of the macroinvertebrates that were considered non-grazers were

probably eating some of the diatoms (as most macroinvertebrates are generalist to a certain extent (Mihuc 1997)). Thus, it could be useful to perform gut content analysis on macroinvertebrates to determine exactly what happens via the food chain and exactly which diatoms actually are the preferred food source.

In order for freshwater ecosystems to be protected and maintained monitoring and understanding the biotic interactions that structure the assemblages is extremely important. Increases in understanding should help in determining the correct management options to maintain sustainable freshwater ecosystem services. Overall, links between macroinvertebrates and diatoms need to be taken into account when monitoring as they can influence each other through the food chain. This demonstrates the importance of biotic interactions in structuring a system and other ecosystems could be similarly influenced by biotic as well as abiotic interactions. Although the groups were not linked through responses to the same environmental gradient, environmental influences, e.g. disturbance events on one group, could act on the other through changes in structure and/or abundance that could be mediated through the food chain.

Appendix 1

Table A1: Species list of diatoms found in stream experiment 1. Relative abundances of diatoms species found at 2% or more in at least 1 stream section is given, in differently grazed treatments, with Baetidae as the mayfly grazer. Different letters mean significantly different by tukey's test, n.s. means non-significant.

Diatom species	**Average**				**ANOVA**		
	Baetidae	Control	Both	Hydrobiidae	R^2	P	F
Planothidium lanceolatum	0.39 (0.089)	0.49 (0.102)	0.32 (0.059)	0.41 (0.092)	n.s	n.s	n.s
Achananthidium minutissimum	64.31 (1.94)[a]	38.26 (2.54)[b]	51.53 (2.72)[c]	49.74 (2.02)[c]	33.47	<0.001	20.96
Encyonema silesiacum	0.22 (0.05)	0.35 (0.07)	0.34 (0.09)	0.23 (0.05)	n.s.	n.s.	n.s.
Gomphonema parvulum	1.87 (0.25)	2.65 (0.31)	2.1 (0.25)	3.02 (0.41)	4.09	<0.05	2.69
Reimeria sinuata	0.65 (0.10)	0.91 (0.16)	0.62 (0.08)	0.88 (0.18)	n.s	n.s	n.s
Asterionella formosa	2.48 (0.39)	3.01 (0.51)	2.65 (0.49)	2.02 (0.32)	n.s	n.s	n.s
Fragilaria capucina	12.52 (1.41)[a]	27.36 (1.43)[b]	19.10 (1.57)[c]	21.14 (1.13)[c]	31.19	<0.001	18.68
Synedra ulna	3.46 (0.63)[a]	17.10 (1.09)[b]	8.55 (1.03)[c]	11.18 (1.13)[c]	44.55	<0.001	32.87
Navicula minima	0.38 (0.12)	0.22 (0.06)	0.27 (0.12)	0.16 (0.05)	n.s	n.s	n.s.
Nitzschia capitellata	3.24 (0.41) [a]	2.63 (0.38)[a,b]	2.34 (0.30)[a,b]	1.85 (0.24) [b]	4.62	<0.05	2.91
Nitzshia draviensis	6.90 (0.96)[a,b]	3.48 (0.84)[b]	8.61 (1.40)[a]	6.63 (1.43)[a,b]	5.3	<0.05	3.22
Nitzschia paleacea	0.86 (0.24)	0.28 (0.13)	0.86 (0.28)	0.25 (0.10)	4.64	<0.05	2.93

Surirella brebissoni	0.44 (0.10)	0.39 (0.13)	0.32 (0.10)	0.35 (0.09)	n.s	n.s	n.s
Achnanthes oblongella	<1.0	<1.0	<1.0	<1.0	-	-	-
Cocconeis pedicus	<1.0	<1.0	<1.0	<1.0	-	-	-
Cocconeis placentula	<1.0	<1.0	<1.0	<1.0	-	-	-
Cymatopleura solea	<1.0	<1.0	<1.0	<1.0	-	-	-
Diploneis oblongella	<1.0	<1.0	<1.0	<1.0	-	-	-
Eunotia exigua	<1.0	<1.0	<1.0	<1.0	-	-	-
Frustulia vulgaris	<1.0	<1.0	<1.0	<1.0	-	-	-
Encyonema minutum	<1.0	<1.0	<1.0	<1.0	-	-	-
Gomphonema truncatum	<1.0	<1.0	<1.0	<1.0	-	-	-
Gomphonema pumilum	<1.0	<1.0	<1.0	<1.0	-	-	-
Rhoicosphenia abbreviata	<1.0	<1.0	<1.0	<1.0	-	-	-
Diatoma tenuis	<1.0	<1.0	<1.0	<1.0	-	-	-
Staurosira leptostauron	<1.0	<1.0	<1.0	<1.0	-	-	-
Hannea arcus	<1.0	<1.0	<1.0	<1.0	-	-	-
Meridion circulaire	<1.0	<1.0	<1.0	<1.0	-	-	-
Placoneis clemetis	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula cincta	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula lanceolata	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula lacentula	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia dissipata	<1.0	<1.0	<1.0	<1.0	-	-	-
Tryblionella levidensis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia linearis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia pussila	<1.0	<1.0	<1.0	<1.0	-	-	-
Surirrella angusta	<1.0	<1.0	<1.0	<1.0	-	-	-

Table A2: Species list of diatoms found in stream experiment 2. Relative abundances of diatoms species found at 2% or more in at least 1 stream section, in differently grazed treatments, with Ephemerellidae as the mayfly grazer. Different letters mean significantly different by tukey's test, n.s. means non-significant.

Diatom species	**Average (SE)**				**ANOVA**		
	Ephemerellidae	Control	Both	Hydrobiidae	R^2	P	F
Achananthidium minutissimum	61.13 (1.16)[a]	41.14 (2.05)[b]	56.28 (1.73)[a]	44.77 (2.24)[b]	39.07	>0.05	26.22
Encyonema silesiacum	0.642 (0.667)	0.62 (0.12)	0.58 (0.15)	0.896 (0.225)	n.s.	n.s.	n.s.
Gomphonema parvulum	0.823 (0.104)	1.12 (0.195)	1.04 (0.27)	1.329 (0.253)	n.s.	n.s.	n.s.
Asterionella formosa	0.237 (0.0779)	0.20 (0.062)	0.24 (0.08)	0.2175 (0.0442)	n.s.	n.s.	n.s.
Fragilaria capucina	14.09 (0.773)[a]	31.23 (1.23)[b]	17.0 (1.27)[a]	27.73 (1.08)[b]	57.97	<0.05	55.7
Synedra ulna	3.152 (0.417)[a]	6.99 (0.75)[b]	4.11 (0.47)[a]	5.890 (0.598)[b]	17.01	<0.05	9.13
Navicula minima	0.522 (0.101)	0.67 (0.13)	0.69 (0.11)	0.368 (0.102)	n.s	n.s.	n.s.
Nitzcshia capitellata	4.637 (0.424)	4.377 (0.54)	3.92 (0.36)	4.083 (0.519)	n.s.	n.s.	n.s.
Nitzschia draviensis	1.528 (0.232)	1.60 (0.30)	1.76 (0.30)	1.701 (0.348)	n.s.	n.s.	n.s.
Nitzschia paleacea	11.281 (0.808)	9.23 (0.87)	11.412(0.97)	9.924 (0.947)	n.s.	n.s.	n.s.
Achnanthes	<1.0	<1.0	<1.0	<1.0	-	-	-

oblongella							
Cocconeis pedicus	<1.0	<1.0	<1.0	<1.0	-	-	-
Eunotia exigua	<1.0	<1.0	<1.0	<1.0	-	-	-
Frustulia vulgaris	<1.0	<1.0	<1.0	<1.0	-	-	-
Encyonema minumtum	<1.0	<1.0	<1.0	<1.0	-	-	-
Amphora ovalis	<1.0	<1.0	<1.0	<1.0	-	-	-
Gomphonema truncatum	<1.0	<1.0	<1.0	<1.0	-	-	-
Gomphonema pumilum	<1.0	<1.0	<1.0	<1.0	-	-	-
Rhoicosphenia abbreviata	<1.0	<1.0	<1.0	<1.0	-	-	-
Diatoma tenuis	<1.0	<1.0	<1.0	<1.0	-	-	-
Staurosira leptostauron	<1.0	<1.0	<1.0	<1.0	-	-	-
Hannea arcus	<1.0	<1.0	<1.0	<1.0	-	-	-
Meridion circulaire	<1.0	<1.0	<1.0	<1.0	-	-	-
Placoneis clementus	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicuala cincta	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula lanceolata	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula lacentula	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia dissipata	<1.0	<1.0	<1.0	<1.0	-	-	-
Tryblionella levidensis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia linearis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia pussila	<1.0	<1.0	<1.0	<1.0	-	-	-
Surrirella angusta	<1.0	<1.0	<1.0	<1.0	-	-	-
S. brebissoni	<1.0	<1.0	<1.0	<1.0	-	-	-
Cocconeis placentula	<1.0	<1.0	<1.0	<1.0	-	-	-

Table A3: Species list of diatoms found in stream experiment 3. Relative abundances of diatoms species found at 2% or more in at least 1 stream section, in differently grazed treatments, with Heptagenidae as the mayfly grazer. Different letters mean significantly different by tukey's test, n.s. means non-significant.

Diatom species	**Average**				**ANOVA**		
	Heptagenidae spp	Control	Both	Hydrobiidae	R^2	P	F
Planothidium lanceolatum	0.51 (0.08)	0.70 (0.11)	0.85 (0.18)	0.588 (0.106)	n.s.	n.s.	n.s.
Achnanthidium minutissimum	59.50 (1.14)	33.31 (1.52)	54.78 (1.89)	37.54 (1.92)	59.92	<0.05	60.31
Encyonema silesicum	0.63 (0.12)	0.74 (0.115)	0.85 (0.18)	0.588 (0.106)	n.s.	n.s.	n.s.
Gomphonema parvulum	1.35 (0.20)	1.51 (0.35)	1.526 (0.32)	1.574 (0.330)	n.s.	n.s.	n.s.
Reimeria sinuata	0.62 (0.098)	0.57 (0.10)	0.66 (0.14)	0.684 (0.118)	n.s.	n.s.	n.s.
F. capucina	10.08 (0.62)	31.09 (1.56)	13.58 (0.85)	26.40 (1.71)	60.85	<0.05	62.65
Synedra ulna	2.25 (0.33)	8.85 (1.16)	2.56 (0.48)	8.17 (1.05)	29.96	<0.05	17.96
Navicula minima	1.60 (0.29)	1.77 (0.29)	1.80 (0.25)	1.584 (0.252)	n.s	n.s.	n.s.
Nitzschia capitellata	4.71 (0.45)	4.92 (0.34)	4.47 (0.47)	5.409 (0.433)	n.s.	n.s.	n.s.
Nitzschia draviensis	1.23 (0.19)	0.62 (0.11)	1.10 (0.24)	0.792 (0.197)	n.s.	n.s.	n.s.
Nitzschia paleacea	15.91 (1.15)	13.66 (1.09)	16.15 (1.27)	14.68 (1.24)	n.s.	n.s.	n.s.
Achnanthes oblongella	<1.0	<1.0	<1.0	<1.0	-	-	-
Cocconeis pedicus	<1.0	<1.0	<1.0	<1.0	-	-	-
C. placentula	<1.0	<1.0	<1.0	<1.0	-	-	-
Diploneis oblongella	<1.0	<1.0	<1.0	<1.0	-	-	-
Epithemia adnata	<1.0	<1.0	<1.0	<1.0	-	-	-

Eunotia bilunaris	<1.0	<1.0	<1.0	<1.0	-	-	-
Amphora ovalis	<1.0	<1.0	<1.0	<1.0	-	-	-
Frustulia vulgaris	<1.0	<1.0	<1.0	<1.0	-	-	-
Encyonema minutum	<1.0	<1.0	<1.0	<1.0	-	-	-
Gomphonema truncatum	<1.0	<1.0	<1.0	<1.0	-	-	-
Gomphonema pumilum	<1.0	<1.0	<1.0	<1.0	-	-	-
Rhoicosphenia abbreviata	<1.0	<1.0	<1.0	<1.0	-	-	-
Asterionella formosa	<1.0	<1.0	<1.0	<1.0	-	-	-
Diatoma hyemale	<1.0	<1.0	<1.0	<1.0	-	-	-
Diatoma tenuis	<1.0	<1.0	<1.0	<1.0	-	-	-
Meridion circulaire	<1.0	<1.0	<1.0	<1.0	-	-	-
Tabellaria floculosa	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula cincta	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula lanceolata	<1.0	<1.0	<1.0	<1.0	-	-	-
Navicula subrotundata	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia dissipata	<1.0	<1.0	<1.0	<1.0	-	-	-
Tryblionella levidensis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia linearis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia pussila	<1.0	<1.0	<1.0	<1.0	-	-	-
Surrirella angusta	<1.0	<1.0	<1.0	<1.0	-	-	-
Surrirella brebissoni	<1.0	<1.0	<1.0	<1.0	-	-	-

Table A4: Species list of diatoms found in stream experiment with 'moderate/poor' diatom assemblage. Relative abundances of diatoms species from the 'poor/moderate' starting assemblage found at 2% or more in at least 1 stream section, in differently grazed treatments, with Baetidae as the mayfly grazer. Different letters mean significantly different by tukey's test, n.s. means non-significant.

