The impact of sewer misconnection effluents on diatom communities

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Abstract

Sewer misconnections lead to direct discharge of foul sewage to surface waters, which conflicts with one of the fundamental aims of a separate sewer system - to prevent discharge of foul sewage to surface waters. Though misconnections are often thought to be a threat to receiving water bodies, and to achieving Water Framework Directive targets, they have received little study to date, and their ecological impacts remain unknown. Review of current knowledge of misconnections and their potential impacts in receiving waters identified key areas of research which are required to improve understanding of the misconnection problem. To fully understand the impacts of misconnection effluents in receiving waters, their impacts in biological communities must be considered. Diatoms, a group of organisms which are commonly used for ecological assessments, play an important role in freshwater ecosystems and exhibit many characteristics which make them ideal for use in investigating response to intermittent stresses. This thesis investigates the impacts of misconnection effluents in the diatom communities of receiving waters.

Current methods to identify misconnection effluents in sewer systems lack sensitivity to misconnection effluents specifically, and suffer from issues of low sampling frequency. An inexpensive, passive, method for detecting misconnection effluents in sewer systems using optical brighteners produced very promising results, and is highly appropriate for use by the Environment Agency and Water companies, pending further validation.

Exposure of diatom communities to detergent effluents in a microcosm study show that detergents cause significant decrease of algal total abundance and notable change in community composition a closed laboratory system. These effects correlate well with high surfactant concentration and alkalinity in the detergents, leading to the conclusion that these are key drivers of responses in the diatom community. Specific diatom species are identified as potential indicators of detergent pollution, showing strong increases in abundance when exposed to high detergent concentrations.

Diatom communities exposed to misconnection effluents in the field in one catchment showed a shift in community composition toward more motile species, indicative of exposure to organic pollution discharged from the misconnected outfalls, however communities in three other catchments did not show any such response. Common diatom based monitoring measures do not respond to effluents from misconnected sewer outfalls, and diatom

communities do not show the decrease in abundance or shifts in species composition which were observed in the laboratory.

Misconnection effluents have potential to cause significant impacts in diatom communities. However, low frequency of discharge, short exposure period, and high dilution of effluents, prevent significant responses in the diatom community. In the upland streams investigated, misconnections do not pose a significant threat to diatom communities.

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Abbreviations

ANOVA - Analysis of Variance

ASPT - Average Score Per Taxon

BOD - Biochemical Oxygen Demand

BDI - Biological Diatom Index

BMWP - Biological Monitoring Working Party

COD - Chemical Oxygen Demand

CSO - Combined Sewer Overflow

DEFRA - Department for Environment, Food, and Rural Affairs

DIY - Do It Yourself

DO - Dissolved Oxygen

FIO - Fecal Indicator Organisms

IDG - Indice Biologique Diatomique

IPS - Indice de Polluosensibilite

OB - Optical Brightener

PAH - Polyaromatic Hydrocarbons

PSWO - Polluted Surface Water Outfall

RIVPACS - River Invertebrate Prediction and Classification System

SPI - Specific Pollution Index

TDI - Trophic Diatom Index

Tukey's HSD - Tukey's Honestly Significant Difference

UKWIR - UK Water Industry Research

UV - Ultra Violet

UV-VIS - Ultra Violet Visible Spectrometry

WFD - Water Framework Directive

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Chapter 1

Thesis introduction

1.1 Urban diffuse pollution overview

A particular threat to urban streams and rivers is urban diffuse pollution, which does not have a specific source, but is generated throughout urban catchments, and enters water courses by a wide variety of routes (Walsh, 2000). This is associated with aspects of the urban stream syndrome such as increased nutrients and pollutants, reduced biotic richness, and proliferation of tolerant taxa (Walsh *et al.*, 2005; Maltby *et al.*, 1995a,b). The wide range of potential pollutants and pollution sources which may contribute to urban diffuse pollution make it extremely difficult to tackle, as no single solution can be employed to rectify all sources (D'Arcy *et al.*, 2000). It is therefore crucial to identify major sources of urban diffuse water pollution, and their impacts in receiving waters, in order to target major threats with remediation.

In the UK there are two basic sewer system structures: Combined sewer systems, and separate sewer systems, both of which have benefits and drawbacks in terms of their impacts in receiving waters (Brombach *et al.*, 2005). Combined sewer systems use a single pipe which conveys wastewater from households and rain water from surface water run-off, and conveys them to be treated at municipal sewage treatment works. At the treatment works, wastewater is treated to a high standard, and discharged to local watercourses. Significant work on reducing point source pollution has substantially reduced the threat of these inputs to surface waters (Oliveira & Goulder, 2006). Along the course of combined sewer systems, combined sewer overflows (CSOs) exist, which are designed to discharge excess sewage from the pipe when there is sufficiently high flow to overwhelm the treatment works. As such, CSOs should only discharge during periods of particularly high flow in the sewer. This may occur because of intense rainfall events which significantly increase the flow in the sewer system, though increased pressure on increasingly antiquated sewer systems can lead to more frequent CSO discharges (Thomas & Crawford, 2011). Though CSOs lead to raw sewage being discharged directly to surface waters, they should only discharge during high flows, and therefore any sewage discharged should be extremely dilute. However, these diluted discharges can still have significant impacts in receiving water biology and chemistry (Rochfort *et al.*, 2000).

Separate sewer systems consist of a foul water sewer, which discharges wastewater directly to sewage treatment works, and a surface water sewer, which discharges surface run-off directly to local water courses. This leads to a relatively constant flow of wastewater, preventing the threat of treatment works becoming overwhelmed, and preventing the problem of intermittent discharges of diluted raw sewage to surface waters. However, surface water sewers do not discharge entirely clean water. Urban run-off discharging from roads can contain a wide variety of pollutants including oil, fertilisers, metals such as lead and zinc, and organic pollutants such as poly-aromatic hydrocarbons (PAHs) and pesticides, the concentrations of which may show major temporal variations (Boxall & Maltby, 1995, 1997; Goulding *et al.*, 2010). However, as discharge from surface water sewers undergoes little or no treatment before discharge to receiving waters, any polluted water discharged to surface water sewers will be conveyed directly to streams and rivers.

1.2 Misconnected sewer systems

Direct discharge to surface water sewers from human activities such as on-the-street car washing, improper disposal of waste, duel manholes, and sewer misconnections, can lead to a wide range of pollutants, generally linked to domestic activities being released into surface water sewers. Misconnections are defined in many different ways, and the term can be used to refer to industrial and domestic discharges to surface water sewers, including those of toilets (Chapter 2). In this thesis misconnections are defined as "direct discharge of domestic wastewater or greywater to surface water sewers", and therefore industrial misconnections are not investigated, though some industries such as laundrettes are expected to have similar impacts to those of domestic inputs, if larger in scale. These discharges are expected to contain a large quantity of soaps and detergents, nutrients, and many potentially toxic organic compounds (UKWIR, 2012). Though these discharges of wastewater and grey water are generally lower in volume than CSO discharges, they are often undiluted, and therefore may lead to similar impacts to CSOs (De Toffol *et al.*, 2007; Welker, 2007). As CSOs are designed into combined sewer systems, it is expected that they will discharge sewage at times of high flow. In contrast, polluted discharge from surface water sewers defeats the aim of the separate sewer system, and renders it ineffective.

With estimates ranging from 0.2% (DEFRA, 2009) to 20% (Environment Agency, 2007) of properties in the UK having misconnected appliances, these discharges may be major contributors of environmentally important pollutants to ecological impacts in river ecosystems. Misconnections are expected to contribute to locally significant increases in nutrient concentrations, toxic pollutants such as nonyl-phenols and triclosan, soaps and detergents (UKWIR, 2012). They therefore may affect the ability to achieve goals set by the European Water Framework Directive (2000/60/EC) (WFD), which aims to achieve good ecological status in all surface water bodies, as well as identifying and assessing major pressures on ecosystems, including diffuse pollution sources (Kallis & Butler, 2001). A key component of monitoring for the WFD is monitoring of the biota of ecosystems, separated into phytoplankton, macrophytes and periphyton, benthic invertebrates, and fish (Pollard & Huxham, 1998). In order to determine whether misconnection effluents pose a real threat to WFD goals, their impacts in freshwater communities must be investigated.

1.3 Investigating the ecological impact of misconnections.

Organisms within rivers are important providers of ecosystem services such as nutrient cycling, primary production, biodiversity, and pollution remediation (Dudgeon *et al.*, 2006). Discharge of pollutants to surface waters can have major impacts in the structure and function of freshwater ecosystems, leading to impoverished ecosystems which may suffer from reduced biological functioning (Cardinale, 2011). Biological communities can also be used to investigate the impacts of pollution in surface water ecosystems. They respond dynamically to the presence of pollutants, and give an integrated indication of the recent chemical history of the water course (Li *et al.*, 2010). The structure of these communities can give a strong indication of pressures upon the ecosystem, as species respond characteristically to different stresses (Holt & Miller, 2011).

For an intermittent diffuse issue like misconnections, biological communities are ideally suited to identify and investigate impacts which would often not be observed in instant water chemistry analyses. Diatoms and macroinvertebrates are groups of organisms which are commonly used to investigate the ecological status and impacts of pollution such as sewage in freshwater ecosystems (Menezes *et al.*, 2010; Smucker & Vis, 2011b; McCormick & Cairns Jnr, 1994). Though fish are also a key group of organisms in river ecosystems (Holmlund & Hammer, 1999), they tend to have a patchy distribution, are difficult to sample, and naturally vary over greater spatial scales than individual misconnections would be expected to impact.

1.3.1 Macroinvertebrates

Macroinvertebrates are important organisms in the cycling of nutrients in river systems and decomposition, as well as an important link between primary producers and higher trophic levels (Malmqvist, 2002). Invertebrate indices such as RIVPACS (River Invertebrates Prediction and Classification System) (Wright *et al.*, 1998), BMWP (Biological Monitoring Working Party) and ASPT (Average Score Per Taxon) (Hawkes, 1998), are commonly used to determine the ecological quality of water bodies. These are measures of overall ecological quality, and do not specify impacts from particular pollutants.

Invertebrates have been used for biomonitoring from the individual species level, up to the community level (Bonada *et al.*, 2006; Hering *et al.*, 2006) for many years. They have the benefit that many are long lived organisms (Resh, 2008), and therefore individuals may be exposed to repeated intermittent discharge of misconnections for long periods of time. They also respond to the organic and nutrient pollution which is expected to be a major component of misconnection discharges (Blanco & Becares, 2010; Guilpart *et al.*, 2012).

However, with long generation times it can take a long time for species to recover from a single major event, as they may not be able to recolonise habitat until reproduction is possible. Therefore the community may not be representative of the current state of the river chemistry, and instead reflect a previous major event in the river. Invertebrate communities also respond strongly to catchment factors such as imperviousness of surface, river bed composition, and flow (Sonneman *et al.*, 2001; Walsh *et al.*, 2001) which may mask effects of potentially low pollutant concentrations.

1.3.2 Diatoms

One of the key groups of organisms used to determine ecological status of water courses are macrophytes and phytobenthos, plants and algae which live within the river channel (Pollard & Huxham, 1998). These are commonly characterised by the diatoms, a diverse group of unicellular algae (Kelly *et al.*, 2008). Diatoms often constitute the majority of primary producer biomass in river ecosystems (Power, 1990). Factors which have a strong effect on diatom communities can affect the energy available in the ecosystem, and thus impact throughout aquatic food webs.

Diatom communities have been used to monitor impacts from a broad range of pollutants in freshwater ecosystems across Europe (Kelly *et al.*, 1995; Potapova & Charles, 2002; Dixit *et al.*, 1992; Coste *et al.*, 2009), leading to the development of indices including the trophic diatom index (TDI) (Kelly *et al.*, 1995, 2001, 2008), specific pollution sensitivity index (SPI) (Coste & Ayphassorho, 1982), and biological diatom index (BDI) (Lenoir & Coste, 1996).

Diatoms are particularly useful when measuring short term impacts in rivers due to their short life cycles (Fore & Grafe, 2002; Sonneman *et al.*, 2001), limited mobility (Resh, 2008), and rapid, accurate, cost effective data collection (Round *et al.*, 1990), meaning that the community which is present is representative of recent conditions at that point in the river. Communities quickly adapt with changes in water quality (Stevenson & Pan, 1999), and are among the first organisms to respond to environmental change in freshwater ecosystems (Lavoie *et al.*, 2008). This means that impacts from intermittent inputs such as misconnection effluents will quickly be realised in the community structure (Juttner *et al.*, 2012). Diatom species show species specific responses to different pollutants (Kelly, 1998), but also communities respond broadly to more generalised pollution inputs (Hering *et al.*, 2006).

1.4 Thesis aims

The aim of this thesis is to investigate the impacts of sewer misconnections in diatom communities, a key group of organisms in freshwater ecosystems, and to develop current methods for indicating the presence of misconnection effluents in sewer systems and receiving waters. This aim is achieved using four key objectives:

1.*Identification and review of current knowledge and potential impacts of sewer misconnections (Chapter 2).* The aim of this chapter is to review the academic and grey literature related to misconnections. Important challenges which are currently facing misconnection research are considered using the limited publications which are available on the subject. Wider literature review is then used to investigate the potential problems which misconnections may cause.

2. *Development of a low cost method to detect polluted surface water outfalls and misconnected drainage (Chapter 3).* This section aims to test and develop a new monitoring method for identifying misconnection effluents in surface water sewer systems, using optical brighteners as an indicator of pollution. This allows rapid, inexpensive and unmistakable indication, providing new opportunities for active misconnection identification. This also allows the identification of misconnected sewer systems in the field, in order to perform the work in Chapter 5.

3.*Investigation of the response of diatom communities to artificial detergent effluents (Chapter 4).* The aim of this section is to determine the response of natural benthic diatom communities to detergent effluents, which are indicated as unique and common pollutants in misconnection discharges, using a microcosm experiment. This shows the impacts which misconnection effluents may cause in natural communities, and demonstrates the effectiveness of diatoms as indicators of pollution which is expected in many misconnection discharges.

4. *Investigation of the impact of sewer misconnections in natural diatom communities (Chapter 5).* This section aims to investigate the response of diatom communities in natural ecosystems to real misconnection effluents. This tests whether effects observed in Chapter 4 can be observed in the field, and whether natural communities were impacted more severely by misconnected sewer outfalls than correctly connected outfalls.

In chapter 6, the thesis concludes that misconnection effluents generally do not contribute significantly to the impacts of surface water sewer systems in diatom communities. Laundry detergent effluents, expected to be a common component of misconnection effluents, impact algal communities by reducing total abundance and altering community composition. In the field, specific impacts of detergent effluents were generally not observed, but some misconnected outfalls show shifts in community structure toward more motile species, indicating organic pollution entering the water course. Misconnection effluents were successfully identified in surface water sewer systems using cotton samplers to adsorb optical brighteners. Future work should focus on the use of other biological communities in freshwater ecosystems, such as macroinvertebrates, and should thoroughly identify the individual misconnections on each sewer system.

Chapter 2

Domestic sewer misconnections: a review of current knowledge

2.1 Introduction

Sewer misconnections are the connection of domestic appliances to surface water sewers (Figure 2.1), a source of urban diffuse pollution. Though misconnections have been a problem on separate sewer systems since they were first introduced in the 1950s (Butler & Davies, 2004), there has been very little study of the problem to date.

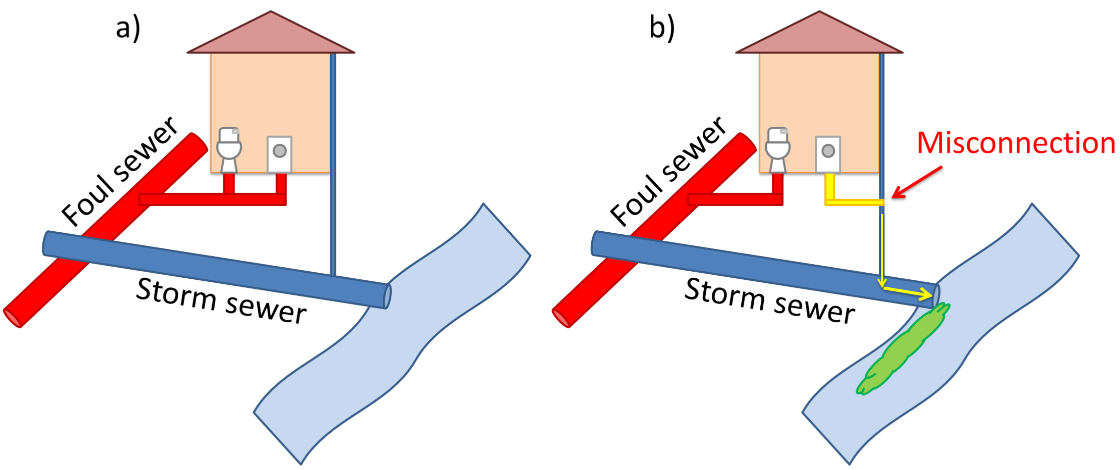

Figure 2.1: a) Correctly connected separate sewer system. b) misconnected separate sewer system

There are a wide range of terms used to describe misconnections and similar problems, summarised in table 2.1. This can lead to confusion over the precise issue which is being referred to in individual cases (Dunk *et al.*, 2008) and there is currently no unified terminology even within specific countries to define these issues. In this thesis misconnections are defined as "direct discharge of domestic wastewater or greywater to surface water sewers".

Table 2.1: Summary of terminology relating to misconnections and similar problems.

Term	Region used	Definition	Example
Misconnection	UK	Wastewater released into surface water sewers from domestic appliances	(Dunk *et al.*, 2008, 2012; Edmonds-Brown & Faulkner, 1995)
	UK	Connection of domestic and industrial appliances and properties to surface water sewers	(Marsden & Mackay, 2001)
	UK	not specifically defined	(Baker *et al.*, 2003)
Wrong connection	UK	Wastewater released into surface water sewers from domestic appliances	(D'Arcy *et al.*, 2000)
	The Netherlands	Wastewater released into surface water sewers from domestic appliances	(Kluck.J. & F., 2008)
Cross connection	UK	Mixing of effluent from foul sewer and surface water sewers	(Environment Agency and Water UK, 2014)
	USA	Discharge from a foul sewer to a surface water sewer	(Pitt *et al.*, 2004)
	UK	not specifically defined	(Baker *et al.*, 2003)
	USA	Connection of a potable water supply with a non-potable water system	(Georgia Water and Sewer Bureau, 2001)
Polluted surface water outfall	UK	Wastewater released into surface water sewers from domestic appliances	(Dunk *et al.*, 2012)
Non-agricultural diffuse water pollution (NADWP)	UK	Overarching term referring to any pollution from dispersed urban sources	(Environment Agency, 2007)
Illicit discharge	USA	Surface water outfalls with dry weather flow containing pollutants and/or pathogens	(Pitt *et al.*, 2004)
	USA	Presence of anything in a surface water sewer which is not surface water run-off	(Braun, 2011; Corson-Knowles, 2005)
Illicit connection	The Netherlands	Wastewater released into surface water sewers from domestic appliances	(de Haan *et al.*, 2011; Hoes *et al.*, 2009)

This shows that similar problems can be caused in the sewer system and the receiving water by a wide range of sources, both industrial and domestic. In addition to this complexity, surface water run-off can contain high levels of pollutants (Brombach *et al.*, 2005) which may lead to similar effects to those of misconnections. These sources of variation in discharge from surface water sewers makes it difficult to differentiate the problems of sewer misconnections from those of other aspects of the sewer system.

This chapter reviews current knowledge on five important aspects of misconnection research - The magnitude of the misconnection problem, the cause of misconnections, identifying misconnection discharges and sources, the impact of misconnection effluents, and correcting misconnections. Recommendations are then made on future developments in each of these key areas to maximise the benefit of advances in the field.

2.2 The magnitude of the misconnection problem

A number of studies have investigated and/or estimated the percentage of misconnected properties in different regions and catchments (table 2.2). These show a wide range of values, produced largely from data in the London area, and some data from Severn Trent water, or from unknown data sources. This brings into question the applicability of these data sets for use in broad scale estimations at a larger spatial scale, as the rate of misconnections may vary between regions throughout the country.

Table 2.2: Estimates of number of misconnections in separate sewer catchments in the UK.

Size of study	Estimate (percentage of properties)	Reference	Additional information
England and Wales	20%	Environment Agency (2007)	Origins of data unclear
England and Wales	0.6-2%	DEFRA (2009)	Origin of data unclear
England and Wales	1 million properties (approximately 4.3%)	Consultants in Environmental Services Ltd., (1999)	Cannot access original report (information from Dunk *et al.* (2008))
95,130 surveyed properties	3.9%	UKWIR (2012)	Data from Severn Trent Water
7 catchments, Torbay	1%	UKWIR (2012)	Data from South West Water, number of properties in catchments is unclear
Thames region	10%	Dunk *et al.* (2008)	
Milk street, Bromley	3%	Dunk *et al.* (2008)	misconnections included; 3 toilets, 11 baths, 17 hand basins, 14 dishwashers, 2 showers, 26 kitchen sinks, 29 washing machines
Pyrles Lane, Loughton	3.5%	Dunk *et al.* (2008)	Misconnections included; 5 toilets, 49 washing machines, 14 dishwashers, 27 kitchen sinks, 23 hand basins, 8 sinks, 9 showers, 7 baths, 4 water softeners, 1 bidet, 1 swimming pool

Individual studies at the catchment scale have been performed and the number of polluted outfalls in each catchment, identified by visual inspection, are shown in table 2.3. While these studies show a large number of visibly polluted outfalls in sewer catchments, these are not necessarily directly associated with misconnection discharge, and may be polluted due to other problems such as industrial discharges or incorrect disposal of waste. There is no indication of whether these catchments were randomly selected, or whether they were selected because large quantities of pollution were previously known about, therefore bringing into question the accuracy of estimates based on extrapolation from these examples.

Table 2.3: Estimates of number of polluted surface water outfalls in catchments in the UK.

Size of study	Number of polluting outfalls	Study	Additional information
Mayes Brook (30 surface water outfalls)	21 (70%)	UKWIR (2012)	Data from Thames Water
Roxbourne River (24 surface water outfalls)	14 (58%)	UKWIR (2012)	Data from Thames Water
Wealdstone Brook (96 surface water outfalls)	32 (33%)	UKWIR (2012)	Data from Thames Water
Moselle Brook (19 surface water outfalls)	12 (63%)	UKWIR (2012)	Data from Thames Water
3 catchments on the south coast of the UK (63 surface water outfalls in total)	34 (54%)	UKWIR (2012)	Data from Southern Water, number of properties in catchments is unclear, and which catchments they are is unclear
River Rom at Collier Row, Romford (30 surface water outfalls)	12 (40%)	Internal report from Thames Water	Found 131 misconnections from various household appliances over the period 2005-2010

Ellis (2013) observed a major mismatch between the number of misconnections which are occurring, and those which are known about. This strongly indicates that a larger data set is required before accurate estimations of the number of misconnected properties can be calculated. This larger data set should incorporate data from multiple regions of the UK, and multiple catchments within those regions, while not focussing exclusively on known severely polluted cases.

2.3 The cause of misconnections

Misconnections are generally reported as being caused by bad initial construction, badly trained or DIY plumbers, the addition of extensions to existing properties, and other practices (UKWIR, 2012). These reports are often anecdotal, and relatively few in number. Investigation into the sources of misconnected drainage which are particularly common would allow focussed prevention measures to target appropriate user groups, therefore reducing the problem at the source.

There are publicly available resources relating to the problem of sewer misconnections in the form of the newly constructed *www.connectright.org.uk* website, which provides information on how to identify and correct sewer misconnections for home-owners and businesses. While this is a valuable resource for disseminating knowledge regarding misconnections and misconnection rectification, it is unlikely that people will visit the site unless they are already interested in misconnections. This then raises the question of how to spread knowledge of the misconnection problem.

Misconnections are not a widely discussed subject, and are rarely investigated unless they are known to be causing contamination in receiving waters. There is a general lack of knowledge of sewerage systems among the public. Though improved provision of information by water companies to their customers may help to improve knowledge, novel techniques may be required to successfully inform the public (Dunk *et al.*, 2012). This has been tried in other diffusion pollution areas, such as the yellow fish scheme which involves painting yellow fish by roadside drains in order to indicate that those drains discharge directly to natural habitats (Environment Agency, 2012), however there is no evidence that this novel approach had successful results.

Without substantial preventative developments, the threat posed to receiving waters by sewer misconnections will only increase, as reactive rectification inherently cannot maintain pace with the introduction of misconnections to sewer systems. Proactive methods such as public education have great potential to increase awareness of the problem and therefore reduce the threat of misconnections, however performing this in an engaging and inspirational manner may prove difficult. Misconnections will always be a threat to receiving waters, without prevention at their source - the plumbers, builders, and members of the public engaging in DIY who introduce them.

2.4 Identifying misconnection discharges and sources

Whilst not a new development, sewer misconnections have only relatively recently started to attract focussed attention as an important source of pollution in surface waters. As such, there is not a large body of information about misconnections, such as exists for point source and other diffuse pollution issues. Misconnections pose a diverse range of threats to surface waters due to the wide variety of pollutants which may be discharged depending on the specific misconnected appliances, and the specific substances used in those appliances. This means that methods used to identify and monitor one misconnection effluent, may fail to identify another. Ellis (2013) found that the vast majority of misconnections present in sewer systems remain undetected, and therefore developing methods to detect misconnections should be a priority.

Detection of misconnection effluents in receiving waters is an area which is currently far from ideal. A method to detect misconnection effluents should take into account the intermittent nature of the problem and be relatively insensitive to pollutants from other sources which may occur alongside misconnections. The most common water sampling technique used at present is dip sampling (Roig *et al.*, 2007), which is unlikely to identify misconnection discharges unless they are occurring at the time of sampling. Cassidy & Jordan (2011) suggest that only constant monitoring of surface waters will account for the observed large natural variation in water quality. Misconnections will only increase this variability, making it even more crucial that frequent or constant monitoring be applied to account for misconnection discharges in water quality values. Conversely, current monitoring for the Water Framework Directive is recommended once every three months for surface waters, or once every month for priority substances (Facchi *et al.*, 2007). This is clearly not sufficient to detect inputs from sewer misconnections, which may vary greatly within a very short space of time (Almeida *et al.*, 1999).

There are a range of methods currently used to identify misconnection discharges in sewer systems and receiving waters (Environment Agency and Water UK, 2014; Center for Watershed Protection & Pitt, 2004). These include visual inspection for aesthetic indicators of pollution (Hickey, 1988; Pitt *et al.*, 2004; Environment Agency and Water UK, 2014), distributed temperature sensing (de Haan *et al.*, 2011; Hoes *et al.*, 2009), and optical brightener detection using fluorometers (Braun, 2011). Once misconnection discharge has been identified in a sewer system, dye testing can be used to identify specific misconnected appliances (Hoes *et al.*, 2009; Environment Agency and Water UK, 2014). This is a labour intensive method, requiring access to polluting properties, however at present it is the only accurate method to identify specific polluting appliances. The application of these methods is discussed in more detail in Chapter 3.

To directly investigate the impact of misconnections in the field, another integrated method is to use biological communities such as invertebrate or diatom communities. These can be used as measures of ecosystem quality, as they are constantly exposed to discharges, and are widely used as indicators for pollutant inputs to surface waters, including nutrients and organic pollutants (Wright *et al.*, 1998; Blanco & Becares, 2010; Kelly *et al.*, 2008; Coste *et al.*, 2009). This may be a relatively simple method to detect misconnection impacts, using skills which are common throughout both the Environment Agency and ecological consultancies. However, biological investigation of misconnected sewer outfalls has not been thoroughly investigated to date.

An alternative method which relies less on the sewerage provider, would be to enshrine testing for misconnections in the condition report or building survey used when buying and selling houses, so that when a property changes hands, it can be tested for misconnections. This would allow piecemeal but thorough investigation of misconnections. Between 2009 and 2013, an average of 837,000 property transactions occurred per year in England (HMRC press office, 2014). With 23.4 million properties in England and Wales (Office for national statistics, 2014), this suggests that on average each property will change hands approximately once every 28 years, and therefore in 28 years the vast majority of misconnections could be corrected. This would also allow constant re-testing to ensure that if misconnections occur during ownership, they are fixed relatively quickly. However, this may be difficult to establish in survey requirements due to additional expense of sewer investigations.

In the UK, misconnections are generally only investigated once a member of the public has reported a pollution problem. By the time pollution from misconnections is sufficiently severe to cause aesthetic impacts, they may have already been discharging for long periods of time. To effectively identify misconnections, more pro-active methods are required. Pro-active methods should be inexpensive to allow wide application, and should be specific to misconnection pollution.

2.5 The impact of misconnection effluents

Sewer misconnections are expected to discharge a wide range of different compounds and chemicals depending on the appliances which are misconnected. This section aims to summarise impacts which have been observed in the few misconnection studies which have taken place, and identify common components of misconnection discharge, and their potential impacts in receiving waters.

Reports from Environment Agency projects briefly summarise the outcomes of misconnection rectification operations (Table 2.4). These are often qualitative, demonstrating benefits which rectifying misconnections can bring to local water courses. Though these are vague in content, they do show that misconnection investigations are often instigated due to water quality problems, relating to the Bathing Water and Water Framework directives. These reports also highlight a general lack of follow up investigations after most projects. Confirmation of the outcomes of misconnection rectification projects is crucial to determine their effects in receiving waters, otherwise change in the quality of the receiving water cannot be measured, and therefore the impact which misconnection discharges had cannot be quantified.

Table 2.4: Summaries of internal Environment Agency reports.

Site	Scale of project	Reason for project	What was done	Outcome
Bixley Heath SSSI - Ipswich	500 houses	Water quality	Identified/rectified 30 misconnections	Water quality improved
Torbay	Not known	Bathing water quality	Identified/rectified 105 misconnections	Improved bathing water quality, public education
South West region	12 urban sub-catchments	Bathing water quality	Identified 170 misconnections	Not known
Hart dyke, Ashford	Not known	Education/ Investigation	Identified/rectified misconnections	Removed pollution, public education
Kings Lynn, Heacham and Hunstanton	Not known	Bathing water failure	Identified/rectified various polluting sources	Not known
Mablethorpe	Not known	Bathing water failure	Identified pollution sources	Not known
Buck beck, Cleethorpes	Not known	WFD failure	Identified polluted outfalls	Not known
Haverigg, Haysham	Not known	Bathing water failure	Identified sources of pollution	Not known
Herefordshire	Not known	WFD failure	Identified 6 polluted surface water outfalls	Not known
Loddon and Wandle catchments	Not known	Ecological status	Investigation of phosphorus discharge	Insignificant P inputs, no misconnections found
Newbury	Not known	WFD failure	Identified polluted outfalls	Not known

Notes: the reports used in this table were provided by - Katy Bray - South Suffolk Environment Management, and Rob Dryden - FRB (Bixley Heath SSSI, Ipswich), Mike Ingham, South West Water, and Torbay Council (Torbay bathing waters project), Nick Smart (South West Region, UK), Barrie Neaves (Park farm, Hart dyke, Ashford), Ian Mears and Anna Pearce (Kings Lynn, Heacham and Hunstanton), Chris Martin (Mablethorpe, Buck Beck, Cleethorpes), Paul Simmons (Haverigg, Heysham, and Morecambe), Simon Worrall and Andrew Osbaldiston (Herefordshire), Lars Akesson (Loddon and Wandle catchments), Mark Barnett (Newbury).

The impacts of misconnections can be inferred by looking at the pollutants which are expected to be present in discharges, however there have been few studies to validate the contribution of misconnections to those pollutants in surface waters. This is partially due to the difficulty in separating the impacts of misconnection effluent from those of the run-off from the surface water outfall, which can be significant (Payne & Hedges, 1990), and partially due to historically greater threats such as point source pollution, being more severe and therefore demanding a greater importance. A small number of studies however have considered misconnection discharges as sources of pollution.

The Pymmes brook is a tributary to the River Lee in London, which has both combined and separate sewer systems discharging to it. The brook had suffered from sewage fungus and offensive odours (Faulkner *et al.*, 2000), both of which can be indicators of misconnections. Edmonds-Brown & Faulkner (1995) highlight misconnections alongside foulwater contamination as expected causes of the worst diffuse pollution in the catchment. This was confirmed after benthic invertebrate sampling showed distinct drops in Biological Monitoring Working Party (BMWP) scores at outfalls which were suspected of containing misconnections, though there is no evidence of attempts to separate the impact of the misconnection effluents from those of polluted run-off. Misconnections were reported at an outfall on Lawton Road, which caused "very poor water quality" at high flows due to a first flush effect from the sewer sediment (Faulkner *et al.*, 2000). Edmonds-Brown & Faulkner (1995) suggested a complete replacement of the sewer was required to ensure pollution events were prevented, however Dunk *et al.* (2008) show that even when misconnections are rectified on a system, they can quickly be reintroduced. Therefore it may be more appropriate to perform rigorous and repeated monitoring of outfalls, with focussed rectification campaigns rather than whole catchment replacement.

Hoes *et al.* (2009) investigated two catchments using distributed temperature sensing, one consisting of residential land use, and one industrial. In the residential catchment, 6 locations were identified with warm water, indicative of misconnection discharge, entering the sewer system. These were later verified by deliberately pouring warm water down the suspect appliances, where the same spike in temperature was observed. In a similar study, de Haan *et al.* (2011) found a discharge which was linked to 5 misconnected static caravans at a caravan park in the Ede catchment and a household misconnection caused by a renovation in an adjacent catchment, which discharged approximately once every 2 hours between 9:30am and 16:15pm, on a daily basis, attributed to a washing machine, dishwasher, or similar appliance. Even though these are relatively small misconnection problems, they still caused an impact which was significant enough to warrant investigation (de Haan *et al.*, 2011). This highlights the potential visual and environmental impact which even few misconnections can cause.

Snook & Whitehead (2004) found tributaries of the River Lee to have over 100 times the bacterial coliforms and 574 times the faecal coliforms allowed under the EC Bathing

Waters Directive (76/160/EEC). With no sewage treatment works on those tributaries, these values were attributed to combined sewer overflows and misconnections. Similarly the River Brent suffered from poor water quality which was attributed to misconnections, amongst other problems (Eden & Tunstall, 2006). However, there is no evidence in either of these studies to show that any work was carried out which specifically identified misconnections as a cause. Misconnections are often suggested as a cause of observed pollution when the source is unknown.

With the exception of the distributed temperature sensing studies, all of these peer reviewed studies have taken place in parts of London. This highlights the need for studies elsewhere in the U.K., to determine how well observations in the capital correlate with misconnection impacts in other parts of the country.

2.5.1 Contributions to pollutant loads from sewer misconnections

The volume of discharge and composition of effluent from misconnections is highly variable, depending on the type and number of misconnected appliances in a sewer catchment. Data available on misconnected discharges is limited to a small number of reports which have relatively small sample sizes (UKWIR, 2012; Ellis, 2013). However, misconnections are expected to be potential sources of many pollutants, including poly aromatic hydrocarbons (PAHs), sediments, metals, organic pollution, and nutrients (Ellis & Mitchell, 2006).

The most commonly misconnected appliances are expected to be washing machines and sinks (figure 2.2) (Dunk *et al.*, 2008; UKWIR, 2012), which also consume a large portion of the water used by a household (figure 2.3). Therefore much of the water discharged from misconnections is expected to be grey water (figure 2.4). Grey water contains discharge from sinks, washing machines, dishwashers, showers, and baths (Li *et al.*, 2009) but not foul water discharge from toilets. It constitutes approximately 75% of sewage from domestic households (Eriksson *et al.*, 2003).

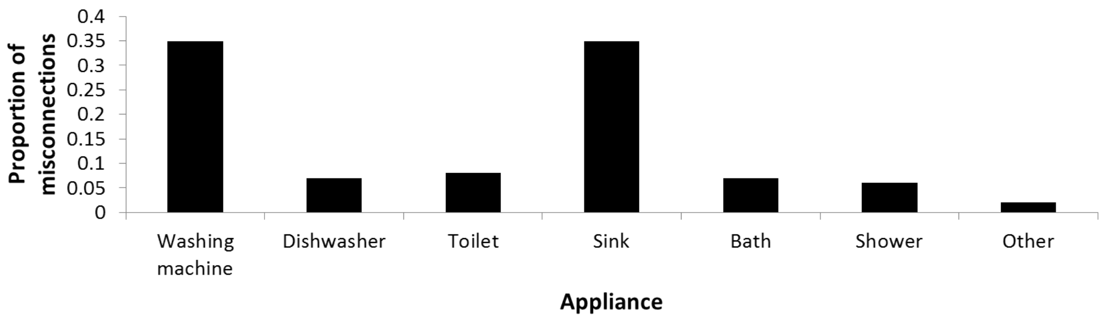

Figure 2.2: Distribution of misconnections among appliances in the UK. Data from UK-WIR (2012).

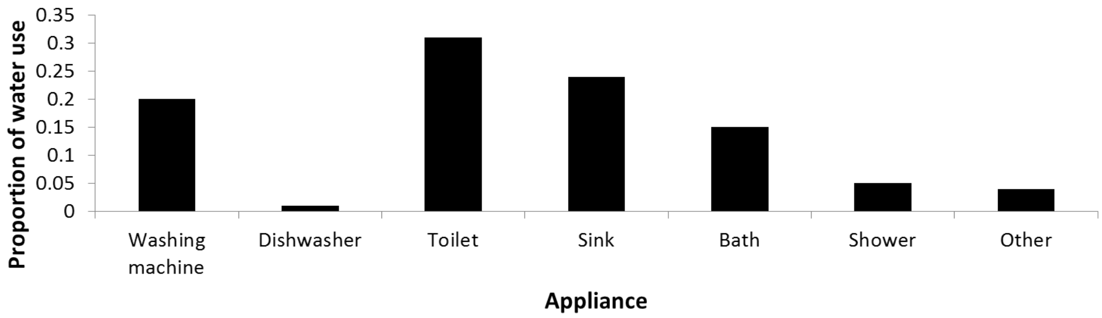

Figure 2.3: Average distribution of water consumption among appliances in a house in the UK over one year (data adapted from Anglian Water in Post (2000)).

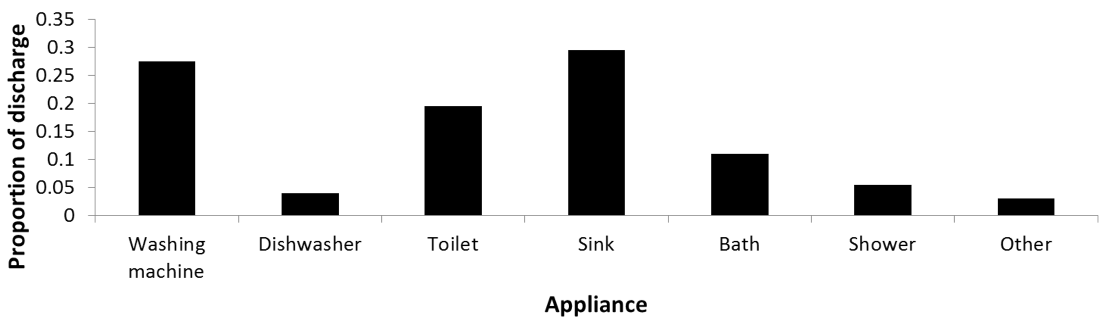

Figure 2.4: Expected proportion of misconnection effluent discharged from appliances in the UK. Calculated using data from Post (2000) and UKWIR (2012).

Bascombe *et al.* (1988) found that even minor combined sewer overflow events could lead to a notable decrease in diversity scores immediately downstream of outfalls, and Ellis (1995), Bascombe *et al.* (1988), and Seager & Maltby (1989) found decreased diversity up to 300m downstream of combined sewer overflows. These were attributed to toxicity, changes in flow dynamics, and sediment load from the additional inputs. Combined sewer overflows and misconnections, both result in a direct discharge of untreated wastewater to surface waters, and so impacts could be expected to be similar. The major difference is that CSOs may discharge large volumes of dilute sewage in a single event, whereas misconnections are likely to regularly discharge smaller volumes of concentrated sewage.

2.5.2 Nutrients

Nutrients are essential chemicals which are required for growth and development in organisms. The major nutrients which limit primary production in freshwater ecosystems are phosphorus and nitrogen. Although phosphorus is often present in stormwater discharge at relatively low concentrations (Duncan, 1999), raised levels of phosphorus in surface waters can originate from wastewater treatment plants, industry, and diffuse sources (Wind, 2007). As phosphorus removal methods at wastewater treatment works improve and become more widely used (Oehmen *et al.*, 2007), diffuse phosphorus sources from urban pollution become more important (Mainstone & Parr, 2002). UKWIR (2012) found phosphate to be a good indicator of pollution from misconnected domestic appliances, as it can be present at high concentrations in the major effluents expected to be present in misconnection discharges. High phosphate concentrations are expected to be characteristic of toilet, washing machine, and sink effluents (Butler *et al.*, 1995). Nitrate and nitrite can also be at raised levels in sewer discharges, particularly from kitchen sink and shower discharges (Almeida *et al.*, 1999), leading to potential eutrophication issues similar to those of phosphate, depending on which nutrient is limiting growth in the system.

Soluble reactive phosphorus is generally considered to be directly bioavailable to primary producers (Millier & Hooda, 2011). Other forms of phosphorus generally require transformation to orthophosphate before they become bioavailable (Reddy *et al.*, 1999).

House (2003) summarised these interactions:

Physical processes:

1. Inflow of P from the floodplain
2. Remobilisation of P-rich sediment and release of dissolved P from porewater
3. Deposition of sediment P on the river bed during stable and falling river discharge
4. Storage of sediment associated P in floodplain deposits

Chemical and biochemical processes:

1. Uptake of soluble reactive P and total dissolved P to sediments via sorption processes.

2. Desorption of P from sediments as the soluble reactive P in water decreases

3. Conversion of total dissolved P to soluble reactive P through breakdown of organophosphates and inorganic polyphosphates

4. Formation of total dissolved P from decomposition of plants and detritus

5. Biological uptake of SRP by macrophytes, phytoplankton, and benthic algae

Combination of physical and chemical processes:

1. Interaction of soluble reactive P with P-deficient material eroded from riverbanks

2. Retention of soluble reactive P in alluvial deposits on flood plains

Ammonia is expected to be a major component of discharge from toilets, but not discharge from other appliances (Almeida *et al.*, 1999). Ammonia and ammonium the ion in which form it is usually found, can be toxic to freshwater organisms at concentrations as low as 0.001-0.006mg/l NH_3-N (Passell *et al.*, 2007; USEPA, 1999). They can also contribute to acidification and eutrophication in surface waters, and can cause increased biochemical oxygen demand via nitrification, leading to the loss of sensitive species, and alterations in microbial processing ability (Camargo & Alonso, 2006). Soonthornnonda & Christensen (2008) found that foul sewage contributed over 58% of the ammonia which was detected in combined sewer overflows in the US. Ammonium is a useful indicator of the presence of foul sewage in storm sewer discharge, and therefore a possible indicator of misconnections, though toilets are expected to be infrequently misconnected, so this may not be ideal.

The impacts of excess nutrients in rivers are well known, including loss of species which are sensitive to high nutrient concentrations, and eutrophication, which can lead to hypoxic or anoxic conditions in severe situations (Carpenter *et al.*, 1998). However, though nutrients are expected to be present in almost all misconnection discharges, misconnections are not expected to discharge exceptionally large quantities of nutrients except in extremely severe cases. A more probable outcome is that nutrient levels may be moderately increased, leading to biological communities becoming dominated by species which are more nutrient tolerant.

2.5.3 Organic pollution - general

Organic pollutants are present in surface water, foul water, and grey water, and are therefore likely to be present in effluent from misconnections. Ellis & Mitchell (2006) highlight suspended solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD), and poly-aromatic hydrocarbons (PAHs) as key issues related to organic matter from diffuse urban pollution. Under normal circumstances, much of the organic matter from foul sewage would be degraded at a wastewater treatment works (Robson & Neal, 1997), however misconnections bypass this, and therefore these organic compounds may be important threats posed by misconnections.

The presence of excess suspended solids can increase turbidity in receiving waters, leading to reduced light levels in the water which in turn can reduce primary production in the system (Hill *et al.*, 1996). A colmation layer may form on the river bed depositing a layer of fine sediment which covers the underlying natural substrate. This can cause major structural changes to benthic habitats, and can affect invertebrate, macrophyte, algae, and fish populations (Heppell *et al.*, 2009; Clarke & Wharton, 2001; Acornley & Sear, 1999) by smothering habitats, and reducing the heterogeneity of the river bed.

The degradation of organic matter through microbial metabolism can lead to an increased BOD, and therefore reduced dissolved oxygen (DO) (Dodds, 2007). Release of untreated sewage, particularly into relatively small receiving waters can cause severe anaerobic conditions (Daniel *et al.*, 2002), favouring species which are tolerant of anaerobic conditions (Parr & Mason, 2003). Unimpacted surface waters usually have between 70% and 100% dissolved oxygen saturation, and a minimum of 30% is usually required to support large organisms such as fish (Dunk *et al.*, 2008), and therefore untreated discharge from misconnections may affect large organisms directly.

2.5.4 Xenobiotic organic compounds

Xenobiotic organic compounds are compounds which would not normally be present in UK waters, including components of detergents, soaps, shampoos, perfumes, preservatives, dyes, tea, coffee, dairy products, and pharmaceuticals (Eriksson *et al.*, 2002). As these are not usually present, native organisms may not have the appropriate physiology to tolerate them.

Nonyl phenols and their ethoxylates, tributyl tin, and tryclosan are believed to be common and potentially harmful components of misconnection discharge (personal communication with Ian Myers, EA misconnections team). Nonyl phenols are endocrine disruptors, present in some pharmaceuticals, which interfere with the hormonal system of organisms, and can reduce fertility in many organisms (Soares *et al.*, 2008). Tributyl tin and tryclosan are biocides, used as preservatives in clothing and personal care products, which are toxic to many organism groups, particularly algae (Singer *et al.*, 2002; Orvos *et al.*, 2002). These could be expected to be discharged from washing machines, and sinks and showers respectively, though not necessarily in all cases.

Surfactants are amphiphilic molecules, featuring a polar head, and a non-polar tail, providing hydrophilic and hydrophobic regions respectively (Mungray & Kumar, 2009). There are four distinct groups of surfactants: anionic (overall negatively charged), cationic (overall positively charged), amphoteric (both positive and negative charges on different parts of the head region) and non-ionic (no overall charge). Of those groups cationic and amphoteric surfactants make up a very small percentage of the total surfactants in sewage (Myers, 2005). Surfactants increase the solubility of organic particles, causing them to move into solution more readily. Detergents often contain complex mixtures of many different surfactants, homologues, isomers, and/or ethoxymers (Lara-Martin *et al.*, 2008a), each of which may cause varying impacts in freshwater ecosystems.

At high concentrations, surfactants aggregate into micelles; clusters of molecules which naturally form due to the reduction in entropy caused by moving the hydrophobic tails from contact with water, while the hydrophilic heads form hydrogen bonds with water molecules (Haigh, 1996). In the presence of organic molecules, the same process causes the hydrophobic tail region to adsorb to the particle surface, increasing the solubility of the organic matter. The sorption capacity increases with length of the alkyl chain (Kiewiet *et al.*, 1996).

Sorption and degradation are key processes in determining how long a surfactant remains in the environment (Lara-Martin *et al.*, 2008a). Once they enter a water course, anionic and non-ionic surfactants are estimated to have half lives between hours and days within the water column, depending on the individual surfactants and the physical and chemical conditions in the water course (Lara-Martin *et al.*, 2008a). However, surfactants can have significantly longer half lives if they become sorbed to sediments, where they may become buried and exposed to anaerobic conditions (Lara-Martin *et al.*, 2008b; Ying, 2006).

Surfactants are organic molecules, and in many cases are readily degraded by microbial metabolism. In wastewater treatment works, approximately 99% of linear alkylbenzene sulphonate is degraded before it is released to the receiving water (Leon *et al.*, 2006). In the case of misconnections, this degradation does not occur, and though surfactants are naturally degraded aerobically (Perales *et al.*, 1999), this can take days, and may result in high biochemical oxygen demand.

Builders are molecules which bind calcium and magnesium ions from water, allowing more suitable conditions for surfactants to function, improved solubility of other detergent components, and increased alkalinity (Glennie *et al.*, 2002). Many detergents contain high levels of phosphorus due to builders such as sodium tripolyphosphate (Wind, 2007), though phosphorus free detergents in which zeolites replace builders, are becoming increasingly common. Zeolites have very little impact in receiving waters, both in their production and when released into the environment (Glennie *et al.*, 2002). Bleaches and enzymes are also used in detergents to degrade particularly organic matter, to ensure particles are small, in order to assist the action of surfactants (Glennie *et al.*, 2002).

2.5.5 Micro-organisms

Microorganisms fall into two broad categories: naturally occurring microorganisms which are important in cycling and processing of matter in freshwater ecosystems; and potentially disease causing sewage related microorganisms which are short lived, but indicate the presence of domestic effluents. Organic matter and nutrients in misconnection effluents provide an abundant energy source which allows growth of heterotrophic microorganisms such as fungi and bacteria. These form the biofilm in sewers and river beds, commonly referred to as "sewage fungus" (Hickey, 1988). Bacterial biomass often increases immediately downstream of sewer treatment works, and can be significant downstream of combined sewer overflows (CSOs), where no treatment takes place (Rechenburg *et al.*, 2006). Misconnections may also therefore be a major source of bacterial biomass to receiving waters.

Sercu *et al.* (2009) found surface water outfalls to be an important source of faecal indicator organisms (FIOs) to rivers in southern California. FIOs should not be present in large quantities in storm water, though animal waste in surface run-off can contribute some FIOs

(Sercu *et al.*, 2009). However grey water can be an important source of FIOs (Eriksson *et al.*, 2002), as is foul wastewater, and therefore misconnections may be a key source of FIOs to surface waters (O'Keefe *et al.*, 2005). Whilst not a problem in their own right, FIOs are used as indicators of the presence of faecal pollution, which can include pathogenic organisms which pose a threat to human health, and large quantities of organic matter.

2.6 Correcting misconnections

There are legal guidelines which require misconnections to be corrected in the UK (table 2.5), as well as pressure to achieve the targets set by the EU Water Framework Directive (2000/60/EC), Revised Bathing Waters Directive (76/160/EEC), and Shellfish Waters Directive (2006/113/EEC), which require a reduction in non-agricultural diffuse water pollution (Environment Agency, 2010). This legislation allows sewerage providers and environmental regulators to enforce correction of sewer misconnections, however in practice, enforcement can prove difficult due to the communication between water providers, environmental regulators, local authorities, and householders. At present there is not sufficient data to determine whether misconnections significantly affect water quality targets for these directives, and there are no studies of the ecological impacts of sewer misconnections, making the realised benefits of misconnection correction in receiving waters difficult to define (UKWIR, 2012; Ellis, 2013). This means that misconnection correction is performed purely on the basis that misconnections are illegal. Further development of understanding of the environmental impact of sewer misconnections may improve the drive to correct misconnections.

Table 2.5: Summary of relevant legislation for misconnections - information from UKWIR (2012).

Source	Section	Summary of relevant points
Water Industry Act 1991	106	Right to discharge foul wastewater and surface water to public sewer system.
	106 subsection (2)	Where there are separate systems, rights of connection are restricted only to the appropriate sewer type.
	109	It is a criminal offence to connect a private sewer with a public sewer and not adhere to the requirements of section 106.
	113	A sewerage undertaker may carry out work on private drains they believe to be misconnected, though they pay the costs.
Building Act 1984	59	Local authorities must serve notice to fix the problem on the owner of any building in which the private sewer systems connect to the public sewer system, and are in any way defective, prejudicial to health, or a nuisance. This means that the owner must redirect the pipe to the correct sewer system.
Environmental Protection Act 1990	79 and 80	Local authorities must serve an abatement notice on any person causing a 'statutory nuisance' to carry out work to abate the nuisance.
Water Resources Act 1991	161A	If polluting matter is present in controlled waters, the Environment Agency can serve a works notice requiring the polluter to carry out work to prevent the pollution entering the controlled waters.
Environmental Permitting (England and Wales) Regulations 2010	12 and 38	It is a criminal offence to cause or knowingly permit any poisonous, noxious or polluting matter or solid waste to enter inland freshwaters unless an Environmental Permit has been obtained. Continuing to discharge this effluent after prohibition notice has been served is also an offence.
Sewage (Scotland) Act 1968	12	Scottish Water has power to remove unlawful connections.
	15, subsection (1)	Scottish Water and Local authorities have power to issue notices requiring owners or occupiers to fix drainage defects, or fix them themselves and charge the owners.
Building (Scotland) Act 2003	28	If drainage is sufficiently problematic, the local authority can serve a defective building notice, which requires the owner to fix the drainage or face fines.
Public Health (Ireland) Act 1878	107 and 112	Local authorities can serve an abatement notice or prohibition notice to an owner or occupier if a nuisance is occurring or is likely to occur. The local authority may fix the nuisance and charge the owner if the owner does not fix it themselves. It is a criminal offence to not comply with the requirements of the notice.
Water (Northern Ireland) Order 1978	Article 7 (1) (a)	This is the main legislation controlling polluting discharges to waterways in Northern Ireland.

Once a misconnection is identified, water companies should notify the home-owner of what needs to be done to correct it. However this is not always a simple process (figure 2.5). While initial approach of property owners is performed by water companies who have an obligation to correct sewer misconnections, enforcement is performed by local authorities who have no requirement to correct misconnections, and thus it may be low on their list of priorities (Ellis, 2013). Local authorities then need to report back to the water company once correction has been performed to confirm correction. In addition to this, the whole process must be performed in under six months, or data is considered out of date and unreliable (Environment Agency and Water UK, 2014), meaning that identification of the misconnection must be performed again. This process makes it very difficult for misconnections to be corrected, as there are many links and transfers in the chain which may fail for a wide range of reasons, leading to data becoming out dated, and the cost of correction increasing significantly in terms of processing costs.

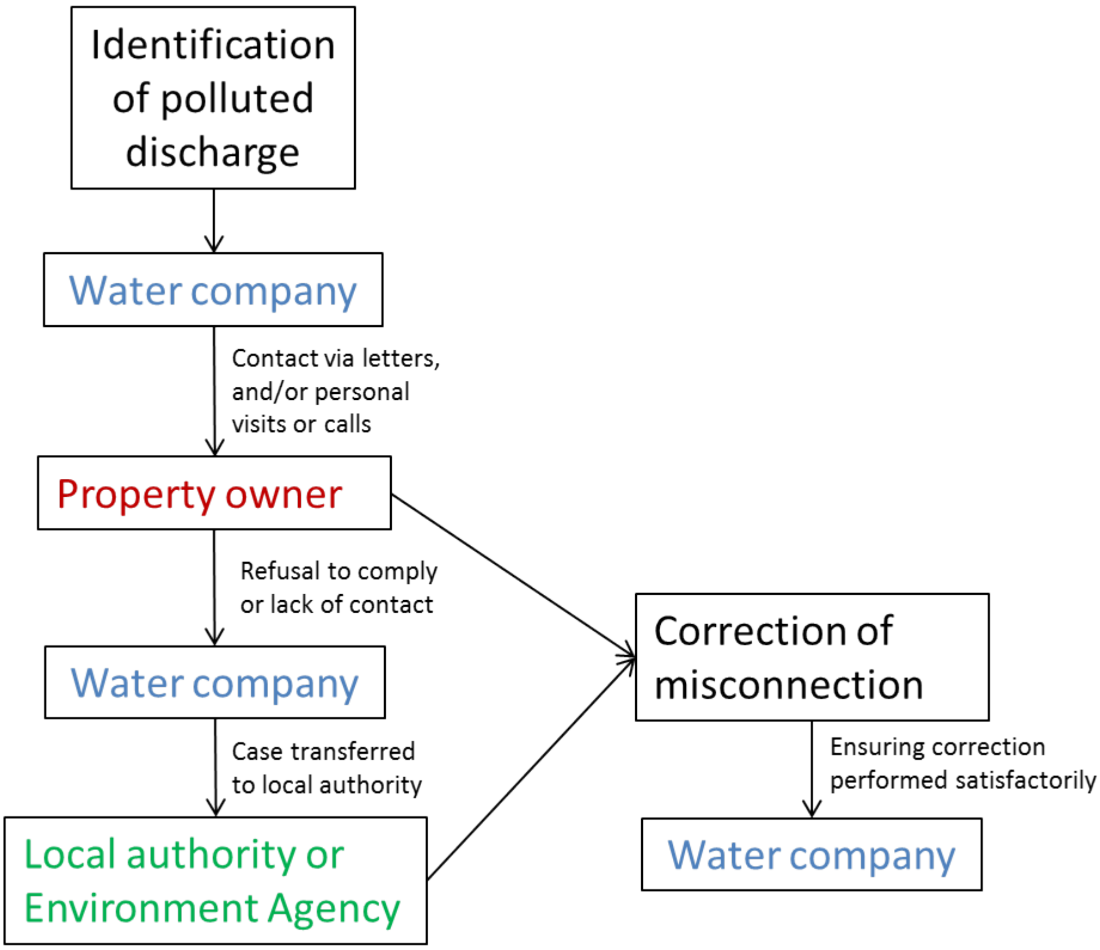

Figure 2.5: Flow chart of the current process required for misconnection correction (information from Environment Agency and Water UK (2014)).

Though there is a relatively large body of grey literature relating to the problem of identifying and rectifying misconnections (Environment Agency and Water UK, 2014; Center for Watershed Protection & Pitt, 2004; Irvine *et al.*, 2011), there is very little peer reviewed literature considering the problem and its impacts. This is likely due to the fact that point source pollution has generally been viewed as a more important threat to surface waters in urban areas (D'Arcy *et al.*, 2000). However, years of focussed attention on point source pollution have rectified many major sources, and put in place sufficient legislation to help prevent future problems (D'Arcy *et al.*, 2000). Therefore diffuse urban pollution sources such as misconnections are now becoming seen as more important as sources of pollution to watercourses.

2.7 Further developments of misconnection research

From the literature which has been reviewed in this chapter some promising areas for future developments in the field of misconnections are suggested in table 2.6. Two of these areas - Identifying misconnection effluents, and investigating the impact of misconnections in receiving waters - were selected for further investigation in this thesis. Chapter 3 presents a method to identify misconnection effluents in sewer systems. Chapters 4 and 5 present investigations into the response of diatoms, an important group of organisms in freshwater ecology, to determine the impacts of misconnection effluents in freshwater ecosystems.

Table 2.6: Suggestions for primary objectives required to develop a better understanding of misconnection problems.

	Primary objectives	Barriers
Magnitude of the misconnection problem	• Collect detailed data from multiple regions	• Cost related to detailed characterisation of sewer systems • Ensure no bias towards known polluted catchments
Cause of misconnections	• Perform anonymous surveys of separate sewer system users regarding knowledge of misconnections to identify risk groups • Develop education methods to improve misconnection knowledge in target groups • Add warnings to plumbing supplies labels.	• May be difficult to engage public with education of sewer systems • Large scale education campaign would be expensive
Identification of misconnection effluents	• Develop inexpensive, misconnection specific, pro-active methods to identify sewer misconnections • Investigate potential of biological indicator organisms as misconnection indicators	• No single method will identify all misconnection effluents • Conflicts between cost and accuracy of methods may lead to compromises
Impact of misconnections in receiving waters	• Investigation into the ecological impact of misconnection effluents • Constant water chemistry monitoring may allow high resolution characterisation of effluents	• Separating the effect of misconnection effluent inputs from those of other discharges may prove difficult
Correcting misconnections	• Improve fluidity of the misconnection identification to enforcement process	• Changing any legislative barriers is difficult • Improved co-ordination between water companies and local authorities may be an easier way to improve fluidity of the process

2.8 Conclusions

This chapter has reviewed the current literature regarding sewer misconnections, and drawn on knowledge of similar problems in order to develop understanding of the problem of misconnections in the UK, and their potential threats in the environment. The study has focussed on five key aspects of misconnection research - the magnitude of the misconnection problem, the cause of misconnections, identifying misconnection discharges and sources, the impact of misconnection effluents, and correcting misconnections. This review generally found that the body of peer reviewed literature on sewer misconnections is extremely limited, and grey literature on the subject proved a better source of information in almost all cases.

The number and severity of misconnections across the UK is currently unknown, with the few estimates which exist being based on relatively small studies. Almost all of the current studies used to estimate wider misconnection rates come from the London area, and therefore do not reflect potential variation in the frequency and severity of misconnections in other regions of the UK. This is a crucial aspect in determining the contribution of misconnection effluents to Water Framework Directive, Bathing Waters Directive, and Shellfish Waters Directive targets across the UK.

There is no data at present on the major sewer user groups which contribute to misconnections. This is an important area in which developments are required, as preventing misconnections from occurring is at the heart of the process of tackling misconnections. Without prevention measures being put in place, misconnections will continue to be introduced to sewer systems, and therefore the problem cannot be solved until prevention is in place.

Methods for identifying misconnections are currently inefficient, and generally rely on indicators which may be present due to other pollution sources. This does not allow pro-active misconnection identification. Generally misconnection identification relies on reports from the public to identify particularly badly polluted outfalls, at which generic techniques can be employed to identify specific misconnected appliances.

There is a general lack of follow up investigations in the few studies where misconnections have been corrected, and therefore the success of these projects is extremely difficult to judge. Though the pollutants which are likely to be present in sewer misconnection discharges are relatively well known, the wide range of potential volumes of discharge, as well as the intermittent nature of the discharge make it difficult to predict the impact which misconnections may have in receiving waters. Identifying the impact of misconnections in receiving waters is a critical step toward determining the environmental value of rectifying misconnections, as it is an expensive and time consuming process. Biological communities and passive water chemistry sampling are promising methods to begin investigating the impact of misconnections in surface waters.

While there is a large body of legislation with which to enforce misconnection correction, current methods and procedures require a great deal of interaction between water providers, local authorities and the Environment Agency, which can make it extremely difficult to progress from a misconnection being identified to rectification taking place. This means that even known misconnections can be difficult to correct in reality, and a single misconnection incident may continue for long periods of time, requiring relatively high levels of resources before completion.

Chapter 3

A low cost method to detect polluted surface water outfalls and misconnected drainage

3.1 Introduction

Polluted surface water outfalls (PSWOs) can be major sources of faecal indicator organisms (O'Keefe *et al.*, 2005), nutrients, and toxic compounds (Environment Agency, 2007; UKWIR, 2012), which can significantly impact receiving waters. Sewer misconnections are a key contributor of pollution to PSWOs, and can discharge a wide range of pollutants (UKWIR, 2012; Ellis, 2013) (Chapter 2). Misconnections discharge intermittently and therefore pose problems for monitoring, as impacts may only be observable during discharge.

Monitoring PSWO effluents generally takes the form of either spot sampling; taking an instant sample at a point which can be stored for later analysis, or continuous monitoring; placing a sampler or sensor in situ which will collect samples over time. Due to the intermittent nature of misconnection discharges, continuous monitoring is the most promising method to identify these discharges, as spot sampling will only identify effluent if it is present at the time of sampling.

Commonly monitored components include nutrients, sewer solids, bacterial growth, biochemical oxygen demand (BOD), ammonia, phosphorus and pH, among others (Environment Agency and Water UK, 2014). These components are present in a wide range of discharge, and so the value of them for specifically identifying and tracing misconnections is limited. Though misconnections are expected to discharge a wide variety of pollutants which could be used as indicators, many of them are not expected to be present in the majority of discharges, therefore limiting their functionality.

Optical brighteners (OBs) in particular are a promising indicator of misconnection effluent in surface water sewers, as they are found in many components of misconnection effluent. This chapter presents the first UK trial of a cheap, simple, passive sampler for OBs in surface water sewers.

3.2 Current and developing practice

Aesthetic indicators such as turbidity, sewage fungus, and solids are common results of polluted discharge, which are easily identified and develop quickly following exposure to polluted discharge (Hickey, 1988; Pitt *et al.*, 2004). These are either observed on natural substrates, or can be sampled in sewers using caging to trap solids (Environment Agency and Water UK, 2014). However visual indicators are not always present in misconnection discharge, and are not uniquely a result of misconnection discharges, they can be present as a result of other inputs to sewer systems, and therefore do not definitively indicate the presence of misconnections on a sewer system.