Diatom species	**Average (standard error)**				**ANOVA**		
	Baetis spp	Control	Both	Hydrobidae	R^2	P	F
Planothidium lanceolatum	4.306 (0.405)	3.335 (0.379)	3.703 (0.320)	3.589 (0.271)	n.s.	n.s.	n.s.
Achananthidium minutissimum	52.32 (2.46)^{a,b}	47.80 (1.88)^{a}	55.45 (1.90)^{b}	51.59 (1.42)$^{a.b}$	3.88	0.055	2.6
Cocconeis pedicus	0.664 (0.117)	0.600 (0.114)	0.448 (0.101)	0.417 (0.083)	n.s.	n.s.	n.s.
Navicula gregaria	8.137 (0.720)	7.408 (0.576)	7.082 (0.65)	7.239 (0.461)	n.s.	n.s.	n.s.
Navicula lanceolata	10.44 (1.20)	9.807 (0.923)	7.890 (0.687)	9.006 (0.719)	n.s.	n.s.	n.s
Amphora pedicus	1.265 (0.158)^{a}	2.720 (0.356)^{b}	1.361 (0.216)^{a}	2.389 (0.122)^{b}	18.46	<0.001	9.98
Gomphonema pumilum	0.356 (0.087)	0.473 (0.134)	0.159 (0.05)	0.3057 (0.0572)	n.s.	n.s.	n.s.
Gomphonema angustum	0.369 (0.0657)^{a}	0.864 (0.161)^{b}	0.4983 (0.0907)^{b}	0.521 (0.101)^{b}	6.29	<0.05	3.66
Rhoicosphenia abbreviata	0.1227 (0.0437)	0.278 (0.088)	0.127 (0.0465)	0.1667 (0.0516)	n.s.	n.s.	n.s.
Fragilaria	0.959 (0.155)^{a}	4.263 (0.336)^{b}	1.505	3.250 (0.180)^{c}	54.95	<0.001	49.38

vaucherie			(0.145)[a]				
Synedra ulna	0.1897 (0.0457)[a]	0.936 (0.151)[b]	0.4097 (0.081)[b]	0.7043 (0.0951)[b]	19.46	<0.001	10.59
Diatoma problematica	0.365 (0.069)	0.370 (0.082)	0.243 (0.058)	0.484 (0.105)	n.s.	n.s.	n.s.
Diatoma vulgare	0.384 (0.0815)	0.437 (0.086)	0.382 (0.083)	0.476 (0.071)	n.s.	n.s.	n.s.
Navicula minima	5.807 (0.327)	5.547 (0.440)	6.530 (0.526)	5.967 (0.330)	n.s.	n.s.	n.s.
Nitzschia capitellata	3.912 (0.278)	3.944 (0.325)	4.243 (0.324)	4.052 (0.260)	n.s.	n.s.	n.s.
Nitzschia dissipata	0.703 (0.145)	0.798 (0.121)	0.742 (0.152)	0.760 (0.112)	n.s.	n.s.	n.s.
Nitzschia paleacea	5.270 (0.327)	5.687 (0.500)	6.141 (0.584)	5.680 (0.402)	n.s.	n.s.	n.s.
Nitzschia pussilla	1.353 (0.149)	1.920 (0.333)	1.147 (0.326)	1.396 (0.341)	n.s.	n.s.	n.s
Surrirella brebissoni	0.998 (0.173)	0.987 (0.143)	0.682 (0.118)	0.836 (0.112)	n.s.	n.s	n.s.
Nitzschia bergii	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia linearis	<1.0	<1.0	<1.0	<1.0	-	-	-
Nitzschia palea	<1.0	<1.0	<1.0	<1.0	-	-	-
Diatoma vulgaris	<1.0	<1.0	<1.0	<1.0	-	-	-
Encyonema minutum	<1.0	<1.0	<1.0	<1.0	-	-	-
Encyonema silasiacum	<1.0	<1.0	<1.0	<1.0	-	-	-
Reimeria sinata	<1.0	<1.0	<1.0	<1.0	-	-	-
Rhoicosphenia abbreviata	<1.0	<1.0	<1.0	<1.0	-	-	-
Surrirella brebissoni	<1.0	<1.0	<1.0	<1.0	-	-	-

Appendix 1.1 Preliminary water quality experiment

Can a 'poor/moderate' diatom assemblage be maintained in laboratory streams in 'good' water for 2 weeks?

Table A5: Water chemistry for sites used for water and diatoms in experiment on grazer influences on diatom assemblages indicating poor water quality.

River	River Rivelin ("good" diatoms used in previous study)	River Don ("Poor" diatoms)	River Hipper (Rother catchment – "poor" diatoms)
Phosphate (mg/l PO_4)	0.20	0.46	0.72
Phosphate (mg/l P)	0.066 (Low)	0.15 (Moderate – High)	0.24 (High)
Nitrate (mg/l N)	0.035	0.040	0.13
Nitrate (mg/l NO_3)	0.15 (1)	0.18 (1)	0.57 (1)
Nitrite (mg/l N)	0.030	0.034	0.051
Ammonia (mg/l N)	0.11	0.07	0.30

Alkalinity (mg/l $CaCO_3$)	60	30	95

- River Rivelin has lower values for all nutrients but has higher alkalinity than the river don
- Phosphate concentrations are high/high to moderate for the sites with "poor" diatom assemblage but low for the good quality site
- Nitrate is 1 (lowest for E.A. classification) for all sites but is higher for the poorer sites.

Diatom seeding solution (Composition of diatom assemblage at beginning of trial)

- Diatom trait make up
- 27.23% Prostrate
- 54.44% Motile
- 14.74% Stalked
- 3.59% Erect

- TDI – 63.33

- 31 different species present
- 15 different genera present
- 12 species make up more than 1% of the assemblage

- Dominant species:
- *Navicula lanceolata* (Motile)
- *Achnanthidium minutissimum* (Prostrate)
- *Navicula gregaria* (Motile)
- *Amphora pedicus* (Erect)
- *Planothidium lanceolatum* (Prostrate)

Initial streams after 1 week

- Average TDI for "Good" water was 55.96; Average TDI for "bad" water was 54.87.
- There was no significant difference between the TDI for diatom assemblages grown in different qualities of water.
- Average high profile diatoms for "good" water were 19.88; Average for "poor" water was 13.95.
- There was no significant difference between Percentage high profile diatoms for diatom assemblages grown in different qualities of water.
- Average motile diatoms for "good" water were 59.28; Average for "poor" water was 59.88.
- There was no significant difference between percentage motile diatoms for diatom assemblages grown in different qualities of water.

- Figure 7.1 shows the distribution of growth forms of diatoms grown in either "good" quality water or "poor" quality water.

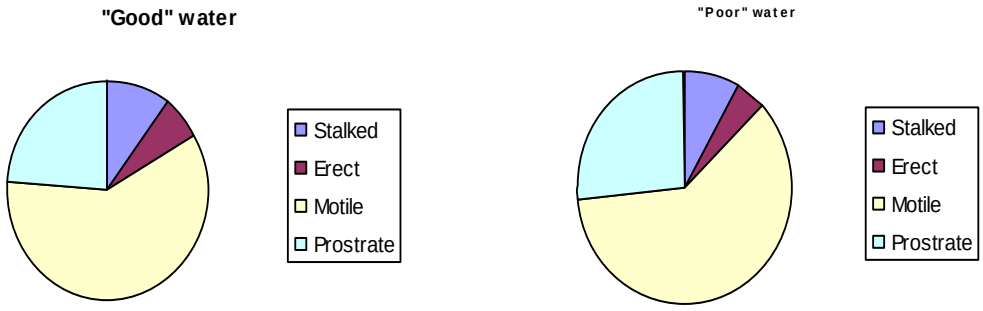

Figure A1: Trait composition of diatoms in assemblage grown in good or poor water after 1 week growth in artificial streams.

- Dominant species for diatom assemblages grown in "good" water were: *Achnanthidium minutissimum, Navicula gregaria, Navicula minima, Amphora pedicus, Navicula* lanceolata and *Surrirella brebissoni*.
- Dominant species for diatom assemblages grown in "poor" water were: *Achananthidium minutissimum, Navicula gregaria, Navicula minima, Amphora pedicus, Nitzschia capitellata* and *Surirella brebissoni*.
- The maximum number of species found in a "good" sample was 30 and the minimum was 26 (Genera 16 and 13).
- The maximum number of species found in a "poor" samples was 26 and the minimum was 21 (Genera 14 and 10).

Initial streams after 2 weeks

- Average TDI for "Good" water was 57.53; Average TDI for "bad" water was 58.29.
- There was no significant difference between the TDI for diatom assemblages grown in different qualities of water.
- Average high profile diatoms for "good" water were 20.38; Average for "poor" water was 17.58.
- There was no significant difference between Percentage high profile diatoms for diatom assemblages grown in different qualities of water.
- Average motile diatoms for "good" water were 56.86; Average for "poor" water was 67.26.
- There was a significant difference between percentage motile diatoms for diatom assemblages grown in different qualities of water. There was significantly more motile diatoms when the assemblage was grown in "poor" water ($P = 0.004$)
- Figure 7.2 shows the distribution of growth forms of diatoms grown in either "good" quality water or "poor" quality water.

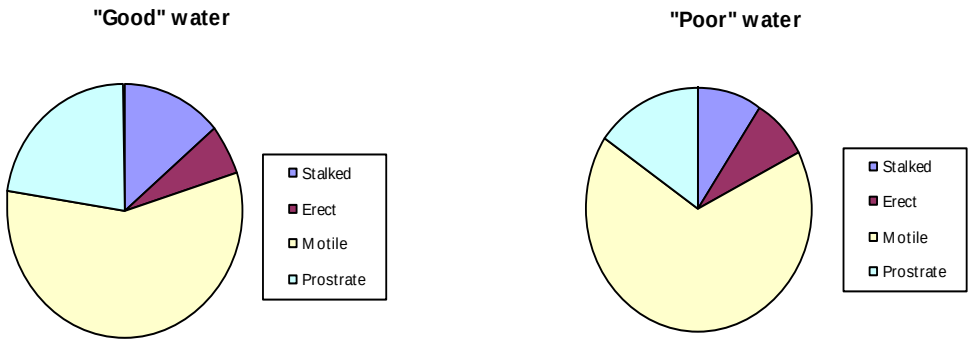

Figure 7.2: Trait composition of diatoms in assemblage grown in good or poor water after 2 weeks growth in artificial streams.

- Dominant species for diatom assemblages grown in "good" water were: *Achnanthidium minutissimum, Navicula gregaria, Navicula minima, Amphora pedicus, Navicula lanceolata, Nitzshia palea* and *Surirella brebissoni*.
- Dominant species for diatom assemblages grown in "poor" water were: *Navicula gregaria, Navicula lanceolata, Navicula minima, Amphora pedicus, Nitzshia capitellata, Nitzshia palea, Achnanthidium minutissimum* and *Surirella brebissoni*.
- The maximum number of species found in a "good" sample was 28 and the minimum was 24 (Genera 14 and 12).
- The maximum number of species found in "poor" samples was 28 and the minimum was 26 (Genera 14 and 11).

Conclusions

- TDI is similar after 2 weeks growth, whether grown in "good" or "poor" water.
- Percentage high profile diatoms is similar between the two treatments
- Motile diatoms make up significantly more of the assemblage when grown in "poor" water (by approx. 10%)
- Assemblage grown in "good" water would still be classified as poor after 2 weeks growth from the seeding solution being put into artificial streams
- Assemblages remain similar to the seeding solution.
- Dominant species are similar between the two treatments
- In this closed system, for a short term experiment a "poor" diatom assemblage can be maintained in "good" quality water with only minor structural changes.

Appendix 2

Table A6: Diatom assemblage at naturally grazed (un-caged) treatments at 10 sites.