Distributed temperature sensing uses fibre optic cables, temporarily inserted into sewer systems, to detect changes in temperature of water entering sewer systems (de Haan *et al.*, 2011). This can be very time-efficient, but is also expensive, and requires considerable technical knowledge to operate the temperature sensor (Hoes *et al.*, 2009). While this method is rarely used at present, if costs can be reduced it may become more widely accessible.

Passive water chemistry samplers can be used in rivers to observe changes in concentrations of chemicals over time periods from days to months (Namiesnik *et al.*, 2005; Vrana *et al.*, 2005; Zhang & Davison, 2000). These are inexpensive, do not require external power, and do not require regular maintenance (Zabiegala *et al.*, 2010), however they have not been tested for monitoring misconnection effluents, and may only be sufficiently sensitive to identify large, or constant, discharges.

Dye testing involves pouring fluorescent dye into appliances in households, which can then be detected in the surface water sewer system if the appliance is misconnected (Hoes *et al.*, 2009; Environment Agency and Water UK, 2014). Dye testing is only used once a region of the sewer system suffering from misconnections is identified using other methods, as it is a relatively slow process, visiting individual properties to perform testing. However, this is the only method at present which unambiguously identifies specific appliances which are discharging to the surface water system, and therefore is needed in the final stage of misconnection correction actions.

Further information on these and other less commonly used methods for tracing and correcting sewer misconnections in the UK and USA can be found in Environment Agency and Water UK (2014) and Center for Watershed Protection & Pitt (2004) respectively.

3.3 Passive sampling for optical brighteners

3.3.1 Method

Optical brighteners (OBs) are chemicals which fluoresce under ultraviolet (UV) light and do not occur naturally in the environment. They have a high affinity for fabrics such as cotton, and are commonly used in laundry detergents, toilet paper, and cleaning products (Burres, 2011). These are components which are expected to be present in the majority of misconnection effluents (UKWIR, 2012). OBs have been used to identify illicit discharge to surface water sewers (Braun, 2011), usually using a fluorometer to measure the fluorescence of discharged water. Fluorometers are relatively inexpensive, though they require flow in the sewer, so will not detect a response if there is no flow or no optical brighteners discharged at the time of sampling. Therefore the ability to identify misconnection effluents is limited. To overcome this limitation, an in situ passive method has been tested and developed to identify misconnection effluents in the field.

For sampling OBs in situ, tampons were fixed in surface water sewers, either by tying to a suitable point in the sewer, or tied to lengths of bamboo cane which could then be wedged in the sewer (figure 3.1) so that they lay in the invert of the sewer. If there was flow in the sewer at the time of sampling, the tampon was briefly exposed to the flow and tested for fluorescence on site using an inexpensive UV light, if suitable darkness could be achieved to accurately identify fluorescence. If a positive response was not observed instantly, tampons were left in situ for a three day period, to ensure polluted discharge was not missed. Three days was found to be the optimum time to leave a sample in place to avoid fouling, but ensure a good exposure time. When samplers were removed from the sewers, they were placed in individual zip-lock bags, and stored in darkness to avoid contamination between samples, and photodecay of OBs, until samples could be exposed to a UV light to test for fluorescence.

Figure 3.1: A representation of a passive OB sampler consisting of a tampon tied to a length of bamboo cane, in situ at a surface water outfall.

Laboratory testing was performed to determine concentrations of detergent at which fluorescence would be observed. OBs adsorbed to tampons within seconds of contact with detergent solutions, and were identified at 1μl of detergent per litre of water, up to 30 days after initial exposure (figure 3.2).

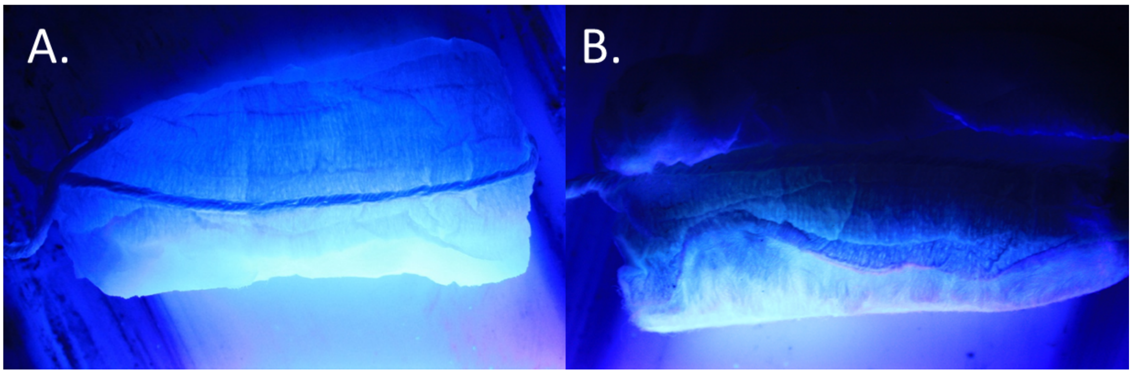

Figure 3.2: Samplers exposed to UV light to show fluorescence. A. Fluorescing tampon sampler. B. Tampon sampler showing reflection of UV light, but no fluorescence.

3.3.2 Field trial

Tampon sampling was performed in 16 surface water sewer outfalls across three catchments in the Sheffield area in March 2013. Nine of the 16 outfalls were indicated as discharging OBs over a week of sampling. Further investigation was performed by technical staff at Yorkshire Water on four sewer systems, using the method in accessible manholes to trace OB containing effluent to its source. Samplers were returned to the laboratory at Yorkshire Water and tested for OBs using an inexpensive UV light. Where OBs were found below a section of sewer, but not above it, a misconnection was indicated between the two points, and therefore an area of the system to be dye tested could be identified (figures 3.3 and 3.4). The cost of initial purchase of raw materials (UV light, cotton, apparatus to attach them in place) in this investigation was approximately 20 pence per sampler.

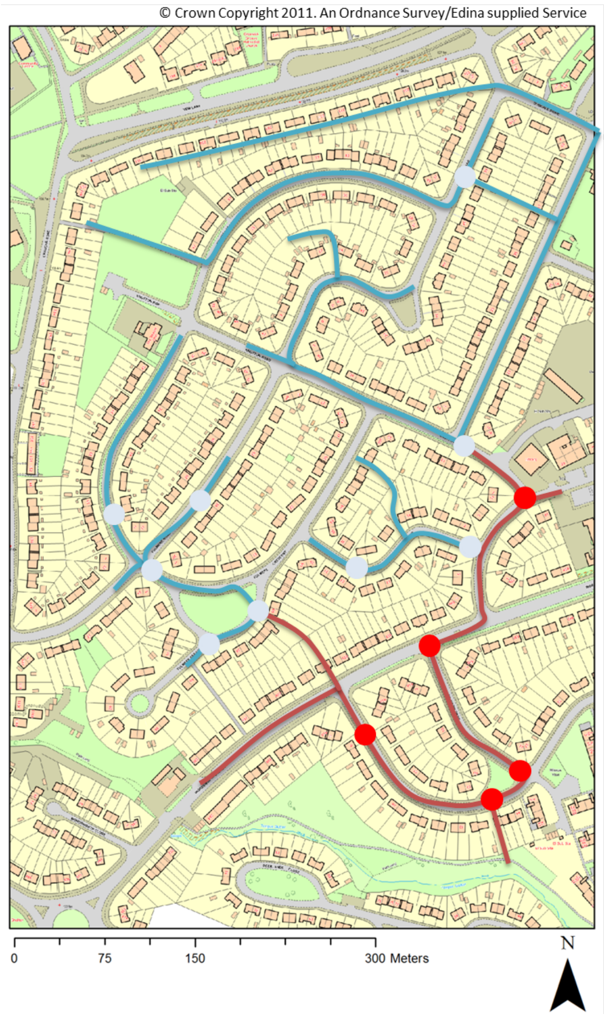

Figure 3.3: Sewer system 1. Red circles show samplers indicating OB presence, red areas indicate potential misconnection effluent affected areas of the sewer system. White circles show samplers indicating OB absence, blue areas indicate misconnection free areas of the sewer system.

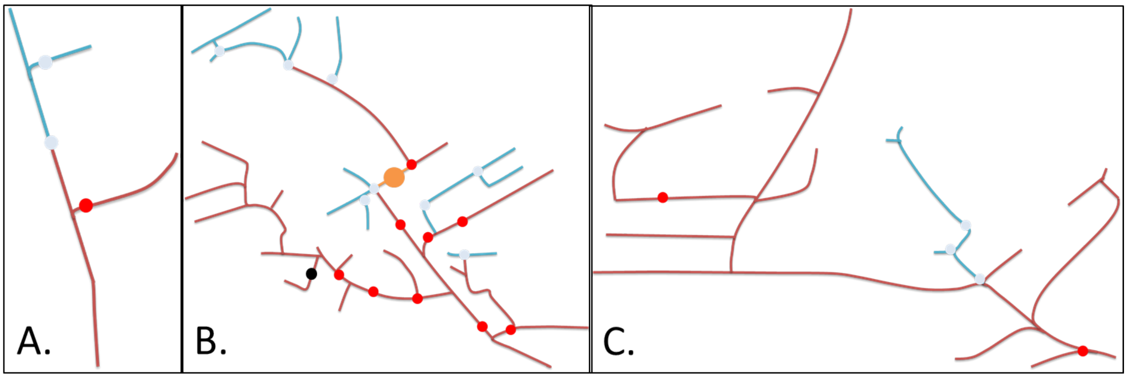

Figure 3.4: A. Sewer system 2. B. Sewer system 3. C. Sewer system 4. Red circles show samplers indicating OB presence, red areas indicate potential misconnection effluent affected areas of the sewer system. White circles show samplers indicating OB absence, blue areas indicate misconnection free areas of the sewer system. The black circle in sewer system 3 (B) indicates where validation has been performed and misconnections observed. The orange circle in sewer system 3 (B) indicates where a conflict was observed between indication of upstream and downstream samples.

The method successfully identified areas of the sewer systems in which further investigation using dye and visual misconnection inspection could be performed. This significantly reduced the area in which detailed investigation was required, and thus reduced cost of follow up investigations. Samples corroborated well, with indicated misconnected points joining up, and correctly connected points joining up. The method showed only one conflict over 4 catchments where a sewer was indicated as correctly connected at one point, but misconnected further up the catchment (figure 3.4b).

Visual inspection of properties was performed in part of sewer system 3 (figure 3.4b). A sink and a soil stack were found misconnected in this area. These misconnections were corrected, though additional sampling could not be performed, to determine whether other misconnection problems existed in the system after correction, due to budgetary constraints.

3.3.3 Practical issues

When large quantities of suspended solids are present in sewer systems, tampon samplers can become fouled, and fluorescence masked to the extent that if OBs are present, fluorescence is not observable. Once significantly fouled, washing the sampler did not remove enough of these solids to allow analysis to be performed on the sampler. A shortened period of exposure reduced the risk of this problem, however to ensure the same exposure period as samplers on other outfalls, samplers were replaced more frequently, which increased the cost for those points.

At some sewer outfalls, samplers were vandalised by members of the public. This only occurred when sampling outfalls, and only at sites which were close to footpaths, even though they were generally not visible from the footpath. This may be avoided by inserting the samplers further into the outfall, though in the present study this was not possible without contravening health and safety requirements.

There is a risk of misinterpretation of fluorescence due to the presence of oil (Lambert *et al.*, 2003) or surface discharges of OB containing compounds, such as from car washing with soaps. Oil, which also should not be present in the surface water sewer system, will leave a coating on the sampler, and therefore should be easily identified. Surface discharge of OB containing compounds are not expected to be a frequent occurrence, but may cause confusion where they do occur.

The major limitation of the method is that some misconnections may not discharge compounds containing OBs, and therefore will not be detected using the method. Combining the optical brightener method with other established methods, such as visual inspection methods, allows an integrated sampling strategy so that a weight of evidence approach can be taken to identify systems which require further investigation.

3.3.4 Further development

This study demonstrated that the method successfully identified misconnection discharge in surface water sewer systems, however budget limitations prevented full validation of the sewer systems from being performed. The next development of the method should be to perform a full validation of the method, including full tampon sampling throughout several sewer systems, and thorough dye testing to ensure that where misconnections are indicated, they are found, and where they are not indicated, they are not found. This would give a better indication of the accuracy of the method.

Following thorough method validation, the main improvement which could be made to the method is to develop a way to protect the sampler from sewer solids. Fouling is a major problem for the method at present, limiting the time that samplers can be left in situ, yet it is one of the easiest limitations to overcome. Solving this may require development of a protective barrier to block solids, or a cleaning process to clean off solids, and leave OBs in place on the sampler. This would reduce the number of visits required, and therefore reduce costs of sampling, though it would increase the cost of individual samplers.

3.4 Conclusion

This chapter presented the first UK investigation of an inexpensive and simple passive method to identify sewer misconnection effluents using cotton samplers onto which optical brighteners bind. The method successfully identified optical brighteners in surface water sewer systems, and limited validation showed misconnections were present where they were indicated. This proved a very promising method for identifying sewer misconnections, and, pending further validation, is recommended for investigation of sewer misconnections in surface water sewer systems.

Chapter 4

Diatom community response to detergent effluents

4.1 Introduction

Laundry detergents are composed of surfactants and builders (which remove magnesium and calcium ions), but can also contain a wide range of additional compounds such as bleaches, optical brighteners, and opacifiers. Surfactants are amphiphilic molecules, which can be toxic to algae (Lewis, 1992; Pavlic *et al.*, 2005; Sibila *et al.*, 2008), invertebrates (Pettersson *et al.*, 2000), and fish (Lewis, 1992). Surfactants are also major toxicants in municipal sewage discharges (Ankley & Burkhard, 1992; Pettersson *et al.*, 2000). Though surfactants are degraded naturally over time, this can take weeks, during which time they damage the ecosystem (Pettersson *et al.*, 2000).

Detergents can enter rivers and streams by many routes, including combined sewer overflows (CSOs), sewer misconnections, and direct discharges such as irresponsible disposal of wastewater from car washing (Sablayrolles *et al.*, 2010). Combined sewer systems carry waste water and surface water in a single pipe to sewage treatment works, and contain CSOs, which discharge wastewater to rivers during high flow conditions, to prevent treatment works from becoming overwhelmed. CSOs should only discharge when sewage is highly diluted due to high precipitation levels. Separate sewer systems have a wastewater pipe, which delivers foul sewage from properties to sewage treatment works, and a surface water sewer, which discharges rain water direct to rivers and streams. Sewer misconnections occur where domestic appliances are connected to a surface water sewer, leading to undiluted sewage, often containing a large proportion of detergent rich effluent (UKWIR, 2012), being discharged regularly to rivers and streams. The extent of pollution from these misconnections is largely unknown, with only a small number of studies looking at impacts in water chemistry (UKWIR, 2012; Ellis, 2013) and invertebrates (Edmonds-Brown & Faulkner, 1995) currently published . There has been very little investigation into the response of ecological communities to misconnection effluents, a large proportion of which are expected to contain laundry detergent effluents (Chapter 2).

Among the ecological communities which may be affected by misconnection discharges are algal biofilms - important primary producers in riverine ecosystems (Vannote *et al.*, 1980). Diatoms are a group of algae commonly used in monitoring (Fore & Grafe, 2002), and form a major component of these biofilms (Battin *et al.*, 2003). Diatom communities respond rapidly to changing water conditions including nutrients (Kelly & Whitton, 1995), organic pollutants (Rott *et al.*, 1998), and metals (Gold *et al.*, 2003a,b; De Jonge *et al.*, 2008). They are also ubiquitous throughout freshwater ecosystems (Round *et al.*, 1990), important primary producers in riverine habitats, have short generation times, and show species specific responses to many pollutants (Kelly *et al.*, 1995), making them ideal as rapid indicators of ecological impacts and water quality in freshwater ecosystems (Juttner *et al.*, 1996; Stevenson *et al.*, 2008). There have been a number of single species investigations into algal response to surfactants, a major component of detergents, showing that surfactant toxicity varies greatly between different species (Lewis, 1990; Pavlic *et al.*, 2005; Azizullah *et al.*, 2011). Diatom communities therefore have potential for use in observing effects of detergent effluents in freshwater ecosystems, and use as indicators of the presence of detergent effluents in surface waters, thereby indicating the presence of effluents from sources such as sewer misconnections. However the response of algal communities to full detergent formulations at the community level has not been investigated to date.

Diatoms are traditionally identified to species level due to a general high variance in response of species within genera to particular pollutants. Kociolek (2005) found that moving from species to genus level caused a significant change in the prediction of water condition, and Ponader & Potapova (2007) found that within a small number of *Achnanthidium* species there was considerable differentiation in responses to water chemistry. However, genus level analysis can show broad responses, but also has the advantage that it requires less refined training, and is significantly easier than species level analysis. This means that it can be learnt quickly, and performed quickly, reducing the time required to analyse a sample, one of the major issues related to diatom based analyses. Within a genus, individual species may show similar responses to abiotic changes due to a common ancestry resulting in common solutions to stress (Hermant *et al.*, 2012). Performing analyses at the genus level can help to remove noise in the data set by reducing the influence of very rare species which may explain little of the community response.

In this study, a laboratory experiment is presented in which field sourced diatom communities were exposed to a range of concentrations of two different detergents. Using the laboratory design allows control of factors which could not be controlled in the field ensuring that any effects observed are caused by different treatments, and not co-varying factors. This allows unambiguous identification of the impacts of detergent based effluents in diatom communities. The aim of the experiment was to (i) examine the effects of detergents on community structure and composition, (ii) determine whether time savings in analysis could be achieved without major loss of information by identifying communities to genus level, and (iii) investigate whether diatom communities might contain useful indicators of detergent discharges by identifying species which give strong responses to the presence of detergents.

4.2 Methods

4.2.1 Collection and culturing of diatoms

Five cobbles coated in biofilm were collected at each of five random points along the River Rivelin, a small upland river which flows into Sheffield, UK. The collection points were in the 2km stretch before the river reaches major urban areas, before which the river is entirely rural. Samples were collected in June 2012. These were stored in the dark during sampling, and taken to the lab within 3-4 hours of collection. A toothbrush was used to brush biofilms from the cobbles, into a litre container of modified Chu No. 10 major nutrient solution based on that used in Debenest *et al.* (2009) (Table 4.1). This was homogenised by stirring vigorously, and poured into a large tray lined with 150, 4.5cm^2 ceramic tiles. A further 6L of modified Chu No. 10 major nutrient solution, and 1L of trace nutrient solution (Table 4.1) were then added to the tray. The solution was stirred to ensure homogeneous distribution of individuals throughout, and aerated using needles connected to a pressurized air source. Algal communities were allowed to colonise the tiles for 72 hours following the recommendations of Debenest *et al.* (2009). These were maintained at 19°C ±2°C and 12 hour light/dark cycles in artificial light.

Two simulated misconnection effluents were created using Persil^{TM} 'small and mighty non-bio' liquid detergent (solution P) and Ecover^{TM} 'concentrated non-bio' liquid detergent (solution E) respectively. These are two common laundry detergents available in the UK. One millilitre of detergent was mixed with 2L of tap water, approximating the concentration of detergent recommended for use for each detergent solution. One gram of composting soil was added to each, simulating the interaction of detergents with organic matter in a washing machine and sewer pipe, which may lead to sequestering of components of the detergent formulations. The solutions were then heated and stirred continuously for 30 minutes to replicate conditions in a washing machine. Tiles coated with biofilm were then randomly selected from the large tray, and placed into separate aerated glass dishes containing 100ml of one of four different treatments for each of the two detergents (ten tiles in each treatment for each detergent) (Table 4.2, Figure

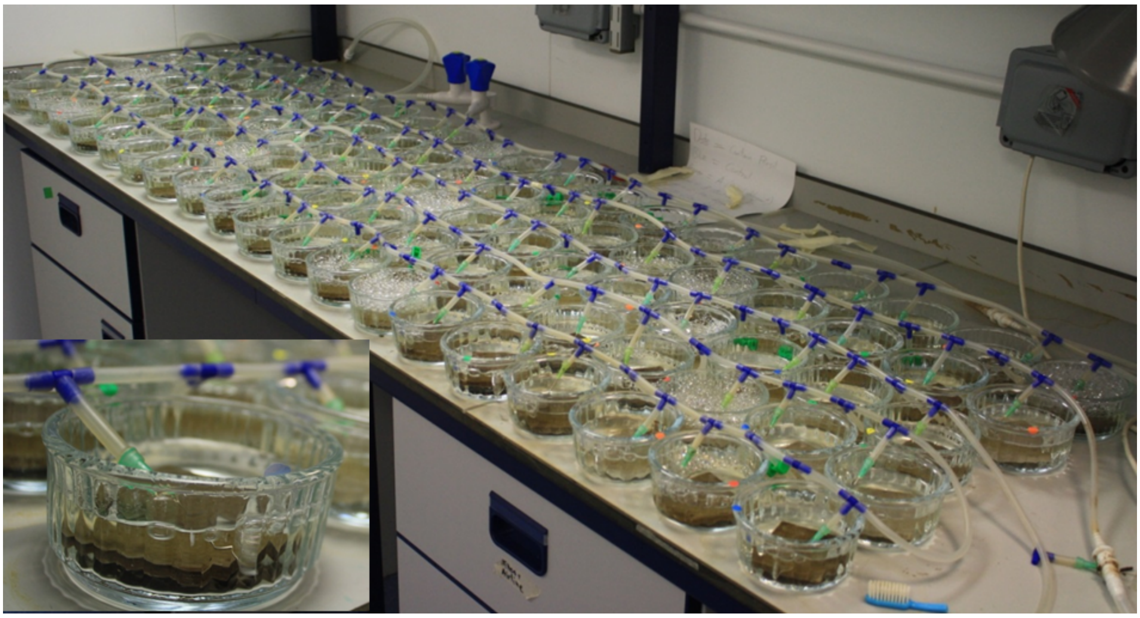

Figure 4.1: Microcosms containing algal communities on tiles, nutrient solutions and artificial detergent effluents, aerated using needles connected to air supply. Inset closer picture of microcosm

Table 4.1: Modified Chu No. 10 major nutrient solution and trace nutrient solution composition, based on that used by Debenest *et al.* (2009).

Solution	Nutrient source	Quantity (mM/L)
Major nutrient solution	$Ca(NO)_3$	0.0420
	K_2HPO_4	0.0032
	Na_2CO_3	0.1880
	$MgSO_4$	0.1000
	$Na_2SiO_3.5\,H_2O$	0.2020
Soil extract	1g/L filtered compost solution	

Table 4.2: Components of microcosm solutions for each concentration treatment.

Treatment	Effluent (ml)	Soil extract (ml)	Nutrient solution (ml)	Replicates
Control	0	5	95	10
A	0.1	5	94.9	10
B	1	5	94	10
C	10	5	85	10
D	50	5	45	10

For the rest of this chapter, treatments are coded using the detergent type, followed by the concentration treatment. For example, the highest concentration of the Persil detergent is referred to as PD, the lowest concentration of the Ecover detergent is referred to as EA.

4.2.2 Cleaning and fixing diatoms for counting

After 28 days, biofilms were brushed off tiles into individual bottles containing 50ml of tap water. These were stirred and shaken, and clumps of algae were separated thoroughly, to ensure a homogeneous distribution of diatoms throughout the solution. Forty five millilitres of this suspension was used for chlorophyll *a* analysis, and 5ml was transferred to a boiling tube to be cleaned for community analyses.

Five millilitres of hydrogen peroxide was added to each sample for community analysis. They were then placed in a waterbath at 80°c for approximately eight hours, and allowed to cool over night. Two drops of hydrochloric acid were added to neutralise any remaining hydrogen peroxide, and the samples transferred to centrifuge tubes. Samples were centrifuged three times at 3000rpm for five minutes. Each time the supernatant was removed and the sample re-suspended with distilled water. Finally samples were re-suspended and a few drops of this suspension were placed on a microscope slide cover slip and allowed to dry, before being mounted on a microscope slide using Naphrax^{TM} mountant. For each of five tiles for each treatment, four hundred individuals were identified using a microscope at 1000x magnification.

4.2.3 Shannon diversity calculation

The Shannon diversity is a measure of community structure which is commonly used in ecological studies (equation 4.1). Diversity combines measures of the species richness and species evenness in order to give more complete characterisation of the structure of a community, accounting for both the number of species and relative abundance of those species in a single easy to understand value.

$$Shannon\, diversity\, (H) = -\sum_{i=1}^{s} P_i ln P_i \qquad (4.1)$$

Where s = total number of species in the community, and P_i = the abundance of species i.

4.2.4 Chlorophyll *a* analysis

Chlorophyll *a* content is a measure of total algal abundance, which was measured using an adapted method from Horne (2009) and Gregor & Marsalek (2004). Samples were filtered using Whatmann No.1 filter paper (11μm pore size), and frozen over night in order to rupture the algal cell membranes. These were then thawed, placed in boiling tubes, and heated with 90% ethanol in a waterbath to 80^{o}c in order to extract the chlorophyll. Filter paper was then removed from the boiling tubes, and the ethanol filtered. This was analysed using a spectrophotometer at 664nm and 750nm wavelengths. The readings at these wavelengths were then used in equation 4.2, based on the methods of Horne (2009) and Gregor & Marsalek (2004), to calculate the chlorophyll *a* content.

$$Chlorophyll\, a\, (\mu g/cm^2) = E \times \frac{A_{664nm} - A_{750nm}}{Area} \times V_{extract} \times Dilution\, factor \times L \qquad (4.2)$$

Where E = extinction coefficient for chlorophyll in 90% ethanol at 664nm (12.8), A_{664nm} = absorption at 664nm, A_{750nm} = absorption at 750nm, $V_{extract}$ = Volume of extract in ml (10), DF = dilution factor, Area = area sample taken from, L = cuvette path length.

4.2.5 Treatment solution analysis

Five replicate samples of each treatment solution were analysed for water chemistry variables. Major ion analysis was performed on the treatment solutions using a Dionex DX-120 ion chromatograph. Alkalinity was measured using a Hach AL-DT alkalinity test kit to perform titration with bromcresol green - methyl red indicator powder and 0.16 molar nitric acid.

Total surfactants were measured using methylene blue chloroform extraction. Five millilitres of each sample was added to 5ml of chloroform, and 100μl of methylene blue dye. These were shaken thoroughly 3 times for 20 seconds each to ensure the sample and chloroform mixed thoroughly. The chloroform was then removed and absorption measured at 650nm wavelength using UV-VIS spectrometry on a UV-2401 PC spectrophotometer.

4.2.6 Data analysis

Concentrations of nitrate, phosphate, ammonium, surfactants, and a measure of alkalinity were used to characterise the water chemistry of the treatments, as these are components which are expected to influence diatom growth. Chlorophyll *a* was used as a measure of total algal abundance. Species richness, species evenness, and the Shannon diversity index were calculated to characterise broad community structure. One-way ANOVA was used to investigate the response of these measures to different concentrations within each detergent type separately. Two-way ANOVA was used to investigate the response of these measures to different detergent type, and concentration levels, and to determine interactions between detergent type and concentration level. Tukey's HSD was used to identify differences between treatments. Statistical tests were performed using the R statistics program (R Core Team, 2014).

Community composition was investigated using correspondence analysis. Ordination techniques such as correspondence analysis allow simple visualisation of many dimensions of data, reducing noise, and preventing issues associated with performing multiple individual comparisons of species data (Van Wijngaarden *et al.*, 1995). Data are represented by a number of axes explaining increasingly lower percentages of the variation in the data. These can then be used to plot the taxa and samples in biplots, in which the relative positions of species and samples indicate the species which are characteristic of given samples. Correspondence analysis is an indirect gradient analysis, allowing identification of the underlying major gradients in ecological data without attributing it specifically to any particular factors (Gauch, 1982), meaning that even if important environmental gradients have not been measured, they can still be observed in the ecological data. Correspondence analysis can be influenced by change in relative abundance of rare species to a greater degree than change in relative abundance of more common species (Legendre & Gallagher, 2001), therefore species which did not constitute more than 1% of the population of any individual sample were removed prior to correspondence analysis, excluding 73 species from the analysis, leaving 38 species in the correspondence analysis.

Correspondence analysis was used to investigate community composition in response to detergents at both the species and genus level. This allowed investigation into the potential benefits and drawbacks of counting diatoms to different taxonomic levels.

4.3 Results

4.3.1 Water chemistry

When performing the surfactant analysis, one of the PD samples showed no surfactant present, and one became significantly contaminated with surfactants, containing 94.6mg/L of surfactant compared with an average of 9.4mg/L in the other samples. These were removed from the analysis due to clear errors. Measures for all other variables use all five samples.

Expected concentrations of nitrate, phosphate, and ammonium were calculated from the amounts of each component put into the core solutions, and the amount contributed from soil solutions and detergent solutions. Multiple treatments contained no ammonium or no surfactant, meaning that ANOVA analysis could not be performed on these variables, as the assumption of a non-zero variance in all treatments was not met. However, in these cases major differences between treatments were generally visually clear.

The control, A, and B concentrations for both detergents did not differ significantly from each other in any water chemistry measures, and did not vary greatly from expected values (figure 4.2). Surfactant concentration was higher in the PC, EC, PD, and particularly the ED treatments, than in all other treatments (figures 4.2m., 4.2n., and 4.2o.). The EC and PD treatments show very similar total surfactant concentrations (figure 4.2o.). There was a significant interaction between detergent type and concentration level in both nitrate ($F_{(3,32)} = 24.76$, $p<0.001$) (figure 4.2c.) and alkalinity ($F_{(3,32)} = 10.48$, $p<0.001$) (figure 4.2l.). The ED treatment contained significantly lower nitrate than the other Ecover treatments and the controls ($F_{(4,20)} = 22.15$, $p<0.001$) (figure 4.2b.). The EC treatment contained significantly higher alkalinity than the control, EA, and EB treatments, and the ED treatment contained higher alkalinity than the EC treatment ($F_{(4,20)} = 96.36$, $p<0.001$) (figure 4.2k.). The PC treatment contained significantly higher alkalinity than the control, PA, and PB treatments, and the PD treatment contained higher alkalinity than the PC treatment ($F_{(4,20)} = 65.03$, $p<0.001$) (figure 4.2j.). Ammonium concentration was higher in the PC treatment than all other treatments except the PD treatment, which contained a substantially higher concentration than the PC treatment (figures 4.2g. and 4.2i.).

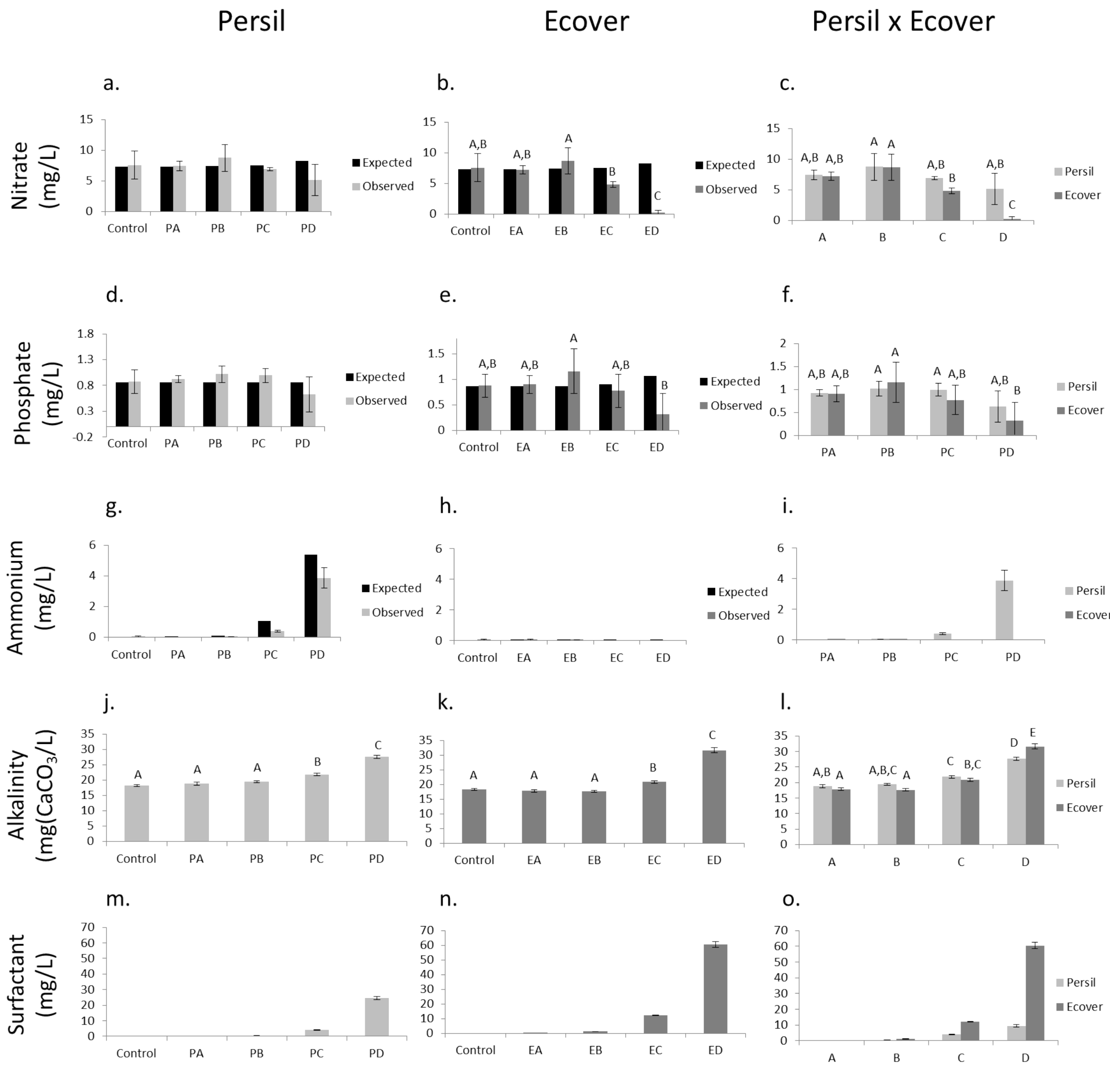

Figure 4.2: Nitrate, phosphate, ammonium, alkalinity, and surfactant concentration plots for Persil and Ecover detergents. Error bars show standard deviation. Within each plot, letter codes are given if results from the ANOVA were significant, treatments with the same letter code do not differ significantly from each other (Tukey HSD test, p>0.05). Expected values were calculated using the known value of nutrients added to the nutrient solutions, and values measured from detergent and soil solutions (see appendix 7).

4.3.2 Diatom abundance

The Chlorophyll *a* concentration in all Ecover treatments was significantly lower than in the control treatment ($F_{(4,45)} = 5.19$, $p < 0.01$) (figure 4.3a.). The Persil treatments also showed significantly lower chlorophyll *a* content than the control treatment, with the exception of the EC treatment which showed significantly higher chlorophyll *a* concentration than the other effluent treatments ($F_{(4,45)} = 11.3$, $p < 0.001$) (figure 4.3b.). The Ecover treatments resulted in significant higher chlorophyll *a* content than Persil treatments ($F_{(1,72)} = 20.38$, $p < 0.001$), and there was a significant interaction between detergent and concentration ($F_{(3,72)} = 4.04$, p <0.05) (figure 4.3c.). The EC treatment showed significantly higher chlorophyll *a* content than the PC treatment (figure 4.3c.).

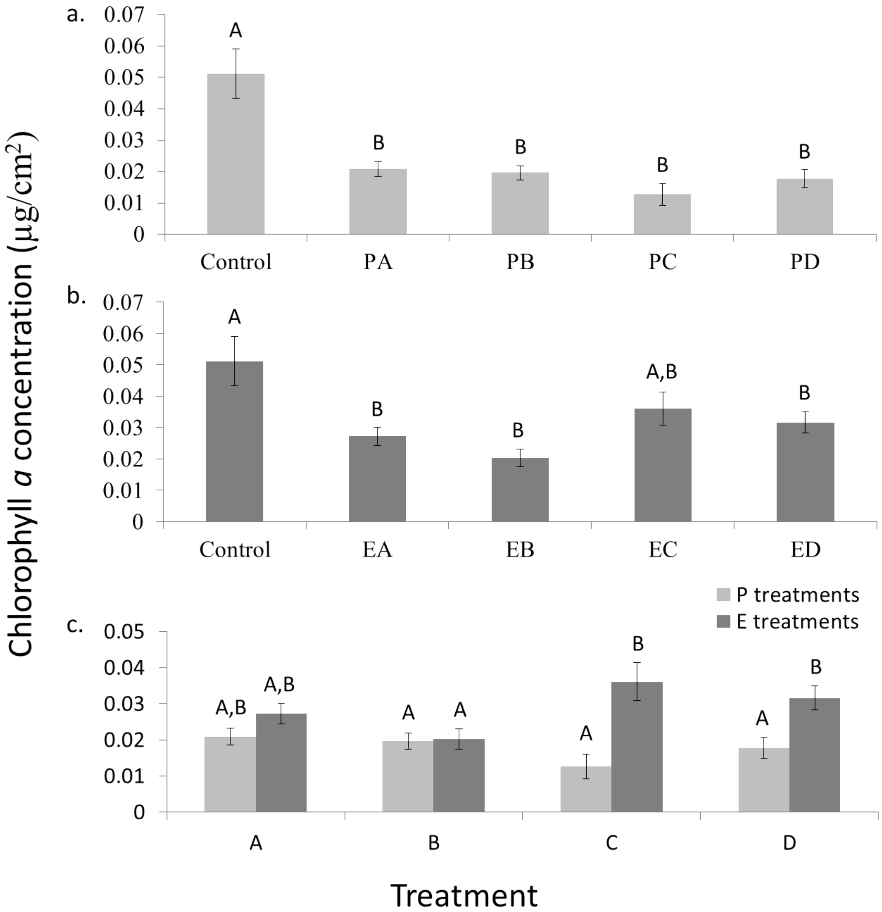

Figure 4.3: Chlorophyll *a* concentrations for control and effluent treatments for a. Ecover detergent, b. Persil detergent, and c. effluent concentration treatments only for both detergents. Error bars show standard error. Within each plot, treatments with the same letter code do not differ significantly from each other (Tukey HSD test, p>0.05).

4.3.3 Diversity measures

Two-way ANOVA showed the D treatments had significantly higher species richness than the A treatments ($F_{(3,32)} = 2.93$, p<0.05) (figure 4.4c.), and significantly lower species evenness than the A, B, and C treatments ($F_{(3,32)} = 10.03$, p<0.001) (figure 4.4f.). The PB treatment showed significantly lower species evenness than the controls and PA treatment ($F_{(3,32)} = 2.93$, p<0.05) (figure 4.4d.). The ED treatment showed significantly lower species evenness than the controls and all other Ecover treatments ($F_{(3,32)} = 2.93$, p<0.05) (figure 4.4e.).

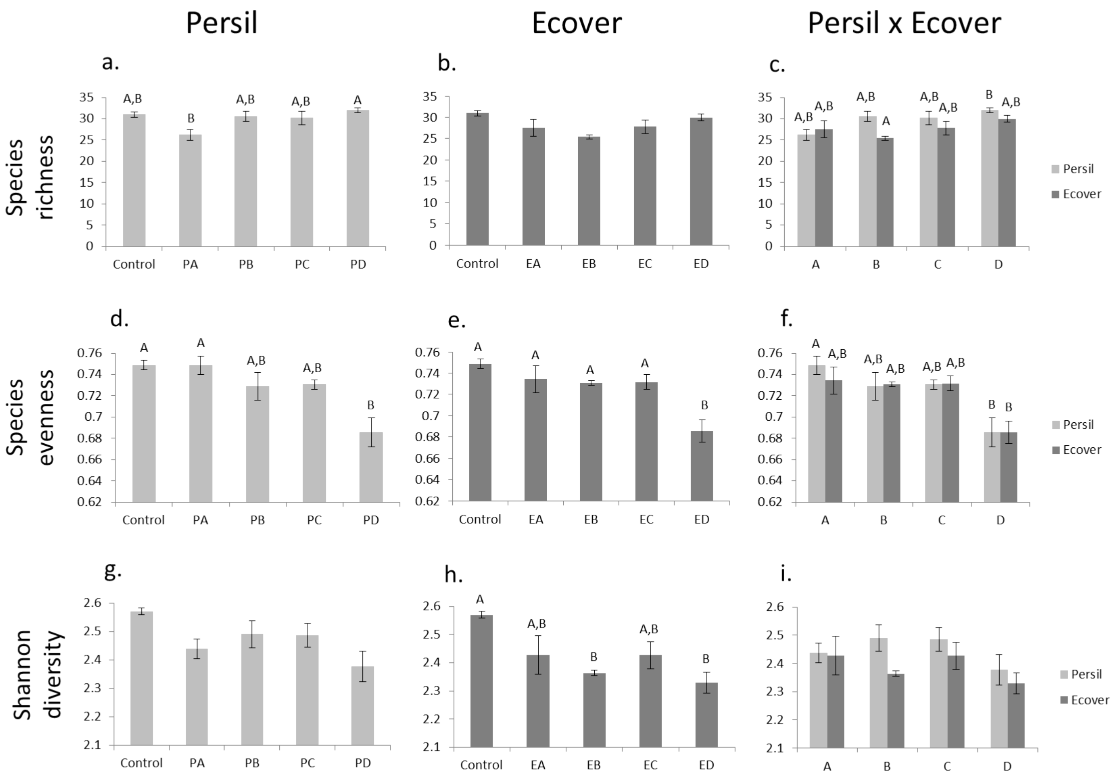

Figure 4.4: Species richness, species evenness, and Shannon diversity plots for Persil and Ecover detergents. Letter codes are given if results from the ANOVA were significant, Letters indicate non-significant difference (Tukey HSD, p<0.05).

4.3.4 Diatom community composition

Correspondence analysis was used to investigate species responses to the detergent treatments (Figure 4.5). The position of a species name on the plot indicates increasing relative abundance of that species in that direction from 0. Axes are unscaled, and thus while spatial positioning around 0 is comparable within each plot, axes values are not. The first and second axes explained 36% of the variation within the community.

The control, A, and B samples clustered high on the first correspondence analysis axis and low on the second axis, and were characterised by higher relative abundance of *Navicula gregaria*, *Planothidium lanceolatum*, *Planothidium frequentissimum*, and *Navicula minima*. The first axis separated the D treatments, low on the axis, from the lower concentration treatments, high on the axis (Figure 4.5a.), driven by higher relative abundance of *Achnanthes oblongella*, *Cocconeis placentula* (Figure 4.5b.). The C treatments also, broadly, cluster higher on the second axis, driven by increasing relative abundance of *Cocconeis placentula*, *Achnanthes oblongella*, *Nitzschia perminuta*, *Nitzschia dissipata*, and *Nitzschia inconspicua* with the majority of the PC treatment occurring higher on the first axis than the EC treatment (figure 4.5).

The second axis separates the EA samples, characterised by higher relative abundance of *Nitzschia palea*, *Nitzschia perminuta*, *Nitzschia dissipata*, and *Nitzschia inconspicua*, from the PA samples and controls, characterised by higher *Planothidium frequentissimum*, *Planothidium lanceolata*, *Amphora pediculus*, and *Reimeria sinuata* (figure 4.5b.).

Water chemistry values were then averaged across treatments, and correlated to the correspondence analysis axes (figure 4.5a.) using the envfit function of R (R Core Team, 2014), which allows variables to be correlated with the underlying treatment groups in the ordination space. The direction of arrows indicate increase in that variable, and the length of the arrows indicate the strength of relationship between that variable and the correspondence analysis values. Correlation was observed between the axes and nitrate ($r^2 = 0.45$, $p<0.001$), phosphate ($r^2 = 0.48$, $p<0.001$), ammonium ($r^2 = 0.30$, $p<0.01$), alkalinity ($r^2 = 0.67$, $p<0.001$), and surfactants ($r^2 = 0.39$, $p<0.001$) (figure 4.5a.). These show that increase in surfactant concentration and particularly alkalinity, and decrease in nitrate and phosphate concentration correlated strongly with the separation of the D treatments from the lower concentration treatments.

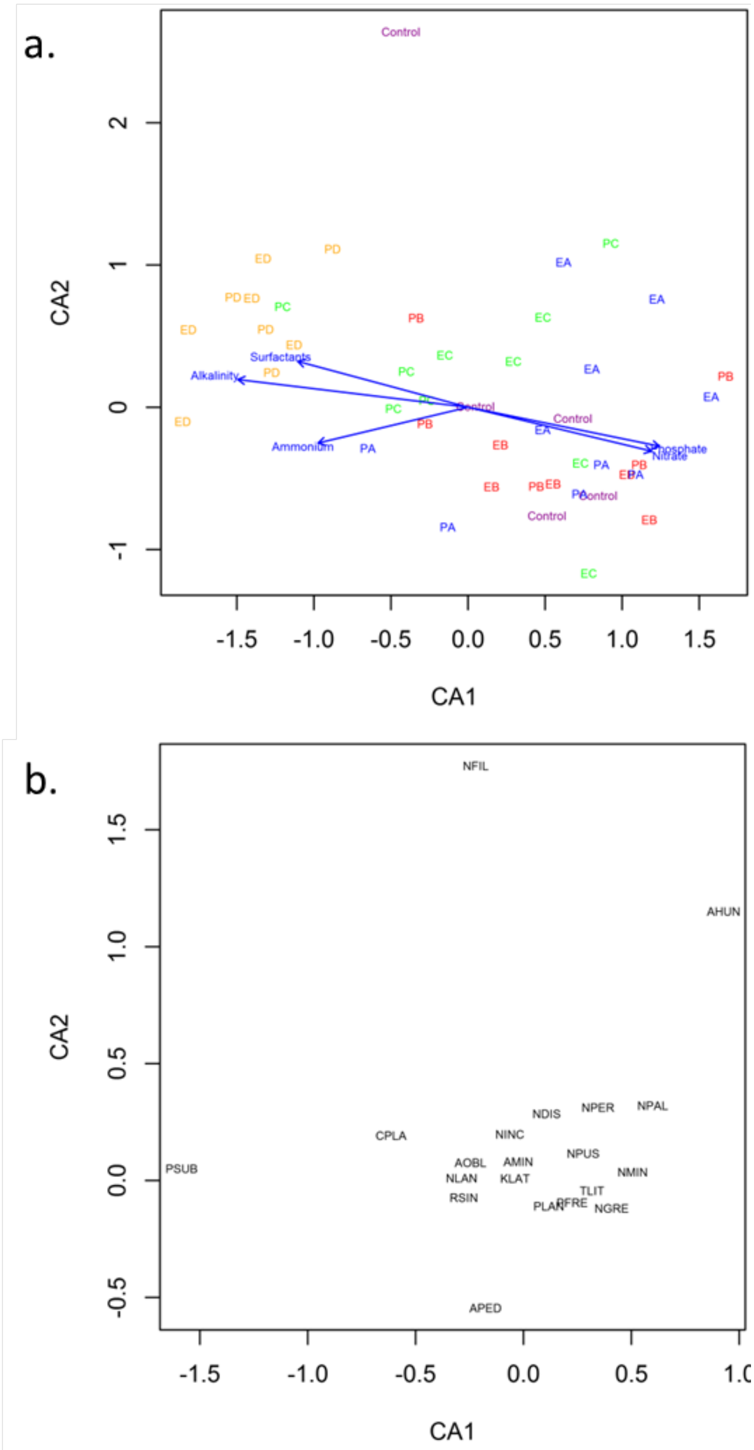

Figure 4.5: Correspondence analysis of all species and all control and effluent treatment samples. Axis 1 describes 25% of the variation within the data set. Axis 2 describes 11% of the variation within the data set. For clarity, the figure has been separated into; **a.** sample positioning on the ordination axes, purple = control, blue = A treatments, red = B treatments, Green = C treatments, orange = D treatments; **b.** species relative placement in the correspondence analysis (only species which constituted at least 2% of one sample are shown for clarity). Species codes are - AHUN: *Achnanthes hungarica*, AOBL: *Achnanthes oblongella*, AMIN: *Achnanthidium minutissimum*, ALIB: *Amphora libyca*, APED: *Amphora pediculus*, CPLA: *Cocconeis placentula*, KLAT: *Kolbesia laterostrata*, NGRE: *Navicula gregaria*, NLAN: *Navicula lanceolata*, NMIN: *Navicula minima*, NDIS: *Nitzschia dissipata*, NFIL: *Nitzschia filiformis*, NINC: *Nitzschia inconspicua*, NPAL: *Nitzschia palea*, NPUS: *Nitzschia pusilla*, NPER: *Nitzschia perminuta*, PSUB: *Pinnularia subcapitata*, PFRE: *Planothidium frequentissimum*, PLAN: *Planothidium lanceolata*, RSIN: *Remeria sinuata*, TLIT: *Tryblionella littoralis*. One of the PD samples and *Amphora Libyca* heavily influence each other, and both occur extremely low on both the first and second axes, leading to difficulties in interpreting the plot, they have therefore been removed from the plot for easy interpretation.

Achnanthes oblongella and *Cocconeis placentula* show potential as indicators of the presence of high concentrations of detergent pollution. At lower concentrations it is extremely difficult to identify key species as the separation from the control treatments is difficult to identify. *A. oblongella* abundance increased steadily through increasing concentrations of both detergent treatments, up to approximately twice the abundance present in the control samples in the ED treatment (figure 4.6). *C. placentula* shows a less clear trend, however the average abundance of the controls is heavily influenced by a single sample which contained 83 individuals, and therefore taking this into account, there is generally a similar increase in the Persil samples (figure 4.7). The Ecover samples show a less strong response in the EA, EB, and EC treatments, but show a very strong response in the ED treatments (figure 4.7).

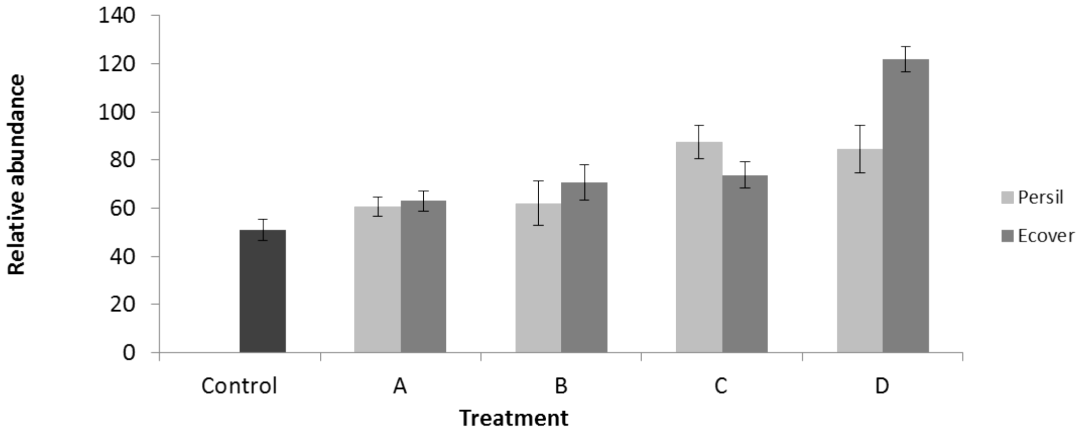

Figure 4.6: Relative abundances of *Achnanthes oblongella*.

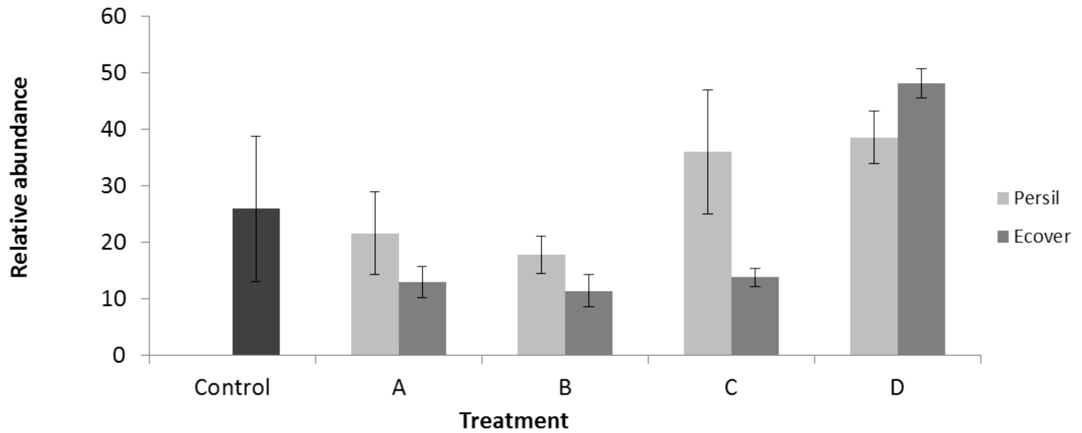

Figure 4.7: Relative abundances of *Cocconeis placentula*.

Correspondence analysis was then used at the genus level to determine how taxonomic resolution affects observed responses (figure 4.8). Axis 1 (figure 4.8) is inverted compared with axis 1 in the species level analysis (figure 4.5), but axis 2 (figure 4.8) corresponds directly with axis 2 in the species level analysis (figure 4.5).

The majority of the control, PA, PB, and EB samples cluster relatively low on the first and second axes (figure 4.8). The EA samples grouped away from this cluster, high on the second axis, driven by higher relative abundance of *Nitzschia*, *Fragilaria*, *Navicula*, and *Achnanthidium*. The ED and PD treatments clustered separately from the lower concentration samples, high on the first axis, driven by increasing relative abundance of *Cocconeis* and *Achnanthes*. The EC and PC samples clustered between the low concentration samples and the D treatment cluster, though there was high variation in the EC samples.

Correlation was then used to link water chemistry values to the correspondence analysis axes in the same way as at species level. Correlation was observed between the CA axes and nitrate ($r^2 = 0.48$, $p<0.001$), phosphate ($r^2 = 0.49$, $p<0.001$), ammonium ($r^2 = 0.22$, $p<0.01$), alkalinity ($r^2 = 0.67$, $p<0.001$), and surfactants ($r^2 = 0.41$, $p<0.001$) (figure 4.8a.). These show the same correlations as the species level analysis, with surfactants and alkalinity correlated with the higher concentration treatments, and nitrate and phosphate negatively correlated with the higher concentration treatments.

Achnanthes and *Cocconeis* showed potential as indicators. As both are characterised almost exclusively by a single species, these show the same responses as *A. oblongella* (figure 4.6) and *C. placentula* (figure 4.7).

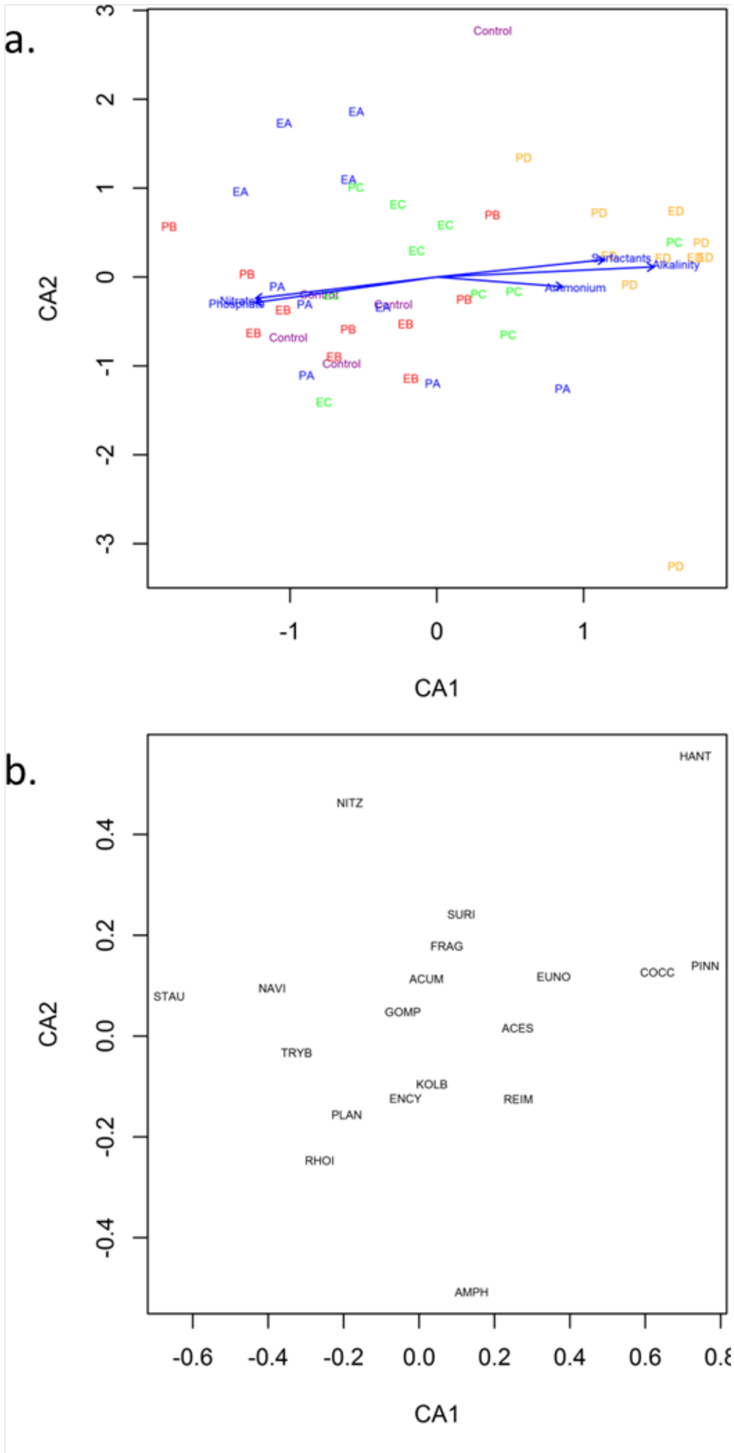

Figure 4.8: Correspondence analysis for all genera and all control and effluent treatment samples. Correspondence analysis axis 1 explained 36% of the variation in the data set. Axis 2 explained 15% if the variation in the data set. For clarity, the figure has been separated into; **i)** sample positioning on the ordination axes, purple = control, blue = A treatments, red = B treatments, Green = C treatments, orange = D treatments; **ii)** genera (only genera which constituted at least 2% of one sample are shown for clarity). The orange oval indicates the D treatment main grouping, the green oval indicates the C treatment main grouping, and the blue oval indicates main grouping of the EA treatments. Genus codes are - ACES: *Achnanthes*, ACUM: *Achnanthidium*, AMPH: *Amphora*, COCC: *Cocconeis*, ENCY: *Encyonema*, EUNO: *Eunotia*, FRAG: *Fragilaria*, GOMP: *Gomphonema*, HANT: *Hantzschia*, KOLB: *Kolbesia*, NAVI: *Navicula*, NITZ: *Nitzschia*, PINN: *Pinnularia*, PLAN: *Planothidium*, REIM: *Reimeria*, RHOI: *Rhoicosphenia*, STAU: *Stauroneis*, SURI: *Surirella*, TRYB: *Tryblionella*.

4.4 Discussion

This laboratory experiment investigated the response of field collected diatom communities to a range of environmentally relevant concentrations of simulated effluent containing two different detergents. Detergent concentration caused a clear shift in species composition at high concentrations. The effect at lower detergent concentration was less distinct, though detergent type had a greater influence on community structure at low concentrations. The presence of any detergent resulted in a significant reduction in total algal abundance.

4.4.1 Effects of detergents on diatom community structure and function

Community composition

Results from species level correspondence analysis revealed that high detergent concentrations caused considerable change in community composition. The controls and low concentration detergent treatments contained high relative abundance of *Navicula gregaria*, *Navicula minima*, *Planothidium frequentissimum*, and *Planothidium lanceolata*. These are generally alkiliphilous species, regarded as tolerant of phosphorus concentrations up to around 1mg/l in the field, and tolerant of heavy organic pollution (Kelly *et al.*, 2005). In contrast, communities at high detergent concentration were characterised by higher relative abundance of *Achnanthes oblongella* and *Cocconeis placentula*. These species have similar optima for nutrient concentrations and pH as those indicated in lower concentration samples, but are tolerant of lower concentrations of organic pollution. These samples also contained the highest concentrations of surfactants, and the highest alkalinity in the experiment, as well as the lowest nitrate and phosphate concentrations. The C treatment samples showed higher relative abundance of *Nitzschia dissipata* and *Nitzschia inconspicua* than the lower concentration samples. These species have similar niches, in moderate nutrient concentrations, moderate to heavy organic pollution concentrations, and high pH (Kelly *et al.*, 2005).

Many organic pollutants are directly toxic to freshwater organisms such as algae and invertebrates (Hellawell, 1988; Azizullah *et al.*, 2011; Soares *et al.*, 2008). The Ecover detergent contained significantly higher concentrations of surfactants than the Persil detergent. Similar concentrations of total surfactants were present in the PD and EC treatments, and therefore if total surfactants were driving species composition changes, these two treatments would be expected to have caused similar responses. Surfactants can cause highly variable toxic impacts in different algal species (Lewis, 1991), and so the different surfactants used in each detergent formula could have been expected to cause varying species responses, which may have led to this difference in community response at the PD and EC treatments, even though total surfactant concentrations were equal. However, if the different surfactants present in each detergent had significantly different effects, species specific responses would be expected, rather than simple changes in the magnitude of the response.

As both detergents resulted in similar diatom communities at the highest detergent concentration, a common factor across both detergents is likely to have caused the change in community composition. Alkalinity, a measure of carbonates dissolved in the water, correlated strongly with increasing concentration of both detergents. This was likely due to builders in both detergents, which remove cations from the water to ensure optimum conditions for detergents to function. While alkalinity is not toxic (Lewis, 1992), some species show sensitivity to it (Soininen, 2007). Smucker & Vis (2011a) found that diversity increased with alkalinity. In this study no response was observed in the Shannon diversity, although as Blanco *et al.* (2012) note, diversity measures may not be particularly sensitive indicators of change in diatom communities. However, species evenness did decrease in the D treatments compared with the controls and the A treatments, indicating that the D treatments produced a less equitable spread of species abundance, probably due to the D treatments containing much larger populations of *Cocconeis placentula* and *Achnanthes oblongella*. This would also explain the smaller response of the diatom communities in the low detergent concentration treatments, as the alkalinity did not differ from the controls in the low concentration treatments.

The lowest detergent concentration treatments (A) showed a separation in community structure, associated with detergent type. The Ecover treatment resulted in communities with high relative abundance of *Nitzschia palea*, *Nitzschia dissipata*, *Nitzschia perminuta*, and *Nitzschia pusilla*. The Persil treatment grouped close to the control treatment samples, with higher relative abundance of *Navicula gregaria*, *Planothidium lanceolata*, *Planothidium frequentissimum*, and *Amphora pediculus*. These species all inhabit similar ecological niches, favouring moderate nutrient concentrations and moderate to heavy organic pollution (Kelly *et al.*, 2005). The measured water chemistry variables for both detergents were similar at this low concentration of detergent, and therefore did little to clarify the cause of this separation, indicating that a difference in specific toxicity of each detergent may have had an effect at low concentration. Low concentrations of detergents such as these are highly likely to be observed in effluents from sewer misconnections in the field due to the potentially high dilution factor of an effluent entering a water course. This demonstrates that the particular detergent formulations discharged from a misconnection may affect the response observed in the receiving water. Though further detailed chemical analysis to elucidate the specific cause of this separation was not possible in this study, it does indicate that not only the type of substances, but specific formulations discharged from misconnections, may affect community responses.

The EC and PC treatments showed a subtle separation in community structure. The majority of the PC treatment grouped closer to the high concentration detergent samples, while the EC treatment grouped nearer to the low concentration samples. This shift in species composition correlated with higher ammonium concentration in the PC samples, which may have caused additional toxicity in the community. Hurlimann & Schanz (1993) found that addition of ammonium concentrations similar to those found in the PC and PD treatments led to changes in community composition and total abundance, therefore this could explain the separation between the PC and EC treatments, though a similar separation was not observed between the PD and ED treatments, where it could be expected to be more pronounced. Alternatively, the separation of the C treatments may have been due to different components in the respective detergents, as observed at lower concentrations.