	Peakshole water			Rivelin			Loxley			River Noe			Sheath			Porter brook			Storrs brook	Oldhay brook			South of fulwood hall			Upstream of damflask		
Rep	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Achnanthidium biasoletiana	0	0	0	7	1	0	0	4	2	8	2	2	2	1	2	2	0	1	1	0	2	1	3	2	1	1	0	0	0
Achnanthes oblongella	0	1	0	55	12	29	16	13	1	59	0	6	11	1	1	3	4	2	4	1	9	16	13	12	39	27	2	1	0
Achnanthes daui	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	5	0	0	0	0
Planothidium delicatulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planothidium lanceolatum	10	43	17	6	4	7	3	1	1	14	13	9	12	5	9	6	10	0	9	17	2	1	4	8	37	17	7	4	1
Achnanthidium minutissimum	165	85	132	97	147	118	124	140	131	77	148	140	63	92	27	24	36	123	117	138	74	73	57	106	91	88	105	103	140
Caloneis silicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticular accomoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis pediculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis plancentula	4	11	9	11	11	30	25	19	0	14	0	10	86	95	144	99	76	24	22	9	104	82	104	21	8	5	16	11	3
Eunotia bilunaris	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0	0	0	2	1	0	1	0	2	0	2
Eunotia exigua	0	0	0	1	1	3	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	5	3	0	0	2	0	4	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia bebisoni	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0	1	0	0	1	0	0	0	0	1	1	1	0	4	1
Stauroneis pseudosubobstusides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pinularia rupestris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0
Diploneis oblongella	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Diploneis ovalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0
Navicular angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicular cari	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Navicular capitatoradiata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A6. continued

Navicula cinta	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	11	4	5	6	0	1	4	7	7	2	3	4	5	0	0	11	17	4	11	4	1	2	7	12	3	5	31	10	10	
Navicula ignita	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula lanceolata	3	2	5	6	1	2	11	21	39	4	5	6	6	3	3	13	18	4	8	2	4	3	5	5	2	9	10	8	9	
Navicula minima	10	18	10	6	3	4	0	3	1	4	5	5	5	10	0	6	1	12	6	7	2	1	0	4	6	4	1	2	4	
Navicula protractor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
Navicula tripunctata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	3	0	0	0	0	1	0	0	0	0	0	0	0	
Nitzschia bergii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Nitzschia capitelata	9	29	6	11	10	2	6	16	45	5	0	5	3	2	1	3	5	10	7	10	4	12	10	7	0	6	6	10	7	
Nitzschia draveienis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	
Nitzschia dissipata	1	3	1	2	0	1	2	2	13	4	3	7	0	5	0	1	0	0	3	0	0	3	0	1	0	0	4	5	0	
Nitzschia hantzschina	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	3	0	0	0	
Nitzschia inconspicua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Nitzshia lacuum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Nitzschia ignita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Tryblionella levidensis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	2	0	0	
Nitzschia linearis	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	
Nitzschia paleacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Nitzschia palea	1	5	0	1	6	4	3	4	0	4	16	5	0	0	0	0	5	11	0	2	1	0	1	5	3	3	1	4	0	
Nitzchia pusila	0	0	0	0	0	1	1	0	0	0	0	0	0	3	0	2	4	0	0	2	2	1	0	0	1	1	0	0	0	
Nitzschia sociabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Sellaphora pupula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Surirella amphyioxis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Surirella angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	
Suriella brebisoni	4	5	0	1	0	1	3	3	8	0	1	0	1	1	1	6	1	3	5	1	1	0	1	0	0	2	2	0	1	
Surirella pinnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Amphora ovalis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Amphora montana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Amphora pediculus	2	11	2	3	2	3	0	1	4	3	2	7	6	0	5	37	23	2	10	3	3	2	1	0	19	11	3	2	0	
Asterionella spp	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	1	0	0	0	0	
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table A6. continued

Cymbella affinis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella caespitosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella gracilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cymbella microcephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella pusilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema silesiaca	15	11	7	2	0	2	3	4	1	3	9	3	5	2	0	1	1	0	3	1	2	0	1	0	0	1	1	2	4		
Gomphonema parvalum	0	1	1	2	6	3	0	1	2	4	6	4	0	0	0	2	0	4	6	4	0	2	1	0	4	3	2	1	4		
Gomphonema pumilum	7	9	13	0	0	0	0	0	0	2	0	0	3	1	1	3	6	1	2	2	0	0	0	7	1	0	1	2	0		
Gomphonema truncatum	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0		
Encyonema minutum	0	0	0	0	0	2	0	1	3	1	0	0	0	3	0	1	0	0	3	0	1	0	0	4	1	1	3	2	1		
Diatom hyemale	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0		
Diatoma vulgaris	0	0	1	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Diatoma problematica	0	0	0	0	0	0	5	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Diatoma tenuis	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Meridion circulare	0	0	4	0	0	2	0	0	0	0	1	3	0	0	0	1	1	1	1	0	0	0	1	1	0	1	0	0	2		
Fragilaria arcus	0	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
F. capucina	5	10	6	12	7	10	9	7	6	1	7	5	3	2	0	2	3	4	4	8	8	10	9	3	4	6	8	6	5		
F. vaucherie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Synedra ulna	1	1	0	6	5	5	2	1	1	0	2	2	1	0	0	2	3	3	1	4	1	2	2	3	6	2	5	4	4		
Staurosira leptostauron	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1		
Tabellaria floculossa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0		
Rhoicosphenia abbreviata	1	0	0	0	0	0	0	1	0	0	0	0	2	0	0	2	4	0	1	0	0	0	0	0	0	0	0	1	1		
Reimeria sinuata	3	18	5	0	1	4	0	1	0	29	6	2	5	7	5	2	0	7	2	1	5	4	5	7	7	3	1	2	2		

Table A7: Diatom assemblage at grazed (caged) treatments at 10 sites.

Site	Peakshole water			Rivelin		Loxley			River Noe			Sheath			Porter brook			Storrs brook			Oldhay brook		South of fulwood hall		Upstream of damflask		
Rep	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**1**	**1**	**1**	**2**	**3**
Achnanthidium biasolettiana	0	0	0	0	0	4	2	4	4	4	5	1	1	0	0	0	0	1	3	1	0	3	0	2	0	0	0
Achnanthes oblongella	0	0	0	17	16	21	9	15	16	66	44	3	3	2	3	0	2	1	2	0	10	48	17	10	0	0	0
Achnanthes daui	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Planothidium delicatula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planothidium lanceolatum	25	24	45	4	10	8	5	1	10	13	9	14	3	4	12	5	10	11	10	4	5	8	12	3	1	7	4
Achnanthidium minutissimum	138	160	106	85	95	134	160	156	108	64	124	22	19	7	41	34	53	115	134	152	65	64	116	133	143	143	109
Bacillaria paxillifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis amphisbaena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis molaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Camphylodiscus hibernicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticular accomoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis pediculus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis plancentula	10	11	11	10	4	6	1	6	16	27	28	143	181	110	95	127	69	15	24	23	98	98	29	8	2	6	4
Neidium binodeforme	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hannaea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Denticula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithemia adnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia bilunaris	0	0	0	2	6	0	1	0	1	0	0	2	1	0	0	0	0	0	0	0	0	1	1	0	1	4	1
Eunotia exigua	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	4
Placoneis clementis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia bebisoni	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0
Pinularia subgibba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A7. continued

Pinularia rupestris	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0		1	0	1	0
Pinnularia subcapita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia pectinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithemia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia rhombiscus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia cruzburgensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia saxena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Navicula angustula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula atomus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cari	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
Navicula capitatoradiata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cinta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptonella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	9	1	8	1	0	4	3	8	5	1	2	6	1	1	11	3	20	10	11	4	9	0	7	7	9	8	29
Navicula ignita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula lancelata	4	2	7	0	4	5	9	11	5	2	0	8	9	1	15	5	10	3	4	3	8	1	4	0	6	8	16
Navicula lenzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula novaesiberica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicular minima	6	17	8	5	5	6	0	1	2	3	4	0	1	1	2	0	1	23	11	10	0	2	1	3	1	2	2
Navicula oligatrapheta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula protractor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1
Navicula reinharti	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula recens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula rhynchotella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula tripunctata	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Navicular splendica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula veneta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia bergii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia capitelata	20	5	3	8	12	11	11	4	10	3	0	3	0	3	2	2	7	10	11	6	9	2	4	8	5	10	11
Nitzschia debilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzcshia draveienis	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0

Table A7. continued

Nitzschia dissipata	2	9	4	0	1	3	4	3	3	2	1	0	0	0	0	0	1	2	1	0	1	1	0	0	4	4	11
Nitzschia flexa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzshia frustulium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschicia hantzschina	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschis inconspicua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia lacuum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia ignita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryblionella levidensis	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Nitzscia linearis	2	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1	1	0	2	1	0	1	0	1	0	1	1
Nitzschia paleaformis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia paleacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	1	7	8	1	0	0	10	8	4	0	0	0	3	0	3	12	5	4	5	1	3	1	1	2	6
Nitzschia pusila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	2	0	1	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sigma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sociabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellaphora bacillum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellaphora pupula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellaphora seminulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis pseudosubobstusides	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella amphyioxis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella angusta	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella crumena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surrirella linearis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suriella terricola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suriella brebisoni	8	6	4	0	2	7	2	0	0	1	0	3	0	0	1	0	2	0	4	3	0	0	0	0	0	1	1
Surirella pinnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora libica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	4	1	3	1	4	4	1	1	6	0	2	11	2	1	25	15	18	4	1	3	1	2	3	14	1	2	1
Asterionella spp	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	2	0
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella affinis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A7. continued

Cymbella caespitosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella gracilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	4
Cymbella crocephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella pusilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella silesiaca	2	8	5	1	3	4	7	4	1	0	1	0	2	0	0	0	0	0	4	0	1	0	1	0	2	3	3	
Cymbella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonma silesiacum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enconema gracile	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema minutum	1	1	1	2	3	1	7	6	0	1	0	1	1	0	0	0	0	0	0	0	5	1	1	1	0	0	2	
Gomphonema angustum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abbreviata	0	0	0	0	1	0	0	1	1	0	0	0	1	0	5	2	1	0	0	0	0	0	0	0	0	0	0	0
Reimeria sinata	4	1	5	0	0	2	0	0	14	29	4	2	0	7	3	0	1	2	0	0	1	12	1	0	2	0	0	
Gomphonema acuminatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaccum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema minutum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema parvalum	0	0	1	3	4	2	1	0	6	2	6	2	1	1	3	2	1	0	1	2	1	0	4	2	7	3	8	
Gomphonema pumilum	2	2	2	0	0	0	0	1	2	0	1	2	0	0	7	2	0	2	0	0	0	0	0	2	0	0	0	
Gomphoneam truncatum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Diatom hyemale	1	2	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	
Diatoma vulgaris	1	0	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
Diatoma problematica	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Diatoma tenuis	0	0	0	0	0	2	0	3	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	
Diploneis oblongella	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	
Diploneis ovalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fragilaria arcus	0	0	0	0	0	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fragilaria capucina	5	6	8	3	15	12	6	9	3	3	2	2	2	1	1	1	2	2	2	2	7	2	5	2	4	7	11	
F. vaucherie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Synedra ulna	4	1	4	2	4	1	3	0	1	1	0	2	1	0	2	3	2	1	1	0	1	3	2	3	6	3	2	

Synedra parisitica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria pinnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurosira leptostauron	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Meridion circulare	6	1	5	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	3	3	1			
Tabellaria floculossa	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Tabellaria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			

Table A8: Diatom assemblage at un-grazed (caged) treatments at 10 sites.

Site	**Site**	**Peakshole water**			**Rivelin**			**Loxley**			**River Noe**			**Sheath**			**Porter brook**			**Storrs brook**			**Oldhay brook**			**South of fulwood hall**			**Upstream of damflask**		
	Rep	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Achnanthidium biasoletiana		0	0	0	2	1	1	5	3	2	7	2	9	1	0	0	0	0	0	3	1	0	2	5	4	0	1	0	0	0	
Achnanthes oblongella		0	0	0	12	16	21	20	46	25	29	6	108	6	10	18	1	2	1	0	3	0	15	21	14	10	11	0	0	0	
Achnanthes daui		0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
Planothidium delicatula		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	
Planothidium lanceolatum		33	25	16	4	8	3	5	2	2	6	4	3	0	5	4	4	18	13	22	10	4	13	6	7	11	11	5	4	2	
Achnanthidium minutisimum		111	96	108	121	110	110	100	74	97	108	95	75	24	51	72	41	32	31	90	80	110	36	39	36	137	130	104	142	99	
Bacillaria paxillifer		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Caloneis amphisbaena		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Caloneis molaris		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Caloneis silicula		0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	
Camphylodiscus hibernicus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Craticula accomoda		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cocconeis pediculus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cocconeis plancentula		5	6	6	17	6	8	5	5	6	14	54	7	176	109	95	60	82	49	16	68	23	105	105	103	7	4	15	3	3	
Denticular		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Diploneis		0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	

Table A8. continued

oblongella																													
Diploneis ovalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema silesiacum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enconema gracile	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema minutum	0	0	0	6	3	1	5	5	5	0	0	1	0	1	2	0	0	0	1	0	0	0	0	0	3	3	1	0	3
Epithemia adnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia bifunaris	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	6	2	1
Eunotia exigua	0	0	0	0	3	1	2	0	0	0	0	0	0	0	4	0	0	0	0	0	0	1	1	0	0	0	4	2	3
Eunotia pectinalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithemia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia rhombiscus	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia cruzburgensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia saxena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	3	2
Gyrosigma acuminata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hannaea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula angustula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula atomus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cari	4	2	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula capitatoradiata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cinta	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptonella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	8	4	6	5	7	9	10	2	2	3	0	1	0	4	3	12	14	17	12	2	8	1	2	2	4	6	14	6	28
Navicula ignita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula lancelata	5	6	12	6	4	3	2	10	4	0	2	0	3	12	6	8	8	17	2	2	6	4	0	3	1	3	11	6	19
Navicula lenzi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula novaesiberica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula minima	21	16	16	4	4	2	2	1	2	4	4	1	2	1	3	6	0	1	8	6	3	2	3	1	4	3	4	3	6
Navicula oligatrapheta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A8. continued

Navicula protractor	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0
Navicula reinharti	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula recens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula rhynchotella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula tripunctata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0
Navicula splendica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicular veneta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neidium binodeforme	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia bergii	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia capitelata	7	9	3	3	14	9	0	7	6	1	9	2	1	4	5	4	5	1	15	8	7	1	3	8	4	5	13	6	16
Nitzschia debilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitszcshia draveienis	0	0	0	0	0	0	0	1	1	0	4	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Nitzschia dissipata	17	12	14	0	1	0	1	4	1	1	5	1	0	0	0	2	0	0	1	0	0	1	2	0	1	0	3	3	12
Nitzschia flexa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia hantzschina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	1	0	0	0
Nitzschia inconspicua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia lacuum	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Nitzschia ignita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Tryblionella levidensis	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzshcia linearis	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0
Nitzschia paleaformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia paleacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	4	2	11	1	3	3	2	7	4	0	1	0	0	1	0	0	6	4	0	0	1	3	4	0	0	1
Nitzschia pusila	0	0	0	1	5	1	0	1	0	0	0	0	0	0	0	4	2	0	3	1	0	1	0	0	3	0	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sigma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sociabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A8. continued

Placoneis clementis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia bebisoni	0	0	0	0	1	1	0	0	1	1	0	0	0	2	0	1	0	1	2	0	0	0	0	0	0	1	2	0	2
Pinularia subgibba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinularia rupestris	0	0	0	0	0	0	0	0	0	0	0	0		0	0	2	0	1	0	0	0	0	0	0	5	0	1	1	0
Pinnularia subcapita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellaphora bacillum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sellaphora pupular	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Sellaphora seminulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis pseudosubobstus ides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella amphyioxis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Surirella crumena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Surrirella linearis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suriella terricola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Suriella brebisoni	3	3	5	4	2	0	2	1	1	0	0	0	0	3	2	2	1	4	2	3	5	0	2	1	0	0	1	1	0
Surirella pinnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora libica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	8	6	8	5	0	1	3	4	0	6	8	4	0	3	0	0	43	64	0	7	4	5	6	5	8	12	0	3	0
Amphora montana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Asterionella spp	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	3	0	2	0	0	1	0	0	0	1	0	0	0	0
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella affinis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella caespitosa	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	8	0	3	0	0	0	0	0	0	0	0	0	0	0
Cymbella gracilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	5	3	5
Cymbella microcephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella pusilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A8. continued

Cymbella silesiaca	26	13	13	5	4	1	3	8	2	2	2	2	0	2	1	0	0	0	0	0	5	2	0	0	1	1	5	2	5
Cymbella spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom hyemale	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Diatoma vulgaris	1	1	1	0	0	0	3	0	3	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	1	0	0	0	0
Diatoma problematica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatoma tenuis	0	0	0	0	0	2	10	7	1	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	1	0	0	0	0
Fragilaria arcus	0	0	0	3	2	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina	22	19	18	22	27	22	45	35	33	8	8	4	13	17	21	11	13	11	12	22	14	25	10	17	11	15	23	30	21
F.vaucherie	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synedra ulna	5	5	1	31	14	14	5	4	11	6	6	1	0	6	9	4	5	7	11	4	8	2	8	6	7	10	12	7	5
Fragilaria parisitica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurosira pinnata	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurosira leptostauron	0	0	0	0	3	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0
Gomphonema angustum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema acuminatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema minutum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema parvalum	0	4	0	3	4	5	4	1	3	3	0	3	0	0	1	4	0	1	0	2	2	2	2	2	9	7	2	7	9
Gomphonema pumilum	7	14	6	0	0	0	1	0	0	2	0	0	0	0	1	10	11	3	0	2	0	2	0	0	0	0	0	0	0
Gomphoneam truncatum	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Meridion circulare	11	7	12	1	0	3	2	1	1	0	0	0	0	0	0	2	1	2	1	1	4	0	1	0	0	1	4	1	2
Rhoicosphenia abreviata	0	0	1	0	0	0	0	0	1	1	1	0	0	1	0	0	6	5	1	0	0	0	1	0	0	0	0	0	0
Reimeria sinata	1	1	0	1	1	0	0	0	0	16	4	10	0	5	0	0	9	0	1	2	2	2	5	2	5	4	1	3	2
Tabellaria floculossa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Tabellaria spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3

Table A9. Average physical and chemical characteristics of sites sampled in July 2006, standard deviations in brackets.

Site	Width (cm)	Depth (cm)	Flow (m/s)	Alkalinity (mg/L)	Phosphate (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	pH(pH units)	Conductivity (µS)	Temperature (˚C)	Dissolved oxygen content (mg/L)
Alton	173 (49.33)	12 (2.64)	0.06 (0.02)	168.0 (0.0)	0.36 (0.02)	0.0 (0.0)	0.007 (0.001)	7.05 (0.02)	337.7 (7.51)	19.20 (0.26)	8.07 (0.06)
Hipper	713 (35.12)	13 (2.64)	0.14 (0.06)	57.33 (2.31)	0.11 (0.01)	0.01 (0.0)	0.012 (0.001)	7.46 (0.01)	687 (12.17)	19.13 (0.25)	10.03 (0.06)
Netherloads	100 (10)	11 (4.17)	0.04 (0.02)	228.0 (5.0)	0.17 (0.08)	0.06 (0.0)	0.025 (0.0)	6.90 (0.01)	202 (3.61)	18.30 (0.0)	5.93 (0.06)
Lathkill	657 (7.64)	35 (7.21)	0.13 (0.06)	164.67 (2.89)	0.17 (0.01)	0.16 (0.03)	0.001 (0.0)	8.16 (0.02)	493.7 (2.08)	15.10 (0.0)	16.33 (0.06)
Peakshole water	413 (15.28)	10 (1.91)	0.07 (0.02)	181 (3.46)	0.02 (0.0)	0.00 (0.0)	0.003 (0.0)	7.27 (0.06)	383.67 (19.55)	13.90 (0.0)	11.17 (0.23)
Redleadmill brook	153 (15.28)	10 (2.5)	0.07 (0.01)	173.0 (0.0)	0.14 (0.0)	0.0 (0.0)	0.008 (0.001)	7.73 (0.01)	415 (17.78)	16.80 (0.17)	9.03 (0.06)
River Noe	657 (92.92)	24 (13.1)	0.24 (0.23)	32.67 (4.62)	0.12 (0.0)	0.13 (0.0)	0.0 (0.0)	8.18 (0.03)	136 (20.78)	15.87 (0.06)	10.93 (0.32)
Oughtibridge	333 (15.28)	8.3 (3.02)	0.15 (0.09)	38.67 (1.15)	0.37 (0.01)	0.31 (0.08)	0.004 (0.0)	7.82 (0.07)	230 (1.73)	16.70 (0.0)	9.60 (0.53)
South of fulwood hall	260 (26.46)	19 (6.01)	0.13 (0.04)	56.0 (0.0)	0.20 (0.02)	0.49 (0.14)	0.05 (0.0009)	6.44 (0.01)	717 (0.0)	13.50 (0.0)	9.97 (0.15)
River Wye	693 (12.58)	26 (12.29)	0.26 (0.27)	136.67 (2.89)	0.27 (0.01)	0.63 (0.04)	0.004 (0.0)	7.72 (0.00)	439 (9.64)	15.73 (0.06)	14.40 (0.26)
Stubbing court	252 (2.89)	12 (3.54)	0.03 (0.02)	298.33 (7.64)	0.06 (0.01)	0.22 (0.0)	0.009 (0.001)	6.97 (0.01)	425.67 (0.58)	13.87 (0.06)	10.17 (0.06)
Brindwoodgate	53 (7.22)	7.22(2.64)	0.05 (0.05)	253.33 (2.89)	0.27 (0.04)	0.25 (0.08)	0.09 (0.007)	7.80 (0.00)	334 (4.0)	18.77 (0.06)	9.47 (0.06)

Table A9. continued

Berrymoor	162 (14.43)	5.2 (2.39)	0.11 (0.12)	222.0 (6.56)	0.27 (0.01)	0.16 (0.01)	0.001 (0.0)	7.60 (0.02)	416 (14.0)	17.97 (11.2)	11.13 (0.06)
Rivelin	200 (55.68)	26 (16.16)	0.3 (0.13)	44.67 (12.7)	0.05 (0.02)	0.0 (0.0)	0.12 (0.043)	7.72 (0.02)	113.33 (0.58)	16.8 (0.72)	9.93 (0.06)
Milthorpe	255 (13.23)	16 (1.32)	0.09 (0.04)	193.67 (40.08)	0.21 (0.06)	0.34 (0.03)	0.08 (0.033)	8.43 (0.06)	194.33 (1.53)	16.03 (0.06)	10.17 (0.06)
Brookside beck	170 (10)	11 (4.17)	0.09 (0.06)	181 (3.46)	0.29 (0.01)	0.14 (0.0)	0.017 (0.001)	7.90 (0.01)	377.0 (0.0)	16.73 (0.06)	9.47 (0.06)
Holymoorside	227 (66.58)	24 (11.4)	0.12 (0.09)	86.67 (2.89)	0.01 (0.01)	0.0 (0.0)	0.007 (0.0)	6.52 (0.03)	150.33 (0.58)	15.70 (0.0)	9.90 (0.0)
Loxley	997 (41.63)	47 (10.9)	0.39 (0.17)	72.67 (12.5)	0.17 (0.01)	0.5 (0.07)	0.007 (0.008)	7.74 (0.02)	182.67(20.65)	13.13 (0.25)	11.50 (0.0)
Redmires	330	42 (7.95)	0.42 (0.17)	42.0 (0.0)	0.06 (0.02)	0.12 (0.05)	0.001(0.001)	8.46 (0.05)	150 (0.0)	17.03 (0.55)	10.73 (0.06)
Upstream of Damflask	403 (159.48)	41 (15.77)	0.19 (0.26)	130.67 (12.10)	0.07 (0.05)	0.33 (0.12)	0.007 (0.0)	7.49 (0.02)	190.67 (3.21)	13.60 (0.0)	11.63 (0.15)
Thurgoland	830 (20)	44 (12.44)	1.41 (0.91)	60.33 (4.51)	0.89 (0.57)	0.90 (0.05)	0.016 (0.003)	6.70 (0.03)	1009.33(7.23)	12.13 (0.15)	9.20 (0.26)
Meersbrook	140 (26.46)	13 (6.67)	0.02 (0.01)	234.33 (9.81)	1.64 (0.25)	0.04 (0.0)	0.005 (0.002)	8.60 (0.0)	262.33(14.01)	13.07 (0.06)	9.20 (0.0)
Cranemoor	134 (33.86)	7 (3.57)	0.06 (0.06)	164.67 (2.89)	0.37 (0.01)	0.02 (0.0)	0.001 (0.0)	7.71 (0.07)	1203.0(153.6)	16.73 (0.06)	12.10 (0.10)
Endcliffe park	520 (20)	9 (5.29)		100.33 (9.29)	0.10 (0.03)	0.41 (0.39)	0.004 (0.001)	6.44 (0.02)	355	12.9	9.97 (0.15)

Table A10: Macroinvertebrate family abundance for 24 sites sampled in July 2006. Abbreviations: A = East of Alton, H= Hipper, N=Netherloads, L=Lathkill, P=Peakshole water, Re=Redleadmill brook, RN= River Noe, O=Oughtibridge, SF=South of Fulwood hall, W=River Wye, S=Stubbing Court, Br= Brindwoodgate, Be=Berrymoor, Ri=Rivelin, M=Millthorpe, BB=Brookside beck, Ho=Holymoorside, Lo=Loxley, Rm=Redmires, UD=Upstream of Damflask, T= Thurgoland, Me=Meersbrook, C=Cranemoor, E=Endcliffe park.

Site	A	H	N	L	P	Re	RN	O	SF	W	S	Br	Be	Ri	M	BB	Ho	Lo	Rm	UD	T	Me	C	E
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Ancyclidae	3	2	0	3	3	0	1	0	0	7	1	0	0	1	1	0	0	6	0	0	4	2	0	0
Asellus	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	11	0	0	0	0	36	0	0	0
Atrichopogonae	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	129	54	14	332	250	0	12	23	15	453	0	96	10	15	3	58	40	75	9	38	78	20	19	73
Capnidae	0	88	9	17	0	4	0	60	2	0	238	0	70	13	42	22	40	25	0	0	1	39	0	64
Chironomidae	31	56	10	21	27	35	32	279	0	10	36	25	76	17	0	41	15	27	5	10	0	49	52	71
Dixinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Dytiscidae	0	0	0	0	5	0	12	1	8	0	0	7	1	0	0	0	0	2	0	0	0	1	1	0
Elmidae (A)	13	0	0	9	5	0	12	1	0	0	0	0	3	0	2	0	0	0	0	0	0	12	1	1
Elmidae (L)	6	17	0	59	10	8	0	1	11	3	25	0	6	2	5	5	6	4	3	2	0	8	0	9
Epherellidae	36	43	1	340	236	3	30	21	6	95	8	5	3	30	14	32	26	150	15	1	71	46	0	226
Ephemeridae	6	0	0	0	0	0	6	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	2	2
Erpodbellidae	6	7	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0
Gammaridae	123	0	100	66	5	82	0	15	80	124	9	48	78	13	4	72	110	66	7	6	0	61	276	89
Glossosomidiae	4	26	1	7	1	5	1	1	0	0	25	0	1	0	3	5	1	0	4	0	0	0	1	0
Glossiphonidae	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Halacaridae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Helodidae	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	1	0
Heptageniidae	0	13	1	0	110	4	25	0	3	0	6	0	1	2	11	1	0	1	0	0	0	0	0	3
Hydrobidae	3	1	0	95	45	42	0	5	0	1	75	69	0	5	1	14	0	0	0	0	2	51	342	7
Hydrophilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	10	0	0	1	0	0	0	0
Hydropsychidae	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	6	0	2	0	0	1
Lepitoceridae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebidae	0	0	4	0	0	0	1	0	12	0	39	7	3	0	11	6	0	0	0	0	0	24	0	5
Leuctridae	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	28	0	144	0	0

Table A2. continued

Limnephilidae	0	0	0	14	2	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
Limoniidae	0	12	3	0	9	0	9	0	0	0	11	3	9	3	3	8	8	0	1	3	0	0	0	5
Nematoda	0	0	0	0	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	42	0	0	0
Noterinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OLIGOCHAETE	24	8	6	2	0	0	0	5	2	0	6	5	4	5	0	28	0	1	10	0	32	0	4	8
Planorbidae	0	0	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Polycentropidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1
Ptychopteridae	0	0	0	0	0	11	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	12	8	0
Rhyacophilidae	6	0	0	2	4	0	7	5	4	6	0	0	10	7	1	5	2	13	0	7	3	0	1	5
Sphaeridae	0	1	0	0	1	13	0	3	2	1	1	1	0	4	1	0	0	1	0	0	2	4	8	6
Simulidae	16	6	7	16	0	0	12	55	7	11	7	44	0	0	0	4	22	0	3	5	1	2	1	1
Taenipteridae	0	0	0	0	251	0	33	0	0	0	0	0	0	0	22	0	0	10	0	0	0	0	0	0
Tipulinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Tubificidae	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Velidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Table A11: Diatom species counts for 24 sites sampled in July 2006. Abbreviations: A = East of Alton, H= Hipper, N=Netherloads, L=Lathkill, P=Peakshole water, Re=Redleadmill brook, RN= River Noe, O=Oughtibridge, SF=South of Fulwood hall, W=River Wye, S=Stubbing Court, Br= Brindwoodgate, Be=Berrymoor, Ri=Rivelin, M=Millthorpe, BB=Brookside beck, Ho=Holymoorside, Lo=Loxley, Rm=Redmires, UD=Upstream of Damflask, T= Thurgoland, Me=Meersbrook, C=Cranemoor, E=Endcliffe park.