Reduction of nitrate and phosphate was observed at the higher concentrations of both detergents, and a particularly strong response was observed in the nitrate concentration in the ED treatment. This reduction was highly unexpected, and suggests sequestration of these nutrients by components of the detergent. However, the removal of nutrients from the systems could not be further investigated in this study, and there is no known mechanism by which this sequestration should occur. The scale of reduction in nutrient concentrations could be expected to affect the response of the diatom community. The fact that the D treatments clustered closely in the correspondence analysis indicates that they contained very similar communities despite a significant difference in nitrate concentration, suggesting that other factors are likely to have caused the observed response. Similarly, if nutrient concentrations were causing major shifts in the communities, the EC and PD treatments would be expected to group closely in the correspondence analysis, as they contained very similar nutrient concentrations.

When species data were combined into genera, the results showed similar, but more pronounced responses, to those of the species level analysis. Many major species in the analysis were either the sole representative of that genus, such as *Cocconeis placentula*, or highly dominant in the genus, such as *Achnanthes oblongella*. Within a genus, species may show similar responses to abiotic changes due to a common ancestry resulting in common solutions to stress (Hermant *et al.*, 2012), as was observed with *Nitzschia inconspicua*, *N. dissipata*, *N. perminuta*, and *N. palea*, all showing similar responses. This strongly influenced the positioning of the genus *Nitzschia* in the genus level analysis. However, this was not observed in all cases. *Navicula lanceolata* was characteristic of the high detergent concentration treatments, whereas *Navicula minima* and *Navicula gregaria* were more closely associated with the low detergent concentration samples. When combined, the genus *Navicula* was grouped at the low detergent concentrations due to *N. lanceolata* having low relative abundance in the samples compared with that of *N. minima* and *N. gregaria* combined. Therefore performing the analysis at the genus level helped to remove noise in the data set which did little to explain the community responses.

The results of this study strongly indicate that responses of communities to detergents are similar at the species and genus levels. This may, however, have been a limitation of the microcosm experimental design, which inherently prevents species which may be better suited to conditions in certain treatments from entering the system, as would occur in the natural situation. This means that the only observed change will be sensitive species lost from the system, and change in relative abundance of tolerant species, and therefore the system will tend toward a structure with relatively few species per genus.

Total algal abundance

Almost all detergent concentrations resulted in a major reduction in algal total abundance compared with that of the controls. As this effect was observed even in the lowest detergent concentrations, it is clear that it must be caused by the presence of one or more components of the detergents, which need only be present at very low concentrations to cause significant impact in the community. Pavlic *et al.* (2005) found that concentrations of surfactants similar to those in the low concentration detergent treatments of this study had significant ecotoxicity effects on two freshwater green algae, and 2 marine diatom species, though they did find the effect to vary between species by an order of magnitude. Surfactants interact with proteins of the cell wall, reducing control of substance movement and permeability of cells, and thus contributing to the toxicity of other compounds, in addition to the toxicity of the surfactants themselves (Lewis, 1990). This may explain the decrease in algal abundance due to increased toxicity from surfactants, and increased susceptibility to toxicity. However, if this was the case, one would expect to also see changes in the community structure and diversity in all detergent treatments compared with the control treatments, as some species would be more susceptible than others. As strong shifts from the community of the control treatment were not observed in all treatments, this suggests that the detergents may have a major impact in a common aspect of diatom physiology, thus causing non-species specific effects.

Algal biofilms form the base of many foodwebs in streams, and are consumed by many macroinvertebrate and fish species (Dixit *et al.*, 1992; McCormick & Cairns Jnr, 1994). If the total abundance of algae is significantly reduced, this will reduce the availability of energy to these higher trophic levels in the field, posing potentially major threats to freshwater ecosystems.

4.4.2 Diatoms as indicators of misconnection effluents

Diatom communities have many attributes which make them ideal for biomonitoring of misconnections. They are ubiquitous in river systems (McCormick & Cairns Jnr, 1994), constantly exposed to potential misconnection discharges (Chapter 1), and short life cycles mean diatom communities respond quickly to short term effects such as intermittent discharge of sewers (Fore & Grafe, 2002). Diatoms are very useful indicators of nutrient pollution (Kelly & Whitton, 1995), and can be used to robustly identify organic pollution as well (Hering *et al.*, 2006), so have a wide applicability for monitoring. One of the major drawbacks of using diatom communities for monitoring is that identification to species level can be difficult to learn, and time consuming to perform. However, as previously shown in this chapter, and Rimet & Bouchez (2012), counting diatoms to genus level can lead to considerable time savings, with relatively little loss of information.

Total algal abundance was found to be a strong indicator of the presence of detergent effluents, being heavily impacted by even the lowest concentrations of detergent. Chlorophyll *a* is also a relatively simple variable to measure, making it an ideal indicator. Though total algal abundance in the field is likely to be affected by many aspects, such as light intensity, water turbidity, invertebrate grazing intensity, and potentially other chemical impacts, the strength of the response seen in this study suggests that at small spatial scales, a reduction in abundance downstream of misconnected outfalls due to detergent pollution should be observable. In the field, this would require comparison of the chlorophyll *a* content of the unaffected community upstream of an outfall, and the affected community downstream, to observe change in algal abundance caused by the discharge.

In the species analysis, high abundance of *A. oblongella* and *C. placentula*, were good indicators of the presence of high concentrations of detergent pollution, found at high relative abundances. However, responses of these species were only observed at high concentrations, which are not likely to be common in the field. The *Nitzschia* species which were indicative of the EA treatment and C treatments are tolerant of many common pollutants such as nutrients and organic matter, therefore, while responses to detergent pollution may be observed, it would be difficult to differentiate these from the impacts of other pollutants if significant background pollution levels were present in a water course. Species which were particularly sensitive to detergents could not be identified, as the control samples showed highly similar communities to the low concentration detergent treatments

At the genus level, in addition to *Nitzschia* as previously discussed, *Cocconeis* showed particularly strong responses to detergent pollution. Cocconeis is a pioneer species (Davie *et al.*, 2012; Kelly *et al.*, 2005), being among the first diatoms to colonise substrates. Pioneer species quickly colonise substrates in advantageous conditions, and therefore *Cocconeis* is likely to respond quickly to misconnection effluents. *Cocconeis* is also a very easy genus to identify in samples, making it potentially ideal as an indicator.

4.5 Conclusions

Field sourced diatom communities were exposed to a range of concentrations of two different artificial laundry detergent effluents in a laboratory microcosm experiment.

Detergents were found to have a strong impact in diatom community composition at high concentrations driven by high alkalinity, leading to communities containing increased relative abundance of species which are generally considered pollution sensitive. At low concentrations, the two detergents led to slightly different community compositions, both containing species which are generally pollution tolerant. The presence of any detergent led to a strong decrease in the total abundance of algae, indicating a major impact which may have severe consequences in the case of detergents discharged to natural systems. At different taxonomic resolution (species and genus), communities showed extremely similar responses, indicating that analysis of genera may be a valid alternative to more common species level identification, with the benefit of reducing analysis time significantly. The genus *Nitzschia* as well as *Achnanthes oblongella* and *Cocconeis placentula* are promising indicators of detergent pollution in surface waters.

This study concludes that diatom communities are strongly affected by detergents in misconnection effluents. Detergents caused strong reduction in algal abundance, and affected community composition at high detergent concentrations. They also show potential as indicators of the presence of detergent pollution in natural surface waters.

Chapter 5

Impact of misconnected sewer outfalls on diatom communities

5.1 Introduction

Separate sewer systems consist of surface water sewers which carry rain water to rivers and streams, and foul sewers which carry domestic wastewater to sewage treatment works. Sewer misconnections occur where domestic appliances are connected to surface water sewer systems. They circumvent the design of the separate sewer system, leading to intermittent direct discharge of sewage to receiving waters. Though the effects are likely to be similar to those of combined sewer overflows (CSOs), CSOs discharge only during periods of particularly high rainfall, therefore discharging diluted sewage into high flowing rivers (Welker, 2007). In contrast, misconnections discharge in all weather conditions, therefore the most severe effects of sewer misconnections are expected to be during periods of low rainfall, where sewage will be undiluted and discharged into low flow conditions in the receiving water.

Misconnections are high on priority lists of urban diffuse pollution problems (DEFRA, 2012), are sources of a wide range of pollutants (Ellis & Mitchell, 2006), and are a threat to both water and biological quality in surface waters (UKWIR, 2012; Environment Agency and Water UK, 2014; Zielinski & Brown, 2003). A surface water sewer system may suffer multiple misconnections from different appliances. The impacts which they cause in receiving waters depend on the components of the discharge, frequency of discharge, and concentration of pollutants, but may range from fertilisation by addition of nutrients, to damage caused by toxic compounds (Chapter 2).

Diatom communities are ideally suited to investigating the impacts of sewer misconnections due to their dynamic response to a wide range of pollutants and ubiquitous distribution in freshwater ecosystems (Smucker & Vis, 2011a), short generation time, and key role as the major primary producers in many river ecosystems (Lowe & Pan, 1996). They are also frequently used to investigate the impacts of nutrients and organic matter entering surface waters (Kelly *et al.*, 2008). The response of diatom communities to detergents, a common component of sewer misconnections (UKWIR, 2012), has been investigated in a microcosm study (Chapter 4), showing that common effluents from sewer misconnections can impact biological communities, and therefore ecosystem functioning, and showing that aspects of the diatom community may be useful indicators of misconnection effluents at concentrations which could be expected in urban freshwater ecosystems. In the field, misconnection effluents are expected to be highly variable, however, containing a wide range of pollutants including nutrients and organic pollution, to which diatom communities are known to respond. However, in the natural setting, wider abiotic and biotic factors such as background nutrient and organic pollution (Rott *et al.*, 1998; Birkett & Gardiner, 2005), light availability, and grazing (Lange *et al.*, 2011; McCormick & Stevenson, 1998; Rosemond *et al.*, 2000), can influence the diatom community, and therefore responses to detergent effluents may not be as clear as in Chapter 4.

This chapter presents the first large scale investigation of the response of diatom communities to misconnected sewer discharges. The objectives were to investigate (i) whether sewer misconnection impacts would be observed using common monitoring methods, (ii) whether impacts observed in the laboratory could be seen in the field, and (iii) whether misconnected outfalls cause other impacts in natural diatom communities.

5.1.1 Introduction to catchments

This study was performed in catchments in Totley (SK 317803), Parson Cross (SK 351924), and Chapeltown (SK 344967), in Sheffield, UK (figure 5.1). In each catchment, 4-6 surface water outfalls which were safely accessible for sampling, high in the respective catchments, were selected for investigation. Outfalls were tested for misconnections using visual inspection and optical brightener testing as detailed in Chapter 3.

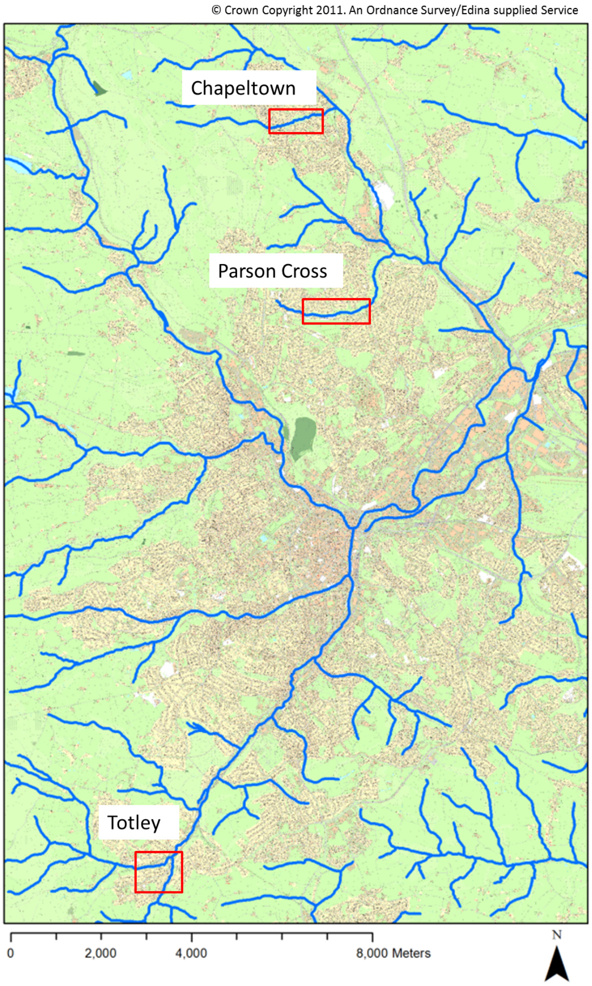

Figure 5.1: Position of sampled catchments in Sheffield, UK.

The Parson Cross catchment contained four outfalls, three of which had suspected misconnections (Figure 5.2). There was one inaccessible outfall between P1 and P2, and one inaccessible outfall between P3 and P4. Parson Cross contained two combined sewer overflows, downstream of outfall P3, but upstream of outfall P4 (figure 5.2). The major land use in all sewer catchments was housing, with two supermarkets, a sports centre, and a waste recycling plant on sewer system P1, a community centre and a working men's club on sewer system P2, and a community centre on sewer system P4. The catchments in Parson Cross served between 5 and 600 properties. The stream bed at all sites was a combination of cobbles, bedrock, and fine gravel. The stream was surrounded by riparian woodland and parkland. Where misconnections could be identified and investigated in Parson Cross, they were found to be sinks and soil stacks, which could be expected to discharge soaps and detergents, therefore responses such as those observed in Chapter 4 could be expected.

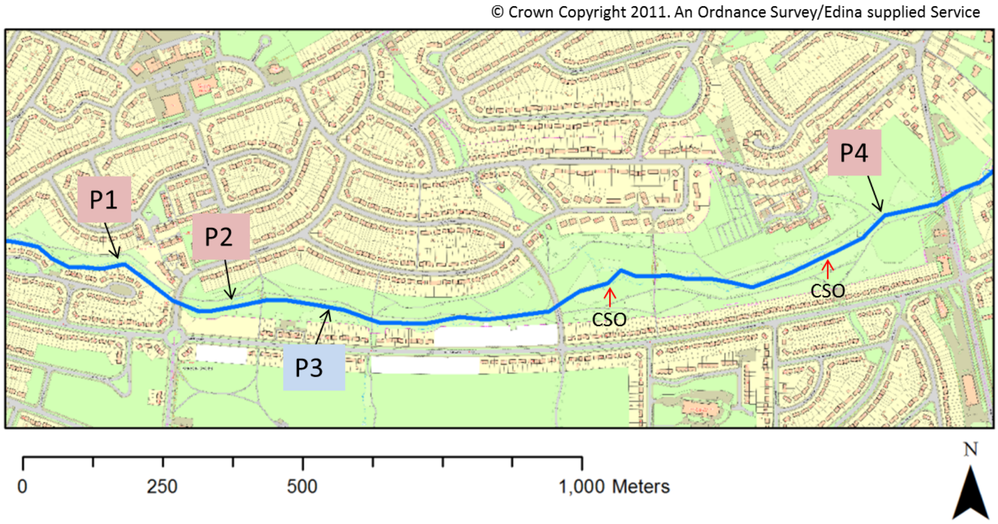

Figure 5.2: Map of outfalls in the Parson Cross catchment. Blue shows the suspected clean outfall (P3), red shows suspected misconnected outfalls (P1, P2, and P4).

The Totley catchment was separated into two subcatchments, the Oldhay Brook and Totley Brook, consisting of two and three outfalls respectively, and containing two suspected misconnected outfalls, one in each subcatchment (Figure 5.3). Site T4 comprised of two separate sewer outfalls, one on each side of the stream. Both outfalls are indicated as being misconnected, and both enter the stream at the same point. The effects of one could not be separated from those of the other therefore the two outfalls were treated as a single outfall for the study. The major land use on all sewer catchments was housing. Sewer system T4 also served a school. There were three surface water outfalls which could not be safely accessed between sites T4 and T5. There was a single surface water outfall between T1 and T2, and one between T2 and T3 which could not be sampled due to issues with access. The number of houses served by the sewer catchments in Totley ranged from approximately 18, to approximately 200. The stream bed at all sites was a combination of cobbles and bedrock, with the exception of T4, which was a concrete channel with an occasionally broken bed, where cobbles and broken concrete were typical. The riparian area in Totley was gardens and woodland. There were no combined sewer overflows in the Totley catchment.

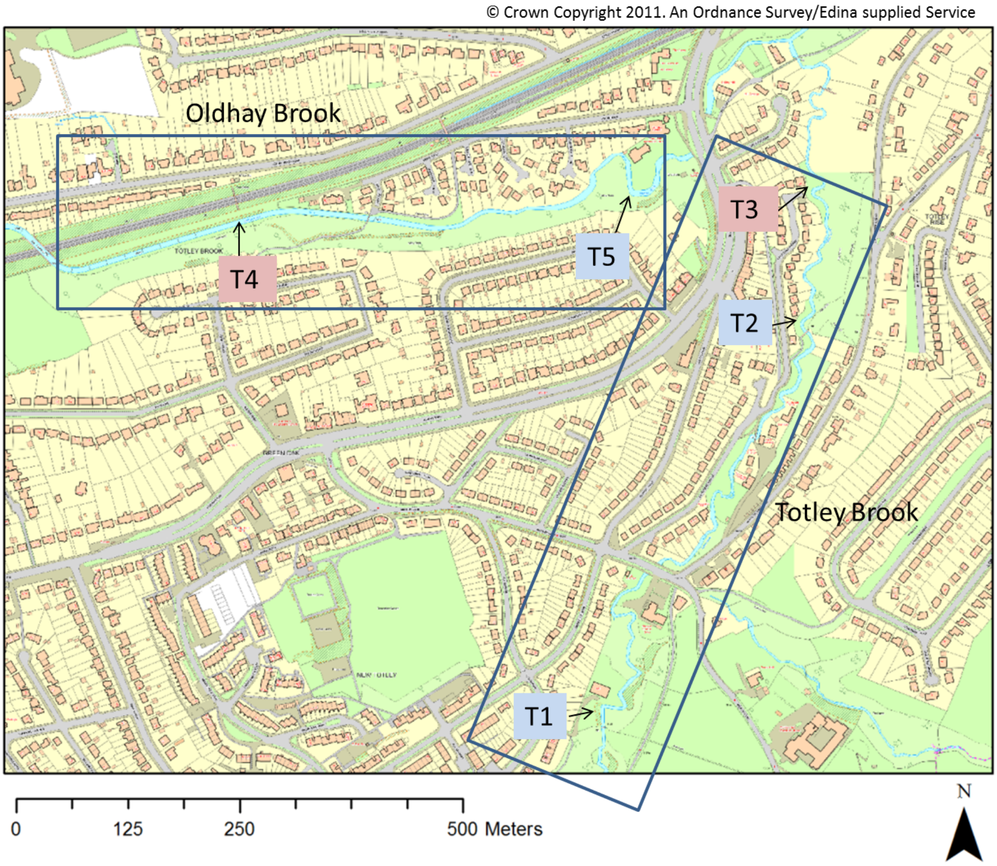

Figure 5.3: Map of outfalls in the Totley catchment. Blue shows suspected clean outfalls (T1, T2, and T5), red shows suspected misconnected outfalls (T3 and T4).

The Chapeltown catchment contained six outfalls, three of which had suspected misconnections (Figure 5.4). Outfall C4 discharged approximately 10m from the main stream, and flowed to the stream through a wooded riparian area. The downstream sample site for outfall C4 was the upstream site for outfall C4b, as they were in close proximity. The major land use was housing, with one school on sewer catchment C5. The number of properties served by sewer systems varied from approximately 5 to 250 across the Chapeltown catchment, though the sewer system of outfall C4b was not mapped, so its size is unknown. There were no combined sewer overflows in the Chapeltown catchment. The stream bed at all sites was a combination of cobbles and bedrock. The riparian area was woodland, and parkland.

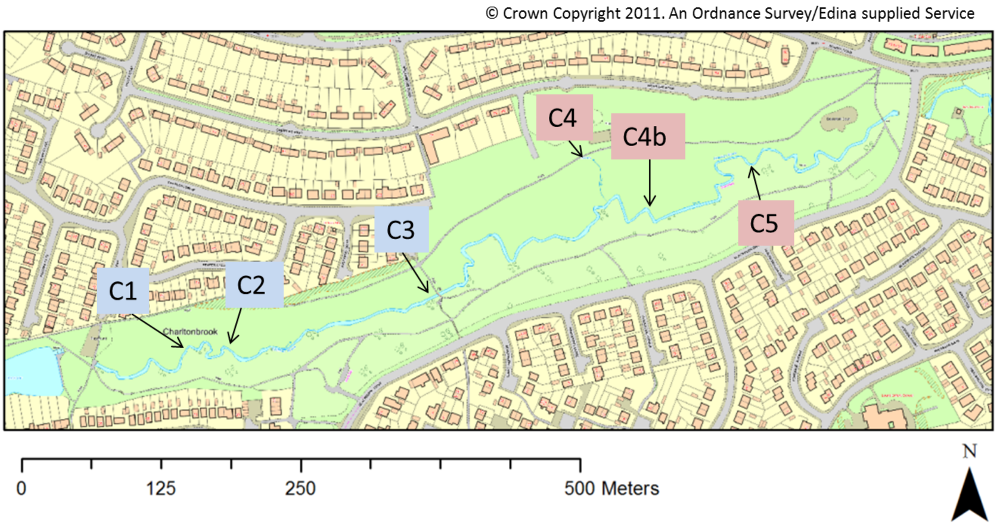

Figure 5.4: Map of outfalls in the Chapeltown catchment. Blue shows suspected clean outfalls (C1, C2, and C3), red shows suspected misconnected outfalls (C4, C4b, and C5).

5.2 Methods

5.2.1 Analysis of physical and chemical variables

At each site, temperature, dissolved oxygen, conductivity and pH were measured, using a portable meter. Three replicate 50ml water samples were collected at each site. Water samples were filtered, and analysed for major ions using a Dionex DX-120 ion chromatograph. Alkalinity was measured using a Hach AL-DT alkalinity test kit to perform titration with bromcresol green - methyl red indicator powder and 0.16 molar nitric acid. Water chemistry data was used to characterise the broad chemistry of the catchments rather than identify impacts of misconnected outfalls, as this is unlikely to be observed using point sampling (Chapter 3).

Percentage canopy cover, a surrogate for light intensity, was estimated by taking a wide angle photograph directly upwards from the level of the river using a Canon EOS 1000d camera. Pixels were then reduced to either black or white based on a threshold value between foliage and background sky for each photograph, black representing foliage, and white representing the sky. The percentage cover of black pixels was then used as the percentage cover of foliage.

5.2.2 Diatom sampling and identification

Stream width and depth were between 1-2 m and 10-40 cm respectively at all sites. At each outfall, samples were collected from the first riffle upstream, and the first riffle downstream at which effluent was judged to have mixed across the cross section of the river, ensuring that all sampled communities were exposed to any effluent from the outfall. Samples were collected on the 15th, 17th, and 19th of October 2012 at Totley, Parson Cross, and Chapeltown respectively. Rainfall was relatively low prior to sampling with the exception of a significant rainfall event three weeks before sampling took place (figure 5.5). The relative lack of rainfall prior to sampling ensured that communities would be exposed to pollutants from misconnections at high concentrations, thus maximising the chance of an effect being observed in the diatom community.

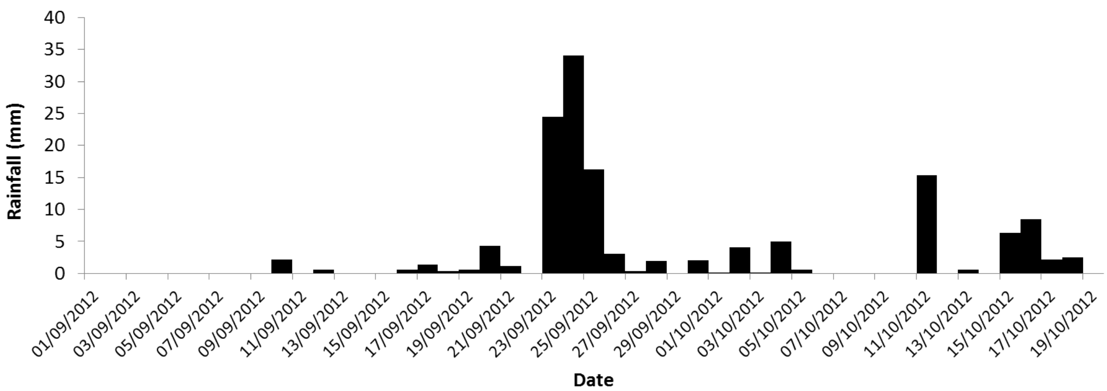

Figure 5.5: Precipitation in the six weeks preceding sampling. Data collected by Western Park weather station, Museums Sheffield.

At each site, five cobbles of approximately 10-20cm diameter were selected, and the biofilm from a 5x5cm area scrubbed into a 50ml bottle using a toothbrush. Water was added, and bottles were stored in darkness and returned to the laboratory within five hours of collection. A subsample of 5ml from each sample was removed for community analyses, and a further 5ml subsample removed and preserved in Lugol's iodene in case further analyses were necessary.

Five millilitres of hydrogen peroxide was added to each of the samples for community analyses. They were then placed in a waterbath at 80^{o}c for approximately eight hours, and allowed to cool over night. Two drops of hydrochloric acid were added to neutralise any remaining hydrogen peroxide, and the samples transferred to centrifuge tubes. Samples were centrifuged three times at 3000rpm for five minutes. Between each time in the centrifuge, the supernatant was removed and the sample re-suspended with distilled water. Finally samples were re-suspended and a few drops of the suspension were placed on a cover slip and allowed to dry, before being mounted on a microscope slide using Naphrax^{TM} mountant. Communities were counted to 400 individuals, using a microscope at 1000x magnification.

5.2.3 Chlorophyll *a* analysis

The remaining 40ml of each sample were used for chlorophyll *a* analysis, a measure of total algal abundance. Samples were filtered using Whatman number 1 filter paper, and the filter papers frozen over night to rupture the cell membranes. Filter papers were then defrosted and heated with 10ml of ethanol for 10 minutes to extract the chlorophyll. A spectrophotometer was used to measure the absorbance at 664nm and 750nm, and equation 5.1, based on the methods of Horne (2009) and Gregor & Marsalek (2004), used to calculate the quantity of chlorophyll.

$$Chlorophyll\ a\ (\mu g/cm^2) = E \times \frac{A_{664nm} - A_{750nm}}{Area} \times V_{extract} \times Dilution\ factor \times L \quad (5.1)$$

Where E = extinction coefficient for chlorophyll in 90% ethanol at 664nm (12.8), A_{664nm} = absorption at 664nm, A_{750nm} = absorption at 750nm, $V_{extract}$ = Volume of extract in ml (10), DF = dilution factor, Area = area sample taken from, L = cuvette path length.

5.2.4 Data analysis

Water chemistry results were combined within each catchment, and analysed using one-way ANOVA analysis. Shannon diversity, species richness, species evenness, and chlorophyll *a* were compared using two-way ANOVA analyses at the site level, to investigate changes between outfalls and upstream or downstream sites at each outfall.

The Trophic Diatom Index (TDI) is a common index used to monitor trophic status, an indication of nutrient levels, in surface waters using diatom community compositions, and species tolerance to nutrient exposure (Kelly & Whitton, 1995; Kelly, 1998; Kelly *et al.*, 2008). It is based on the weighted mean sensitivity equation of Zelinka & Marvan (1961) (equation 5.2).

$$Weighted\,mean\,sensitivity = \frac{\sum_{j=1}^{n} a_j v_j i_j}{\sum_{j=1}^{n} a_j v_j} \quad (5.2)$$

Where a_j = abundance of species j in sample, v_j = the indicator value (1-3) of species j, i_j = pollution sensitivity (1-5) of species j.

However, in the most recent iteration of the TDI, indicator value has been shown to have little effect on the weighted mean sensitivity, and so has been removed from the equation (Kelly *et al.*, 2008). The weighted mean score is then converted into the trophic diatom index using equation 5.3.

$$TDI = (Weighted\,mean\,sensitivity \times 25) - 25 \quad (5.3)$$

This converts the weighted mean sensitivity into values between 0, low trophic status, and 100, high trophic status, though values of the TDI do not directly link to concentrations of nutrients, and are used as an indicator of total trophic status in the river.

The percentage of motile taxa is a measure used to account for changes in environmental variables which may correlate with nutrient concentrations. A change in the percentage of motile taxa may indicate a change in substrate or biofilm properties, which can be associated with suspended solids, or other particulate matter caught in the biofilm (Kelly, 2006). Motility in taxa allows them to move through the biofilm, therefore ensuring optimum light conditions for photosynthesis, while non-motile taxa are less able to adapt to changing conditions.

Correspondence analysis was used to analyse species and genus composition as described in Chapter 4. Sites were analysed using individual samples, and pooling the samples at each site to allow more general comparisons. Both approaches gave similar results, and therefore only the individual samples plots are presented here as they show greater detail in the result. Taxa were removed from the analysis if they did not constitute at least 1% of at least one sample in the catchment in order to reduce the exaggerated effect which rare species have on the response of correspondence analysis.

5.3 Results

5.3.1 Catchment variables

The majority of sites showed phosphate concentrations below the detection limit of 0.3mg/L and therefore were not investigated further. There were significant differences in alkalinity ($F_{(3,82)} = 1338$, $p<0.001$) (figure 5.6a.), dissolved oxygen ($F_{(3,25)} = 63.01$, $p<0.001$) (figure 5.6b.), temperature ($F_{(3,25)} = 130.9$, $p<0.001$) (figure 5.6c.), pH ($F_{(3,25)} = 21.88$, $p<0.001$) (figure 5.6d.), conductivity ($F_{(3,25)} = 671.3$, $p<0.001$) (figure 5.6e.), and nitrate ($F_{(3,82)} = 315.8$, $p<0.001$) (figure 5.6f.). Each catchment was treated separately for all other analyses due to these major differences in the fundamental background water chemistry.

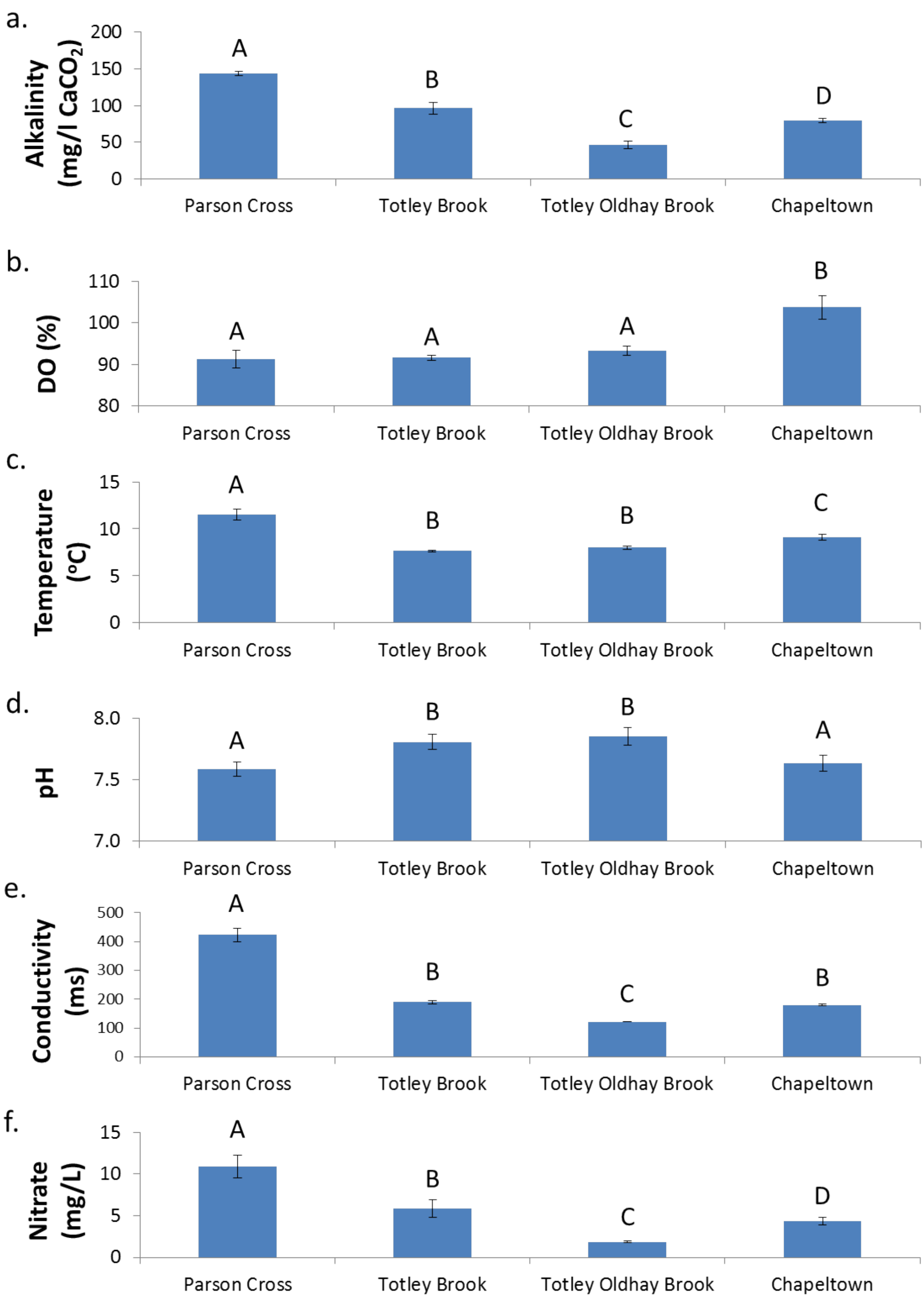

Figure 5.6: Water chemistry values for each catchment. Error bars show the standard deviation of values. Within each plot, letters are given if the ANOVA result was significant, treatments with the same letter code do not differ significantly from each other (Tukey HSD, $p>0.05$).

The percentage cover of foliage was similar upstream and downstream of each outfall in Chapeltown and Totley (figure 5.7). In Parson Cross, outfall P1 had 47% more cover downstream of the outfall than upstream, and P2 had 64% more cover upstream of the outfall than downstream.

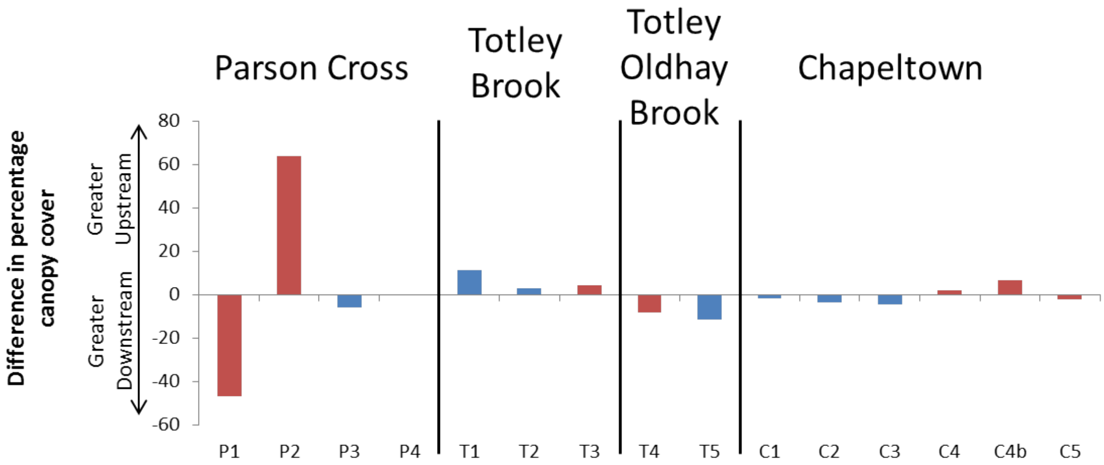

Figure 5.7: Differences between canopy cover upstream and downstream of outfalls in each catchment. Red indicates misconnected outfalls, blue indicates correctly connected outfalls.

There was a high variability in the number of properties served by sewer systems within each catchment (figure 5.8). In Parson Cross these ranged from 5 to 600, in Totley they ranged from 18 to 200, and Chapeltown ranged from 5 to 250 properties.

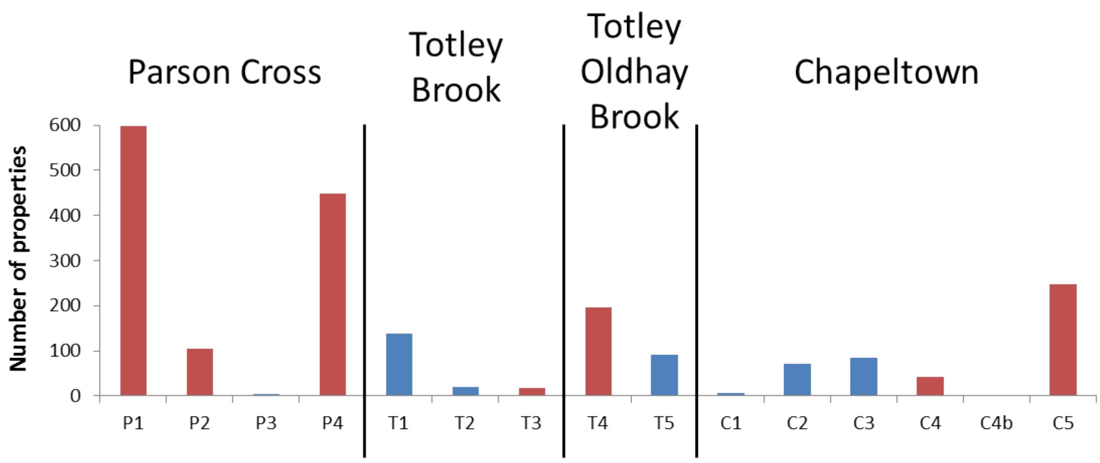

Figure 5.8: Estimated number of properties served by each sewer system. Red indicates misconnected outfalls, blue indicates correctly connected outfalls.

5.3.2 Diversity, abundance, and TDI

One hundred and forty five species representing 44 genera were found in the data set of 90 samples and 36,000 individuals. The most common species across all catchments were *Cocconeis placentula (var. euglypta)*, *Amphora pediculus*, *Planothidium frequentissimum*, *Achnanthes oblongella*, and *Achnanthidium minutissimum*.

There were no significant difference between upstream and downstream sites, or between outfalls, in species richness (figure 5.9a), evenness (figure 5.9b), Shannon diversity (figure 5.9c) or chlorophyll *a* content (figure 5.9d.). All outfalls showed no major change in the Trophic Diatom Index between upstream and downstream sites except outfall C3, which showed significantly lower TDI downstream of the outfall ($F_{(5,24)} = 3.251$, $p<0.022$) (figure 5.9e.). Percentage motile taxa showed no response in any catchments (figure 5.9f.).

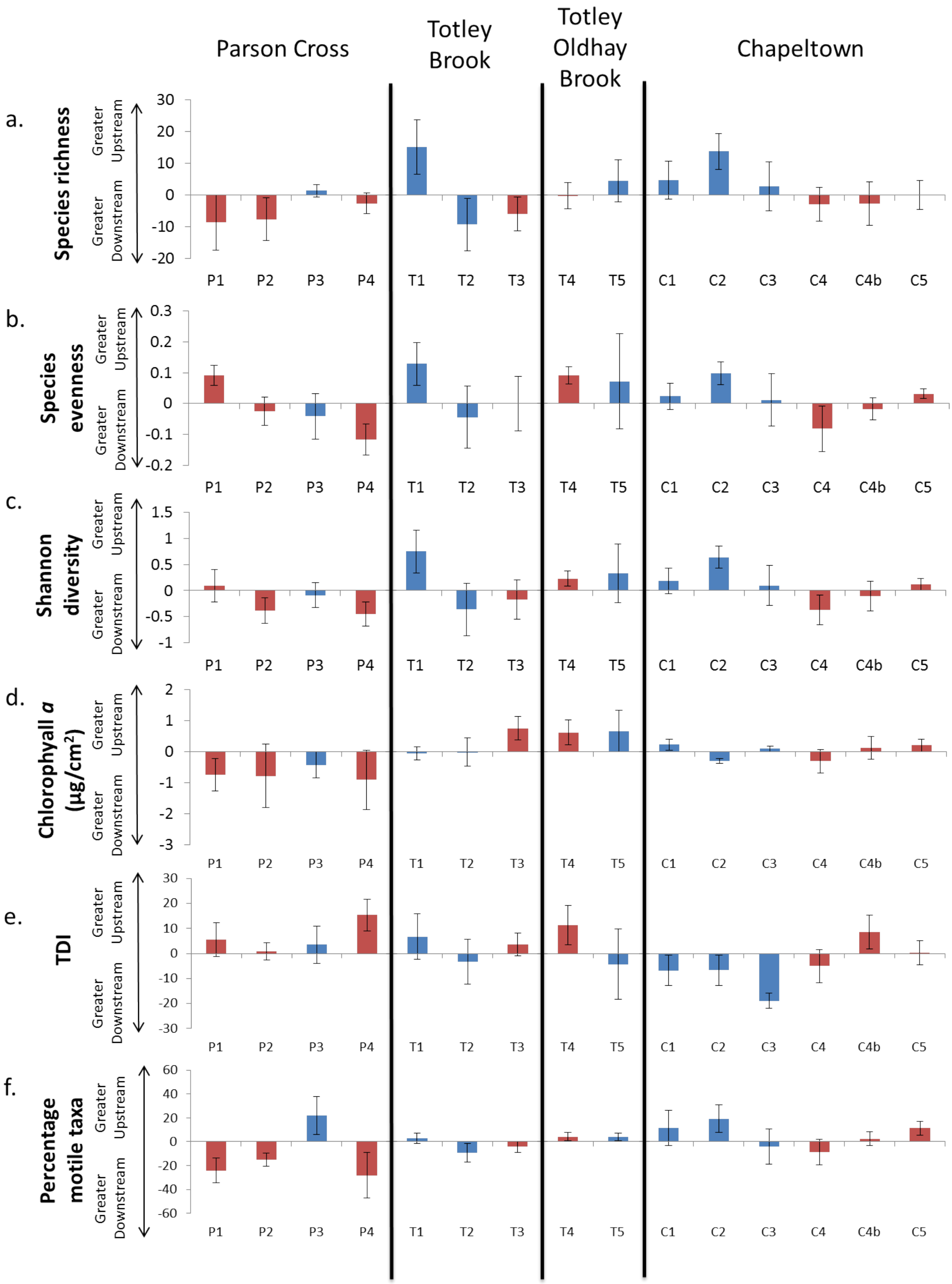

Figure 5.9: Differences in diatom community measures between upstream and downstream sites at each outfall. Red indicates misconnected outfalls, blue indicates correctly connected outfalls. Error bars show standard error.

5.3.3 Diatom community structure

Correspondence analysis was used to investigate responses of community composition to sewer outfalls at the species and genus level for each catchment.

Parson Cross

At the species level, correspondence analysis axis 1 showed a gradient driven by increasing relative abundance of *Cocconeis placentula var. lineata*, *C. placentula var. euglypta*, *Planothidium frequentissimum*, and *Planothidium lanceolata* high on the axis, and high relative abundance of *Navicula gregaria*, *Navicula lanceolata* and many rare species low on the axis (figure 5.10b.). The second axis was characterised by high abundance of *Navicula gregaria*, *Cocconeis placentula var. lineata*, *Planothidium frequentissimum* and many rare species, high on the axis, and *Amphora pediculus*, *Rhoicosphenia abbreviata*, and *Navicula minima* low on the axis (figure 5.10b.).

Though there were no major trends across all outfalls in Parson Cross, individual outfalls did show some response in community structure. Outfall P1 showed close clustering of both upstream and downstream samples, as did outfall P2 (figure 5.10a.). Outfall P3, the only correctly connected outfall in the catchment, showed a high variability in community composition between samples, and no clear trends in response. Outfall P4 showed a major separation of upstream and downstream samples on the second axis, with upstream samples strongly characterised by higher relative abundance of *Amphora pediculus*, *Navicula minima*, and *Rhoicosphenia abbreviata*, and downstream samples characterised by higher relative abundance of *Navicula gregaria*, *Navicula lanceolata* and small changes in rare *Navicula* and *Nitzschia* species (figure 5.10).

At the genus level, correspondence analysis axis 1 showed a gradient driven by high relative abundance of *Cocconeis* and *Planothidium* low on the axis, and high abundance of *Nitzschia*, *Navicula*, *Rhoicosphenia*, and *Amphora* higher on the axis (figure 5.11b.). The second axis was driven by high relative abundance of *Cocconeis*, *Rhoicosphenia*, and *Amphora* low on the axis, and increasing relative abundance of *Nitzschia* and *Navicula* high on the axis (figure 5.11b.).

Samples upstream and downstream of misconnected outfalls grouped more clearly at the genus level than at the species level. Upstream samples were broadly characterised by higher abundance of *Amphora*, *Cocconeis*, and *Rhoicosphenia*, and downstream samples less clearly characterised by higher abundance of *Navicula*, and *Nitzschia* (figure 5.11). Outfall P3, the correctly connected outfall, showed little clustering in the upstream and downstream samples at the genus level (figure 5.11a.).

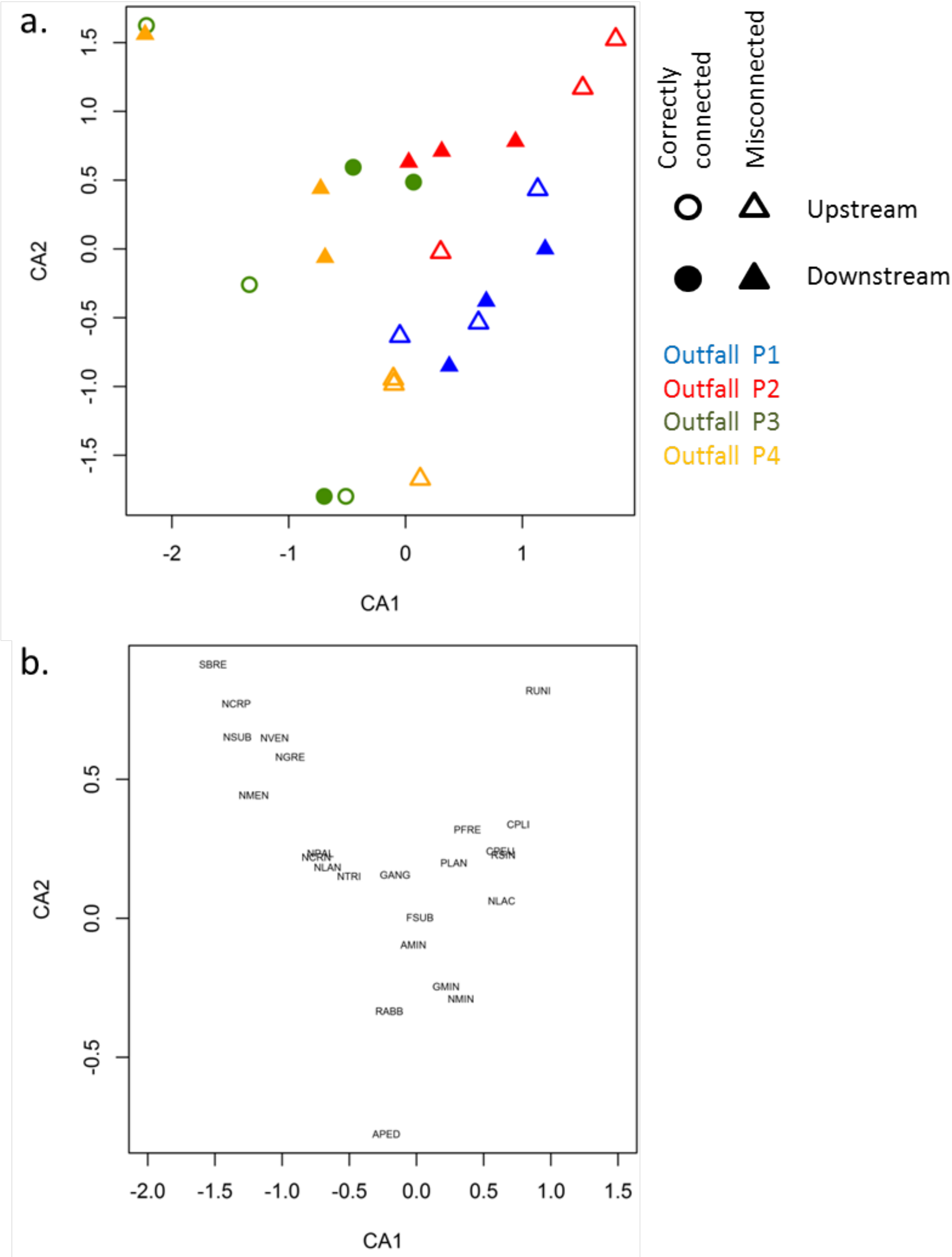

Figure 5.10: Correspondence analysis of species assemblage at Parson Cross. Axis 1 explains 23% of the variation of the community. Axis 2 explains 16% of the variation of the community. Species codes are - AMIN: *Achnanthidium minutissimum*, APED: *Amphora pediculus*, CPEU: *Cocconeis placentula var. euglypta*, CPLI: *Cocconeis placentula var. lineata*, FSUB: *Fallacia subhamulata*, GANG: *Gomphonema angustatum*, GMIN: *Gomphonema minutum*, NCRP: *Navicula cryptocephala*, NCRN: *Navicula cryptonella*, NGRE: *Navicula gregaria*, NLAN: *Navicula lanceolata*, NMIN: *Navicula minima*, NMEN: *Navicula menisculus*, NTRI: *Navicula tripunctata*, NVEN: *Navicula venata*, NLAC: *Nitzschia lacuum*, NPAL: *Nitzschia palea*, NSUB: *Nitzschia sublinearis*, PFRE: *Planothidium frequentissimum*, PLAN: *Planothidium lanceolata*, RSIN: *Remeria sinuata*, RSIN: *Remeria uniseriata*, RABB: *Rhoicosphenia abbreviata*, SBRE: *Surirella brebisonii.*

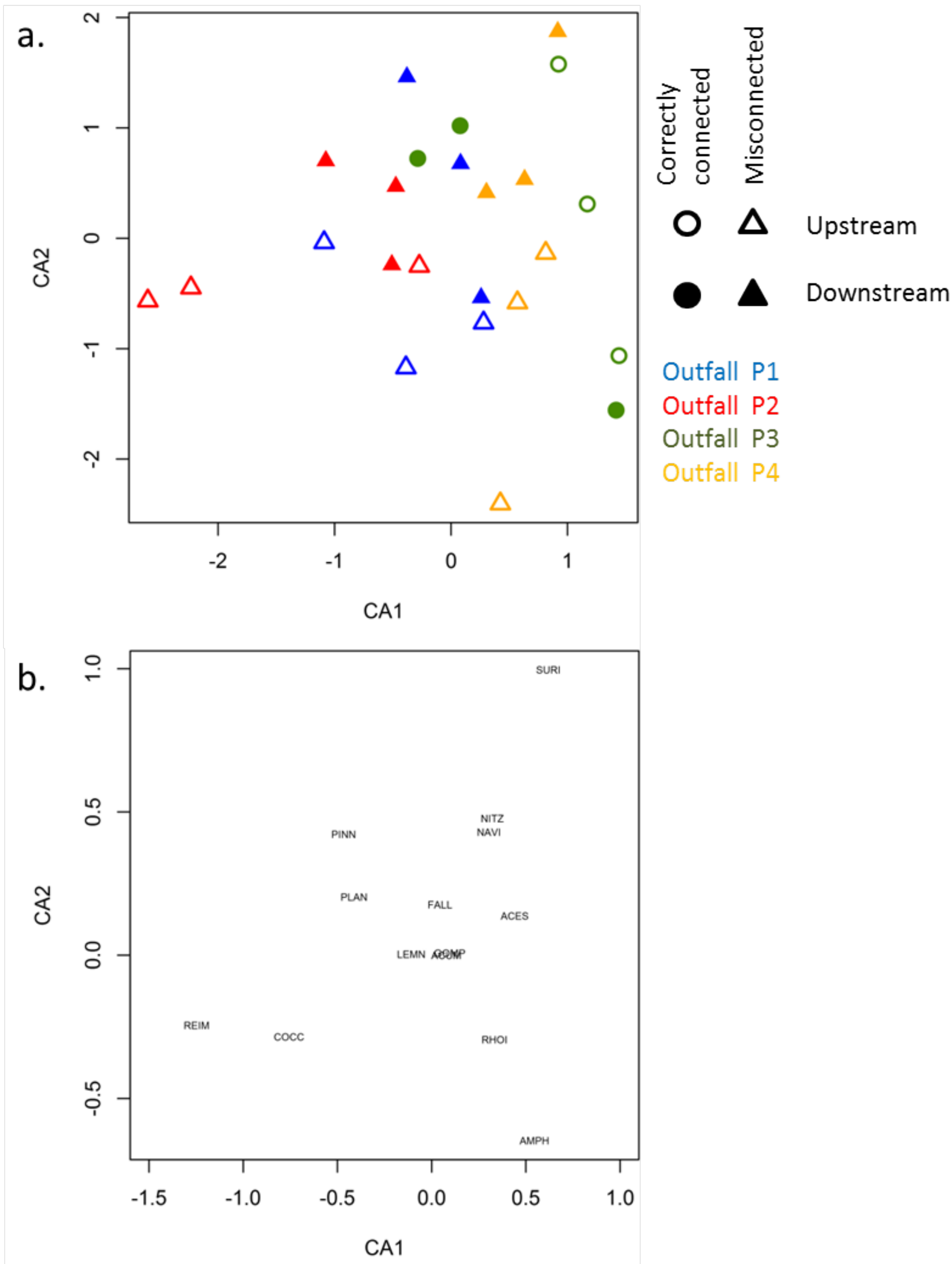

Figure 5.11: Correspondence analysis of genus assemblage at Parson Cross. Axis 1 explains 37% of the variation of the community. Axis 2 explains 25% of the variation of the community. Genus codes are - ACES: *Achnanthes*, ACUM: *Achnanthidium*, AMPH: *Amphora*, COCC: *Cocconeis*, FALL: *Fallacia*, GOMP: *Gomphonema*, LEMN: *Lemnicola* NAVI: *Navicula*, NITZ: *Nitzschia*, PINN: *Pinnularia*, PLAN: *Planothidium*, REIM: *Reimeria*, RHOI: *Rhoicosphenia*, SURI: *Surirella*.

Totley Brook

In the Totley Brook at the species level, axis 1 was characterised by high relative abundance of *Cocconeis placentula var. lineata*, and *Cocconeis placentula var. euglypta* high on the axis, there was little separation of species lower on the axis (figure 5.12b.). Axis 2 was characterised by high relative abundance of *Achnanthidium minutissimum*, *Navicula minima*, and *Navicula gregaria* low on the axis, and higher abundance of *Amphora pediculus* and many rare species, high on the axis.

Upstream and downstream samples for both misconnected and correctly connected outfalls showed no clustering on either axis (figure 5.12a.).

At the genus level, the first axis was characterised by high relative abundance of *Cocconeis* and *Reimeria* high on the axis (figure 5.13b.) with no major definition lower on the axis. The second axis showed high abundance of *Achnanthidium* low on the axis and *Achnanthes* and *Amphora* high on the axis (figure 5.13b.).

At the genus level there was a similar lack of trends in the response of particular samples as at the species level (figure 5.13a.).

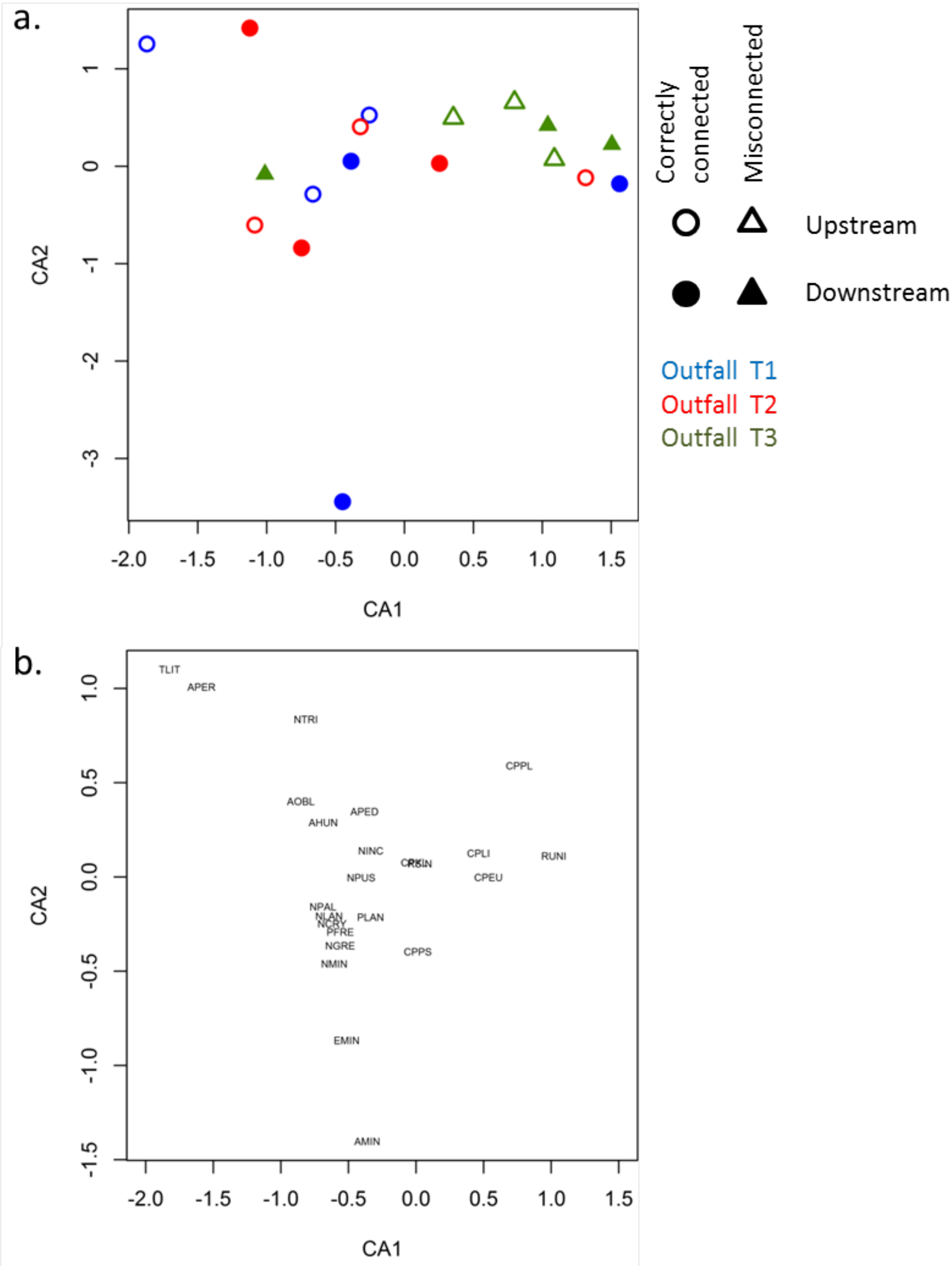

Figure 5.12: Correspondence analysis of species assemblage at Totley Brook. Axis 1 explains 28% of the variation of the community. Axis 2 explains 20% of the variation of the community. Species codes are - AHUN: *Achnanthes hungarica*, AOBL: *Achnanthes oblongella*, APER: *Achnanthes pericava* AMIN: *Achnanthidium minutissimum*, APED: *Amphora pediculus*, CPEU: *Cocconeis placentula var. euglypta*, CPKL: *Cocconeis placentula var. klinographis*, CPLI: *Cocconeis placentula var. lineata*, CPPL: *Cocconeis placentula var. placentula*, CPPS: *Cocconeis placentula var. pseudolineata*, EMIN: *Encyonema minutum*, NCRY: *Navicula cryptonella*, NGRE: *Navicula gregaria*, NLAN: *Navicula lanceolata*, NMIN: *Navicula minima*, NTRI: *Navicula tripunctata*, NINC: *Nitzschia inconspicua*, NPAL: *Nitzschia palea*, NPUS: *Nitzschia pusilla*, PFRE: *Planothidium frequentissimum*, PLAN: *Planothidium lanceolata*, RSIN: *Remeria sinuata*, RUNI: *Remeria uniseriata*, TLIT: *Tryblionella littoralis*.

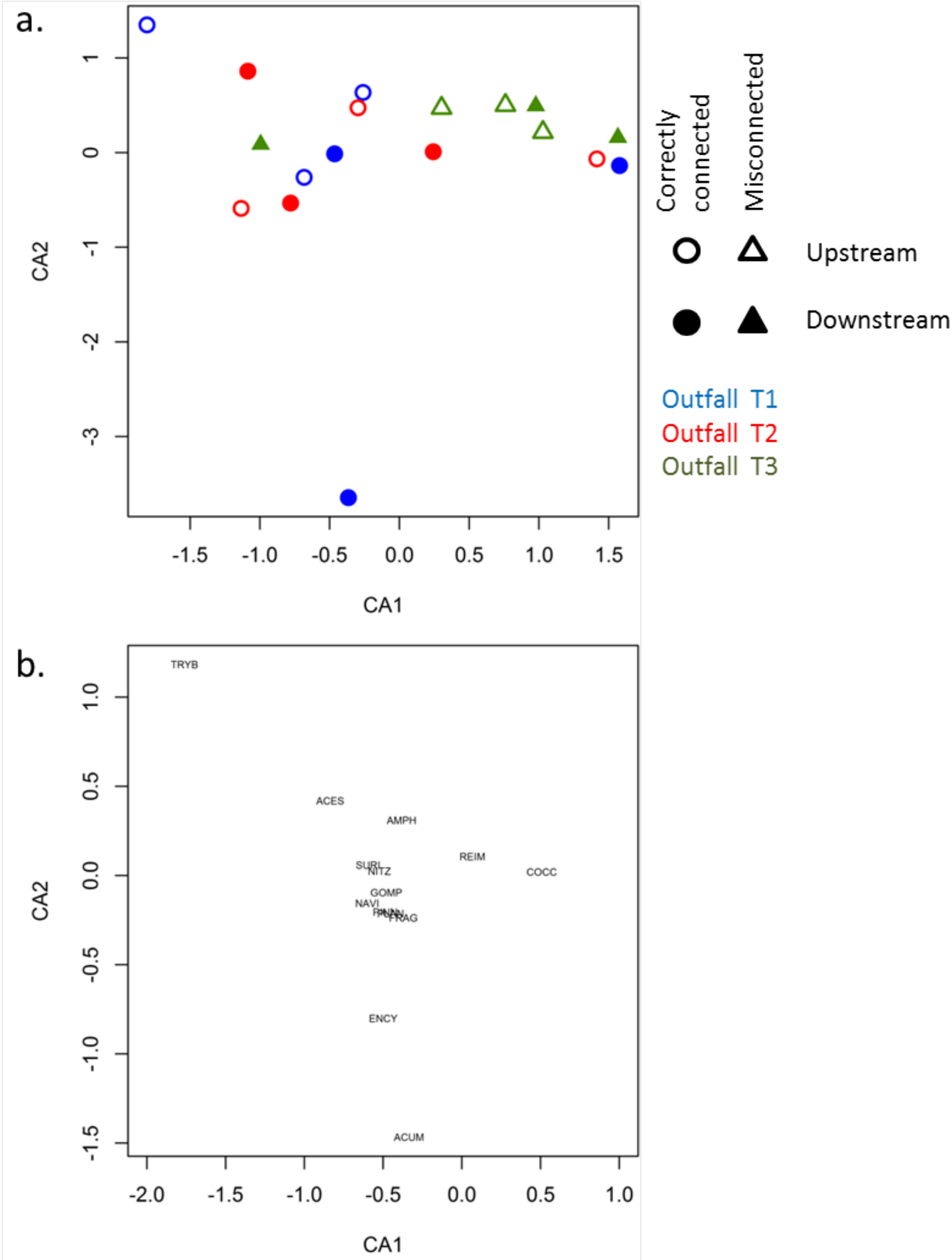

Figure 5.13: Correspondence analysis of genus assemblage at Totley Brook. Axis 1 explains 39% of the variation of the community. Axis 2 explains 26% of the variation of the community. Genus codes are - ACES: *Achnanthes*, ACUM: *Achnanthidium*, AMPH: *Amphora*, COCC: *Cocconeis*, ENCY: *Encyonema*, FRAG: *Fragilaria*, GOMP: *Gomphonema*, NAVI: *Navicula*, NITZ: *Nitzschia*, PINN: *Pinnularia*, PLAN: *Planothidium*, REIM: *Reimeria*, SURI: *Surirella*, TRYB: Tryblionella.