Site	**A**	**H**	**N**	**L**	**P**	**Re**	**RN**	**O**	**SF**	**W**	**S**	**Br**	**Be**	**Ri**	**M**	**BB**	**H**	**Lo**	**Rm**	**UD**	**T**	**Me**	**C**	**E**
Planothidium delicatulum	1	0	6	0	0	0	0	2	0	0	0	0	0	0	0	0	2	2	0	1	0	0	0	0
Planothidium lanceolatum	12	11	0	0	9	6	7	26	4	0	0	6	0	0	2	0	8	2	0	9	17	12	0	0
Achnanthidium minutissimum	17	14	76	32	49	29	158	72	108	95	78	43	91	77	1	104	49	161	90	75	9	12	112	165
Achnanthes oblongella	6	0	1	11	1	4	4	56	24	0	0	10	0	0	1	0	54	53	31	1	0	8	0	0
Achnanthes conspicua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0
Amphora pedicus	16	3	8	0	1	8	14	12	4	0	0	17	0	0	0	0	7	5	0	35	1	0	5	7
Asterionella Formosa	3	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	22	0	0	0	0	0
Caloneis silicula	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis pedicus	70	0	53	3	0	38	3	30	0	0	74	0	35	85	135	104	0	0	0	0	0	0	36	14
Cocconeis placentula	14	109	0	0	11	60	14	0	4	25	0	91	71	0	21	0	1	1	0	1	46	16	0	5
Cymbella cymbelliformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Encyonema caespitosum	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella prostate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Encyonema silesiacum	0	0	0	0	1	0	0	0	0	0	0	4	1	0	0	0	10	0	0	0	0	0	1	2
Diatoma tenue	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
D. hyamale	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	5	0	0	0	0	0

Table A11. continued

Diatoma problematica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0
Diatoma vulgare	0	0	0	1	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diploneis oblongella	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	21	0	0	1	0	0	0	0
Encyonema minutum	0	0	3	0	7	2	0	0	0	0	23	7	20	18	0	44	2	22	7	7	0	0	18	16
Eunotia bilunaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Eunotia exigua	1	0	0	0	0	0	1	4	0	0	0	0	0	5	0	0	3	0	5	2	0	0	0	0
Eunotia minor	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria bidens	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staurosira construens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Fragilaria capucina	0	0	1	2	5	1	2	0	8	0	0	1	0	0	0	0	6	0	0	0	7	0	0	0
Staurosira leptostauron	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Synedra ulna	0	1	0	3	0	0	2	0	0	0	0	0	0	8	0	0	0	1	0	2	0	0	1	0
Frustulia rhomboides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Frutulia vulgaris	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0
Gomphonema angusta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Gomphonema parvulum	0	0	0	0	2	2	0	0	8	5	0	3	0	4	0	0	3	2	0	7	0	0	2	1
Gophonema pumilum	4	0	1	3	1	9	1	0	0	0	0	4	4	0	0	0	0	3	0	24	0	0	0	0
Gomphonema truncatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Gyrosigma acuminata	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	4	0	0	1

Table 11. continued

Hannaea arcus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Hanitzscia	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Meridion circulaire	1	0	0	0	6	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Navicula cari	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Navicula cinta	0	0	1	1	0	1	0	10	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	3	18	1	21	1	8	11	32	28	0	0	7	9	0	0	40	0	15	3	13	82	4	8	17
Navicula inconspicua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Navicula laterostrata	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula lanceolata	1	2	2	1	0	3	3	20	20	60	20	20	6	19	2	12	8	34	2	17	23	4	0	4
Navicula minima	0	1	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0
Navicula recens	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula reinhardti	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula tripunctata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Navicula wiesneri	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia capitellata	0	0	0	0	0	0	1	2	0	0	0	1	3	0	0	0	4	0	0	1	1	0	3	4
Nitzschia dissipata	1	0	0	0	0	0	4	0	0	0	3	1	0	0	0	0	1	15	0	7	6	0	0	0
Nitzschia draviensis	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryblionella levidensis	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Nitzschia linearis	0	0	1	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	6	0	0	0	0	45	0	0	0	4	0	24	0	5	6	0	0	0	0	0
Nitzschia paleaformis	2	0	0	0	32	0	5	0	2	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0

Table 11. continued

Nitzschia pussila	1	8	1	0	5	0	0	8	0	0	0	2	0	0	0	0	1	3	0	0	10	0	0	0
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	1	0	0	0
Nitzschia thermaloides	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia brebissoni	0	0	1	0	0	0	1	1	0	35	8	0	5	9	0	16	0	1	0	0	0	0	0	0
Pinnularia subcapitata	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Reimeria sinata	2	0	14	0	2	0	9	4	0	0	0	10	0	9	3	20	1	0	0	4	2	4	0	0
Rhoicosphenia abbreviata	0	0	0	1	0	0	1	1	4	0	15	5	0	0	1	0	0	0	0	3	0	0	0	0
Surirella angusta	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0
Surirella brebissoni	0	0	0	0	46	0	0	10	0	2	0	0	0	1	0	8	0	1	1	1	1	0	0	0
Surirella pinata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Stauroneis phoncentrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Tabellaria floculosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0
Number of Species	19	9	15	14	21	18	23	19	16	9	8	22	11	12	9	10	19	27	14	24	22	8	9	11

Appendix 4

Table A12. Macroinvertebrate family counts for 10 sites sampled in May 2007 (Pre-flood).

Site	Peakshole water	Rivelin	Loxley	River Noe	Sheath	Porter brook	Brookside beck	Hipper	South of Fulwood hall	U/S Damflask
Aeshnidae	2	0	0	0	0	0	0	0	0	0
Ancyclidae	0	2	0	0	0	0	0	0	1	0
Aselidae	0	0	0	0	2	0	3	5	0	0
Astacdae	0	0	0	0	0	0	0	0	0	0
Baetidae	1579	96	53	343	112	77	125	56	312	59
Capnidae	68	6	11	98	22	2	2	2	23	12
Ceratopoginae	8	2	4	4	2	2	2	8	2	2
Chironomidae	26	255	59	135	45	11	19	18	21	25
COLLEMBOLA	0	0	0	0	0	0	0	0	0	0
Curculionidae	0	0	0	0	0	0	0	0	0	0
Dytiscidae	3	0	2	5	0	0	1	0	0	1
Dytiscidae (l)	0	0	0	0	0	0	0	0	0	0
Elmidae (a)	2	0	1	2	0	0	2	0	3	0
Elmidae(l)	10	4	0	14	12	0	4	2	8	0
Empididae	0	0	0	0	0	0	0	0	0	0
Ephemerellidae	249	19	0	85	23	25	8	5	0	0
Ephemeridae	1	3	2	0	0	0	0	0	0	0
Gammaridae	20	170	3	2	12	61	43	4	7	0
Glossosomatidae	8	5	2	0	3	1	2	1	0	2
Halacaridae	0	0	0	0	0	0	1	0	0	0
Helodidae	0	0	0	0	0	0	0	1	0	0
Hetageniidae	174	7	6	55	67	34	5	7	42	2
Hirudinidae	0	0	1	0	0	0	0	4	0	0
Hydrpsychidae	0	4	5	0	0	0	2	0	0	0
Hygrobiidae	0	0	0	0	0	0	0	0	0	1

Table A12. continued

Hydroptilidae	1	8	0	4	0	0	0	0	0	0
Leptophlebidae	5	0	0	13	0	0	0	0	0	0
Leuctidae	0	0	0	0	0	0	0	0	0	0
Limnaeidae	1	0	0	0	0	0	0	0	0	0
Limnephilidae	20	0	0	2	0	0	0	0	0	0
Nemouridae	0	0	4	0	0	0	0	0	3	34
Nematoda	0	0	0	0	0	0	0	0	0	0
OLIGOCHEATE	11	2	13	2	2	9	7	34	3	3
Perlodidae	0	0	3	3	0	0	0	0	3	0
Philotamidae	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	1	1	0	0	0	0	0	0	0
Hydrobiidae	3	90	0	0	3	19	12	1	0	0
Psychodidae	0	0	0	0	0	0	0	0	0	0
Rycophilidae	6	5	9	8	2	3	6	0	2	6
Simulidae	105	11	19	45	2	8	0	11	5	11
Spaerium	0	3	0	1	0	1	2	0	2	0
Taetnopterygidae	1	0	0	15	4	8	0	0	2	7
Tipulinae	0	0	0	0	0	0	0	0	0	0

Table A13. Macroinvertebrate family counts for 10 sites sampled in July 2007 (Post-flood)

Site	**Peakshole Water**	**Rivelin**	**Loxley**	**River Noe**	**Sheath**	**Porter Brook**	**Brookside beck**	**Hipper**	**South of Fulwood hall**	**U/S Damflask**
Aeshnidae	0	0	0	0	0	0	0	0	0	0
Ancyclidae	3	0	0	0	0	0	0	0	0	0
Aselidae	0	0	0	0	1	2	3	10	0	0
Astacidae	0	0	0	0	0	1	0	0	0	0
Baetidae	136	36	24	10	101	17	46	34	61	82
Capnidae	43	13	11	15	58	10	0	2	0	19
Ceratopoginae	8	17	3	7	4	1	8	6	1	3
Chironomidae	3	1	3	9	50	22	26	0	99	3
COLLEMBOLA	0	0	0	0	0	0	0	0	0	0

Table A13. continued

Curculionidae	0	0	0	0	0	0	0	0	0	0
Dytiscidae	2	0	2	1	4	0	1	0	2	0
Dytiscidae (l)	0	0	0	0	0	0	3	0	0	0
Elmidae (a)	2	0	2	1	9	0	2	0	0	0
Elmidae(l)	0	0	0	7	34	1	1	2	7	0
Empididae	0	0	0	0	0	0	0	0	0	0
Ephemerellidae	51	9	0	0	26	15	16	8	16	0
Ephemeridae	0	5	0	4	0	0	0	0	0	0
Gammaridae	10	33	1	0	31	52	20	1	14	25
Glossosomatidae	0	1	1	0	2	0	3	6	0	4
Halacaridae	0	0	0	0	0	0	2	0	1	0
Helodidae	0	0	0	0	0	0	0	0	0	0
Hetageniidae	31	0	0	12	10	0	0	0	1	0
Hirudinea	0	0	0	0	0	0	0	5	0	0
Hydropsychidae	0	0	0	0	0	0	0	0	0	0
Hygrobidae	0	0	0	0	0	0	2	0	0	3
Hydroptilidae	0	3	1	0	0	1	0	0	0	0
Leptophlebidae	0	0	0	0	0	0	0	0	1	0
Leuctidae	0	0	0	0	0	0	0	0	2	0
Limnaeidae	1	0	0	0	0	0	0	0	0	0
Limnephilidae	0	0	0	0	0	0	0	0	0	0
Nemouridae	0	0	0	0	0	0	0	0	0	0
Nematoda	0	0	0	1	0	0	0	0	0	0
OLGOCHEATE	12	4	4	0	36	5	18	30	6	0
Perlodidae	0	0	0	0	0	0	0	0	0	0
Philotamidae	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	1	0	0	0
Hydrobiidae	1	20	0	0	3	1	31	1	0	0
Psychodidae	0	0	0	0	0	0	0	0	0	0
Rycophilidae	4	7	4	1	1	0	1	0	5	4
Simulidae	3	7	1	4	38	21	2	0	70	0
Spaeridae	0	0	0	0	0	0	3	4	0	0
Taetnopterygidae	9	0	14	6	0	17	0	0	0	0

Table A14. Macroinvertebrate family counts for 10 sites sampled in May 2008.