Totley Oldhay Brook

The first correspondence analysis axis at the Oldhay Brook in Totley showed higher relative abundance of *Cocconeis placentula var. lineata*, *var. pseudolineata*, and *var. euglypta*, low on the axis, with little definition of species higher on the axis (figure 5.14b.). Axis 2 showed high relative abundance of *Achnanthes oblongella*, and *Navicula gregaria*, low on the axis, and high relative abundance of *Amphora pediculus*, *Planothidium frequentissimum*, *Planothidium lanceolata*, and *Achnanthidium minutissimum* high on the axis (figure 5.14b.).

Outfall T5, the correctly connected outfall, showed a high variation, with samples scattered across the plot (figure 5.14a.). Outfall T4, the misconnected outfall on the Oldhay brook, showed lower variation and some clustering of upstream and downstream samples on the second axis. The upstream samples were positioned higher on the second axis, characterised by marginally higher abundance of *Cocconeis placentula var. pseudolineata* and *Navicula cryptocephala*(figure 5.14). The downstream samples positioned lower on the second axis, characterised by higher abundance of *Achnanthes oblongella* (figure 5.14).

At the genus level, axis 1 was characterised by high relative abundance of *Cocconeis* low on the axis with little separation of other species high on the axis (figure 5.15b.). The second axis was characterised by high relative abundance of *Achnanthes* low on the axis, and high abundance of *Amphora*, *Achnanthidium*, and *Planothidium* high on the axis (figure 5.15b.).

The samples showed a similar response to those at the species level, with outfall T5 showing a high variation between samples, and outfall T4 showing a minor separation on the second axis (figure 5.15a.), driven by higher abundance of *Achnanthidium*, *Amphora*, and *Planothidium* in the upstream samples, and higher abundance of *Achnanthes* downstream of the outfall (figure 5.15).

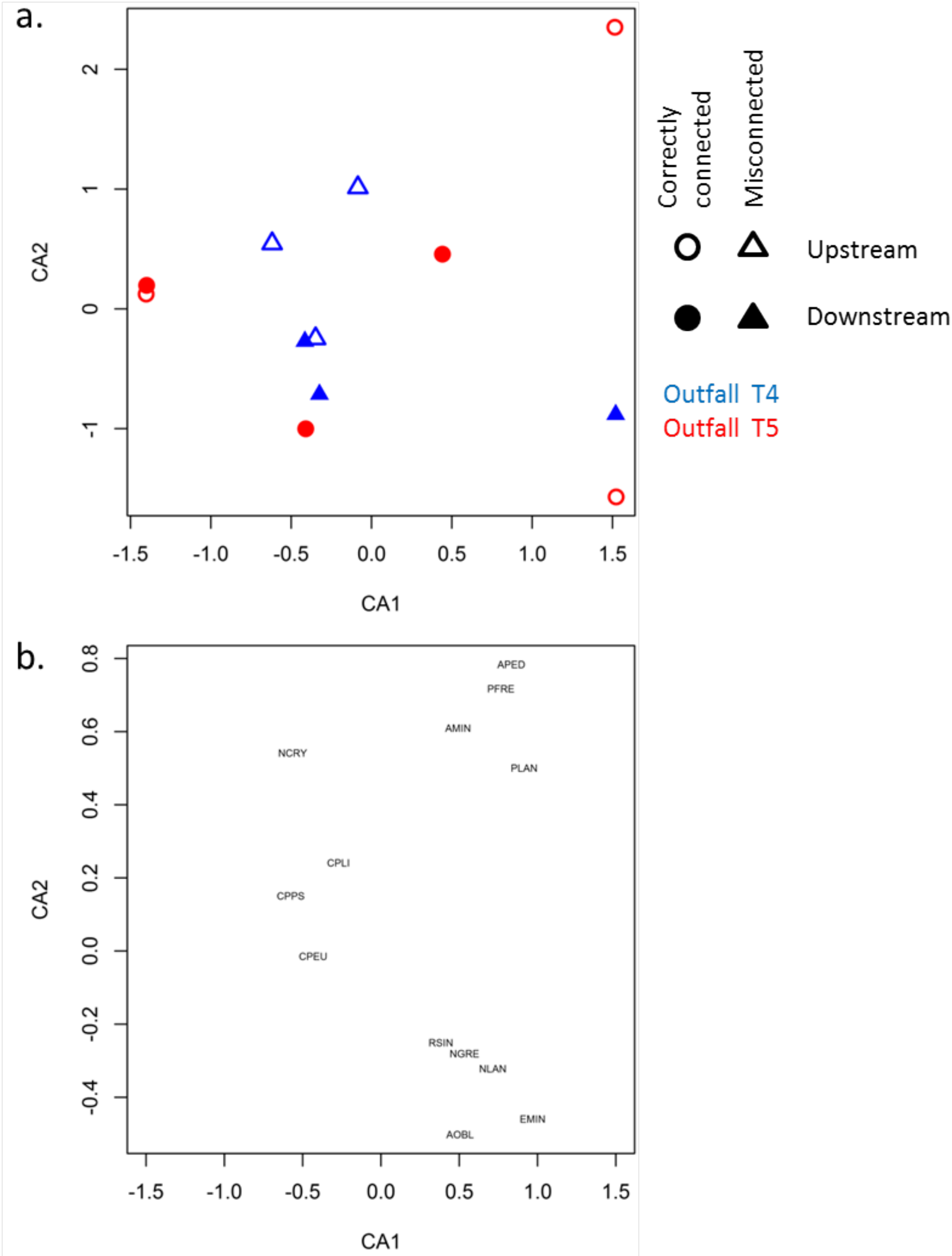

Figure 5.14: Correspondence analysis of species assemblage at Totley Oldhay brook. Axis 1 explains 44% of the variation of the community. Axis 2 explains 24% of the variation of the community. Species codes are - AOBL: *Achnanthes oblongella*, AMIN: *Achnanthidium minutissimum*, APED: *Amphora pediculus*, CPEU: *Cocconeis placentula var. euglypta*, CPLI: *Cocconeis placentula var. lineata*, CPPS: *Cocconeis placentula var. pseudolineata*, EMIN: *Encyonema minutum*, NCRY: *Navicula cryptocephala* NGRE: *Navicula gregaria*, NLAN: *Navicula lanceolata*, PFRE: *Planothidium frequentissimum*, PLAN: *Planothidium lanceolata*, RSIN: *Remeria sinuata*.

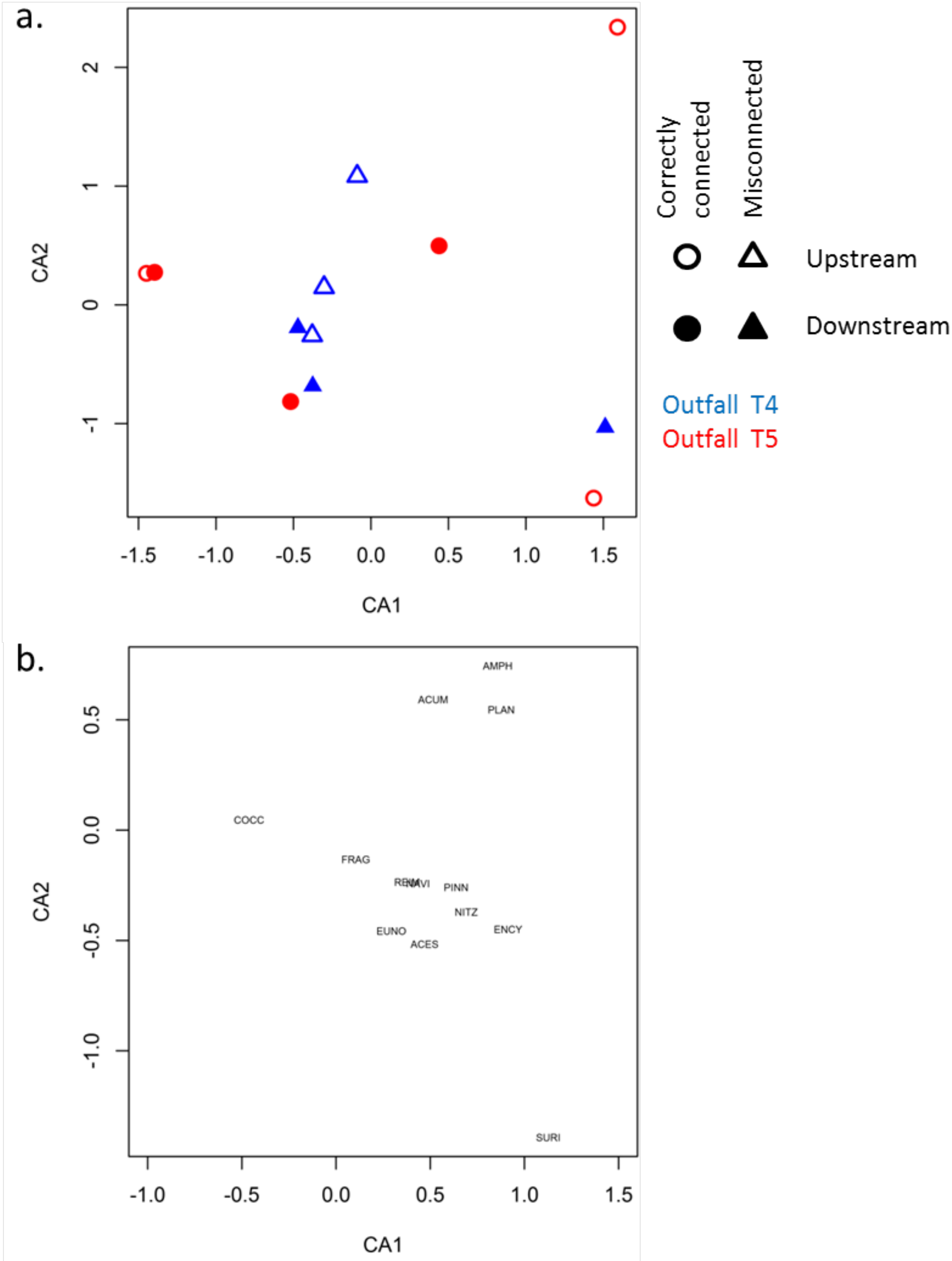

Figure 5.15: analysis of genus assemblage at Totley Oldhay brook. Axis 1 explains 51% of the variation of the community. Axis 2 explains 26% of the variation of the community. Genus codes are - ACES: *Achnanthes*, ACUM: *Achnanthidium*, AMPH: *Amphora*, COCC: *Cocconeis*, ENCY: *Encyonema*, EUNO: *Eunotia*, FRAG: *Fragilaria*, NAVI: *Navicula*, NITZ: *Nitzschia*, PINN: *Pinnularia*, PLAN: *Planothidium*, REIM: *Reimeria*, SURI: *Surirella*.

Chapeltown

In Chapeltown the first correspondence analysis axis showed a gradient primarily separating *Cocconeis placentula var. euglypta* and *C. placentula var. lineata* high on the axis, from the other species low on the axis (figure 5.16b.). The second axis was characterised by higher relative abundance of *Rhoicosphenia abbreviata*, *Amphora pediculus*, and *Navicula lanceolata* high on the axis, and *Navicula minima*, *Planothidium lanceolata*, *Planothidium frequentissimum* and *Nitzschia inconspicua* low on the axis (figure 5.16b.).

The first axis did little to describe any patterns within the sites. The upstream and downstream samples for the correctly connected outfalls, all of which were in the upper catchment, broadly separated on the second axis (figure 5.16a.). The upstream samples occurred lower on the second axis, indicating higher abundances of *Achnanthes oblongella*, and a large number of rare species (figure 5.16). Downstream samples were less tightly clustered, but were largely driven by increasing abundance of *Amphora pediculus* and a number of rare species (figure 5.16). Misconnected outfalls did not show strong trends in community composition (figure 5.16a.).

At the genus level in Chapeltown, the first axis showed a strong gradient of *Cocconeis* low on the axis, and the other species higher on the axis (figure 5.17b.). Axis 2 showed a strong gradient of increasing relative abundance of *Amphora* and *Rhoicosphenia* low on the axis. The higher end of the axis showed a strong influence of very rare genera such as, *Luticola*, which were found at low abundance in a small number of samples. More abundant genera such as *Planothidium* and *Nitzschia* occurred relatively near the origin, indicating that separation on the second axis was largely driven by the presence or absence of *Amphora* and *Rhoicosphenia* (figure 5.17b.).

Genera showed a similar pattern of sites to that produced at the species level analysis, with little separation on the first axis, and a less pronounced separation of the upstream and downstream sites of the correctly connected outfalls on the second axis (figure 5.17a.). This separation was driven by increasing abundance of *Amphora* and *Rhoicosphenia* downstream. Misconnected sites did not show a strong separation (figure 5.17a.).

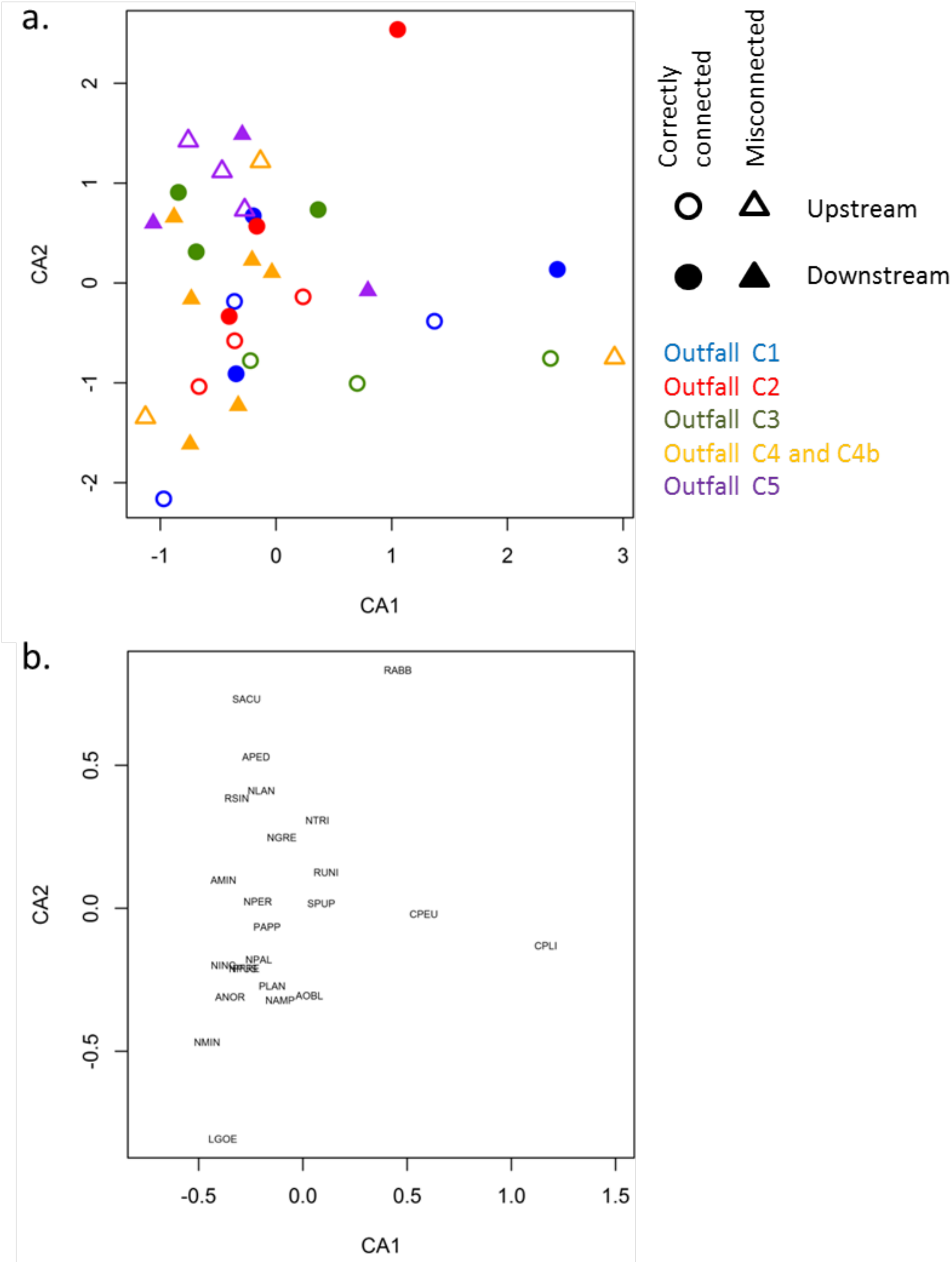

Figure 5.16: Correspondence analysis of species assemblage at Chapeltown. Axis 1 explains 26% of the variation of the community. Axis 2 explains 15% of the variation of the community. Species codes are - AOBL: *Achnanthes oblongella*, AMIN: *Achnanthidium minutissimum*, ANOR: *Amphora normanii*, APED: *Amphora pediculus*, CPEU: *Cocconeis placentula var. euglypta*, CPLI: *Cocconeis placentula var. lineata*, LGOE: *Luticola goeppertiana*, NGRE: *Navicula gregaria*, NLAN: *Navicula lanceolata*, NMIN: *Navicula minima*, NTRI: *Navicula tripunctata*, NAMP: *Navicula amphibia*, NINC: *Nitzschia inconspicua*, NPAL: *Nitzschia palea*, NPUS: *Nitzschia pusilla*, NPER: *Nitzschia perminuta*, PAPP: *Pinnularia appendiculata*, PFRE: *Planothidium frequentissimum*, PLAN: *Planothidium lanceolata*, RSIN: *Remeria sinuata*, RUNI: *Reimeria uniseriata*, RABB: *Rhoicosphenia abbreviata*, SPUP: *Sellaphora pupula*, SACU: *Synedra acus*.

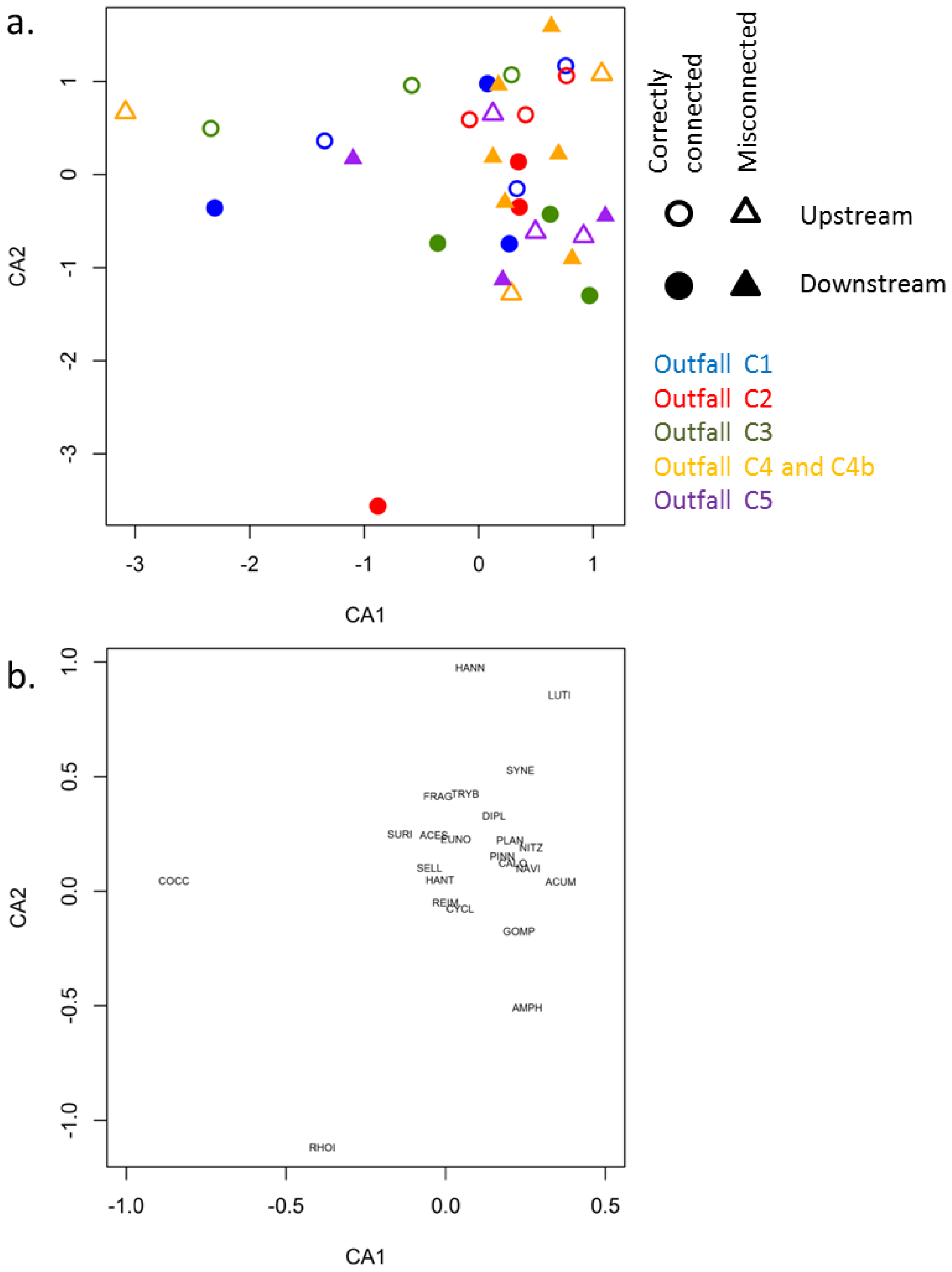

Figure 5.17: Correspondence analysis of genus assemblage at Chapeltown. Axis 1 explains 32% of the variation of the community. Axis 2 explains 18% of the variation of the community. Genus codes are - ACES: *Achnanthes*, ACUM: *Achnanthidium*, AMPH: *Amphora*, CALO: *Caloneis*, COCC: *Cocconeis*, CYCL: *Cyclotella*, DIPL: *Diploneis*, EUNO: *Eunotia*, FRAG: *Fragilaria*, GOMP: *Gomphonema*, HANN: *Hannea*, HANT: *Hantzschia*, LUTI: *Luticola*, NAVI: *Navicula*, NITZ: *Nitzschia*, PINN: *Pinnularia*, PLAN: *Planothidium*, REIM: *Reimeria*, RHOI: *Rhoicosphenia*, SELL: *Sellaphora*, SURI: *Surirella*, SYNE: *Synedra*, TRYB: Tryblionella.

5.4 Discussion

Investigation of diatom communities above and below 15 surface water sewer outfalls across three urban catchments in Sheffield revealed limited and variable responses to sewer misconnections. No response was observed in diversity and abundance measures, and the Trophic Diatom Index and percentage motile taxa also showed little response. Community composition was highly variable within sites in all catchments. Separation of upstream and downstream sites was observed across correctly connected outfalls in the Chapeltown catchment, and misconnected outfalls in Parson Cross and the Oldhay Brook in Totley. Genus level investigation showed very similar responses to the species level analyses, and helped to strengthen responses observed in the Parson Cross catchment, indicating that genus level analysis may be appropriate for misconnection investigation. Potential indicators of misconnected effluents, identified in Chapter 4, showed little response to misconnected outfalls.

5.4.1 Response of common monitoring methods to misconnections

The first aim of this investigation was to determine whether common monitoring methods, including diversity indices, chlorophyll *a* content and the Trophic Diatom Index (TDI), could be used to indicate impacts of misconnected sewer systems.

Diversity indices showed no significant changes between upstream and downstream samples at any outfalls, correctly connected or misconnected. This confirms the findings of Chapter 4, as well as observations made by Blanco *et al.* (2012), that diversity measures are not robust indicators of environmental change in diatom communities. However, some studies have observed marked increases in community diversity with pollution levels (Stevenson, 1984; Lavoie *et al.*, 2008), attributed to redundancy of multiple dominant species in the community, rather than communities dominated by relatively few species, changes in which would drastically affect the community diversity. Juttner *et al.* (2003) found that diversity increased with concentrations of nitrate, sulphate and potassium, but decreased with concentrations of aluminium, iron, surfactants and phenols. This indicates that diversity measures are not robust measures in diatom communities, and if responses are observed, these need to be considered carefully when multiple stressors may be acting upon them.

Chlorophyll *a*, a measure of total algal abundance, showed no significant changes between upstream and downstream sites at individual outfalls. This was unexpected as the presence of detergents, a pollutant which is expected to frequently be present in misconnection effluents (UKWIR, 2012), was found to have strong negative effects on total algal abundance in Chapter 4, even at low concentrations. Total algal abundance also responds to changes in nutrient concentrations (Foster *et al.*, 1997), suspended sediment (Jones *et al.*, 2014), and grazer intensity (Hillebrand & Kahlert, 2001), all of which could be affected by sewer misconnection discharges. Over the distances between upstream and downstream sites used in this study, background water chemistry would not be expected to change significantly, and therefore only substances discharged from the outfalls should have affected algal abundance. This shows that discharges from the sewers did not affect abundance immediately downstream of the outfalls.

The Trophic Diatom Index (TDI), a measure of trophic status in freshwaters, showed little response to the presence of sewer outfalls, either correctly connected or misconnected. It is recommended that multiple samples be taken through multiple years to gain a well rounded indication of the trophic status of rivers (Kelly, 2006), and one sample date is not recommended. However, given the nature of this investigation, one date when impacts of misconnections would be expected to be at their strongest was deemed adequate as an indication of the response of the TDI to misconnection effluents. The TDI is designed to respond to nutrient levels in streams and rivers, and therefore a lack of response does not necessarily show an absence of effect in the diatom community, but simply a lack of response to nutrient pollution from these outfalls. Nutrient pollution is expected to be a regular component of sewer misconnections (Chapter 2). The fact that no clear response was observed in the TDI, suggests that individually the studied sewer outfalls do not contribute a large quantity of nutrients to the receiving waters. Nutrients are likely to be dissolved in the discharge, and therefore there could be an accumulation effect further downstream. This study suggests that the nutrients discharged from misconnected sewer outfalls are not at sufficiently high concentration to affect the TDI, and therefore the TDI is not a strong indicator of misconnection effluents.

The percentage motile taxa, a measure which is used alongside the TDI to indicate responses which could be explained by factors other than nutrients (Kelly, 2006), showed no response in the Chapeltown and Totley catchments. In Parson Cross, percentage motile taxa increased downstream of each of the misconnected outfalls, and decreased downstream of the correctly connected outfall, though non-significantly. Motile taxa tend to favour conditions with low light levels or regular inundation with sediment, as they can freely move higher in the biofilm to ensure optimum light conditions, where non-motile taxa cannot (Hay *et al.*, 1993; Dickman *et al.*, 2005). This indicates that factors such as colmation of the stream bed may be a problem downstream of these outfalls. Colmation tends to occur where sediments are discharged into a river, and deposited across the river bed, leading to a relatively homogeneous sediment based habitat (Descloux *et al.*, 2014), this will benefit species which are motile as they can ensure maximum exposure to light within the biofilm (Kelly, 2006). This suggests that low levels of sediment may have been discharged from the misconnected outfalls in Parson Cross, leading to a non-significant shift in the percentage of motile taxa.

5.4.2 Response of diatom communities to misconnection effluents

In all catchments, the first correspondence analysis axis separated samples which contained high abundance of *Cocconeis* species from those containing lower abundance of *Cocconeis*, though this effect was subtle in Parson Cross. *Cocconeis* species can dominate biofilms in the summer months (Kelly *et al.*, 2005), and their high abundance in some samples may have been an effect of this. However, changes in the abundance of *Cocconeis* species generally were not consistent between upstream and downstream samples in a given catchment.

No response was observed in diatom community composition at correctly connected outfalls in any catchments apart from a minor effect in Chapeltown. This was not unexpected, given the low rainfall over the preceding month, these outfalls are expected to have not discharged a large amount of run-off, and therefore there should not have had strong impact downstream of the outfalls. This therefore suggests that the presence of a sewer system did not inherently impact the diatom community, and therefore any impacts observed in the diatom communities at misconnected outfalls are likely to be due to misconnections on those sewer systems.

In Parson Cross, while species analysis failed to separate upstream and downstream sites at most outfalls, genus level analysis showed a strong separation of samples at the misconnected outfalls. Upstream samples contained higher relative abundance of *Amphora*, *Cocconeis*, and *Rhoicosphenia*, genera which are largely composed of pollution sensitive species (Kelly *et al.*, 2005). The downstream samples contained higher relative abundance of *Nitzschia*, *Navicula*, and *Planothidium*, genera which are broadly pollution tolerant (Kelly *et al.*, 2005). This strongly suggests that pollutants from these misconnected outfalls caused changes in diatom community structure downstream of the outfalls. However, with the exception of outfall P4, this effect was only observed at the genus level, not the species level, indicating that variation in the species data masked the higher level responses. Kelly *et al.* (2009) found a high similarity in response of a species based diatom pollution index (the Indice de Polluosensibilite, IPS), and a genus based index (the Indice Biologique Diatomique, IDG) used on the same data set, stating that key traits such as motility, and physiological characteristics may be associated with the genus level, rather than species level. *Nitzschia* and *Navicula* are largely composed of motile taxa, and their abundance downstream of these outfalls suggests that particulate organic matter discharged from these outfalls may have impacted the downstream communities.

The fact that the upstream samples at each outfall in Parson Cross had similar community compositions indicates that the impact of misconnected outfalls did not persist far downstream of each outfall, and so community recovery could take place. A relatively short spatial impact in the river tends to indicate particulate organic matter which can settle out of the water (Kelly, 2006). This further reinforces the conclusion that misconnected sewers were discharging organic matter at Parson Cross.

Outfalls P1 and P4 served the largest number of properties in the study (600 and 450 respectively). It is therefore expected that there would be a larger effect of pollutants associated with surface water run-off in communities downstream of these outfalls than at smaller systems. However, there is also expected to be a higher number of misconnections on larger systems, as there are more properties which may have misconnected appliances. Though sampling took place following a relatively dry period to minimise the effect of run-off, there was still some precipitation, and therefore surface run-off was discharged prior to sampling. This could lead to discharge of suspended sediments washed off surfaces. It is therefore difficult to separate the effects of polluted surface water run-off in large catchments from those of misconnection discharges in this instance.

The Oldhay Brook in Totley showed a similar response to that of Parson Cross, with samples downstream of the misconnected outfall, T4, showing a shift in community composition from upstream, but the correctly connected outfall samples showed no response. The major separation of the upstream and downstream samples was determined by substantially higher abundance downstream of *Achnanthes oblongella*. *A. oblongella* is sensitive to nutrients and organic pollutants (Kelly *et al.*, 2005) and is not a motile species (Kelly, 2006), and therefore its increased abundance downstream of outfall T4 indicates that these were not a major component of the discharge from the sewer system. *A. oblongella* showed a strong response to detergent effluents in Chapter 4, and this unexpected increase in abundance could indicate the presence of detergents at this outfall. However, a shift in the abundance of a single species at a single outfall does not provide a strong indication of the cause of that shift.

In Chapeltown a weak separation was observed in the correctly connected outfalls. The upstream samples contained higher relative abundance of *Achnanthes oblongella*, while the downstream samples were characterised by higher abundance of *Amphora pediculus*, though both were also associated with small changes in large numbers of rare species. Both *A. oblongella* and *A. pediculus* are sensitive to organic and nutrient pollution (Kelly *et al.*, 2005). Surface water outfalls, even when not misconnected, are expected to intermittently discharge a range of pollutants including metals, nutrients, poly-aromatic hydrocarbons (PAHs), and organic compounds, which may lead to increased toxicity and chemical and biochemical oxygen demand (COD and BOD respectively) (Ellis & Revitt, 2008). It is therefore hypothesised that if surface water outfalls affect diatom communities, the components of discharge should select for more tolerant species. Invertebrate and diatom communities have been found to show marked responses to pollutants found in discharge from surface water outfalls (Newall & Walsh, 2005; Gold *et al.*, 2003b; Maltby *et al.*, 1995b,a). However, Ivorra *et al.* (2002) found that while exposure to excess phosphorus caused an increase in the value of the TDI, exposure to phosphorus, zinc, and cadmium simultaneously had the opposite effect. Similarly, Rotter *et al.* (2013) found that prometryn, a herbicide, negatively affected diatom communities, but nutrients and salinity worked antagonistically with prometryn to reduce the impact in the community. This shows not only that multiple pollutants can have synergistic effects on the biota, but they can also have opposing impacts, leading to a reduction in the observed effect of either pollutant. The change in composition observed in Chapeltown was subtle, with few species showing strong responses to sewer outfalls, and did not show increased abundance of pollution tolerant species downstream, indicating that pollutants carried in run-off were not strongly affecting these communities.