Site	Peakshole water	Rivelin	Loxley	River Noe	Sheath	Porter Brook	Brookside Beck	Hipper	South of Fulwood Hall	Upstream of Damflask
Aeshnidae	0	0	0	0	0	0	0	0	0	0
Ancyclidae	2	0	0	0	4	0	0	0	0	0
Aselidae	0	0	0	0	0	0	2	0	0	0
Astacidae	0	0	0	0	0	0	0	0	0	0
Baetidae	1171	287	448	568	379	113	329	603	519	215
Capnidae	61	23	42	32	9	62	0	42	3	141
Ceratopoginae	2	5	6	9	6	16	8	12	6	6
Chironomidae	118	91	17	17	59	421	117	96	146	34
COLLEMBOLA	0	0	0	0	0	0	0	0	0	0
Curculionidae	0	0	0	0	0	0	0	0	0	0
Dytiscidae	0	0	0	17	0	0	0	0	1	0
Dytiscidae (l)	0	0	0	0	0	0	6	0	0	0
Elmidae (a)	1	2	2	8	1	1	3	1	5	0
Elmidae(l)	5	3	0	18	23	2	1	4	0	3
Empididae	0	0	0	0	0	0	0	0	0	0
Ephemerellidae	69	3	0	2	18	28	0	12	0	0
Ephemeridae	0	0	0	0	0	0	0	0	0	0
Gammaridae	5	22	0	1	7	19	22	1	74	0
Glossosomatidae	0	1	0	2	0	0	2	0	7	3
Halacaridae	0	0	0	0	0	0	0	0	0	0
Helodidae	0	0	0	0	0	0	0	0	0	0
Hetageniidae	609	6	37	173	69	22	15	19	34	2
Hirudinea	0	0	1	0	0	0	0	0	0	0
Hydrpsychidae	2	16	11	8	0	0	2	5	0	0
Hygrobidae	0	0	0	0	0	0	0	0	0	0
Hydroptilidae	0	0	0	0	0	0	0	0	0	0
Leptophlebidae	4	0	2	0	0	0	3	0	0	0
Leutidae	4	0	0	0	0	0	0	6	0	0
Limnaeidae	1	20	0	0	0	0	0	0	0	0

Table A14. continued

Limnephilidae	14	2	16	0	1	0	4	9	0	2
Nemouridae	0	1	27	19	4	0	3	0	3	11
Nematoda	0	0	0	0	0	0	0	0	0	0
OLIGOCHEATE	4	9	2	4	11	8	14	0	7	5
Perlodidae	0	0	5	4	0	0	0	0	0	8
Philotamidae	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	0	8	0	0	2	13	41	2	0	0
Psychodidae	0	0	0	0	0	0	0	0	0	0
Rycophilidae	6	5	0	5	7	9	0	5	9	20
Simulidae	51	5	1	86	2	5	2	3	24	3
Spaeridae	0	1	1	1	1	0	1	0	10	1
Taetnopterygidae	0	0	0	0	0	0	0	0	0	0
Tipulinae	0	0	0	0	0	0	0	0	0	0

Table A15. Macroinvertebrate family counts for 10 sites sampled in July 2008.

Site	**Peakshole water**	**Rivelin**	**Loxley**	**River Noe**	**Sheath**	**Brookside Beck**	**Hipper**	**South of Fulwood Hall**	**Upstream of Damflask**
Aeshnidae	0	0	0	0	0	0	0	0	0
Ancyclidae	0	2	0	0	0	0	0	4	0
Aselidae	0	0	0	0	0	8	0	1	0
Astacidae	0	0	0	0	0	0	0	0	0
Baetidae	608	112	87	13	102	76	54	54	51
Capnidae	63	19	11	121	18	0	11	43	86
Ceratopoginae	23	0	0	2	8	5	4	9	5
Chirnomidae	330	67	43	19	65	112	110	30	21
COLLEMBOLA	0	0	0	0	0	0	0	0	0
Curculionidae	0	0	0	0	0	0	0	0	0
Dytiscidae	0	0	0	0	0	0	0	0	0
Dytiscidae (l)	0	0	0	0	0	3	0	0	0

Table A15. continued

Elmidae (a)	0	3	4	6	9	2	6	6	0
Elmidae(l)	0	0	4	17	8	0	3	7	2
Empididae	0	0	0	0	0	0	0	0	0
Ephemerellidae	156	8	7	12	48	2	9	22	0
Ephemeridae	0	0	0	0	0	0	0	0	0
Gammaridae	14	34	1	3	40	65	0	18	0
Glossosomatidae	0	0	0	0	0	3	0	6	0
Halacaridae	0	0	0	0	0	0	0	0	0
Helodidae	0	0	0	0	0	0	0	0	0
Hetageniidae	40	2	8	56	20	4	4	21	0
Hirudinea	1	0	0	0	0	1	0	1	0
Hydropsychidae	0	0	0	0	0	4	3	0	0
Hygrobidae	0	0	0	0	0	0	0	0	0
Hydroptilidae	0	0	0	0	0	0	0	0	0
Leptophlebidae	0	0	0	0	0	1	0	0	0
Leuctidae	2	0	0	2	0	0	0	0	0
Limnaeidae	0	0	0	0	0	0	0	0	0
Limnephilidae	8	1	0	0	0	0	2	1	0
Nemouridae	0	0	14	0	0	0	0	4	7
Nematoda	0	0	0	0	0	0	0	0	0
OLIGOCHEATE	9	3	2	22	12	23	23	19	8
Perlodidae	0	0	2	2	0	0	0	0	4
Philotamidae	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0
Hydrobiidae	0	68	0	0	0	43	0	0	0
Psychodidae	0	0	0	0	0	0	0	0	0
Rycophilidae	0	0	22	11	0	0	2	5	6
Simulidae	98	0	0	121	0	3	11	0	19
Spaeridae	0	0	0	0	0	0	0	6	0
Taetnopterygidae	1	0	2	12	6	0	0	1	0
Tipulinae	0	0	0	0	0	0	0	0	0

Table A16. Diatom species counts for 10 sites sampled in May 2007 (Pre-flood).

Site	Peakshole Water	Rivelin	Loxley	River Noe	Sheath	Porter brook	Brookside Beck	Hipper	South of Fulwood hall	Upstream damflask
Achnanthidium biasolettiana	0	0	0	0	0	0	0	0	0	0
Achnanthes conspicua	0	0	0	0	0	1	0	0	0	0
Achnanthes oblongella	38	0	0	0	5	1	3	6	0	7
Planothidium delicatulum	0	0	0	0	0	0	0	0	0	0
Planothidium lanceolatum	4	0	5	0	7	3	1	0	7	0
Achnanthidium minutisimum	38	148	118	110	34	87	54	47	112	145
Amphora libica	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	0	0	0	0	0	0	0	0	0
Amphora pediculus	8	0	0	0	0	0	0	4	8	0
Asterionella formosa	0	0	0	0	0	0	1	0	0	0
Bacillaria paxillifer	0	0	0	0	0	0	0	0	0	0
Caloneis amphisbaena	0	0	0	0	0	0	0	0	0	0
Caloneis molaris	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Camphylodiscus hibernicus	0	0	0	0	0	0	0	0	0	0
Craticular accomoda	0	0	0	0	0	0	0	0	0	26
Cocconeis pediculus	0	0	0	0	0	0	0	68	2	0
Cocconeis plancentula	0	2	0	5	0	11	87	57	3	0
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0	0
Cymbella affinis	0	10	0	0	0	0	0	0	0	0
Encyonema caespitosum	0	0	0	0	0	0	0	0	0	1
Encyonema gracile	0	0	0	0	0	0	0	0	0	0
Cymbella microcephala	0	0	0	0	1	0	0	0	0	8
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	4

Table A16. continued

Cymbella pusilla	0	0	0	0	0	0	0	0	0	0
Encyonema silesiacum	0	0	0	0	0	0	0	1	2	0
Cymbella spp	0	0	0	0	0	0	0	0	0	0
Denticula	0	1	0	0	0	0	0	0	0	0
Diatom hyemale	5	0	0	2	0	0	0	0	0	0
Diatoma vulgare	0	0	0	0	0	0	0	0	0	0
Diatoma tenue	0	0	0	0	0	0	0	0	0	0
Didymosophenia germinata	0	0	0	0	0	0	0	0	0	0
Diploneis oblongella	0	0	0	0	0	0	0	0	0	0
Diploneis ovalis	0	0	0	0	0	0	0	0	0	0
Encyonema silesiacum	8	0	0	0	0	0	0	0	0	0
Enconema gracile	0	0	0	0	0	0	0	0	0	0
Encyonema minutum	0	0	4	2	0	4	82	7	5	0
Epithemia adnata	0	0	0	0	0	0	0	0	0	4
Eunotia bilunaris	0	0	1	1	0	0	0	0	0	0
Eunotia exigua	4	0	0	0	1	0	0	0	3	5
Eunotia pectinalis	0	0	0	0	0	0	0	0	0	0
Epithemia spp	0	0	0	0	0	0	0	0	0	0
Fragilaria arcus	9	0	0	0	0	0	0	0	0	0
Fragilaria capucina	0	3	1	0	0	0	0	0	6	0
Synedra ulna	7	67	0	3	0	0	0	1	2	8
Fragilaria parisitica	0	0	0	0	0	0	0	0	0	0
Staurosira pinnata	0	0	0	0	0	0	0	0	2	0
Staurosira leptostauron	0	0	0	0	0	0	0	0	0	0
Frustulia rhombiscus	2	0	0	0	0	0	0	0	0	0
Frustulia cruzburgensis	0	0	0	0	0	0	0	0	0	0
Frustulia saxena	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	0	5	0	0	0	3	0	0	0	0
Gomphonema angustum	0	0	0	6	1	0	0	0	0	0
Gomphonema acuminatum	1	0	0	0	0	0	0	0	0	0
Gomphonema olivacum	0	0	1	0	1	0	0	0	0	43

Table A16. continued

Gomphonema minutum	0	0	0	0	0	0	0	0	0	0
Gomphonema parvalum	11	0	3	0	3	0	3	3	4	0
Gomphonema pumilum	0	0	0	0	0	0	0	0	0	0
Gomphonema truncatum	0	0	0	0	0	0	0	0	0	0
Gyrosigma acuminata	0	0	0	0	0	0	0	0	0	0
Hannaea arcus	0	0	0	1	0	1	0	0	0	0
Hantzschia abundans	0	0	0	0	0	0	0	0	0	0
Meridion circulare	0	16	2	1	0	0	0	0	0	6
Navicula angustula	0	0	0	0	0	1	0	0	0	0
Navicula cari	0	0	0	0	0	0	0	1	0	0
Navicula capitatoradiata	0	0	0	0	0	0	0	0	0	0
Navicula cinta	18	0	0	0	17	5	2	0	0	0
Navicula cryptonella	0	0	0	0	0	1	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	10	0	24	17	54	21	14	7	14	5
Navicula ignita	0	0	0	0	0	0	0	0	0	0
Navicula lanceolata	24	1	5	6	2	0	0	0	4	3
Navicula lenzi	0	0	0	0	0	1	1	0	0	0
Navicula novaesiberica	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	2	0	0	0	0	0
Navicula protractor	0	0	0	0	0	4	0	0	0	0
Navicula oligotrapheta	0	0	0	0	0	0	0	0	0	0
Navicula reinharti	0	0	0	0	0	0	0	0	0	0
Navicula recens	0	0	0	0	0	0	0	0	0	0
Navicula rhynchotella	0	0	0	0	0	0	0	0	0	0
Navicula tripunctata	0	0	0	0	0	0	0	0	0	0
Navicular splendica	0	0	0	0	0	0	0	0	0	1
Navicular veneta	0	0	0	0	0	0	0	0	3	0
Neidium binodeforme	0	0	0	0	0	0	0	0	0	0
Nitzschia bergii	0	0	0	0	0	0	0	0	1	0
Nitzschia capitelata	0	0	0	0	0	0	0	2	5	3

Table A16, continued

Nitzschia debilis	0	0	0	0	0	0	0	0	0	0
Nitzcshia draveiensis	0	0	0	0	0	0	0	0	0	0
Nitzschia dissipata	12	6	2	7	0	0	0	0	0	17
Nitzschia flexa	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulium	0	0	0	0	0	0	0	0	0	0
Nitzschiia hantzschiana	2	0	0	0	0	0	0	0	0	0
Nitzschia inconspicua	0	6	0	0	0	1	0	0	0	0
Nitzschia lacuum	0	0	0	0	0	0	0	0	0	0
Tryblionella levidensis	0	0	0	0	0	0	0	0	0	0
Nitzschia linearis	0	0	0	0	0	0	0	0	0	0
Nitzschia paleaformis	9	0	0	0	0	0	0	0	0	0
Nitzschia paleacea	0	0	0	0	7	2	0	1	0	0
Nitzschia palea	9	6	1	18	0	0	0	0	0	0
Nitzschia pusilla	0	0	13	0	0	0	0	1	3	1
Nitzschia sigmoidea	0	0	0	2	0	0	0	0	0	0
Nitzschia sigma	0	0	0	0	0	0	0	0	0	0
Nitzschia sociabilis	0	0	0	0	0	1	0	0	0	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0
Pinnularia brebisoni	0	0	0	1	0	0	0	0	0	0
Pinularia subgibba	0	0	0	0	0	0	0	0	0	0
Pinnularia subcapita	2	0	0	0	0	0	0	0	0	4
Rhoicosphenia abbreviata	1	0	0	0	0	0	0	0	0	0
Reimeria sinata	0	0	5	9	2	2	4	2	0	0
Sellaphora bacillum	0	0	0	0	0	0	0	0	0	0
Sellaphora pupula	0	0	0	0	0	0	0	0	0	0
Sellaphora seminulum	0	0	0	0	0	0	0	0	0	0
Stauroneis pseudosubobstusides	0	0	0	0	0	0	0	0	0	0
Surirella amphyioxis	0	1	0	0	0	0	0	0	0	0
Surirella angusta	0	6	0	0	0	0	2	1	0	0
Surirella crumena	0	0	0	0	0	0	0	0	0	1
Surrirella linearis	0	0	0	0	0	0	0	0	0	0
Suriella terricola	0	0	0	0	0	1	0	0	0	0

Suriella brebisoni	5	0	7	2	2	0	1	1	0	1
Surirella pinnata	0	0	0	0	0	0	0	0	0	0
Tabellaria floculossa	2	0	0	0	0	0	0	0	0	1
Tabellaria spp	0	0	0	0	0	0	0	0	0	0

Table A17. Diatom species counts for 10 sites sampled in July 2007 (Post-flood).