No clear response was observed in the misconnected outfalls in Chapeltown. Though outfall C4 had a relatively small sewer system (40 properties), and the sewer catchment of outfall C4b was not mapped, outfall C5 had a particularly large sewer system (250 properties) and polluted discharge was observed at the outfall. Though there are limitations in comparing sewer systems between catchments due to different water chemistry, the lack of response downstream of outfall C5 shows that a sewer catchment being large does not inherently lead to observable impacts in the river. Therefore factors other than large quantities of run-off may have been important in the impacts observed in Parson Cross and the Oldhay Brook in Totley. Misconnection frequency and severity is likely to be a substantial aspect of this variation in response on these systems where misconnections are known to occur.

A similar lack of response was observed in the Totley Brook, where there was a high natural variation in community composition at each site. The lack of an observed effect at the misconnected outfall in the Totley Brook may be due to it being a small sewer system of around 20 properties, and therefore a potentially small number of misconnections, leading to an infrequent or low concentration discharge to the river.

Urban and rural run-off can carry significant pollutants to streams (Brombach *et al.*, 2005; Newall & Walsh, 2005; Maltby, 1995; Maltby *et al.*, 1995b,a; Stoate *et al.*, 2001), which has the effect of removing sensitive species. Biological monitoring and investigations are based on the theory that particular diatom species have different tolerances to particular pollutants (Pan *et al.*, 1996), and therefore the presence of those pollutants will reduce the abundance of sensitive species, allowing tolerant species to dominate the community. However, if the water course is sufficiently polluted upstream of the investigated sites, sensitive species may be unable to survive, and therefore the impact of further pollution may go unnoticed. Kelly & Wilson (2004) investigated the installation of nutrient removal on a sewage treatment works effluent, but found that the receiving water was sufficiently polluted that improvements in the diatom community could not be identified. They concluded that other inputs higher up the catchment would need to be reduced before a benefit of the nutrient removal could be observed. This may be the case in the Totley Brook and Chapeltown catchments if the misconnections in those catchments are relatively minor discharges. The upper catchments of both Chapeltown and Totley are largely pasture land, which can contribute significantly to nutrient levels (Hooda *et al.*, 2000), however high nutrient levels were not observed in the water chemistry data from this study, so this explanation is unlikely. A more likely possibility is that the concentration and frequency of discharge at the outfalls in these catchments were simply too low to have significant impacts in the diatom communities.

5.4.3 Response of potential indicator species

In Chapter 2, high abundances of *Achnanthes oblongella*, and *Cocconeis placentula* at the species level, as well as *Nitzschia* at the genus level, were shown to have potential as indicators of the presence of detergent pollution in diatom communities. These species were present in the majority of samples in this study, though *Achnanthes oblongella* was generally not present in Parson Cross, and at extremely low abundance in the Totley Brook. In the few cases where misconnections could be validated in this study area, they were found to be sinks and soil stacks (Chapter 3), and therefore soap and detergent discharges could be expected.

In Parson Cross, *Nitzschia* were indicative of the downstream samples of the misconnected outfalls, though they were present at relatively low abundances. *Cocconeis* showed the opposite trend, with high abundances being more closely linked to the upstream samples for those outfalls. *Nitzschia* are broadly considered tolerant of pollution (Kelly *et al.*, 2005), and so are not strong indicators of detergents specifically, however, their increased abundance does indicate the presence of water pollution. This may be a response to increased water turbidity as discussed earlier, as *Nitzschia* are broadly motile, while *Cocconeis* are not (Kelly, 2006). At the species level, the only major response of potential indicators observed at Parson Cross was at site P4, where the downstream samples showed a small increase in *Navicula lanceolata*, but little response in the other indicator species. These responses were not sufficiently strong to draw major conclusions from.

In the Oldhay Brook at Totley, downstream samples at outfall T4, the misconnected outfall of the catchment, were characterised by higher abundance of *Achnanthes oblongella* and *Navicula lanceolata*, at the species level, and *Nitzschia* at the genus level, though abundance of *Cocconeis* showed no response to the outfall. While *N. lanceolata* was present at low relative abundance in these samples, *A. oblongella* showed a strong increase downstream of the outfall. This suggests that this outfall may have discharged detergents, as *A. oblongella* is sensitive to nutrients and organic pollutants, which are associated with broader pollution.

The general lack of response in the indicator species identified in Chapter 4 at misconnected outfalls, suggests that in the field, factors other than detergents can overwhelm indicative responses, or these species are not strong indicators of detergent pollution in the field. Given the size of the sewer catchments present in this study, and the rough estimate of 2% of properties containing a misconnection (DEFRA, 2009; UKWIR, 2012), it is estimated that between one and twelve misconnections were present in the misconnected systems of this study, though this is a coarse estimation. Among those misconnections which were present, some may not have discharged detergents, some may have been extremely infrequent, and some may have discharged extremely low concentrations of pollutants, so it is not surprising that a strong response to detergent pollution was not observed in the community structure of all outfalls. In addition to this, laboratory based studies tend to show stronger responses than those in the field, due to them being simplified systems (Morin *et al.*, 2008). In the field there is significantly less control, and therefore natural variation in the pollutants, diatom communities, and abiotic conditions such as light levels are more likely to mask effects. This study demonstrates that at the majority of misconnected sewer outfalls, the species identified as possible indicators in Chapter 4 are not useful indicators of misconnection discharge. This suggests that the potential pollutants discharged from surface water outfalls are too diverse to identify indicators purely for misconnection discharge.

5.5 Conclusion

This study presented the first investigation into the ecological impacts of sewer misconnections, using diatom communities. It also provided initial investigation into the applicability of potential indicator species of common misconnection components in the field.

Shannon diversity, species richness, species evenness, total algal abundance and the Trophic Diatom Index showed no response to sewer outfalls, either correctly connected or misconnected in any of the test catchments. This showed that using standard monitoring metrics, sewer misconnections would not be observed in receiving waters. Diatom community composition showed shifts toward more pollution tolerant and motile taxa downstream of misconnected outfalls in one catchment, however no response was observed at half of the misconnected outfalls studied, and there was considerable difficulty in separating impacts caused by misconnection effluents from those caused by the effects associated with large sewer systems, such as discharge of sediments. In the few cases where communities did appear to be affected by sewer outfalls, recovery took place a short distance downstream. There was also no accumulation effect of pollutants downstream in the catchments. This study therefore suggests that misconnected sewer outfalls, at least at the density or volume of discharge of the outfalls used in this study, do not pose a major threat to diatom communities in small streams, though localised impacts can occur.

There was little benefit in identifying diatoms to species level, and in fact impacts in the community were more easily identified at the genus level, possibly due to reduced noise in the data set. Potential indicator species, identified in Chapter 4 did not show characteristic community shifts downstream of misconnected outfalls in the majority of cases. Misconnections are expected to discharge a wide range of pollutants, and this may compromise the effectiveness of indicator species used for this purpose. This, however, makes it exceedingly difficult to differentiate the impacts of sewer misconnections from those of surface water run-off, and therefore it may be necessary to perform a detailed characterisation of sewer systems and their associated misconnections before robust investigation into the impact of sewer misconnection effluents can be performed in the field.

Chapter 6

Discussion and conclusions

6.1 Introduction

Sewer misconnections are the connection of domestic appliances to surface water sewers. They are generally perceived to be a threat to water quality and ecosystem functioning, as they discharge untreated sewage directly to receiving waters as a source of diffuse urban water pollution (UKWIR, 2012). The majority of misconnected appliances are expected to be washing machines and sinks, which would theoretically lead to discharge of soaps and detergents in misconnection effluents (Chapter 2). Though the quantity of pollutants discharged from domestic appliances is highly variable, the volume of untreated wastewater discharged from misconnections can be substantial, even at misconnection rates of 3% (Ellis, 2013), and can therefore lead to substantial discharge of nutrients and organic pollutants (Butler *et al.*, 1995). However, the magnitude of the national misconnection problem is currently unknown, with estimates ranging from 0.6% of properties (DEFRA, 2009), up to 20% of properties (Environment Agency, 2007) across England and Wales. Misconnections may therefore contribute to failing of water quality objectives for the Water Framework Directive (WFD), Shellfish Waters Directive, and Bathing Waters Directive (UKWIR, 2012). This has led to pressure to correct misconnections in order to reduce these perceived impacts. However, the extent to which misconnections contribute to impacts in receiving waters has received very little study to date.

Misconnections can lead to substantial aesthetic impacts in receiving waters, including visual impacts such as sewage fungus (figure 6.1), and odours. In these cases it is clear that misconnection effluents have a strong impact in the receiving water, and local residents can identify misconnection effluents as a threat to surface water quality, and can identify the impacts of misconnections in receiving waters (Faulkner *et al.*, 2001). These are often the reason for reporting and correction of misconnections, as current detection methods are inefficient or expensive, so are rarely used pro-actively. However, many aesthetic indicators of pollution only become apparent once misconnection pollution has become extremely severe. Misconnections are likely to contribute to urban diffuse pollution loads at lower concentrations, which are unlikely to cause these clear aesthetic problems, and therefore are more difficult to identify. The ecological impacts of these smaller inputs are unknown, and this thesis set out to investigate their impacts in the diatom communities of receiving waters.

Figure 6.1: A surface water outfall showing significant aesthetic indicators of misconnection pollution, Tongue gutter, Parson Cross, Sheffield.

The chemical constituents of misconnection effluents are expected to impact natural communities, as has been observed from sources other than misconnections (Chapter 2). As biological communities are important indicators of surface water quality, determining the impacts which misconnections have on these organisms is crucial for determining the threat of misconnections to WFD objectives. Invertebrate community structure has previously been found to deteriorate downstream of a particularly badly misconnected outfall on the Pymmes Brook in London (Faulkner *et al.*, 2000), and biological methods were recommended as a cost effective alternative to constant water chemistry monitoring. However, no other biological investigations into the impacts of misconnections have been performed to date, and the example on the Pymmes Brook was particularly severely polluted. Despite this lack of empirical data, it is generally believed that misconnections cause sufficiently strong impacts in freshwater ecosystems that their correction should be a priority in reducing the impact of urban diffuse pollution.

Diatom communities were identified as ideal organisms with which to investigate the impact of sewer misconnections (Chapter 1). Diatoms are often used as indicators of nutrient impacts as well as other chemical inputs in streams and rivers due to their many benefits as biological monitoring tools (Round *et al.*, 1990). Community composition gives a more specific indication of the response of the diatom community to inputs, providing greater ecological detail. Diatoms are generally identified to species level in order to gain maximum information from each sample, and because species can show variable responses to different stressors (Kelly & Whitton, 1995). Therefore from the species level responses, relatively detailed information on the conditions in which that community has grown can be determined. The drawback of this however, is that identification to species level can take a long time, and requires a high level of training. While identifying to genus level does not provide such a high resolution of data, it is significantly easier, and requires much less time, therefore allowing fast, low resolution, identification.

This thesis set out to investigate the impact of misconnection effluents in diatom communities, and to develop methods to identify misconnection effluents discharged into surface water sewer systems. Peer-reviewed and grey literature on the subject of misconnections were reviewed, and key objectives for better understanding the impacts of misconnections in the UK were identified. Two of these - improving methods to identify misconnection effluents, and investigating ecological impacts of misconnections - were then further investigated. A method to identify the presence of misconnection effluents in surface water sewer systems was developed and tested providing many benefits over other current monitoring methods. The response of field sourced diatom communities to artificial laundry detergent effluents was investigated using a laboratory based microcosm study in which significant impacts were observed. Finally the impact of misconnected sewer systems on diatom communities in the field was investigated in multiple catchments in the Sheffield area showing a general lack of response.

6.2 Contributions and implications of findings

6.2.1 Misconnection impacts

Diatoms were used to indicate the response of biological communities to laundry detergent effluents and effluents from misconnected sewer systems in Chapters 4 and 5. Chapter 4 showed that diatom communities give relatively strong responses to dilute laundry detergent effluents, particularly at relatively high concentrations. In the field study detailed in Chapter 5, no such response was observed, suggesting that not only is the contribution of detergent effluents to impacts in receiving waters relatively small, but also misconnections generally showed very little significant impact in the diatom communities of these streams.

Diversity and trophic diatom index

Diversity measures did not show strong responses to either the microcosm experiment (Chapter 4) or the field study (Chapter 5). Diversity, species richness, and species evenness are often used as broad descriptors of biological communities (Stevenson, 2014), however the lack of response observed here indicates that these are not strong measures in diatom communities, even when other measures such as total abundance and community composition are affected. A lack of response in diatom diversity measures has been observed in previous studies of different stressors (Blanco *et al.*, 2012; Hirst *et al.*, 2002), however some studies have found diversity to be a useful measure in diatom communities (Birkett & Gardiner, 2005; Ferreira da Silva *et al.*, 2009; Lavoie *et al.*, 2008). These studies often occur over larger spatial scales than the present study, comparing different catchments or full river lengths, over which water chemistry could be expected to differ to a greater extent, and therefore change in diversity could be expected. However, in Chapter 4, a range of detergent concentrations were used, which could be expected to have strong impacts in the diversity, especially as shifts were observed in total abundance and community composition. This may be explained by a high redundancy of species in the community. As diversity measures do not account for changes in abundance of specific species, but characterise the whole community, the observed increase in dominance of *Achnanthes oblongella* and *Cocconeis placentula* in Chapter 4 had little influence on diversity measures. In the field, samples generally showed smaller responses than the highest concentration samples in Chapter 4, and therefore a lack of response in diversity could be expected. Similarly, communities in both studies showed no response in species richness. Species richness is a coarse measure which would only change if significant numbers of species were lost or gained in one community compared with another. The effect of detergents and total misconnection effluents in diatom communities appears to be related to more subtle changes in species abundance, than loss and gain of whole populations. While these measures are often included in studies as general measures of community structure, the specific taxonomic responses of diatom communities were a better measure of change in the community in this thesis, and may be better suited to other diatom based investigations, as broad diversity measures were unresponsive.

Misconnections are expected to discharge high concentrations of nutrients, associated with discharges from all misconnected appliances, particularly toilets, kitchen sinks, and washing machines (Almeida *et al.*, 1999) which are expected to be major contributors to misconnection effluents (UKWIR, 2012). Increased nutrient concentrations can often lead to an increase in algal abundance, which can cause many issues in river ecosystems, associated with eutrophication (Carpenter *et al.*, 1998). In Chapter 4, nutrient concentrations were unaffected at low detergent concentrations, and actually decreased at higher detergent concentrations. Neither laundry detergent contributed significant nutrient concentrations, thus explaining the lack of an increase in nutrients, however the reduction in nutrients could not be explained by sequestering or sorption of nutrients to organic particles, or by biological removal. No literature could be found explaining this decrease in nutrient concentrations, yet it was a significant and repeatable occurrence in the nutrient solutions. This therefore remains an unexplained response, though definitely characteristic of the high detergent concentration treatments.

In the field, the Trophic Diatom Index, a measure of nutrient status, showed no major effects of nutrient pollution downstream of any of the misconnected outfalls. The Trophic diatom index is one of many diatom indices used to assess trophic status in streams and rivers. Similar indices such as the specific pollution sensitivity index (SPI) (Coste &

Ayphassorho, 1982), and biological diatom index (BDI) (Lenoir & Coste, 1996), developed across Europe, use a similar method, classifying different species based on their tolerance to nutrients and in some cases other pollutants, and using their relative abundances to determine a trophic status for the site. However the TDI is the method generally used in the UK, as it was developed here and has undergone a great deal of refinement to reach its current form (Kelly & Whitton, 1995; Kelly *et al.*, 2001, 2008). Given the relatively low background nutrient concentrations in each of the catchments, a major nutrient discharge would be expected to cause observable effects in the TDI. The fact that no such response was observed at any outfalls strongly indicates that these outfalls did not cause major addition of nutrients. However, the spatial scale of the study presented in Chapter 5 was smaller than most diatom studies, which often either cover multiple catchments, such as Juttner *et al.* (2003), or cover much larger distances on a single river reach, such as Kelly & Wilson (2004). This lack of response indicates that during routine diatom monitoring, which often relies on calculation of the TDI, discharges from sewer misconnections are unlikely to be identified, even in samples close to outfalls. The fact that no response was observed, shows that misconnections did not contribute significant nutrient concentrations to the receiving waters at an individual entry point.

Community composition and abundance

Relative abundance of *Achnanthes oblongella*, and *Cocconeis placentula* showed strong increases in increasing detergent concentrations in Chapter 4. These species are also generally sensitive to nutrient and organic pollution (Kelly *et al.*, 2005), therefore an increase in their abundance in the field would not be confused with responses to other common components associated with misconnection effluents, though conflicting responses could overwhelm effects in discharges containing high nutrient and detergent concentrations. In Chapter 5, downstream of misconnected sewer outfalls these species did not show a similar response to that shown in the high concentration samples of Chapter 4, and many of the misconnected outfalls did not cause any observable change in the diatom community. A similar response was observed in total algal abundance, which was significantly reduced by detergents in Chapter 4, yet showed no response downstream of misconnected outfalls in Chapter 5. There are three possible explanations for an effect of detergents not being observed in the field. The first is that the effects of detergents present in the discharge may have been overwhelmed by other, more influential, factors, such as background water chemistry or grazer intensity. Given the strength of response, particularly in algal abundance, observed in the laboratory, it is unlikely that the effects of detergents present in the studied streams would have been totally negated by other factors, particularly given the relatively clean nature of most of the streams studied. The second is that they may be present at sufficiently low concentrations that they did not influence the water chemistry of the stream ecosystem. However, even at extremely low concentration, detergents still caused a strong response in total algal abundance, therefore a low concentration of detergents being discharged into surface waters is unlikely to have caused no response at all. Finally the intermittent discharge of effluents may have meant that the diatom community was only exposed to detergents for short periods of time, and therefore did not adapt to the change in water conditions as they could resist short term exposure. Though the frequency of discharge could not be monitored in this study, as the other possibilities are unlikely, this leads to the conclusion that the intermittent discharge of misconnection effluents in these catchments is likely to have been too infrequent for the diatom communities to respond to the inputs.

Approximately half of the sewer catchments with indicated misconnections in Chapter 5 were relatively small, serving around 100 properties or less. Using what is generally believed to be the best estimate of 2% of properties having misconnected appliances (DEFRA, 2009), this suggests that these catchments may have contained only one or two misconnections. This may explain the lack of observed response due to infrequent and low concentration discharges to the streams. However, no response was observed at a large catchment of approximately 250 properties in Chapeltown. This catchment may simply have contained a lower than average number of misconnections, however there was a constant discharge and observed pollution at the outfall on multiple occasions, both of which suggest a large number of misconnections present on the system. The fact that no effect was observed at this outfall lends weight to the argument that the discharge of misconnections is sufficiently intermittent or diluted when it enters the river system, that no strong response to any particular pollutant is observed.

As there is no clear cause for the discrepancy between the findings of the laboratory and field based studies, consideration must be given to the design of each study. Laboratory studies allow almost complete control of all conditions, whereas in the field there are many factors which cannot be controlled, and may not be measurable, which may influence the observed response. This means that responses in the field are expected to be less pronounced than those of the laboratory, even in an ideal situation. Total algal abundance is often used as a measure of primary producer abundance in water courses because it is relatively quick and simple to calculate (Stevenson, 2014). However this thesis found that even strong responses may not be observed in the field, possibly due to the frequency of discharge and complexity of interactions in natural ecosystems. The method used in Chapter 4 did not allow intermittent exposure to detergents to be investigated, instead focussing on the concentration of detergent in each treatment. Exposure period may be a critical factor in the impact of these effluents in the field, as it will have an important effect on how the diatom communities perceive effluents. At the most extreme, a single misconnected washing machine might be expected to discharge once per week, meaning that the diatom community must only survive exposure for a few minutes per week, but would otherwise be unaffected by the discharge. Even sensitive species could be expected to tolerate these short periods of exposure, and only chemicals bound to sediment, deposited immediately downstream of the outfall, are likely to have major effects in the community. However, appliances are generally expected to discharge more frequently than this (Butler, 1993), and outfalls are likely to contain more than just one misconnection. This means that multiple discharges could be expected each day, thus leading to a more regular exposure, and therefore impacts more similar to those found in Chapter 4. However this may still not be sufficiently frequent to cause observable impacts in the receiving water. The unknown effect of the frequency of discharge may be a crucial factor in the impact of misconnections, and should be one of the key points for further investigations to consider.

Misconnected outfalls in the Parson Cross catchment caused notable changes in community composition, increasing the abundance of motile *Navicula* and *Nitzschia* species. This is often an indicator of the presence of increased organic matter content, which may be discharged from these outfalls (Kelly, 2006). Organic matter is expected to be present in many misconnection discharges as total suspended solids form a large part of the discharges from most domestic appliances (Almeida *et al.*, 1999). However organic matter can also be associated with surface run-off (Ball *et al.*, 1998; Butler & Davies, 2004), and distinguishing the contribution of each aspect to impacts in the communities was not possible, though sampling occurred after a period at which run-off should have been at a minimum. Addition of organic matter to surface waters tends to be associated with increased biological and chemical oxygen demand, and through this, decreased dissolved oxygen content. It can also lead to changes in habitat structure through colmation of the river bed (Descloux *et al.*, 2014). The impact in the diatom community did not persist downstream, indicating that the organic matter had precipitated out of the water column before the upstream site of the next outfall was reached, as has been observed from combined sewer overflows (Seidl *et al.*, 1998). Personal communication with local residents in Parson Cross and staff at Yorkshire Water suggests that the sewer systems in Parson Cross are particularly badly misconnected, with many properties suffering from misconnections. Though this is anecdotal, the optical brightener testing shown in Chapter 3 does indicate that large areas of the sewer systems contain misconnections. This may be the reason for impacts being observed at these outfalls, however if the sewer systems are particularly badly misconnected in this catchment, and the diatom communities show only shifts in community composition, not large scale loss of species or loss of abundance, this suggests that even in severe cases, the impacts of misconnections are relatively minor. However these shifts in composition may impact the invertebrate community, as different diatom species will benefit different grazer types. Low lying motile taxa such as those found downstream of outfalls in Parson Cross tend to benefit invertebrate species with scraping mouthparts, such as snails (Tall *et al.*, 2006; Horne, 2009), and thus changes in community structure at higher trophic levels may occur, causing wider ranging impacts in the community.

In both Chapter 4 and Chapter 5, genus level analysis showed very similar responses to the species level analyses. While genera are not commonly used in diatom studies due to the perceived variability in response at the species level, these investigations showed that in fact genus level analysis was adequate to describe the major variations in the communities, agreeing with the findings of Rimet & Bouchez (2012). While many of the genera found in these studies were represented by only one or two species, which will show the same response at species level as at genus level, some, such as *Navicula* and *Nitzschia*, contained many species, and therefore using genus level taxonomic resolution would be expected to reduce the explanatory power of these abundant genera. This thesis found that in fact identifying diatoms to the genus level would be an excellent time saving method, which would not lose detail in the analysis. This particularly applies to cases where a general characterisation of the community is required, without the need for more specific species based indices.

In the few cases that misconnections affected the diatom community, the major contribution of the misconnections was a contribution to organic pollution. However, for the most part, there was no effect of misconnections observed in any measures of the diatom community. This therefore leads to the conclusion that misconnections on these upland sewer catchments are not a significant threat to the diatom communities, despite the response observed in the laboratory, most likely due to their intermittent nature.

Sewer systems

Though misconnections did not cause major ecological impact, the early identification of misconnections allows prevention of the problem before they become more severe. The results of Chapter 3 show the optical brightener method to be a highly beneficial low cost method to identify the presence of misconnection effluents in surface water sewer systems. This provides an important benefit over other detection methods, in being extremely low cost, and allowing passive sampling, which removes the need for an operative to be present to perform sampling.

Approximately 50% of the sewer outfalls investigated across four catchments were indicated as containing misconnections, generally with larger sewer systems more likely to contain misconnections than smaller systems. This corresponded well with the percentages of polluted surface water outfalls indicated in catchments in the Thames Water region in UKWIR (2012) (Chapter 2), which ranged from 33% to 70% of outfalls. Though thorough investigation of individual properties could not be performed to determine the specific number of misconnections in each system, this shows that a high proportion of surface water sewer systems across a variety of catchments suffer from misconnections, and therefore misconnections are likely to be present in the vast majority of catchments which contain separate sewer systems across the UK. This supports the view that misconnection effluents can pose a threat to surface water quality in any catchment which contains separate sewer systems.

Aesthetic indicators of misconnections were not present in large quantities at any of the investigated outfalls, though small amounts of sewage fungus and some solids associated with toilet waste were observed at a small number of outfalls. Therefore at the majority of outfalls in these studies, misconnections are unlikely to have been identified and reported by members of the public until further misconnections were introduced and aesthetic indicators developed. This highlights the major benefits of this method in pro-active searching for misconnections on systems which would otherwise be considered clear of misconnections. However, the fact that previous investigations into misconnected sewer systems have not used passive methods such as this to identify more subtle misconnection discharge, suggests that previous estimates of the number of polluted outfalls may be lower than reality. This also suggests that the catchments in which detailed misconnection investigation campaigns have been performed are likely to be biased toward particularly badly polluted sewer systems, where the presence of pollution is clear, therefore estimates extrapolated from these studies may be higher than reality.

The optical brightener method should greatly improve identification of polluted sewer systems, therefore allowing less severely polluted sewer systems containing misconnections to be identified and investigated. This should lead to more accurate estimates of the scale of the misconnection problem across the country, as sewer systems suffering less severely from misconnections can be investigated. This also greatly improves the ease with which misconnected sewer systems can be pro-actively investigated, meaning that there is much better potential for active rectification campaigns, rather than the current approach of piecemeal reactive correction, which will inherently fail to keep pace with the misconnections being introduced to separate sewer systems. This is a big step toward being able to tackle the misconnection problem, making it cost effective and not requiring complex technologies to find misconnections. However the correction of misconnections is still heavily influenced by limitations in the correction process. It can be a long and complex path from identification of misconnections, to correction, requiring local sewerage providers, local authorities, Environment Agency, and the householders, to co-operate quickly in order to ensure correction can be performed within six months of identification, as is required by legislation (UKWIR, 2012). This is often extremely difficult to achieve, as the individual groups do not have the same driving aims. For example, local authorities are not inherently required to correct misconnections, however if householders refuse to correct misconnections, the local authority is the group which has the power to enforce misconnection correction. This means that local sewerage providers must ask local authorities to enforce correction, however this is not high on the list of priorities for the local authorities. This is the next area which requires focus in order to allow correction of misconnections more easily. This may take the form of improving the interactions of the different groups to ensure communications and interactions are as swift as possible. Alternatively, this could require changes in the powers of each group, logically giving the sewerage provider greater power to enforce correction upon the householder.

6.3 Further work and thesis conclusions

While the investigations into detection of misconnections in Chapter 3, and the response of diatoms to laundry detergent effluents presented in Chapter 4 were relatively conclusive, the field investigation in Chapter 5 has generated a series of further areas for development.

At an early stage in this project it was decided to use diatoms to investigate ecological impacts in surface waters due to their many benefits in responses to expected pollutants from misconnections (Chapter 1). The response observed in the field however indicates that diatoms may not be strong indicators and the impacts of misconnections in receiving waters are relatively generic, therefore the use of other organism groups to investigate impacts may show further effects. Macroinvertebrates are another key group of organisms which are commonly used for monitoring and investigating ecological impacts in surface waters (Malmqvist, 2002; Holt & Miller, 2011). Invertebrates fulfil many niches within freshwater ecosystems including grazers, detritivores, predators, and filter feeders, and therefore they can be used to investigate impacts at different trophic levels in the ecosystem (Bonada *et al.*, 2006). Though invertebrates tend to be more mobile than diatoms, communities upstream and downstream of outfalls should still reflect impacts from misconnections. Invertebrate communities can show benefits over diatoms such as reflecting longer term impacts through bioaccumulation (Kelly *et al.*, 2007), however invertebrates tend to be slower to respond to pollutant inputs than diatoms and therefore it may take a long time for the invertebrate community to stabilise to intermittent inputs such as misconnection discharges (Resh, 2008).

A major limitation found in this project was that the contribution of misconnection effluents to observed impacts could not easily be differentiated from that of large sewer systems, at which the most significant observed impacts occurred. Though attempts were made to identify species indicative of the impacts of detergent effluents, these did not prove effective in the field, probably because detergents did not cause a significant pressure in the field. It is therefore critical that future work develops the differentiation between impacts caused by run-off from large sewer systems, and that of sewer misconnections. The best approach to achieving this task would be to perform a study similar to that presented in Chapter 5, using invertebrate communities as well as diatom communities. Misconnections on the sewer systems could then be thoroughly investigated, identified, and corrected in each system. The biological communities would then be allowed to adapt to the misconnection free conditions. Another set of samples would then be collected once communities had reached equilibrium. The change in community composition, allowing for natural differences indicated in control sites, could then be directly related to the removal of misconnections from the system and therefore the contribution of misconnections could truly be identified. However, this approach would likely require cooperation from researchers or ecological consultancies, water companies, and local authorities in order to allow thorough data collection and analysis, and thorough and rapid misconnection identification and rectification within the time limits of a single project, which would be difficult. This would also be an expensive project, however this is the only clear way that the impacts of misconnections could accurately be separated from those of surface water run-off in the field.

6.4 Summary of thesis conclusions

The main conclusions of this project are:

1. Misconnection effluents can be observed and traced through sewer systems using inexpensive sampling of optical brighteners
2. Misconnection effluents can impact total algal abundance and diatom community composition, key components of freshwater ecosystem communities
3. Misconnection effluents did not affect diversity or Trophic Diatom Index values
4. In the field, misconnections generally do not cause strong impacts in diatom communities, due to the low concentrations of pollutants and intermittent nature of discharge
5. Where impacts are observed they occur over short distances downstream of the outfall, but are difficult to separate from background impacts of urban run off
6. Genus level identification of diatoms is often sufficient to observe the major trends in community responses
7. Diatom communities did not prove to be strong indicators of misconnection pollution
8. Individual misconnections are concluded not to be a significant threat to surface water diatom communities

Chapter 7

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Chapter 8

Appendices

A CD provided with this thesis contains the following appendices.

8.1 Appendix 1: Yorkshire Water report from sampling of optical brightener method presented in Chapter 3

A PDF document provided by Tom Gow-Smith and James Harrison at Yorkshire Water, detailing the sewer system sampling presented in Chapter 3.

8.2 Appendix 2: Diatom species data in Chapter 4

A Microsoft Excel spreadsheet of the raw species data collected for analyses presented in Chapter 4.

8.3 Appendix 3: Water chemistry of nutrient solutions in Chapter 4

A Microsoft Excel spreadsheet of the raw water chemistry data collected from nutrient solution analyses presented in Chapter 4.

8.4 Appendix 4: Diatom species data in Chapter 5

A Microsoft Excel spreadsheet of the raw species data collected for analyses presented in Chapter 5.

8.5 Appendix 5: Water chemistry of catchments in Chapter 5

A Microsoft Excel spreadsheet of the raw water chemistry data collected for each catchment in Chapter 5.

8.6 Appendix 6: Catchment variables of catchments in Chapter 5

A Microsoft Excel spreadsheet of the raw water sewer catchment and canopy cover data collected for each catchment in Chapter 5.

8.7 Appendix 7: Calculation of expected nutrient values in Chapter 4

A Microsoft Excel spreadsheet of the calculations of expected values for the nutrient and detergent solutions in Chapter 4.