Site	Peakshole water	Rivelin	Loxley	River Noe	Sheath	Porter brook	Brookside beck	Hipper	South of fulwood hall	Upstream of damflask
Achnanthes biasoletiana	0	0	0	0	0	0	0	0	0	0
Achnanthes conspicua	0	0	0	0	0	0	0	0	0	0
Achnanthes oblongella	39	44	0	0	19	1	5	5	15	0
Achnanthes delicata	0	0	0	0	0	1	0	1	0	0
Achnanthes lanceolata	4	8	0	2	12	19	0	1	17	12
Achnanthes minutisimum	114	90	14	24	53	35	16	3	55	132
Amphora libica	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	0	0	0	0	0	0	0	1	0
Amphora pediculus	19	3	0	2	10	22	7	23	5	0
Asterionella spp	0	0	0	0	0	0	0	0	0	0
Bacillaria paradoxa	0	0	0	0	0	0	0	0	2	0
Caloneis amphisbaena	0	1	0	0	0	0	0	0	0	0
Caloneis molaris	0	0	0	0	0	0	0	0	1	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Camphylodiscus hibernicus	0	0	0	0	0	0	0	0	0	0
Craticular accomoda	0	0	0	3	0	0	0	0	6	0
Cocconeis pediculus	2	4	0	5	28	7	0	40	0	4
Cocconeis plancentula	0	0	0	1	12	30	7	0	8	0
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0	0
Cymbella affinis	0	0	0	0	0	0	0	0	0	0

Table A17. continued

Encyonema caespitosum	3	0	0	0	2	0	0	0	0	0
Encyonema gracile	0	0	0	0	0	0	0	0	0	0
Cymbella microcephala	0	0	0	0	0	0	0	0	1	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Cymbella pusilla	2	0	0	0	0	0	0	0	0	0
Encyonema silesiacum	0	0	0	0	0	0	0	0	0	0
Cymbella spp	0	0	0	0	0	0	0	0	0	0
Denticula	0	0	0	0	0	0	0	0	0	0
Diatom hyamale	0	0	0	0	0	11	2	0	0	0
Diatoma vulgare	0	0	1	0	0	0	0	0	0	0
Diatoma tenue	1	0	0	0	0	0	0	0	0	0
Didymosophenia germinata	0	0	0	0	0	0	0	0	0	0
Diploneis oblongella	0	8	0	0	2	0	1	0	1	0
Diploneis ovalis	0	0	0	0	0	0	0	1	0	0
Encyonmea silesiacum	0	0	0	0	0	0	0	0	0	0
Enconema gracile	0	0	0	0	0	0	0	0	0	0
Encyonema minutum	0	1	0	0	0	0	0	1	6	2
Epithemia adnata	4	0	0	0	0	0	0	0	0	0
Eunotia bilunaris	0	0	0	0	0	0	0	0	0	1
Eunotia exigua	3	6	0	0	1	0	0	0	0	0
Eunotia pectinalis	0	2	0	0	0	0	0	0	0	0
Epithemia spp	0	1	0	0	0	0	0	0	0	0
Fragilaria arcus	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina	1	11	0	0	5	3	0	1	0	12
Synedra ulna	0	4	0	0	0	0	1	0	5	3
Fragilaria parisitica	0	0	0	0	0	0	0	0	0	0
Staurosira pinnata	2	1	0	0	0	0	0	0	2	0
Staurosira leptostauron	0	0	0	0	0	0	0	0	0	0
Frustulia rhombiscus	0	0	0	0	0	0	0	0	0	0
Frustulia cruzburgensis	0	0	0	0	0	1	0	0	0	0
Frustulia saxena	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	0	2	0	0	0	1	0	0	0	0

Table A17 continued

Gomphonema angustum	0	0	0	0	0	0	0	0	0	0
Gomphonema acuminatum	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaccum	2	0	0	3	0	0	0	0	0	0
Gomphonema minutum	0	0	0	0	0	0	0	0	0	0
Gomphonema parvalum	1	1	0	0	2	2	1	1	4	15
Gomphonema pumilum	0	0	0	0	0	0	0	0	0	2
Gomphoneam truncatum	0	0	0	0	0	0	0	0	0	0
Gyrosigma cuminata	0	0	0	0	0	0	0	1	0	0
Hannaea arcus	0	0	0	0	0	0	0	0	0	0
Hantzschia abundans	0	0	0	0	0	0	0	0	0	0
Meridion circulare	4	0	0	0	0	0	0	0	2	0
Navicula angustula	0	0	0	0	0	0	0	0	0	0
Navicula cari	8	0	0	2	0	0	1	0	0	0
Navicula capitatoradiata	0	0	0	0	0	0	0	0	0	0
Navicula cinta	0	3	0	0	4	0	0	0	0	12
Navicular cryptonella	0	0	0	1	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	1	4	3	1	16	18	4	10	13	24
Navicula ignita	10	0	0	0	4	0	0	0	1	0
Navicula lancelata	0	2	1	1	16	3	4	7	1	0
Navicula lenzi	0	0	0	0	1		0	0	0	0
Navicula novaesiberica	0	0	0	0	0	0	0	0	0	0
Navicula minima	0	0	0	0	0	0	0	0	0	17
Navicular protractor	0	0	0	0	1	0	1	0	0	0
Navicula oligotrapheta	0	0	0	0	0	0	0	0	0	8
Navicular reinharti	0	0	0	0	1	0	0	0	0	0
Navicular recens	0	0	0	0	0	0	0	0	0	0
Navicular rhynchotella	0	0	0	0	0	0	0	0	0	0

Navicular tripunctata	0	0	0	0	0	0	0	0	0	0
Navicular splendica	4	0	0	0	3	0	0	0	0	1
Navicular veneta	0	0	0	0	0	0	0	0	0	0
Neidium binodeforme	0	1	0	0	0		0	0	0	0
Nitzshia bergii	8	0	0	0	0	0	0	0	5	0
Nitzschia capitelata	1	8	1	2	29	23	2	5	4	11
Nitzschia debilis	0	0	0	0	0	0	0	0	0	0
Nitzcshia draveienis	0	0	0	0	0	0	0	0	0	7
Nitzschia dissipata	0	0	0	1	1	2	0	0	0	8
Nitzschia flexa	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulium	0	0	0	0	0	0	0	0	0	2
Nitzschiia hantzschina	0	0	0	0	0	0	0	0	0	0
Nitzschia inconspicua	0	0	0	0	0	0	0	0	0	0
Nitzschia lacuum	0	0	0	0	0	0	0	0	0	0
Nitzschia levidensis	1	2	0	0	0	0	0	0	3	0
Nitzschia linearis	0	0	0	0	1	0	0	0	0	3
Nitzschia paleaformis	0	1	0	0	0	0	0	0	1	2
Nitzschia paleacea	0	0	0	0	0	0	0	0	0	0
Nitzschia palea	0	0	0	0	0	0	0	0	0	0
Nitzschia pusila	4	3	1	0	13	8	1	8	7	2
Nitzschia sigmoidea	0	0	0	0	0	0	0	0	0	0
Nitzschia sigma	0	0	0	0	0	0	0	0	0	0
Nitzschia sociabilis	0	4	0	1	0	0	0	0	0	0
Placoneis clementis	1	0	0	0	2	0	0	0	0	0
Pinnularia bebisoni	0	0	0	0	0	0	0	0	0	0
Pinularia subgibba	0	0	0	0	0	0	0	0	0	0
Pinnularia subcapita	1	2	0	0	0	1	0	0	1	0
Rhoicosphenia abreviata	1	0	0	0	1	2	0	0	0	0
Reimeria sinata	32	0	0	3	23	10	3	5	3	2
Sellaphora bacillum	0	0	1	0	0	0	0	0	0	0
Sellaphora pupular	0	0	0	0	0	0	0	0	0	0
Sellaphora seminulum	0	0	0	0	0	0	0	0	0	4
Stauroneis	0	0	0	0	0	0	0	0	0	0

Table A17. continued

pseudosubobstusides										
Surirella amphyioxis	0	0	0	0	0	0	0	0	0	0
Surirella angusta	0	0	0	0	0	2	0	1	0	0
Surirella crumena	0	0	0	0	0	0	0	0	0	0
Surrirella linearis	0	0	0	0	0	0	0	0	0	0
Surirella terricola	0	0	0	0	0	0	0	0	0	0
Surirella brebisoni	0	3	0	1	3	0	0	0	1	2
Surirella pinnata	0	0	0	0	0	0	0	0	0	0
Tabellaria floculosa	0	1	0	0	0	0	0	0	0	0
Tabellaria spp	0	0	0	0	0	0	0	0	0	0

Table A18. Diatom species counts for 10 sites sampled in May 2008

Site	Peakshole water	Rivelin	Loxley	River Noe	Sheath	Porter brook	Brookside beck	Hipper	South of fulwood hall	Upstream of Damflask
Achnanthidium biasolettiana	0	0	0	1	0	0	0	0	0	0
Achnanthes conspicua	0	0	0	0	0	0	0	0	0	0
Achnanthes oblongella	0	18	1	2	0	1	0	0	3	0
Planothidium delicatulum	0	0	0	0	0	0	0	0	0	0
Planothidium lanceolatum	8	14	3	3	6	8	26	32	10	3
Achnantheidium minutisimum	182	164	161	117	80	77	94	163	95	209
Amphora libica	0	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	0	2	0	0	0	36	0	0	0
Amphora pediculus	1	0	7	6	7	19	0	7	3	0
Asterionella formosa	0	0	0	0	0	0	0	1	0	0
Bacillaria paxillifer	0	0	0	0	0	1	0	0	0	0
Caloneis	0	0	0	0	0	0	0	0	0	0

Table A18. continued

amphisbaena										
Caloneis molaris	0	0	0	0	0	0	0	0	0	0
Caloneis silicula	0	0	0	0	0	0	0	0	0	0
Camphylodiscus hibernicus	0	0	0	0	0	0	0	0	0	0
Craticula accomoda	0	0	0	0	0	0	0	0	0	0
Cocconeis pediculus	1	2	2	0	8	9	85	35	1	0
Cocconeis plancentula	0	3	2	3	17	12	5	20	0	0
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0	0
Cymbella affinis	0	0	0	0	0	0	0	0	0	0
Cymbella caespitosa	0	0	0	4	0	0	0	0	0	0
Encyonema gracile	0	0	0	0	0	0	0	0	0	0
Cymbella microcephala	0	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0	0
Cymbella pusilla	0	0	0	0	0	0	0	0	0	0
Encyonema silesiacum	4	3	5	1	0	0	0	0	0	10
Cymbella spp	1	0	0	0	0	0	0	0	0	0
Denticula	0	0	0	0	0	0	0	0	0	0
Diatom mesodon	0	0	1	4	0	0	0	0	0	0
Diatoma vulgare	42	0	0	1	1	10	0	0	0	0
Diatoma tenue	0	1	0	1	0	1	1	0	0	0
Didymosophenia germinate	0	0	0	0	0	0	0	0	0	0
Diploneis oblongella	0	7	0	1	0	0	0	1	0	2
Diploneis ovalis	0	0	0	0	0	0	2	3	0	0
Encyonema silesiacum	0	0	0	0	0	0	0	0	0	0
Encyonema gracile	0	0	0	0	0	0	0	0	0	0
Encyonema minutum	0	4	18	6	1	0	0	3	3	9
Epithemia adnata	0	0	0	0	0	0	0	0	0	0

Table A18. continued

Eunotia bilunaris	0	0	0	0	0	0	0	0	0	0
Eunotia exigua	0	1	0	1	0	0	0	1	0	3
Eunotia pectinalis	0	0	0	0	0	0	0	0	0	0
Epithemia spp	0	0	0	0	0	0	0	0	0	0
Fragilaria arcus	0	5	0	27	0	0	0	0	0	0
Fragilaria capucina	14	15	6	11	8	4	4	5	0	26
Synedra ulna	73	21	2	12	29	5	4	2	2	5
Fragilaria parisitica	0	0	0	0	0	0	0	0	0	0
Staurosira pinnata	0	0	0	0	0	0	0	0	0	0
Staurosira leptostauron	0	0	0	0	0	0	0	0	0	0
Frustulia rhombiscus	0	0	0	0	0	0	0	0	0	0
Frustulia cruzburgensis	0	0	0	0	0	0	0	0	0	0
Frustulia saxena	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	0	0	0	0	0	0	0	0	0	0
Gomphonema angustum	0	0	0	0	0	0	0	0	0	0
Gomphonema acuminatum	0	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0	0
Gomphonema minutum	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	1	2	16	41	0	4	1	0	5	4
Gomphonema pumilum	3	1	1	11	18	7	3	3	0	2
Gomphonema truncatum	0	0	0	1	0	0	0	1	0	0
Gyrosigma acuminata	0	0	0	0	0	0	0	0	0	0
Hannaea arcus	0	0	0	0	0	0	0	0	0	0
Hantzschia abundans	0	0	0	0	0	0	1	0	0	0
Meridion circulare	18	1	4	2	0	0	0	0	0	26
Navicula angustula	0	0	0	0	0	0	0	0	0	0

Table A18. continued

Navicula cari	0	0	0	0	0	0	0	0	0	0
Navicula capitatoradiata	0	0	0	0	0	0	0	0	0	0
Navicula cinta	0	0	0	0	0	0	1	0	0	0
Navicula cryptonella	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0	0
Navicula clementis	0	0	0	0	0	0	0	0	0	0
Navicula gregaria	2	14	22	18	39	69	22	22	9	6
Navicula ignita	0	3	3	2	0	0	0	0	0	1
Navicula lancelata	3	35	25	51	64	44	24	22	13	9
Navicula lenzi	0	0	0	0	0	0	0	0	0	0
Navicula novaesiberica	0	0	0	0	0	0	0	0	0	0
Navicula minima	7	4	11	0	3	3	7	7	0	3
Navicula protractor	0	0	0	1	0	0	0	0	0	0
Navicula oligotrapheta	0	0	0	0	0	0	0	0	0	0
Navicula reinharti	0	0	0	0	0	0	0	0	0	0
Navicula recens	0	0	0	0	0	0	0	0	0	0
Navicula rhynchotella	0	0	0	0	0	0	0	0	0	0
Navicula tripunctata	0	0	0	1	0	1	0	0	0	0
Navicula splendica	0	0	0	0	0	0	0	0	0	0
Navicula veneta	0	0	0	0	0	0	0	0	0	0
Neidium binodeforme	0	0	0	0	0	0	0	0	0	0
Nitzschia bergii	0	0	2	0	0	0	2	2	0	0
Nitzschia capitelata	4	10	7	5	5	3	0	6	3	5
Nitzschia debilis	0	0	0	0	0	0	0	0	0	0
Nitzcshia draveienis	0	0	0	0	0	0	0	1	0	0
Nitzschia dissipata	1	10	1	16	1	4	0	3	0	4
Nitzschia flexa	0	0	0	0	0	0	0	0	0	0
Nitzschia frustulium	0	0	0	0	0	0	0	0	0	0
Nitzschia hantzschiana	0	0	0	0	0	0	0	0	0	0
Nitzschia inconspicua	0	0	0	0	0	0	0	0	0	0

Table A18. continued

Nitzschia lacuum	0	0	0	0	0	0	0	0	0	0
Nitzschia levidensis	0	0	0	0	0	0	0	0	0	0
Nitzshcia linearis	1	0	0	0	1	0	1	0	0	1
Nitzschia paleaformis	3	0	0	1	0	0	0	7	0	0
Nitzschia paleacea	0	8	5	14	0	0	0	0	0	0
Nitzschia palea	0	0	1	0	0	1	8	0	4	3
Nitzschia pusila	0	0	0	0	1	3	5	8	1	0
Nitzschia sigmoidea	0	0	4	0	0	0	0	0	0	0
Nitzschia sigma	0	0	4	1	0	0	0	0	0	0
Nitzschia sociabilis	0	0	0	1	0	0	0	0	1	0
Placoneis clementis	0	0	0	0	0	0	0	0	0	0
Pinnularia brebisoni	0	0	0	1	0	0	0	0	0	0
Pinularia subgibba	0	0	0	0	0	0	0	0	0	0
Pinnularia subcapita	0	0	0	0	0	0	0	0	0	0
Rhoicosphenia abbreviata	1	0	0	0	16	24	1	3	1	0
Reimeria sinata	0	2	6	27	2	0	6	8	0	1
Sellaphora bacillum	0	0	0	0	0	0	0	0	0	0
Sellaphora pupula	0	0	2	0	0	0	0	0	0	0
Sellaphora seminulum	0	0	0	0	0	0	0	0	0	0
Stauroneis pseudosubobstusides	0	0	0	0	0	0	0	0	0	0
Surirella amphyioxis	0	0	0	0	0	0	0	0	0	0
Surirella angusta	0	0	0	0	0	0	0	0	0	1
Surirella crumena	0	0	0	0	0	0	0	0	0	0
Surirella linearis	0	0	0	0	0	0	0	0	0	0
Surirella terricola	0	0	0	0	0	0	0	0	0	0
Surirella brebisoni	1	7	11	12	10	4	10	6	0	6
Surirella pinnata	0	0	0	0	0	0	0	0	0	0
Tabellaria floculossa	0	1	1	0	0	0	0	0	0	0
Tabellaria spp	0	0	0	0	0	0	0	0	0	0

Table A19. Diatom species counts for 10 sites sampled in July 2008

Site	Peakshole water	Rivelin	Loxley	River Noe	Sheath	Porter Brook	Brookside Beck	South of Fulwood hall	Upstream of Damflask
Achnanthidium biasolettiana	0	8	6	0	0	0	0	0	1
Achnanthes conspicua	0	0	1	0	0	0	0	0	0
Achnanthes oblongella	0	79	61	9	3	6	2	5	0
Planothidium delicatulum	0	0	1	0	0	1	0	0	0
Planothidium lanceolatum	9	0	2	7	7	17	11	8	8
Achnanthidium minutisimum	121	149	135	80	124	42	86	205	0
Amphora libica	0	0	0	0	0	0	0	0	0
Amphora ovalis	0	0	0	0	0	0	0	0	4
Amphora pediculus	35	0	0	5	6	93	0	3	2
Asterionella formosa	0	0	1	0	1	0	0	0	0
Bacillaria paxillifer	0	0	0	0	0	0	0	0	0
Caloneis amphisbaena	0	0	0	0	0	0	0	0	0
Caloneis molaris	0	0	0	0	0	0	21	0	0
Caloneis silicula	0	0	0	0	0	0	7	0	0
Camphylodiscus hibernicus	0	0	0	0	0	0	0	0	0
Craticula accomoda	0	0	0	0	0	0	0	0	0
Cocconeis pediculus	0	0	3	4	18	0	0	0	0
Cocconeis plancentula	12	0	0	0	110	120	1	2	16
Cymbella cymbiformis	0	0	0	0	0	0	0	0	0
Cymbella affinis	0	0	0	0	0	0	0	0	0
Encyonema caespitosum	0	0	1	0	0	0	0	0	0
Cymbella gracile	0	0	0	0	0	0	0	0	0
Cymbella microcephala	0	0	0	0	0	0	0	0	0
Cymbella naviculiformis	0	0	0	0	0	0	0	0	0
Cymbella pusilla	0	0	0	0	0	0	0	0	0

Table A19. continued

Cymbella silesiacum	84	0	3	0	0	0	0	1	0
Cymbella spp	0	0	0	0	0	0	0	0	0
Denticula	0	0	0	0	0	0	0	0	0
Diatoma hyamale	0	0	0	0	0	0	0	0	0
Diatoma vulgare	10	0	1	0	0	0	0	18	0
Diatoma tenue	0	0	0	0	0	0	0	0	1
Didymosophenia germinata	0	0	0	0	0	0	1	0	0
Diploneis oblongella	0	0	0	0	0	0	0	0	0
Diploneis ovalis	0	0	0	0	0	0	1	0	0
Encyonema silesiacum	0	0	0	0	0	0	0	0	0
Encyonema gracile	0	0	0	0	0	0	0	0	0
Encyonema minutum	0	0	0	0	1	0	0	0	5
Epithemia adnata	0	0	0	0	0	0	7	0	0
Eunotia bilunaris	0	0	0	0	0	0	0	0	0
Eunotia exigua	0	1	0	3	0	0	19	0	2
Eunotia pectinalis	0	0	0	0	0	0	0	0	0
Epithemia spp	0	0	0	0	0	0	0	0	0
Fragilaria arcus	0	0	0	0	0	0	9	0	0
Fragilaria capucina	50	0	20	14	9	2	0	20	13
Synedra ulna	20	0	0	4	14	2	0	31	5
Fragilaria parisitica	0	0	0	0	0	0	0	0	0
Staurosira pinnata	0	0	0	0	0	0	0	0	0
Staurosira leptostauron	0	0	0	0	0	0	0	0	0
Frustulia rhombiscus	0	0	0	0	0	0	0	0	0
Frustulia cruzburgensis	0	0	0	0	0	0	0	0	0
Frustulia saxena	0	0	0	0	0	0	0	0	0
Frustulia vulgaris	0	0	0	0	0	0	0	0	0
Gomphonema angustum	0	0	0	0	0	0	1	0	0
Gomphonema acuminatum	0	0	0	0	0	0	0	0	0
Gomphonema olivaceum	0	0	0	0	0	0	0	0	0
Gomphonema minutum	0	0	0	0	0	0	0	0	0
Gomphonema parvulum	18	0	2	5	1	3	0	5	9
Gomphonema pumilum	18	0	0	0	2	4	0	8	1

Table A19. continued

Gomphonema truncatum	0	0	0	0	0	0	0	0	0
Gyrosigma acuminata	0	0	0	0	0	0	0	0	0
Hannaea arcus	0	0	0	1	0	0	0	0	0
Hantzschia abundans	0	0	0	0	0	0	0	0	0
Meridion circulare	12	0	0	0	0	0	0	5	3
Navicula angustula	0	0	0	0	0	0	0	0	0
Navicula cari	0	0	0	0	0	0	0	0	0
Navicula capitatoradiata	0	0	0	0	0	0	0	0	0
Navicula cinta	0	2	0	0	2	0	1	0	0
Navicula cryptonella	0	0	0	0	0	0	6	0	0
Navicula cryptocephala	0	0	0	0	0	0	0	0	0
Placoneis clementis	1	0	0	0	0	0	0	0	0
Navicula gregaria	10	5	8	9	9	15	0	5	33
Navicula ignita	0	0	0	0	0	0	0	0	0
Navicula lancelata	11	9	2	2	2	9	0	9	0
Navicula lenzi	1	0	0	0	0	0	0	0	0
Navicula novaesiberica	0	0	0	0	0	0	0	0	0
Navicula minima	3	9	2	1	5	0	0	13	4
Navicula protractor	0	0	0	1	0	1	0	0	0
Navicula oligotrapheta	0	0	0	0	0	0	0	0	0
Navicula reinharti	0	0	0	0	0	0	0	0	0
Navicula recens	0	0	0	0	0	0	0	0	0
Navicula rhynchotella	0	0	0	0	0	0	0	0	0
Navicula tripunctata	0	0	0	0	0	1	0	0	0
Navicula splendica	0	0	0	0	0	0	0	0	0
Navicula veneta	0	0	1	0	0	0	0	0	0
Neidium binodeforme	0	0	0	0	0	0	1	0	0
Nitzschia bergii	0	0	0	0	0	0	0	0	0
Nitzschia capitelata	22	4	5	3	4	7	0	3	14
Nitzschia debilis	0	0	0	0	0	0	0	0	0
Nitzcshia draveilensis	1	0	2	0	0	0	0	0	0
Nitzschia dissipata	8	1	5	0	1	0	0	1	17
Nitzschia flexa	0	0	0	0	0	0	3	0	0

Table A19. continued

Nitzschia frustulium	0	0	0	0	0	0	0	0	0
Nitzschia hantzschina	0	0	0	0	1	0	0	0	0
Nitzschia inconspicua	0	0	0	0	0	0	0	0	0
Nitzschia lacuum	0	0	0	0	0	0	0	0	0
Tryblionella levidensis	1	0	0	0	0	0	0	1	3
Nitzschia linearis	2	0	0	0	0	0	0	0	2
Nitzschia paleaformis	0	0	0	0	0	1	1	0	0
Nitzschia paleacea	0	0	0	0	0	0	0	0	0
Nitzschia palea	9	2	0	6	8	1	*0*	8	3
Nitszchia pusila	0	1	1	0	1	3	*0*	2	0
Nitzschis sigmoidea	0	0	0	0	0	0	*0*	0	0
Nitzschia sigma	0	0	0	0	0	0	*0*	0	0
Nitzschia sociabilis	0	0	0	0	0	0	*0*	0	0
Placoneis clementis	0	0	0	0	0	0	*6*	0	0
Pinnularia brebisoni	0	0	0	0	1	0	*0*	0	0
Pinularia subgibba	0	0	0	0	0	0	*0*	0	0
Pinnularia subcapita	0	0	0	0	0	0	*0*	0	0
Rhoicosphenia abbreviata	0	0	1	1	0	0	*0*	1	0
Reimeria sinuata	12	0	1	9	3	2	*0*	3	4
Sellaphora bacillum	0	0	0	0	0	0	*0*	0	0
Sellaphora pupula	0	0	0	0	0	0	*0*	0	0
Sellaphora seminulum	0	0	0	0	0	0	*0*	0	0
Stauroneis pseudosubobstusides	0	0	0	0	0	0	*0*	0	0
Surirella amphyioxis	0	0	0	0	0	0	*0*	0	0
Surirella angusta	0	0	2	0	0	0	*0*	0	0
Surirella crumena	0	0	0	0	0	0	*0*	0	0
Surrirella linearis	0	0	0	0	0	0	*0*	0	0
Surirella terricola	0	0	0	0	0	0	*0*	0	0
Surirella brebisoni	19	3	3	0	1	1	*22*	0	5
Surirella pinnata	0	0	0	0	0	0	*0*	0	0
Tabellaria floculossa	0	0	2	0	0	0	*0*	0	0
Tabellaria spp	0	0	0	0	0	0	*5*	0	0

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